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August 2010

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Table of Contents

Introduction iii

Bibliography

- **Biochemistry** 1
- **Cancer Research** 57
- **Development** 73
- **Cardiology** 483
- **Genetics** 499
- **Ecology** 1023
- **Hematology** 1041
- **Husbandry** 1077
- **Immunology** 1085
- **Neurology** 1119
- **Pharmacology** 1221
- **Physiology** 1253
- **Research Models** 1337
- **Toxicology** 1395

Selected Web Sites 1543

Introduction

The zebrafish, *Danio rerio,* was first recognized as a vertebrate research model in the 1970's when it was bred and studied in the laboratory of Dr. George Streisinger at the University of Oregon. His initial work led to the establishment of the Zebrafish International Resource Center (ZIRC), (http://zebrafish.org/zirc/home/guide.php) located at the University of Oregon, which is supported by grant P40 RR012546 from the NIH-National Center for Research Resources.

This bibliography organizes the published literature into fourteen areas of research where the zebrafish has been used as a model. They include: biochemistry, cancer research, cardiology, development, ecology, genetics, hematology, husbandry, immunology, neurology, pharmacology, physiology, research models and toxicology. The databases that were searched are national and international in scope and include numerous types of literature such as conference proceedings, abstracts, dissertations, patents, journal articles and others.

A March 2007 article by Angeleen Fleming (NC3Rs #10, March 2007, p. 1-7), *Zebrafish as an alternative model organism for disease modeling and drug discovery: implications for the 3Rs,* (http://www.nc3rs.org.uk/downloaddoc.asp?id=533&page=421&skin=0) provides additional information about the usef of zebrafish as an alternative model in biomedical research.

The following are some areas of research where zebrafish are being studied as a model.

Genetics: Bardot-Biedl Syndrome, Barth syndrome, Caveolin Associated Muscle Disease, Congenital Myopathy, Down Syndrome, Ferroportin mutations, Genomics, Hermansky-Pudlak syndrome, Martsolf Syndrome, Menke's Disease, Polycystic Kidney Disease, Pyruvate dehydrogenase deficiency, Warburg micro syndrome;

Circulation: Aortic Arch Defects, Angiogenesis, Cardia Bifida, Cardiac regeneration, Congenital Heart Disease, Coronary Heart Disease, Embryonic myelopoiesis, Hematologic disorders, Hemophilia, Hemostasis, Iron deficiency, Lethal Cardiac Arrhythmias, Thrombosis;

Neurology: Acupuncture, Alzheimer's Disease, Amyotrophic Lateral Sclerosis, Autism, Developmental neurotoxicity, Molecular neurobiology, Neurotoxicology, Parkinson's Disease, Tauopathy;

Cancer: Apoptosis, Cancer cell migration, Carcinogenesis, Colon cancer, Embryonal Rhabdomyosarcoma , Germ cell anerploidy, Ionizing radiation, Leukemia, Pancreatic cancer;

Vision: Cataracts, Choroideremia, Cyclopia, Eye development, Night blindness, Retinal degeneration, Visual disorders;

Immunology: DiGeorge Syndrome, Goodpasture Syndrome, Immune system, Lymphangiogenesis, Mycobacterium pathogenesis, Septicemia, Vaccines, Viral hemorrhagic septicemia, Viral typhimurium infection, Virology;

Endocrinology: Diabetes, Hyperglycemia, Hypogonadism, Lipid metabolism;

Toxicology: Bisphenol A, Cannabis, Developmental toxicology, Ethanol;

Aging: Aging physiology, Functional aging, Senescence;

Hearing: Hearing loss, Sensorineural hearing loss;

Liver: Hepatitis, Steatohepatitis;

Musculature: Muscular Dystrophy, Spinal Muscular Atrophy;

Digestion: Inflammatory bowel syndrome

Skeletal: Osteoporosis

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Biochemistry

Abe, T., T. Mikekado, S. Haga, Y. Kisara, K. Watanabe, T. Kurokawa, and T. Suzuki (2007). **Identification, cDNA cloning, and mRNA localization of a zebrafish ortholog of leukemia inhibitory factor.** *Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology* 147(1): 38-44. ISSN: 0305 0491.

NAL Call Number: QP501.C6

Abstract: Leukemia inhibitory factor (LIF) maintains embryonic stem cells in an undifferentiated state. To establish stable cultures of zebrafish embryonic stem cells, recombinant zebrafish LIF (zLIF) is needed because the LIF sequence varies greatly between species. In the current study, we identified the zebrafish (Danio rerio) and pufferfish (Tetraodon nigroviridis) orthologs of lif from genomic databases, and we isolated a cDNA encoding zLIF. Synteny analysis and comparison of sequences identified zebrafish and Tetraodon orthologs of human LIF. The cDNA for zLIF encoded a predicted 215-amino acid protein with a putative 32-amino acid signal peptide, two disulfide bonds, and two N-linked glycosylation sites. We found that transcription of zlif starts at the hatching period during embryogenesis and is present in the brain, visceral organs, bone, and skin.

Descriptors: zebrafish, complementary DNA genetics, leukemia inhibitory factor genetics, leukemia inhibitory factor metabolism, RNA transport, amino acid sequence homology, metabolism, amino acid , base sequence, chromosomes genetics, molecular molecular cloning, genetic databases, gene expression profiling , gene expression regulation, genome, leukemia inhibitory factor chemistry, molecular sequence data, messenger RNA genetics and metabolism, sequence analysis, synteny genetics, Takifugu genetics.

Agaba, M.K., D.R. Tocher, Zheng XiaoZhong, C.A. Dickson, J.R. Dick, and A.J. Teale (2005). **Cloning and functional characterisation of polyunsaturated fatty acid elongases of marine and freshwater teleost fish**. *Comparative Biochemistry and Physiology B, Biochemistry and Molecular Biology* 142(3): 342-352. ISSN: 0305 0491.

NAL Call Number: QP501.C6

Abstract: Enzymes that lengthen the carbon chain of polyunsaturated fatty acids are key to the biosynthesis of the highly unsaturated fatty acids, arachidonic, eicosapentaenoic and docosahexaenoic acids from linoleic and alpha -linolenic acids. A Mortierella alpina cDNA polyunsaturated fatty acid elongase sequence identified mammalian, amphibian, zebrafish and insect expressed sequence tags (ESTs) in GenBank. Consensus primers were designed in conserved motifs and used to isolate full length cDNA from livers of several fish species by Rapid Amplification of cDNA Ends (RACE). The amplified cDNAs encoded putative open reading frames (ORFs) of 288-294 amino acids that were highly conserved among the fish species. Heterologous expression in yeast, *Saccharomyces cerevisiae*, demonstrated that all of the ORFs encoded elongases with the ability to lengthen polyunsaturated fatty acid substrates with chain lengths from C18 to C22 and also monounsaturated fatty acids, but not saturated fatty acids. There were differences in the functional competence of the elongases from different fish species. Most of the fish elongases showed a pattern of activity towards different fatty acid substrates in the rank order C18 > C20 > C22, although the tilapia and turbot elongases had similar activity towards 18:4n-3 and 20:5n-3. The fish elongases generally showed greater activity or similar activities with n-3 than with n-6 homologues, with the exception of the cod enzyme which was more active towards n-6 fatty acids. Copyright © 2008 CABI

Descriptors: zebrafish, characterization, complementary DNA, DNA cloning, enzyme activity, enzymes, fatty acids, genes, liver, monoenoic fatty acids, open reading frames, polyenoic

fatty acids, species differences, *clarias gariepinus*, cod, fishes, *gadus morhua*, *Oreochromis niloticus*, *Pagrus aurata*, *pleuronectiformes*, sea bream, turbot, zebrafish.

Airaksinen, S., T. Jokilehto, C.M. Rabergh, and M. Nikinmaa (2003). **Heat- and cold-inducible regulation of HSP70 expression in zebrafish ZF4 cells.** *Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology* 136(2): 275-82. ISSN: 0305 0491.
NAL Call Number: QP501.C6
Abstract: Elevated temperature induces a rapid heat shock transcription factor (HSFs)-mediated expression of heat shock (hsp) genes. The effect of cold exposure on hsp gene expression has hardly been investigated, although ectothermic animals experience both cold and heat stress. We have previously shown in zebrafish that the expression of hsf1a and a unique isoform hsf1b vary in a tissue-specific manner upon heat stress. In the current study, using a zebrafish (Danio rerio) embryonic cell line (ZF4), we have compared the effects of heat shock (28-->37 degrees C) vs. cold shock (28-->20 degrees C) on the expression of ahsf1a, zhsf1b and hsp70. Concomitantly, the suitability of the ZF4 cells as a model system was verified. The expression pattern of HSP70 proteins following heat or cold exposure is distinct, and the total HSP70 level is upregulated or stable, respectively. Moreover, heat exposure specifically increases the ratio of zhsf1a/b expression (10-fold), whereas cold exposure decreases it to one half. These data suggest that the zhsf1a/zhsf1b ratio is regulated in a temperature-dependent manner, and the ratio may be indicative of the stressor-specific HSP70 expression. Furthermore, the response in ZF4 cells upon heat shock resembles the response observed in zebrafish liver and thus, supports the use of this cell line in stress response studies.
Descriptors: zebrafish, cold, gene expression regulation, HSP70 heat shock proteins genetics, cold shock, heat, heat shock response genetics, cell line, protein isoforms , messenger RNA genetics and metabolism, time factors, transcription factors genetics.

Andersen, L., P. Bjerregaard, and B. Korsgaard (2003). **Vitellogenin induction and brain aromatase activity in adult male and female zebrafish exposed to endocrine disrupters**. *Fish Physiology and Biochemistry* 28(1-4): 319-321. ISSN: 0920-1742.
NAL Call Number: QL639.1F583
Descriptors: zebrafish, bioassay, brain enzymes, chemical pollutants, drugs, endocrinology, enzymatic activity, females, males, physiology, pollution effects, sex hormones, toxicity, toxicity testing, toxicity tests, vitellogenesis, water pollution effects, *Danio rerio*, brain aromatase activity, endocrine disruptors.

Andersen, L., K. Kinnberg, H. Holbech, B. Korsgaard, and P. Bjerregaard (2004). **Evaluation of a 40 day assay for testing endocrine disrupters: effects of an anti-estrogen and an aromatase inhibitor on sex ratio and vitellogenin concentrations in juvenile zebrafish (*Danio rerio*)**. *Fish Physiology and Biochemistry* 30(3-4): 257-266. ISSN: 0920-1742.
NAL Call Number: QL639.1F583
Descriptors: zebrafish, aromatase, assay, depleted stocks, differentiation, ELISA, endocrinology, environmental impact, enzyme-linked immunosorbent assay, evaluation, exposure, gonads, inhibitors, reproduction, screening, sex ratio, toxicity tests, vitellogenesis, vitellogenin, water pollution effects , endocrine disruptors, *Danio rerio* .

Ashworth, R., B. Devogelaere, J. Fabes, R.E. Tunwell, K.R. Koh, H. De Smedt, and S. Patel (2007). **Molecular and functional characterization of inositol trisphosphate receptors during early zebrafish development.** *Journal of Biological Chemistry* 282(19): 13984-93. ISSN: 0021-9258.
NAL Call Number: 381J824

Abstract: Fluctuations in cytosolic Ca(2+) are crucial for a variety of cellular processes including many aspects of development. Mobilization of intracellular Ca(2+) stores via the production of inositol trisphosphate (IP(3)) and the consequent activation of IP(3)-sensitive Ca(2+) channels is a ubiquitous means by which diverse stimuli mediate their cellular effects. Although IP(3) receptors have been well studied at fertilization, information regarding their possible involvement during subsequent development is scant. In the present study we examined the role of IP(3) receptors in early development of the zebrafish. We report the first molecular analysis of zebrafish IP(3) receptors which indicates that, like mammals, the zebrafish genome contains three distinct IP(3) receptor genes. mRNA for all isoforms was detectable at differing levels by the 64 cell stage, and IP(3)-induced Ca(2+) transients could be readily generated (by flash photolysis) in a controlled fashion throughout the cleavage period in vivo. Furthermore, we show that early blastula formation was disrupted by pharmacological blockade of IP(3) receptors or phospholipase C, by molecular inhibition of the former by injection of IRBIT (IP(3) receptor-binding protein released with IP(3)) and by depletion of thapsigargin-sensitive Ca(2+) stores after completion of the second cell cycle. Inhibition of Ca(2+) entry or ryanodine receptors, however, had little effect. Our work defines the importance of IP(3) receptors during early development of a genetically and optically tractable model vertebrate organism.
Descriptors: zebrafish, embryo, metabolism, developmental gene expression regulation, inositol 1,4,5 trisphosphate receptors genetics, calcium metabolism, enzyme inhibitors pharmacology, inositol 1,4,5 trisphosphate receptors classification, inositol 1,4,5 trisphosphate receptors metabolism, messenger RNA genetics and metabolism, reverse transcriptase PCR, ryanodine receptor calcium release channel metabolism, thapsigargin pharmacology.

Atilla Gokcumen, G., D.S. Williams, H. Bregman, N. Pagano, and E. Meggers (2006). **Organometallic compounds with biological activity: a very selective and highly potent cellular inhibitor for glycogen synthase kinase 3**. *Chembiochem* 7(9): 1443-1450. ISSN: 1439 4227.
NAL Call Number: QD415.A1C34
Abstract: A chiral second-generation organoruthenium half-sandwich compound is disclosed that shows a remarkable selectivity and cellular potency for the inhibition of . The selectivity was evaluated against a panel of 57 protein kinases, in which no other kinase was inhibited to the some extent, with a selectivity window of at least tenfold to more than 1000-fold at 100 mu m ATP Furthermore, a comparison with organic GSK-3 inhibitors demonstrated the superior cellular activity of this ruthenium compound: wnt signaling was fully induced at concentrations down to 30 nM. For comparison, the well-established organic GSK-3 inhibitors 6-bromoindirubin-3'-oxime (BIO) and kenpaullone activate the wnt pathway at concentrations that are higher by around 30-fold and 100-fold respectively. The treatment of zebrafish embryos with the organometallic inhibitor resulted in a phenotype that is typical for the inhibition of GSK-3. No phenotypic change was observed with the mirror-imaged ruthenium complex. The latter does not, in fact, show any of the pharmacological properties for the inhibition of GSK-3, Overall, these results demonstrate the potential usefulness of organometallic compounds as molecular probes in cultured cells and whole organisms. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, enzymology, zebrafish, biochemistry, molecular biophysics, glycogen synthase kinase 3 (gSK-3).

Azuma, M., R. Toyama, E. Laver, and I.B. Dawid (2006). **Perturbation of rRNA synthesis in the bap28 mutation leads to apoptosis mediated by p53 in the zebrafish central nervous system.** *Journal of Biological Chemistry* 281(19): 13309-16. ISSN: 0021-9258.
NAL Call Number: 381J824
Abstract: Zebrafish is a powerful vertebrate model system for using forward genetics to elucidate

mechanisms of early development. We have used chemical mutagenesis to screen for mutants that show defects in the CNS. Here we describe the isolation of the bap28 mutation that leads to abnormalities in the brain starting at midsomitogenesis stages. Mutant embryos display excess apoptosis primarily in the central nervous system (CNS) and die by days 6-7 after fertilization. The mutation was positionally cloned and shown to affect a gene that encodes a large protein with high similarity to the uncharacterized human protein BAP28 and lower similarity to yeast Utp10. Utp10 is a component of a nucleolar U3 small nucleolar RNA-containing RNP complex that is required for transcription of ribosomal DNA and for processing of 18 S rRNA. We show that zebrafish Bap28 likewise is required for rRNA transcription and processing, with a major effect on 18 S rRNA maturation. We suggest that bap28 is required for cell survival in the CNS through its role in rRNA synthesis and processing. Inhibition of p53 protein expression in bap28 mutants led to embryos with morphologically normal appearance, suggesting that p53 is involved in triggering apoptosis in the bap28 mutant CNS. The bap28 mutation provides a genetic approach to study the role of ribosome biogenesis in the development of a vertebrate embryo.
Descriptors: zebrafish, apoptosis physiology, central nervous system metabolism, RNA, ribosomal biosynthesis, tumor suppressor protein p53 metabolism, brain growth and development, brain metabolism, developmental gene expression regulation, mutation, ribosomal RNA 18s metabolism, ribosomes metabolism, zebrafish.

Bessiere, D., C. Lacroix, S. Campagne, V. Ecochard, V. Guillet, L. Mourey, F. Lopez, J. Czaplicki, P. Demange, A. Milon, J.-P. Girard, and V. Gervais (2008). **Structure-function analysis of the THAP zinc finger of THAP1, a large C2CH DNA-binding module linked to Rb/E2F pathways**. *Journal of Biological Chemistry* 283(7): 4352-4363. ISSN: 0021-9258.
NAL Call Number: 381J824
Descriptors: zebrafish, *Danio rerio*, Drosophila, Nematoda, arginine, cell cycle, cell proliferation, DNA, DNA-binding protein, insertion, lysine, magnetic resonance spectroscopy, mutagenesis, N.M.r., nucleotide sequence, retinoblastoma protein, scanning mutagenesis, structure-function relationships, zinc, zinc finger proteins, double prime E2f protein, double prime p elements, transposase.

Biga, P.R., S.B. Roberts, D.B. Iliev, L.A.R. McCauley, J.S. Moon, P. Collodi, and F.W. Goetz (2005). **The isolation, characterization, and expression of a novel GDF11 gene and a second myostatin form in zebrafish, *Danio rerio***. *Comparative Biochemistry and Physiology B, Biochemistry and Molecular Biology* 141(2): 218-230. ISSN: 0305 0491.
NAL Call Number: QP501.C6
Abstract: In the current study, the first non-mammalian growth/differentiation factor (GDF) 11-like homolog was cloned from zebrafish. At the nucleotide level, zebrafish GDF11 is most similar to human GDF11 (79%), while the peptide is most similar to mouse GDF11 (78%). Phylogenetic analysis showed that the zebrafish GDF11 clusters with mammalian GDF11s. This study also cloned a second MSTN form in zebrafish most similar to Salmonid MSTN2 forms. Based on real time PCR, GDF11 is expressed in multiple adult tissues, with levels highest in whole heads and gonads, and expression is less ubiquitous when compared to MSTN expression. During embryonic development, real time PCR demonstrated increasing GDF11 mRNA levels 10 h post-fertilization (hpf), while MSTN mRNA levels remain low until 48 hpf. This is the first report of a transforming growth factor (TGF)- beta superfamily member in a non-mammalian species that is more closely related to GDF11 than MSTN, and also a second form of MSTN in zebrafish; suggesting that a more complex TGF- beta superfamily array exists in primitive vertebrates than previously thought. Copyright © 2008 CABI
Descriptors: zebrafish, amino acid sequences, characterization, complementary DNA, DNA

cloning, gene expression, genes, isolation, nucleotide sequences, phylogeny, transforming growth factor, *Danio rerio*, fishes.

Bink, R.J., H. Habuchi, Z. Lele, E. Dolk, J. Joore, G.J. Rauch, R. Geisler, S.W. Wilson, J. den Hertog, K. Kimata, and D. Zivkovic (2003). **Heparan sulfate 6-o-sulfotransferase is essential for muscle development in zebrafish.** *Journal of Biological Chemistry* 278(33): 31118-27. ISSN: 0021-9258.
NAL Call Number: 381J824
Abstract: Heparan sulfate proteoglycans function in development and disease. They consist of a core protein with attached heparan sulfate chains that are altered by a series of carbohydrate-modifying enzymes and sulfotransferases. Here, we report on the identification and characterization of a gene encoding zebrafish heparan sulfate 6-O-sulfotransferase (hs6st) that shows high homology to other heparan sulfate 6-O-sulfotransferases. When expressed as a fusion protein in cultured cells, the protein shows specific 6-O-sulfotransferase activity and preferentially acts on the iduronosyl N-sulfoglycosamine. In the developing embryo, hs6st is expressed in the brain, the somites, and the fins; the same structures that were affected upon morpholino-mediated functional knockdown. Morpholino injections significantly inhibited 6-O- but not 2-O-sulfation as assessed by HPLC. Morphants display disturbed somite specification independent of the somite oscillator mechanism and have impaired muscle differentiation. In conclusion, our results show that transfer of sulfate to specific positions on glycosaminoglycans is essential for muscle development.
Descriptors: zebrafish, heparan sulfate proteoglycans metabolism, skeletal muscle embryology, skeletal muscle enzymology, sulfotransferases genetics, sulfotransferases metabolism, amino acid sequence, cell differentiation, developmental gene expression regulation, enzymologic gene expression regulation, molecular sequence data, skeletal muscle cytology, antisense oligonucleotides pharmacology, phenotype, somites enzymology.

BiochemistryMiguel-Queralt, S., M. Knowlton, G.V. Avvakumov, R. Al Nouno, G.M. Kelly, and G.L. Hammond (2004). **Molecular and functional characterization of sexhormone binding globulin in zebrafish**. *Endocrinology* 145(11): 5221-5230. ISSN: 0013 7227.
NAL Call Number: 448.8EN2
Abstract: SHBG (sex hormone binding globulin) transports androgens and estrogens in the blood of vertebrates including fish. Orthologs of SHBG in fish are poorly defined, and we have now obtained a zebrafish SHBG cDNA and characterized the zebrafish SHBG gene and protein through molecular biological, biochemical, and informatics approaches. Amino-terminal analysis of zebrafish SHBG indicated that its deduced precursor sequence includes a 25-residue secretion polypeptide and exhibits 22-27% homology with mammalian SHBG sequences and 41% with a deduced fugufish SHBG sequence. The 356-residue mature zebrafish SHBG (39,243 Da) sequence comprises a tandem repeat of laminin G-like domains typical of SHBG sequences; contains three N-glycosylation sites; and exists as a 105,000+or-8700 Da homodimer. Zebrafish SHBG exhibits a high affinity and specificity for sex steroids. An RT-PCR indicated that SHBG mRNA first appears in zebrafish larva, and SHBG mRNA was localized within the liver and gut at this stage of development by whole-mount in situ hybridization. In adult fish, SHBG mRNA was found in liver, testis, and gut. In the liver, immunoreactive SHBG was present in hepatocytes and concentrated in intrahepatic bile duct cells, whereas in the testis it was confined to cells surrounding the seminiferous tubule cysts. In the intestine, immunoreactive SHBG was present in the stroma and epithelial cells of the villous projections and the surrounding muscle. The production and presence of SHBG in the gut of developing and adult zebrafish suggests a novel role for this protein in regulating sex steroid action at this site. Copyright © 2008 CABI

Descriptors: zebrafish, age differences , androgens, complementary DNA, genes, laminins, larvae, liver, messenger RNA, estrogens, polypeptides, stomach, testes, Danio rerio, zebrafish.

Boesch, S.T., B. Eller, and B. Pelster (2003). **Expression of two isoforms of the vacuolar-type ATPase subunit B in the zebrafish *Danio rerio*.** *Journal of Experimental Biology* 206(Pt 11): 1907-15. ISSN: 0022-0949.

NAL Call Number: 442.8B77

Abstract: In the present study we tested the hypothesis that two isoforms of the regulatory subunit B of vacuolar-type ATPase (V-ATPase) are expressed in the zebrafish Danio rerio. The complete coding sequences for both isoforms, vatB1 and vatB2, were cloned and sequenced. BLASTX analysis revealed the greatest similarity to amino acid sequences of B subunits from the European eel Anguilla anguilla and rainbow trout Oncorhynchus mykiss. The isoforms were expressed in a bacterial system and the recombinant proteins verified using isoform-specific antibodies directed against vatB isoforms of the eel. The distribution of both isoforms in zebrafish tissues was investigated using reverse transcriptase-polymerase chain reaction and western blot analysis. The results revealed that at the RNA level both isoforms were expressed in all tested organs, i.e. the gills, swimbladder, heart, kidney, liver, spleen, intestine and skeletal muscle. At the protein level, however, there were tissue-specific variations in the levels of the two vatB isoforms expressed. The highest amounts of V-ATPase were detected in total protein preparations from gill, heart and liver tissue. In liver tissue, however, the western blot analysis indicated that vatB1 was not as prominent as vatB2, and immunohistochemistry revealed that antibodies directed against vatB1 yielded a very weak staining in a number of cells, while an antibody directed against vatB1 and vatB2 yielded a strong staining in virtually every cell. Similarly, neurosecretory cells of the small intestine were stained with an antibody directed against vatB1 and vatB2, but not with an antibody specific for vatB1. Therefore we conclude that the differential expression of two isoforms of the V-ATPase subunits, which may serve different functions as in several mammalian species, may also be a common phenomenon in teleost fish.

Descriptors: zebrafish, gene expression profiling, vacuolar proton translocating ATPases genetics, vacuolar proton translocating ATPases metabolism, amino acid sequence, Western, DNA primers, immunohistochemistry, isoenzymes genetics, isoenzymes metabolism, molecular sequence data, sequence alignment.

Bolanos Garcia, V.M., J. Fernandez Recio, J.E. Allende, and T.L. Blundell (2006). **Identifying interaction motifs in CK2 beta - a ubiquitous kinase regulatory subunit**. *Trends in Biochemical Sciences* 31(12): 654-661. ISSN: 0968 0004.

NAL Call Number: QH345.T73

Abstract: Casein kinase 2 (CK2) is probably the most ubiquitous serine/threonine kinase found in eukaryotes: it phosphorylates >300 cellular proteins, ranging from transcription factors to proteins involved in chromatin structure and cell division. CK2 is a heterotetrameric enzyme that induces neoplastic growth when overexpressed. The beta subunit of CK2 (CK2 beta) functions as the regulator of the catalytic CK2 alpha and CK2 alpha ‘ subunits, enhancing their stability, activity and specificity. However, CK2 beta also functions as a multisubstrate docking platform for several other binding partners. Here, we discuss the organization and roles of interaction motifs of CK2 beta , postulate new protein-interaction sites and map these to the known interaction motifs, and show how the resulting complexity of interactions mediated by CK2 gives rise to the versatile functions of this pleiotropic protein kinase. Copyright © 2008 CABI

Descriptors: zebrafish, *Arabidopsis thaliana*, *caenorhabditis elegans* , *Danio rerio*, *humans*, *Saccha-*

romyces cerevisiae, *Xenopus laevis*casein kinase, enzyme activity, enzymes, phosphorylation, protein kinase, serine, threonine .

Bosworth, C.A.4., C.W. Chou, R.B. Cole, and B.B. Rees (2005). **Protein expression patterns in zebrafish skeletal muscle: initial characterization and the effects of hypoxic exposure.** *Proteomics* 5(5): 1362-71. ISSN: 1615-9853.

NAL Call Number: QP752.F3PF76

Abstract: Patterns of protein expression were examined in white skeletal muscle from adult zebrafish (*Danio rerio*). High resolution two-dimensional gel electrophoresis resolved between 300 and 400 spots with molecular masses between 20 and 120 kDa and isoelectric points between about 5 and 8. Forty spots, representing a range of protein size, charge, and abundance were excised, digested with trypsin, and subjected to matrix-assisted laser-desorption/ionisation-time of flight mass spectrometry for protein identification. Twenty-nine spots were identified, including enzymes of energy metabolism, contractile proteins, an iron transport protein, and a heat shock protein. In addition, several spots matched theoretical proteins predicted from genome sequencing. These theoretical proteins were tentatively identified by similarity to known proteins. Patterns of muscle protein expression were then measured after zebrafish were exposed to low oxygen (16 torr) for 48 h, an exposure previously shown to increase the survival of zebrafish at more severe reductions in oxygen. Exposure to low oxygen (hypoxia) did not change the general pattern of protein expression but did affect the amounts of six low abundance proteins. The relatively subtle effects of hypoxia on patterns of muscle protein expression contrasts the widespread changes previously documented in mRNA levels in this and other species of fish during hypoxic stress. The difference between protein and mRNA expression illustrates the need to integrate both measures for a more complete understanding of gene expression in fish during hypoxic exposure.

Descriptors: zebrafish, anoxia, muscle proteins analysis, skeletal muscle chemistry, two dimensional gel electrophoresis, molecular sequence data, molecular weight, skeletal muscle anatomy and histology, skeletal muscle physiology, oxygen metabolism, random allocation, spectrometry, mass, matrix assisted laser desorption ionization.

Brennan, C., M. Mangoli, C.E. Dyer, and R. Ashworth (2005). **Acetylcholine and calcium signalling regulates muscle fibre formation in the zebrafish embryo.** *Journal of Cell Science* 118(22): 5181-90. ISSN: 0021 9533.

NAL Call Number: QH301.J6

Abstract: Nerve activity is known to be an important regulator of muscle phenotype in the adult, but its contribution to muscle development during embryogenesis remains unresolved. We used the zebrafish embryo and in vivo imaging approaches to address the role of activity-generated signals, acetylcholine and intracellular calcium, in vertebrate slow muscle development. We show that acetylcholine drives initial muscle contraction and embryonic movement via release of intracellular calcium from ryanodine receptors. Inhibition of this activity-dependent pathway at the level of the acetylcholine receptor or ryanodine receptor did not disrupt slow fibre number, elongation or migration but affected myofibril organisation. In mutants lacking functional acetylcholine receptors myofibre length increased and sarcomere length decreased significantly. We propose that calcium is acting via the cytoskeleton to regulate myofibril organisation. Within a myofibre, sarcomere length and number are the key parameters regulating force generation; hence our findings imply a critical role for nerve-mediated calcium signals in the formation of physiologically functional muscle units during development.

Descriptors: zebrafish, acetylcholine pharmacology, calcium metabolism, calcium signaling physiology, muscle fibers metabolism, drug effects, muscles embryology, embryology, acetylcholine metabolism, amino acid sequence, bungarotoxins pharmacology, calcium channels, l type

metabolism, cholinergic antagonists pharmacology, cytosol metabolism, molecular sequence data, muscle fibers cytology, muscle fibers drug effects, muscles metabolism, phylogeny, cholinergic receptors deficiency, nicotinic receptors, metabolism, ryanodine pharmacology, ryanodine receptor calcium release channel metabolism, sequence alignment, somites drug effects, metabolism.

Buckingham, S.D. and D.W. Ali (2004). **Sodium and potassium currents of larval zebrafish muscle fibres.** *Journal of Experimental Biology* 207(5): 841-852. ISSN: 0022 0949.
NAL Call Number: 442.8B77
Abstract: The steady-state and kinetic properties of Na+ and K+ currents of inner (white) and outer (red) muscles of zebrafish larvae 4-6 days post-fertilization (d.p.f.) are described. In inner muscle, the outward currents were half-activated at -1.0 mV and half-inactivated at -30.4 mV, and completely inactivated within 100 ms of depolarization. The inward currents of inner fibres were half-activated at -7.3 mV and half-inactivated at -74.5 mV and completely inactivated within 5 ms of depolarization. Inner muscle fibres were found to support action potentials, while no action potentials could be evoked in outer muscles. In inner muscle fibres, all tested levels of depolarizing current above a threshold value evoked only one action potential. However, spiking at frequencies of up to 200 cycles s-1 was evoked by the injection of depolarizing pulses separated by short hyperpolarizing currents. We suggest that the properties of the inward sodium and outward potassium currents permit high frequency firing in response to a pulsatile depolarizing input of the kind expected in fast swimming, whilst safeguarding against tetany during a strong depolarization.
Descriptors: zebrafish, *Danio rerio*, ion and water relations, sodium and potassium currents, inorganic substances, steady state and kinetic properties in red and white muscle fibres, functional implications, larvae, skeletal musculature, red and white muscle fibres.

Capaldi, S., M. Guariento, G. Saccomani, D. Fessas, M. Perduca, and H.L. Monaco (2007). **A single amino acid mutation in zebrafish (*Danio rerio*) liver bile acid-binding protein can change the stoichiometry of ligand binding.** *Journal of Biological Chemistry* 282(42): 31008-18. ISSN: 0021-9258.
NAL Call Number: 381J824
Abstract: In all of the liver bile acid-binding proteins (L-BABPs) studied so far, it has been found that the stoichiometry of binding is of two cholate molecules per internal binding site. In this paper, we describe the expression, purification, crystallization, and three-dimensional structure determination of zebrafish (*Danio rerio)* L-BABP to 1.5A resolution, which is currently the highest available for a protein of this family. Since we have found that in zebrafish, the stoichiometry of binding in the protein cavity is of only one cholate molecule per wild type L-BABP, we examined the role of two crucial amino acids present in the binding site. Using site-directed mutagenesis, we have prepared, crystallized, and determined the three-dimensional structure of co-crystals of two mutants. The mutant G55R has the same stoichiometry of binding as the wild type protein, whereas the C91T mutant changes the stoichiometry of binding from one to two ligand molecules in the cavity and therefore appears to be more similar to the other members of the L-BABP family. Based on the presence or absence of a single disulfide bridge, it can be postulated that fish should bind a single cholate molecule, whereas amphibians and higher vertebrates should bind two. Isothermal titration calorimetry has also revealed the presence in the wild type protein and the G55R mutant of an additional binding site, different from the first and probably located on the surface of the molecule.
Descriptors: zebrafish, amino acid substitution, carrier proteins chemistry, cholic acid chemistry , membrane glycoproteins chemistry, metabolism, proteins chemistry, amphibia genetics , amphibia metabolism, amphibian proteins chemistry, amphibian amphibian proteins metabo-

lism, binding sites genetics, carrier carrier proteins metabolism, cholic acid metabolism, crystallography, x ray, disulfides chemistry, ligands, liver chemistry, liver metabolism, membrane glycomembrane glycoproteins metabolism, site directed mutagenesis missense mutation, protein binding, tertiary protein structure, structural protein homology.

Castellano, S., A.V. Lobanov, C. Chapple, S.V. Novoselov, M. Albrecht, D. Hua, A. Lescure, T. Lengauer, A. Krol, V.N. Gladyshev, and R. Guigo (2005). **Diversity and functional plasticity of eukaryotic selenoproteins: Identification and characterization of the SelJ family**. *Proceedings of the National Academy of Sciences, USA* 102(45): 16188-16193. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, *Danio rerio*, echinoidea, heart urchins, sand dollars, sea urchins, developmental stages, evolution, freshwater fish, gene expression, genetic diversity, marine invertebrates, phylogenetics, plasticity, population genetics, proteins, species diversity.

Chambers, S.P., L.V. Anderson, G.M. Maguire, A. Dodd, and D.R. Love (2003). **Sarcoglycans of the zebrafish: orthology and localization to the sarcolemma and myosepta of muscle.** *Biochemical and Biophysical Research Communications* 303(2): 488-95. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: The zebrafish is an established model of vertebrate development and is also receiving increasing attention in terms of human disease modelling. In order to provide experimental support to realize this modelling potential, we report here the identification of apparent orthologues of many critical members of the dystrophin-associated glycoprotein complex (DGC) that have been implicated in a diverse range of neuromuscular disorders. In addition, immunohistochemical studies show the localization of the DGC to the sarcolemma of adult zebrafish muscle and in particular the myosepta. Together, these data suggest that the DGC in adult zebrafish may play a highly conserved functional role in muscle architecture that, when disrupted, could offer insight into human neuromuscular disease processes.
Descriptors: zebrafish, cytoskeletal proteins metabolism, membrane glycoproteins metabolism, muscle proteins metabolism, skeletal metabolism, sarcolemma ultrastructure, amino acid sequence, cytoskeletal proteins analysis, cytoskeletal proteins chemistry, dystroglycans, immunohistochemistry, membrane glycoproteins analysis, membrane glycoproteins chemistry, molecular sequence data, muscle proteins analysis, muscle proteins chemistry, skeletal muscle ultrastructure, phylogeny, sarcolemma metabolism, sequence alignment, amino acid sequence homology.

Chang, M.H., C.J. Huang, S.P. Hwang, I.C. Lu, C.M. Lin, T.F. Kuo, and C.M. Chou (2004). **Zebrafish heparin-binding neurotrophic factor enhances neurite outgrowth during its development.** *Biochemical and Biophysical Research Communications* 321(2): 502-9. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: Heparin-binding neurotrophic factor (HBNF) is a secreted heparin-binding protein containing highly basic and cysteine-rich amino acid residues. In this study, we cloned the full-length HBNF cDNA from zebrafish and determined its genomic structure by bioinformatics analysis. Zebrafish HBNF gene is composed of five exons and four introns spanning approximately 82kb. RT-PCR analysis revealed that zebrafish HBNF transcript was highly expressed in adult brain and intestine tissues while less in other tissues. During embryogenesis, zebrafish HBNF transcript was observed to be moderately expressed at earlier stages with a gradual decline. Higher expression level was observed after hatching and maintaining this level into adulthood. The overall amino acid sequence of zebrafish HBNF shows 60% identity to human HBNF, but with approximately 40% identity to other midkine proteins. Like mammalian homolog, zebrafish HBNF could induce significant neurite outgrowth in PC12 cells without NGF stimulation. In

addition, zebrafish HBNF was able to enhance extensive neurite outgrowth in zebrafish embryos during embryogenesis. In summary, a feasible in vivo assay for neurite outgrowth was established in zebrafish.

Descriptors: zebrafish, carrier carrier proteins metabolism, cytokines genetics, cytokines metabolism, neurites metabolism, amino acid sequence, carrier proteins chemistry, carrier proteins pharmacology, cell size drug effects, molecular cloning, cytokines chemistry, cytokines pharmacology, complementary DNA genetics, embryo cytology, embryo drug effects, embryo metabolism, gene expression profiling, developmental gene expression regulation, molecular sequence data, neurites drug effects, pc12 cells, messenger RNA genetics and metabolism, rats, reverse transcriptase PCR, sequence alignment.

Chen, J., J. Chen, and J. Wu (2003). **Molecular cloning and functional analysis of zebrafish high-density lipoprotein-binding protein.** *Comparative Biochemistry and Physiology Part B Biochemistry and Molecular Biology. 2003 Sept; 136(1): 117 130.: Comparative Biochemistry and Physiology Part B Biochemistry and Molecular Biology* 136(1): 117-130. ISSN: 1096 4959.

NAL Call Number: QP501.C6

Abstract: High-density lipoprotein-binding protein (HBP) plays a pivotal role in the endocrine regulation of both lipids and cholesterol. This first study of the zebrafish (*Danio rerio*) HBP gene in a piscine provides information on the complex molecular events that regulates lipid and cholesterol functions in fish, and allows a comparison with starvation and hormonal regulation. One identical zebrafish HBP cDNA clone was obtained from a 24-h-old zebrafish cDNA library. Zebrafish HBP is composed of 1273 amino acids as residues. The 1273-aa of HBP has 87.8% and 87.0% similarities to human and chicken HBP, respectively. Real-time reverse transcription polymerase chain reaction (RT-PCR) analysis showed that HBP is highly expressed in the 36 h of the developmental stage after fertilization as compared to other stages. As to tissue-specific expression, the HBP is highly expressed in the fin, liver and ovary. In the starvation experiment, results show significant differences between the control group and the group after 3-week starvation. After injecting GH, IGF-I, IGF-II or insulin, no significant differences were shown between the control and the experimental groups. These results suggest that in vivo HBP expression is not regulated by the insulin family or by growth hormone, but other factors present during the starvation may down- or up-regulate the HBP. Although the exact function of the HBP is unknown, its high expression in the liver and ovary suggests a role for this molecule in the cumulative efficiency of fish intake of food or lipid transfer; these results can possibly be applied to aquaculture in the near future.

Descriptors: zebrafish, high density lipoprotein, binding proteins, developmental stages, hormonal regulation.

Chen, X., G. Polleichtner, I. Kadurin, and S. Grunder (2007). **Zebrafish acid-sensing ion channel (ASIC) 4, characterization of homo- and heteromeric channels, and identification of regions important for activation by H+.** *Journal of Biological Chemistry* 282(42): 30406-13. ISSN: 0021-9258.

NAL Call Number: 381J824

Abstract: There are four genes for acid-sensing ion channels (ASICs) in the genome of mammalian species. Whereas ASIC1 to ASIC3 form functional H+-gated Na+ channels, ASIC4 is not gated by H+, and its function is unknown. Zebrafish has two ASIC4 paralogs: zASIC4.1 and zASIC4.2. Whereas zASIC4.1 is gated by extracellular H+, zASIC4.2 is not. This differential response to H+ makes zASIC4 paralogs a good model to study the properties of this ion channel. In this study, we found that surface expression of homomeric zASIC4.2 is higher than that of zASIC4.1. Surface expression of zASIC4.1 was much increased by formation of hetero-

meric channels, suggesting that zASIC4.1 contributes to heteromeric ASICs in zebrafish neurons. Robust surface expression of H+-insensitive zASIC4.2 suggests that zASIC4.2 functions as a homomer and is gated by an as yet unknown stimulus, different from H+. Moreover, we identified a small region just distal to the first transmembrane domain that is crucial for the differential H+ response of the two paralogs. This post-TM1 domain may have a general role in gating of members of this gene family.

Descriptors: zebrafish, ion channel gating physiology, membrane proteins metabolism, nerve tissue proteins metabolism, neurons metabolism, protons, sodium channels metabolism, dimerization, gene expression, membrane multigene family, nerve tissue proteins genetics, tertiary protein structure physiology, sodium channels genetics, *Xenopus laevis*, proteins genetics.

Chen YauHung, Chen WeiLi, Wang YunHsin, Huang MeiYun, and Chern MingKai (2007). **Spatiotemporal expression of zebrafish D-amino acid oxidase during early embryogenesis**. *Fish Physiology and Biochemistry* 33(1): 73-80. ISSN: 0920 1742.

NAL Call Number: QL639.1.F583

Abstract: D-amino acid oxidase (DAO, EC 1.4.3.3) is one of the flavoenzymes in peroxisomes catalyzing the oxidation of D-amino acids to alpha -imino acids. By reverse transcription-polymerase chain reaction, DAO cDNA was cloned from the mRNA of zebrafish embryos. The deduced zebrafish DAO amino acid sequence revealed a 348-amino acid polypeptide containing two FAD-binding sites (amino acids positions 7G to 12G, and 236V to 247W), and a peroxisomal targeting signal sequence (346Ser-347Arg-348Leu) at the C-terminal end. Following comparison of the sequences, we found that the zebrafish DAO polypeptide shared the sequence identities of 93%, 79%, 77%, 77%, 46%, 43% and 25% of the reported DAO of carp, human, mouse, pig, Streptomyces coelicor, Rhodotorula gracilis and Schizosaccharomyces pombe, respectively. Whole-mount in situ hybridization revealed that dao was a maternally inherited gene and that its expression was first observed in the zygote period, gradually down-regulated from the cleavage period to the early blastula stages, and had declined to an undetectable level at 6 h post-fertilization (hpf). At 18-hpf, the zebrafish dao transcripts were detected in a very weak manner, and their expression was restricted in the future head and brain regions as well as in the yolk syncytial layers of the yolk and yolk extension regions. At later stages, dao expression was detected in the brain, gut, swimming bladder, pronephric duct, optic tectum, ventrolateral tract and in the floor plate of the developing spinal cord. The enzymatic activities of DAO were also examined and showed that the DAO enzyme activities were expressed in a dynamic and stage-dependent manner: first it was detected at the 1-cell stage at an extremely low level (0.007+or-0.005 U/mg), reached the maximum amount at 6 days post-fertilization (dpf) (0.323+or-0.04 U/mg), and gradually down-regulated to the basal level (0.019+or-0.005 U/mg) in a 1-month juvenile. From these observations, we conclude that zebrafish dao is a maternally inherited gene, and that its expression was restricted in some neurons, gut, and pronephros and could be used as a good marker for the study of pronephridial development. Copyright © 2008 CABI

Descriptors: zebrafish, amino acid sequences, binding sites, embryogenesis, embryonic development, enzyme activity, enzymes, gene expression, genes, oxidoreductases, peroxisomes, signal transduction , *Danio rerio*.

Cheng, S., T. Shakespeare, R. Mui, T.W. White, and G. Valdimarsson (2004). **Connexin 48.5 is required for normal cardiovascular function and lens development in zebrafish embryos.** *Journal of Biological Chemistry* 279(35): 36993-7003. ISSN: 0021-9258.

NAL Call Number: 381J824

Abstract: Gap junctions are composed of connexin (Cx) proteins and mediate intercellular communication required for many developmental and physiological processes. Here we describe the

isolation and characterization of Cx48.5, a zebrafish connexin with the highest sequence identity to mammalian Cx46. Expression analysis showed that Cx48.5 is expressed in the adult and embryonic lens and heart, adult testis, and transiently in the embryonic otic vesicles. Injection of Cx48.5 cRNA into Xenopus oocytes elicited intercellular electrical coupling with voltage sensitivity similar to mammalian Cx46. In single oocytes, Cx48.5 also induced large outward currents on depolarization, consistent with gap-junctional hemichannels. Disruption of Cx48.5 expression in embryos with antisense morpholino oligos (morpholinos) revealed that Cx48.5 has an essential role in the maintenance of lens homeostasis. The morpholino-treated embryos also developed small lenses and eyes as well as severe cardiovascular abnormalities.
Descriptors: zebrafish, connexins physiology, crystalline lens embryology, amino acid sequence, cardiovascular system embryology, chickens, connexins metabolism, gene library, immunohistochemistry, in situ hybridization, crystalline metabolism, mice, molecular sequence data, oligonucleotides chemistry, oocytes metabolism, complementary RNA metabolism, rats, reverse transcriptase PCR, amino acid sequence homology, testis metabolism, time factors, *Xenopus*, proteins metabolism.

Chinen, A., Y. Matsumoto, and S. Kawamura (2005). **Spectral differentiation of blue opsins between phylogenetically close but ecologically distant goldfish and zebrafish.** *Journal of Biological Chemistry* 280(10): 9460-9466. ISSN: 0021 9258.
NAL Call Number: 381J824
Abstract: Zebrafish and goldfish are both diurnal freshwater fish species belonging to the same family, Cyprinidae, but their visual ecological surroundings considerably differ. Zebrafish are surface swimmers in conditions of broad and shortwave-dominated background spectra and goldfish are generalized swimmers whose light environment extends to a depth of elevated short wavelength absorbance with turbidity. The peak absorption spectrum ([lambda]max) of the zebrafish blue (SWS2) visual pigment is consistently shifted to short wavelength (416 nm) compared with that of the goldfish SWS2 (443 nm). Among the amino acid differences between the two pigments, only one (alanine in zebrafish and serine in goldfish at residue 94) was previously known to cause a difference in absorption spectrum (14-nm [lambda]max shift in newt SWS2). In this study, we reconstructed the ancestral SWS2 pigment of the two species by applying likelihood-based Bayesian statistics and performing site-directed mutagenesis. The reconstituted ancestral photopigment had a [lambda]max of 430 nm, indicating that zebrafish and goldfish achieved short wavelength (-14 nm) and long wavelength (+13 nm) spectral shifts, respectively, from the ancestor. Unexpectedly, the S94A mutation resulted in only a -3-nm spectral shift when introduced into the goldfish SWS2 pigment. Nearly half of the long wavelength shift toward the goldfish pigment was achieved instead by T116L (6 nm). The S295C mutation toward zebrafish SWS2 contributed to creating a ridge of absorbance around 400 nm and broadening its spectral sensitivity in the short wavelength direction. These results indicate that the evolutionary engineering approach is very effective in deciphering the process of functional divergence of visual pigments.
Descriptors: zebrafish, *carassius auratus, Danio rerio*, visual pigments, blue opsins, variation , spectral differentiation.

Chiou, M.J., T.T. Chao, J.L. Wu, C.M. Kuo, and J.Y. Chen (2006). **The physiological role of CTGF/CCN2 in zebrafish notochond development and biological analysis of The proximal promoter region.** *Biochemical and Biophysical Research Communications* 349(2): 750-8. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: During mouse embryogenesis, CTGF/CCN2 is expressed in zones containing hyper-

trophic chondroctyes and calcifying cartilage such as long bones, ribs, vertebral column, and phalanges. But in fish, its expression is yet unclear. Development of the vertebrae is morphologically similar among vertebrates, indicating that the underlying mechanism regulating the process is highly conserved during evolution. Analysis of 3.2kb of the CTGF/CCN2 proximal promoter sequence revealed a consensus TATAA box, putative AP1, Brn-2, CdxA, C/EBP alpha, C/EBP beta, C-Ets-, delta E, HFH-2, and HSF2 binding sites. Transient expression experiments with a 5'-deletion revealed at least 4 regulatory regions in the zebrafish CTGF/CCN2 gene, 2 with a stimulatory effect on transcription and 2 with an apparent inhibitory effect after IGF-I treatment in the ZFL cell line. To study the promoter-specific expression, we constructed a series of CTGF/CCN2 (3.0-, 2.5-, 2.0-, 1.5-, 1.0-, and 0.4-kb) promoter-driven green fluorescent protein (GFP) fragments encoding the GFP cDNA transgene which was microinjected into zebrafish embryos. Morphological studies of transgenic zebrafish indicated that the CTGF/CCN2 promoter-driven GFP transcripts appeared in the notochord. Targeted knockdown of the CTGF/CCN2 gene by two antisense morpholino oligonucleotides resulted in disruptions to notochord development. From a comparative point of view, this study of the CTGF/CCN2 gene in zebrafish may correlate well with those previously published on the mouse. These molecular results suggest that CTGF/CCN2 plays an important role in notochord development and is required for general embryonic development.

Descriptors: zebrafish, developmental gene expression regulation, immediate early, intercellular signaling peptides and, notochord metabolism, promoter regions genetics, complementary DNA metabolism, exons, green fluorescent proteins metabolism, HeLa cells, immediate early proteins biosynthesis, intercellular signaling peptides and proteins biosynthesis, mice, transcription factors metabolism, transgenes.

Ciesielski, F., N. Rochel, A. Mitschler, A. Kouzmenko, and D. Moras (2004). **Structural investigation of the ligand binding domain of the zebrafish VDR in complexes with 1alpha,25(OH)2D3 and Gemini: purification, crystallization and preliminary X-ray diffraction analysis.** *Journal of Steroid Biochemistry and Molecular Biology* 89-90(1-5): 55-59. ISSN: 0960-0760.

NAL Call Number: QD426.A1J6

Abstract: The nuclear receptor of Vitamin D can be activated by a large number of agonist molecules with a wide spectrum in their stereochemical framework. Up to now most of our structural information related to the protein-ligand complex formation is based on an engineered ligand binding domain (LBD) of the human receptor. We now have extended our database, using a wild-type LBD from zebrafish that confirms the previously reported results and allows to investigate the binding of ligands that induce significant conformational changes at the protein level.

Descriptors: zebrafish, calcitriol metabolism, receptors, calcitriol chemistry, amino acid sequence, crystallography, x ray, molecular models and sequence data, protein Conformation, receptors, calcitriol isolation and purification and metabolism, amino acid sequence homology.

Curran, J.E. and R.L. Woodruff (2007). **Passage of 17 kDa calmodulin through gap junctions of three vertebrate species**. *Tissue & Cell* 39(5): 303-309. ISSN: 0040-8166.

NAL Call Number: QH573.T5

Descriptors: zebrafish, *Danio rerio*, *Oryzias latipes*, *Xenopus laevis*, African clawed frog, medaka, amphibiotic species, animal physiology, biochemistry, freshwater fish, molecular structure.

Dahlman, J.M., K.L. Margot, L. Ding, J. Horwitz, and M. Posner (2005). **Zebrafish alpha-crystallins: protein structure and chaperone-like activity compared to their mammalian orthologs.** *Molecular Vision* 11: 88-96. ISSN: 1090-0535.

NAL Call Number: QP475 .M65

Abstract: PURPOSE: The vertebrate small heat shock proteins alphaA- and alphaB-crystallin contribute to the transparency and refractive power of the lens and may also prevent the aggregation of non-native proteins that would otherwise lead to cataracts. We previously showed that zebrafish (Danio rerio) and human alphaB-crystallin have diverged far more in primary structure and expression pattern than the orthologous alphaA-crystallins. In this current study we further compare the structure and function of zebrafish and mammalian alpha-crystallins. METHODS: Near UV CD spectroscopy was used to analyze the tertiary structure and thermal stability of recombinant zebrafish alpha-crystallins. The chaperone-like activities of zebrafish and human alpha-crystallins were compared by assaying their ability to prevent the chemically induced aggregation of several target proteins at temperatures between 25 degrees C and 40 degrees C. RESULTS: Zebrafish and human alphaA-crystallin showed very similar tertiary structures, while the alphaB-crystallin orthologs showed differences related to the presence of additional aromatic amino acids in the zebrafish protein. The denaturation temperatures of zebrafish crystallins were lower than those of mammals. The chaperone-like activities of the two zebrafish alpha-crystallins were highly divergent, with alphaA-crystallin showing much greater activity than alphaB-crystallin. CONCLUSIONS: alphaA-crystallin serves a similar physiological function in both zebrafish and mammals as a lens specific chaperone-like molecule. The reduced chaperone-like function of zebrafish alphaB-crystallin and its lack of extralenticular expression indicates that it plays a different physiological role from its mammalian ortholog. Future comparative studies of alpha-crystallin from closely related vertebrate species can help identify specific structural changes that lead to alterations in chaperone-like activity.
Descriptors: zebrafish, molecular chaperones metabolism, alpha crystallin A chain chemistry, alpha crystallin B chain chemistry, Western blotting, cattle, circular dichroism, polyacrylamide gel electrophoresis, protein denaturation, tertiary protein structure, rabbits, ultraviolet spectrophotometry, structure activity relationship, alpha crystallin A and B chain metabolism.

DiMuccio, T., S.T. Mukai, E. Clelland, G. Kohli, M. Cuartero, Wu TingTing, and Peng Chun (2005).
Cloning of a second form of activin- beta A cDNA and regulation of activin- beta A subunits and activin type II receptor mRNA expression by gonadotropin in the zebrafish ovary.
General and Comparative Endocrinology 143(3): 287-299. ISSN: 0016 6480.
NAL Call Number: 444.8G28
Abstract: Activins are dimeric proteins consisting of two inhibin beta subunits. Homo- and hetero-dimerizations of two isoforms of beta subunits, beta A and beta B, produce three forms of activins, activin-A, -B, and -AB. Recent studies have suggested that activin-A mediates gonadotropin-induced oocyte maturation in the zebrafish. To further understand the physiological role of activin-A in the zebrafish ovary, we have cloned cDNAs for a second isoform of the activin-beta A subunit and the activin type IIA (ActRIIA) receptor and determined their regulation by gonadotropin. Two sequences were obtained during the cloning of activin- beta A subunit, both of which showed high identity to beta A subunits of other species, and were therefore designated as isoform 1 and 2. Real-time PCR quantification was used to measure mRNA levels of activin-beta A1 and - beta A2, as well as two type II receptors, ActRIIA and ActRIIB, in the zebrafish ovary. Activin- beta A1 mRNA levels in stages III and IV follicles were similar and higher than those in stage II while high activin- beta A2 mRNA levels were only found in stage IV follicles. Highest levels of mRNA expression were detected in small and large stage III follicles for ActRIIA and ActRIIB, respectively. Treatment with human chorionic gonadotropin induced dose- and time-dependent increases in mRNA levels of activin- beta A1 and - beta A, as well as ActRIIA and ActRIIB. These findings further support the involvement of the activin signaling cascade in gonadotropin-regulated gonadal activities. Copyright © 2008 CABI

Descriptors: zebrafish, *Danio rerio*, activins, biochemical receptors, complementary DNA, DNA cloning, gonadotropins, hormonal control, messenger RNA, ovarian follicles, ovaries .

Du, C., R. Niu, E. Chu, P. Zhang, and X. Lin (2006). **Sequence analysis and functional study of thymidylate synthase from zebrafish, *Danio rerio*.** *Journal of Biochemistry* 139(5): 913-20. ISSN: 0021-924X.
NAL Call Number: 385 J822
Abstract: The thymidylate synthase (TS), an important target for many anticancer drugs, has been cloned from different species. But the cDNA property and function of TS in zebrafish are not well documented. In order to use zebrafish as an animal model for screening novel anticancer agents, we isolated TS cDNA from zebrafish and compared its sequence with those from other species. The open reading frame (ORF) of zebrafish TS cDNA sequence was 954 nucleotides, encoding a 318-amino acid protein with a calculated molecular mass of 36.15 kDa. The deduced amino acid sequence of zebrafish TS was similar to those from other organisms, including rat, mouse and humans. The zebrafish TS protein was expressed in Escherichia coli and purified to homogeneity. The purified zebrafish TS showed maximal activity at 28 degrees C with similar K(m) value to human TS. Western immunoblot assay confirmed that TS was expressed in all the developmental stages of zebrafish with a high level of expression at the 1-4 cell stages. To study the function of TS in zebrafish embryo development, a short hairpin RNA (shRNA) expression vector, pSilencer 4.1-CMV/TS, was constructed which targeted the protein-coding region of zebrafish TS mRNA. Significant change in the development of tail and epiboly was found in zebrafish embryos microinjected pSilencer4.1-CMV/TS siRNA expression vector.
Descriptors: zebrafish, mice, rats, complementary DNA isolation and purification, thymidylate synthase chemistry, thymidylate synthase genetics, amino acid sequence genetics, molecular cloning, complementary DNA metabolism, *Escherichia coli*, gene silencing, phylogeny, recombinant proteins isolation and purification, recombinant proteins metabolism, sequence alignment, sequence analysis, thymidylate synthase metabolism.

Du, S.J., J. Gao, and V. Anyangwe (2003). **Muscle-specific expression of myogenin in zebrafish embryos is controlled by multiple regulatory elements in the promoter.** *Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology* 134(1): 123-34. ISSN: 0305 0491.
NAL Call Number: QP501.C6
Abstract: Myogenin is a member of the basic Helix-Loop-Helix transcription factor family that play key roles in myoblast specification and differentiation. Myogenin is specifically expressed in developing somite and skeletal muscles in zebrafish embryos. To determine the regulation of myogenin expression, we reported here the characterization of zebrafish myogenin gene and analysis of its promoter activity in zebrafish embryos. Our data showed that a 0.8-kb myogenin promoter was sufficient to direct correct temporal and spatial muscle-specific green fluorescence protein expression in zebrafish embryos. Sequence analysis identified two putative E box sites in the myogenin gene promoter. In addition, a MEF2 recognition site and a MEF3 binding site were also found in the promoter. Mutation of the E boxes, MEF2 or MEF3 binding site individually had little effect on the muscle-specificity and activity of the myogenin promoter. However, mutating these sites in various combinations, e.g. E boxes and MEF2 binding site, or MEF2 and MEF3 sites significantly reduced the activity of the promoter. Moreover, mutating the E boxes, MEF2 and MEF3 sites together almost abolished the activity of the promoter. These data indicate that muscle-specific expression of myogenin in zebrafish embryos is controlled by multiple regulatory elements in the promoter. In addition, because these regulatory elements control myogenin expression in mouse and human embryos, these data suggest that the regulatory mechanism controlling myogenin expression might be conserved during evolution.

Descriptors: zebrafish, embryo, skeletal muscle embryology, myogenin biosynthesis, promoter regions genetics, base sequence, binding sites, DNA binding proteins genetics, proteins metabolism, reporter genes, green fluorescent proteins, in situ hybridization, luminescent proteins metabolism, genetic models, molecular sequence data, Myod protein genetics, metabolism, myogenic regulatory factors, plasmids metabolism, time factors, transcription factors .

Fleming, A., M. Sato, and P. Goldsmith (2005). **High-throughput in vivo screening for bone anabolic compounds with zebrafish.** *Journal of Biomolecular Screening* 10(8): 823-31. ISSN: 1087-0571.

Abstract: Osteoporosis and diseases of bone loss are a major public health problem for the present and the future since longevity and prevalence of the disease are increasing in all parts of the world. The bisphosphonates, widely used in the treatment of osteoporosis, act by inhibiting bone resorption. However, there are few agents that promote or increase bone formation in patients who have suffered substantial bone loss. To facilitate the identification of novel anabolic therapies, the authors have developed a rapid, high-throughput in vivo screen using larval zebrafish (*Danio rerio*) in which they are able to identify agents with anabolic effects in the skeleton within a 6-day time period. Vitamin D3 analogs and intermittent parathyroid hormone (PTH) result in dose-dependent increases in the formation of mineralized bone, whereas continuous exposure to PTH results in net bone loss. Because this model is fast, economical, and genetically tractable, it provides a powerful adjunct to mammalian models for the identification of new anabolic bone agents and offers the potential for genetic elucidation of pathways important in osteoblastic activity.

Descriptors: zebrafish, bone density conservation agents pharmacology, physiologic calcification drug effects, preclinical drug evaluation methods, bone density conservation agents therapeutic use, bone remodeling drug effects, bone remodeling physiology, cholecalciferol analogs and derivatives, cholecalciferol pharmacology, cholecalciferol therapeutic use, etidronic acid pharmacology, etidronic acid therapeutic use, feasibility studies, hydroxymethylglutaryl co A reductase inhibitors pharmacology, hydroxymethylglutaryl co A reductase inhibitors therapeutic use, fish model, osteoblasts drug effects, osteoporosis drug therapy, parathyroid hormone pharmacology, parathyroid hormone therapeutic use.

Fraenkel, P.G., D. Traver, A. Donovan, D. Zahrieh, and L.I. Zon (2005). **Ferroportin 1 is required for normal iron cycling in zebrafish.** *Journal of Clinical Investigation* 115(6): 1532-41. ISSN: 0021-9738.

NAL Call Number: 448.8 J8295

Abstract: Missense mutations in ferroportin1 (fpn1), an intestinal and macrophage iron exporter, have been identified between transmembrane helices 3 and 4 in the zebrafish anemia mutant weissherbst (weh(Tp85c-/-)) and in patients with type 4 hemochromatosis. To explore the effects of fpn1 mutation on blood development and iron homeostasis in the adult zebrafish, weh(Tp85c-/-) zebrafish were rescued by injection with iron dextran and studied in comparison with injected and uninjected WT zebrafish and heterozygotes. Although iron deposition was observed in all iron-injected fish, only weh(Tp85c-/-) zebrafish exhibited iron accumulation in the intestinal epithelium compatible with a block in iron export. Iron injections initially reversed the anemia. However, 8 months after iron injections were discontinued, weh(Tp85c-/-) zebrafish developed hypochromic anemia and impaired erythroid maturation despite the persistence of iron-loaded macrophages and elevated hepatic nonheme iron stores. Quantitative real-time RT-PCR revealed a significant decrease in mean hepatic transcript levels of the secreted iron-regulator hepcidin and increased intestinal expression of fpn1 in anemic weh(Tp85c-/-) adults. Injection of iron dextran into WT or mutant zebrafish embryos, however, resulted in significant increases in hepcidin expression 18 hours after injection, demonstrating that hepcidin expression

in zebrafish is iron responsive and independent of fpn1's function as an iron exporter.
Descriptors: zebrafish, cation transport proteins metabolism, intestines metabolism, iron metabolism, proteins metabolism, anemia, hypochromic genetics, anemia, hypochromic metabolism, hypochromic pathology, antimicrobial cationic peptides biosynthesis, cation transport erythrocytes metabolism, erythrocytes pathology, developmental gene expression regulation drug effects, hemochromatosis genetics and hemochromatosis metabolism, hemochromatosis pathology, intestinal mucosa metabolism, intestinal mucosa pathology, ion transport genetics, iron administration and dosage, liver metabolism, liver pathology, macrophages metabolism, macrophages pathology, missense mutation, proteins genetics.

Ge Wei (2005). **Gonadotropins and their paracrine signaling network in the zebrafish ovary**. *Fish Physiology and Biochemistry* 31(2/3): 209-214. ISSN: 0920 1742.
NAL Call Number: QL639.1.F583
Abstract: Pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), play fundamental roles in vertebrate ovarian development and function. However, there has been an increasing body of evidence that the actions of FSH and LH are mediated or modulated by a variety of locally produced peptide or protein factors, which form an intimate regulatory network within and between the ovarian follicles. In the past few years, a variety of growth factors have been identified and characterized in the zebrafish ovary including activin and epidermal growth factor (EGF), which are important components of the intraovarian communication network. To understand how this local network interacts with the gonadotropins from the pituitary, we have recently cloned and characterized all the subunits of zebrafish FSH and LH from the pituitary as well as their receptors (FSHR and LHR) from the ovary. Using the Chinese hamster ovary (CHO) cells as the bioreactor, we have produced recombinant zebrafish FSH and LH with biological activities. With the recombinant hormones available, the functions of zebrafish FSH and LH in the ovary and their interactions with the local factors will be an important issue to address in the future. This review briefly summarizes some recent work from our laboratory and others on both gonadotropins and their potential intraovarian signaling factors in the zebrafish. Copyright © 2008 CABI
Descriptors: zebrafish, activins, epidermal growth factor, fsh, gonadotropins, growth factors, hormonal control, hormone receptors, hormone secretion, LH, ovarian development, ovarian follicles, ovaries, pituitary, reviews, Danio rerio.

Gensure, R.C., B. Ponugoti, Y. Gunes, M.R. Papasani, B. Lanske, M. Bastepe, D.A. Rubin, and H. Juppner (2004). **Identification and characterization of two parathyroid hormone-like molecules in zebrafish.** *Endocrinology* 145(4): 1634-9. ISSN: 0013 7227.
NAL Call Number: 448.8EN2
Abstract: Zebrafish (*Danio rerio*) have receptors homologous to the human PTH (hPTH)/PTHrP receptor (PTH1R) and PTH-2 receptor (PTH2R) and an additional receptor (PTH3R) with high homology to the PTH1R. To find natural ligands for zPTH1R and zPTH3R, we searched the zebrafish genomic database and discovered two distinct regions that, when translated (zPTH1 and zPTH2), showed high homology to hPTH. Isolation of cDNAs and determination of the intron/exon boundaries revealed genomic structures which were similar to known PTHs. Peptides consisting of the first 34 amino acids after the pre- and prosequences of the zebrafish PTHs (zPTHs) were synthesized and were shown to be fully active at the hPTH1R. zPTH2(1-34) was, however, approximately 30-fold less potent at the zPTH1R than hPTH(1-34), hPTHrP(1-36), and zPTH1(1-34). When tested with zPTH3R, zPTH1(1-34) and hPTHrP(1-36) showed similar potencies, whereas the potency of zPTH2(1-34) was moderately (3-fold) reduced. To determine whether other fishes have multiple PTHs, we searched the genomic data-

base of the Japanese pufferfish (Takifugu rubripes) and identified zPTH1 and zPTH2 homologs. Phylogenetic analysis showed that PTHs from zebrafish and pufferfish are more closely related to each other than to known mammalian PTH homologs or to PTHrP and tuberoinfundibular peptide of 39 residues. This is consistent with evolution of two teleost PTH-like peptides occurring after the evolutionary divergence between fishes and mammals. Overall, the PTH system appears more complex in fishes than in mammals, providing evidence of continued evolution in nontetrapod species. The availability of multiple forms of fish PTH and their receptors provide additional tools for PTH ligand/receptor structure-function studies.
Descriptors: zebrafish, parathyroid hormone genetics, parathyroid hormone metabolism, peptide fragments genetics, peptide fragments metabolism, and metabolism, amino acid sequence, complementary DNA, molecular evolution, genome, molecular sequence data, phylogeny, receptor, parathyroid hormone, type 1 metabolism, receptors.

Gonzalez Nunez, V., G. Toth, and R.E. Rodriguez (2007). **Endogenous heptapeptide Met-enkephalin-Gly-Tyr binds differentially to duplicate delta opioid receptors from zebrafish.** *Peptides* 28(12): 2340-7. ISSN: 0196-9781.
NAL Call Number: QP552.P4P45
Abstract: Met-enkephalin-Gly-Tyr (MEGY) is an endogenous peptide that binds to opioid sites in zebrafish and in rat brain homogenates. The aim of this work is to characterize the binding profile of this opioid ligand on two duplicate delta receptors from zebrafish, ZFOR1 and ZFOR4. Our results show that, while ZFOR1 presents one single binding site for [(3)H]-MEGY (K(D)=4.0+/-0.4nM), the experimental data from ZFOR4 fit better to the two-site binding model (K(D1)=0.8+/-0.2nM and K(D2)=30.2+/-10.2nM). Two other MEGY synthetic analogues, (D-Ala(2))-MEGY and (D-Ala(2), Val(5))-MEGY were also prepared and tested, together with the original peptide MEGY and other opioid ligands, in competition binding assays. While these peptides presented K(i) values on the nanomolar range when using [(3)H]-MEGY as radioligand, these parameters were two orders higher in competition binding assays with the antagonist [(3)H]-diprenorphine. Functional [(35)S]GTPgammaS stimulation analysis has revealed that these two receptors can be activated by several opioid agonists. Our results prove that although the MEGY peptide acts as an agonist on ZFOR1 and ZFOR4, there are subtle pharmacological differences between these two delta opioid receptors from zebrafish.
Descriptors: zebrafish, met-enkephalin-gly-tyr, opioid receptors, heptapeptide, ZfOr1, ZfOr4, zebrafish.

Guerardel, Y., L.Y. Chang, E. Maes, C.J. Huang, and K.H. Khoo (2006). **Glycomic survey mapping of zebrafish identifies unique sialylation pattern.** *Glycobiology* 16(3): 244-57. ISSN: 0959-6658.
NAL Call Number: QP552.G59G593
Abstract: Functional genomics and proteomics studies of the developmental glycobiology of zebrafish are greatly hampered by the current lack of knowledge on its glycosylation profile. To furnish the requisite structural basis for a more insightful functional delineation and genetic manipulation, we have initiated a survey mapping of the possible expression of stage-specific glycoconjugates in zebrafish. High-sensitivity mass spectrometry (MS) analysis in conjunction with the usual array of enzymatic and chemical derivatization was employed as the principal method for rapid differential mapping of the glycolipids and sequentially liberated N- and O-glycans from the total extracts. We demonstrated that all developmental stages of the zebrafish under investigation, from fertilized eggs to hatched embryos, synthesize oligomannosyl types of N-glycans, as well as complex types with additionally beta4-galactosylated, Neu5Ac/Neu5Gc monosialylated Lewis x termini. A combination of collision-induced dissociation (CID)-MS/MS and nuclear magnetic resonance (NMR) analyses led to the identification of an abundant and unusual

mucin-type O-glycosylation, based on a novel sequence Fucalpha1-3GalNAcbeta1-4(Neu5Ac/Neu5Gcalpha2-3)Galbeta1-3GalNAc. This core structure may be further oligosialylated, but exclusively in the earlier development stages. Similarly, MS and MS/MS analyses of the extracted glycolipid fraction revealed the presence of a heterogeneous family of oligosialylated lactosylceramide compounds. In contrast to the O-glycans, these glycolipids only appear in the later development stages, suggesting a complex pattern of regulation for sialyltransferase activities during zebrafish embryogenesis.

Descriptors: zebrafish, n acetylneuraminic acid metabolism, polysaccharides analysis, polysaccharides chemistry, metabolism, glycolipids chemistry, magnetic resonance spectroscopy, spectrometry, mass, matrix assisted laser desorption ionization.

Gutierrez Aguirre, I., P. Trontelj, P. Macek, J.H. Lakey, and G. Anderluh (2006). **Membrane binding of zebrafish actinoporin-like protein: AF domains, a novel superfamily of cell membrane binding domains.** *Biochemical Journal, The* 398(3): 381-92. ISSN: 0264 6021.

NAL Call Number: 382B52

Abstract: Actinoporins are potent eukaryotic pore-forming toxins specific for sphingomyelin-containing membranes. They are structurally similar to members of the fungal fruit-body lectin family that bind cell-surface exposed Thomsen-Friedenreich antigen. In the present study we found a number of sequences in public databases with similarity to actinoporins. They originate from three animal and two plant phyla and can be classified in three families according to phylogenetic analysis. The sequence similarity is confined to a region from the C-terminal half of the actinoporin molecule and comprises the membrane binding site with a highly conserved P-[WYF]-D pattern. A member of this novel actinoporin-like protein family from zebrafish was cloned and expressed in *Escherichia coli*. It displays membrane-binding behaviour but does not have permeabilizing activity or sphingomyelin specificity, two properties typical of actinoporins. We propose that the three families of actinoporin-like proteins and the fungal fruit-body lectin family comprise a novel superfamily of membrane binding proteins, tentatively called AF domains (abbreviated from actinoporin-like proteins and fungal fruit-body lectins).

Descriptors: zebrafish, cell membrane metabolism, membrane proteins chemistry, membrane proteins metabolism, porins metabolism, zebrafish, amino acid sequence, fungi metabolism, lectins metabolism, models, molecular sequence data, phylogeny, porins chemistry, porins genetics, protein binding, protein Conformation, protein structure, tertiary.

Harfe, B.D., M.T. Mcmanus, J.H. Mansfield, E. Hornstein, and C.J. Tabin (2005). **The RNaseIII enzyme dicer is required for morphogenesis but not patterning of The vertebrate limb**. *Proceedings of the National Academy of Sciences of the United States of America* 102(31): 10898-903. ISSN: 0027 8424.

NAL Call Number: 500N21P

Abstract: The RNaseIII-containing enzyme Dicer is believed to be required for the processing of most, if not all, microRNAs (miRNAs) and for processing long dsRNA into small interfering RNAs. Because the complete loss of Dicer in both zebrafish and mice results in early embryonic lethality, it has been impossible to determine what role, if any, Dicer has in patterning later tissues in the developing vertebrate embryo. To bypass the early requirement of Dicer in development, we have created a conditional allele of this gene in mice. Using transgenes to drive Cre expression in discrete regions of the limb mesoderm, we find that removal of Dicer results in the loss of processed miRNAs. Phenotypically, developmental delays, in part due to massive cell death as well as disregulation of specific gene expression, lead to the formation of a much smaller limb. Thus, Dicer is required for the formation of normal mouse limbs. Strikingly, however, we did not detect defects in basic patterning or in tissue-specific differentiation of Dicer-deficient limb buds.

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Descriptors: zebrafish, development, molecular genetics, reproductive system, reproduction, dicer, RNAseIII.

Hayashi, Y., Y. Horibata, K. Sakaguchi, N. Okino, and M. Ito (2005). **A sensitive and reproducible assay to measure the activity of glucosylceramide synthase and lactosylceramide synthase using HPLC and fluorescent substrates**. *Analytical Biochemistry* 345(2): 181-186. ISSN: 0003 2697.
NAL Call Number: 381 An13
Abstract: Glucosylceramide synthase (GlcT) and lactosylceramide synthase (GalT) are key enzymes for the synthesis of major glycosphingolipids of vertebrates. In this article, we report a new reliable method to determine GlcT and GalT activities using the fluorescent acceptor substrates C6-4-nitrobenzo-2-oxa-1,3-diazole (NBD)-ceramide and C6-NBD-glucosyleeramide, respectively, and a normal-phase high-performance liquid chromatography (HPLC). The reaction products, C6-NBD-glucosyleeramide for GlcT and C6-NBD-lactosylceramide for GalT, could be separated from the corresponding acceptor substrates within 6 min under the conditions used. Reaction products were able to be detected quantitatively at concentrations ranging from 50 fmol to 50 pmol, making it possible to determine both activities using the lysate from I X 104 cultured CHOP cells (Chinese hamster ovary cells expressing polyoma LT antigen) and one zebrafish embryo. This method was used successfully to evaluate the degree of knockdown of GlcT and GAT during zebrafish embryogenesis after injection of the morpholino-oligo-based antisense into one- to four-cell embryos. These results indicate that the fluorescence-based HPLC method is a highly sensitive, rapid, and reproducible assay for determining GlcT and GalT activities and is useful for evaluating the activities in gene knockdown experiments. (C) 2005 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, methods and techniques, normal phase high performance liquid chromatography, glucosylceramide and lactosylceramide synthase, glycosphingolipids, hampster, zebrafish.

Ho, S.Y., K. Lorent, M. Pack, and S.A. Farber (2006). **Zebrafish fat-free is required for intestinal lipid absorption and Golgi apparatus structure.** *Cell Metabolism* 3(4): 289-300. ISSN: 1550 4131.
Abstract: The zebrafish fat-free (ffr) mutation was identified in a physiological screen for genes that regulate lipid metabolism. ffr mutant larvae are morphologically indistinguishable from wild-type sibling larvae, but their absorption of fluorescent lipids is severely impaired. Through positional cloning, we have identified a causative mutation in a highly conserved and ubiquitously expressed gene within the ffr locus. The Ffr protein contains a Dor-1 like domain typical of oligomeric Golgi complex (COG) gene, cog8. Golgi complex ultrastructure is disrupted in the ffr digestive tract. Consistent with a possible role in COG-mediated Golgi function, wild-type Ffr-GFP and COG8-mRFP fusion proteins partially colocalize in zebrafish blastomeres. Enterocyte retention of an endosomal lipid marker in ffr larvae support the idea that altered vesicle trafficking contributes to the ffr mutant defect. These data indicate that ffr is required for both Golgi structure and vesicular trafficking, and ultimately lipid transport.
Descriptors: zebrafish, golgi apparatus ultrastructure and chemistry, intestinal absorption, lipid metabolism, vesicular transport vesicular transport, amino acid sequence, endosomes physiology, enterocytes chemistry, enterocytes physiology, fluorescent antibody technique, gene expression regulation, membrane proteins genetics and physiology, confocal microscopy, molecular sequence data, mutation, phenotype, tertiary protein structure, protein transport, recombinant fusion proteins metabolism, transport vesicles, vesicular transport proteins chemistry.

Hong, C.C., Q.P. Peterson, J.Y. Hong, and R.T. Peterson (2006). **Artery/vein specification is governed by opposing phosphatidylinositol-3 kinase and map kinase/erk signaling**. *Current Biology* 16(13): 1366-1372. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: Angioblasts are multipotent progenitor cells that give rise to arteries or veins [1]. Genetic disruption of the gridlock gene perturbs the artery/vein balance, resulting in generation of insufficient numbers of arterial cells [2]. However, within angioblasts the precise biochemical signals that determine the artery/vein cell-fate decision are poorly understood. We have identified by chemical screening two classes of compounds that compensate for a mutation in the gridlock gene [3]. Both target the VEGF signaling pathway and reveal two downstream branches emanating from the VEGF receptor with opposing effects on arterial specification. We show that activation of ERK (p42/44 MAP kinase) is a specific marker of early arterial progenitors and is among the earliest known determinants of arterial specification. In embryos, cells fated to contribute to arteries express high levels of activated ERK, whereas cells fated to contribute to veins do not. Inhibiting the phosphatidylinositol-3 kinase (PI3K) branch with GS4898 or known PI3K inhibitors, or by expression of a dominant-negative form of AKT promotes arterial specification. Conversely, inhibition of the ERK branch blocks arterial specification, and expression of constitutively active AKT promotes venous specification. In summary, chemical genetic analysis has uncovered unanticipated opposing roles of PI3K and ERK in artery/vein specification.
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Descriptors: zebrafish, development, enzymology, cardiovascular system, signal transduction, cell fate decision, artery, vein specification.

Hu, M.C., H.Y. Gong, G.H. Lin, S.Y. Hu, M.H. Chen, S.J. Huang, C.F. Liao, and J.L. Wu (2007). **XBP-1, a key regulator of unfolded protein response, activates transcription of IGF1 and Akt phosphorylation in zebrafish embryonic cell line.** *Biochemical and Biophysical Research Communications* 359(3): 778-83. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: The unfolded protein response (UPR) is a conserved and adaptive cellular response to increase cell survival during ER stress. XBP-1 spliced form (XBP-1S) generated by IRE1 endoribonuclease is a key transcriptional regulator in UPR to activate genes involved in protein folding and degradation to restore ER function. Although Akt activation was suggested to be a pro-survival pathway activated during ER stress, the signal to trigger Akt is still not clear. In this study, we report IGF1 transcription and Akt phosphorylation are enhanced in XBP-1S stably overexpressed clone of zebrafish embryonic cell line (ZF4). In addition, zebrafish IGF1 intron1 with predicted UPRE (XBP-1S binding sites) and ERSE (ATF6/XBP-1S binding site) linked with basal promoter could be activated by XBP-1S, not by XBP-1 unspliced form (XBP-1U). Furthermore, we demonstrate that expression of endogenous IGF1 is transiently induced as XBP-1 splicing during ER stress in parallel to ER chaperone GRP78/Hspa5 and ER resided E3 ubiquitin ligase Synoviolin in ZF4 cells by quantitative PCR. Our results suggest zebrafish XBP-1S not only activates genes responsible for protein folding, transporting, glycosylation and ER associated degradation but also activates anti-apoptosis signal via IGF1/Akt pathway in unfolded protein response to cope with ER stress.

Descriptors: zebrafish embryos, DNA binding proteins genetics, proteins metabolism, insulin like growth factor I genetics, proto oncogene proteins C akt metabolism, transcription factors metabolism, transcription, genetics, metabolism, amino acid sequence, cell line, molecular cloning, DNA binding proteins genetics, proteins chemistry, DNA binding proteins genetics, endoplasmic reticulum metabolism, molecular sequence data, oligonucleotide array sequence analysis, phosphorylation, protein folding, messenger RNA genetics, sequence alignment, amino

acid sequence homology, signal transduction, time factors, transcription factors chemistry, transcription factors genetics, up regulation.

Isken, A., J. Holzschuh, J.M. Lampert, L. Fischer, V. Oberhauser, K. Palczewski, and J. von Lintig (2007). **Sequestration of retinyl esters is essential for retinoid signaling in the zebrafish embryo.** *Journal of Biological Chemistry* 282(2): 1144-51. ISSN: 0021-9258.

NAL Call Number: 381J824

Abstract: For vertebrate development, vitamin A (all-trans retinol) is required in quantitative different amounts and spatiotemporal distribution for the production of retinoic acid, a nuclear hormone receptor ligand, and 11-cis retinal, the chromophore of visual pigments. We show here for zebrafish that embryonic retinoid homeostasis essentially depends on the activity of a leci-thin:retinol acyltransferase (Lratb). During embryogenesis, lratb is expressed in mostly non-overlapping domains opposite to retinal dehydrogenase 2 (raldh2), the key enzyme for retinoic acid synthesis. Blocking retinyl ester formation by a targeted knock down of Lratb results in significantly increased retinoic acid levels, which lead to severe embryonic patterning defects. Thus, we provide evidence that a balanced competition between Lratb and Raldh2 for yolk vitamin A defines embryonic compartments either for retinyl ester or retinoic acid synthesis. This homeostatic mechanism dynamically adjusts embryonic retinoic acid levels for gene regulation, concomitantly sequestering excess yolk vitamin A in the form of retinyl esters for the establishment of larval vision later during development.

Descriptors: zebrafish , Drosophila, eye embryology, eye metabolism, retinoids metabolism, signal transduction physiology, metabolism, acyltransferases genetics, acyltransferases metabolism, aldehyde oxidoreductases genetics, aldehyde oxidoreductases metabolism, amino acid sequence, cell line, egg yolk metabolism, embryos metabolism, esters metabolism, developmental gene expression regulation, homeostasis physiology, molecular sequence data, retinaldehyde metabolism, tretinoin metabolism, vitamin A metabolism.

Juarez, M.A., F. Su, S. Chun, M.J. Kiel, and S.E. Lyons (2005). **Distinct roles for SCL in erythroid specification and maturation in zebrafish.** *Journal of Biological Chemistry* 280(50): 41636-44. ISSN: 0021-9258.

NAL Call Number: 381J824

Abstract: The stem cell leukemia (SCL) transcription factor is essential for vertebrate hematopoiesis. Using the powerful zebrafish model for embryonic analysis, we compared the effects of either reducing or ablating Scl using morpholino-modified antisense RNAs. Ablation of Scl resulted in the loss of primitive and definitive hematopoiesis, consistent with its essential role in these processes. Interestingly, in embryos with severely reduced SCL levels, erythroid progenitors expressing gata1 and embryonic globin developed. Erythroid maturation was deficient in these SCL hypomorphs, supporting that SCL was required both for the erythroid specification and for the maturation steps, with maturation requiring higher Scl levels than specification. Although all hematopoietic functions were rescued by wild-type SCL mRNA, an Scl DNA binding mutant rescued primitive and definitive hematopoiesis but did not rescue primitive erythroid maturation. Together, we showed that there is a distinct SCL hypomorphic phenotype and demonstrated that distinct functions are required for the roles of Scl in the specification and differentiation of primitive and definitive hematopoietic lineages. Our results revealed that Scl participates in multiple processes requiring different levels and functions. Further, we identified an Scl hypomorphic phenotype distinct from the null state.

Descriptors: zebrafish model, basic helix loop helix transcription factors physiology, developmental gene expression regulation, proto oncogene, alternative splicing, basic helix loop helix transcription factors metabolism, cell differentiation, cell lineage, DNA chemistry, DNA binding

proteins genetics, proteins, electrophoresis, agar gel, erythroid specific DNA binding proteins genetics, factors metabolism, hematopoiesis, in situ hybridization, genetic models, mutation, phenotype, tertiary protein structure, proto oncogene proteins metabolism, RNA chemistry, antisense chemistry, messenger RNA metabolism, small interfering RNA metabolism, reverse transcriptase PCR, stem cells, transcription factors.

Kawamura, S., K. Takeshita, T. Tsujimura, S. Kasagi, and Y. Matsumoto (2005). **Evolutionarily conserved and divergent regulatory sequences in the fish rod opsin promoter**. *Comparative Biochemistry and Physiology, Part B: Biochemistry and Molecular Biology* 141(4): 391-399. ISSN: 1096-4959.
NAL Call Number: QP501.C6
Descriptors: zebrafish, *Danio rerio*, brain, crX protein, conserved sequence, cores, electrophoretic mobility, evolution, genes, nucleotide sequence, photoreceptors, phylogeny, promoters, regulatory sequences, opsins .

Kazeto, Y., R. Goto Kazeto, A.R. Place, and J.M. Trant (2003). **Aromatase expression in zebrafish and channel catfish brains: changes in transcript abundance associated with the reproductive cycle and exposure to endocrine disrupting chemicals**. *Fish Physiology and Biochemistry* 28(1/4): 29-32. ISSN: 0920 1742.
NAL Call Number: QL639.1.F583
Abstract: Cytochrome P450-aromatase (CYP19) is the critical steroidogenic enzyme controlling oestrogen biosynthesis. In teleosts, two genes for CYP19 are found with CYP19A1 expressed mainly in the ovary and CYP19A2 expressed in both brain and pituitary. The promoters for these two homologous genes are considerably different. Exposure to estrogenic endocrine disrupting chemicals (EDC) such as ethinylestradiol and nonylphenol resulted in strong up-regulation of the CYP19A2 gene whereas the expression of CYP19A1 was largely unaffected. Moreover, CYP19A2 transcript abundance was shown to vary during the spawning cycle of both the catfish and the zebrafish which implies an association of the CYP19A2 gene in brain-pituitary endocrinology. Together these findings suggest that EDCs could affect the reproductive physiology of fish through disrupting the gene expression of CYP19A2 in the brain. Copyright © 2008 CABI
Descriptors: zebrafish, catfish, brain, cytochrome P450, enzymes, gene expression, genes, hormonal control, estrogen receptors, reproduction, spawning.

Kazeto, Y., R. Goto Kazeto, and J.M. Trant (2005). **Membrane-bound progestin receptors in channel catfish and zebrafish ovary: changes in gene expression associated with the reproductive cycles and hormonal reagents**. *General and Comparative Endocrinology* 142(1/2): 204-211. ISSN: 0016 6480.
NAL Call Number: 444.8G28
Abstract: Membrane-bound progestin receptors (mPRs) are potential intermediaries in meiotic maturation of fish oocytes and other physiological processes. In this study, gene expression of the mPRs in the ovary of catfish and zebrafish during the reproductive cycle and the hormonal regulation of the expression were investigated. The transcript abundance of catfish mPR alpha gradually increased in conjunction with ovarian growth and then decreased prior to spawning period whereas the ovarian gene expression of mPR beta varied little throughout the reproductive cycle. In contrast, mPR gamma gene expression peaked early in the mid-vitellogenic stage. The transcript abundance of zebrafish mPR alpha and beta was low in ovarian follicles at early stages of oogenesis and gradually increased after the onset of vitellogenic growth and, thereafter, the gene expression did not vary. Gonadotropic treatment did not modulate the ovarian expression of mPR alpha and beta genes in either catfish or zebrafish. On the other hand, exposure to 17,20

beta -dihydroxy-4-pregenen-3-one (the maturation-inducing steroid in this species) resulted in the down-regulation of mPR alpha in catfish ovary whereas gene expression was significantly induced by estradiol-17 beta . Taken together, these findings suggest that gonadotropin-induced final oocyte maturation may not require an induction of mPR(s) expression or that the gonadotropin stimulates mPR protein production at the post-transcriptional level, presuming these receptors are indispensable for oocyte maturation. Copyright © 2008 CABI
Descriptors: zebrafish, catfish, *Danio rerio*, *Ictalurus punctatus*, estradiol, gene expression, genes, gonadotropins, hormonal control, hormone receptors, estrous cycle, oocyte maturation, oocytes, oogenesis, ovarian follicles, ovaries, seasonality, spawning season, steroid hormones, vitellogenesis .

Kimura, M., M. Taniguchi, Y. Mikami, T. Masuda, T. Yoshida, M. Mishina, and T. Shimizu (2007). **Identification and characterization of zebrafish semaphorin 6D.** *Biochemical and Biophysical Research Communications* 363(3): 762-8. ISSN: 0006-291X.
NAL Call Number: 442.8B5236
Abstract: The semaphorin gene family contains a large number of secreted and transmembrane proteins; some function as repulsive and attractive cues of axon guidance during development. Here, we report cloning and characterization of zebrafish transmembrane semaphorin gene, semaphorin 6D (sema6D). Sema6D is expressed predominantly in the nervous system during embryogenesis, as determined by in situ hybridization. We also found that Sema6D binds Plexin-A1 in vitro, but not other Plexins. It induces the repulsion of dorsal root ganglion axons, but not sympathetic axons. Consequently, Sema6D might use Plexin-A1 as a receptor to repel specific types of axons during development.
Descriptors: zebrafish, semaphorin gene, transmembrane proteins, sema6d, plexin-A1, ganglion axon.

Kitzmann, M., A. Bonnielu, C. Duret, B. Vernus, M. Barro, C.D. Laoudj, J.M. Verdi, and G. Carnac (2006). **Inhibition of notch signaling induces myotube hypertrophy by recruiting a subpopulation of reserve cells**. *Journal of Cellular Physiology* 208(3): 538-548. ISSN: 0021 9541.
NAL Call Number: 444.8 J822
Abstract: During muscle differentiation, a population of quiescent undifferentiated myoblasts (reserve cells) emerges among mature muscle cells. However, the molecular mechanisms underlying such cell segregation and the characterization of this subpopulation of myoblasts remain to be determined. Notch is known to control the behavior and fate of murine muscle stem cells. In this study, we examined the role of Notch in myoblast segregation. We showed that inhibition of Notch activity by either overexpressing Numb or by using a pharmacological gamma-secretase inhibitor (DAPT) enhanced differentiation of murine and human myoblasts. This effect was not restricted to in vitro culture systems since DAPT-treated zebrafish embryos also showed increased differentiation. Using C2.7 myoblasts as a model, we showed that inhibition of Notch induced myotube hypertrophy by recruiting reserve cells that do not normally fuse. We further showed that endogenous Notch-signaling components were differentially expressed and activated in reserve cells with respect to Notch 1 and CD34 expression. We identified CD34 negative reserve cells as the subpopulation of myoblasts recruited to fuse into myotubes during differentiation in response to Notch inhibition. Therefore, we showed here that the activation of Notch 1 is important to maintain a subpopulation of CD34 negative reserve cells in an undifferentiated state. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, biochemistry, c2.7 myoblast model, Notch signaling, muscle differentiation, myotube hypertrophy, zebrafish.

Krawitz, P., C. Haffner, R. Fluhrer, H. Steiner, B. Schmid, and C. Haass (2005). **Differential Localization and Identification of a Critical Aspartate Suggest Non-redundant Proteolytic functions of the Presenilin Homologues SPPL2b and SPPL3**. *Journal of Biological Chemistry* 280(47): 39515-39523. ISSN: 0021-9258.
NAL Call Number: 381J824
Descriptors: zebrafish, antisense, cell death, central nervous system, endoplasmic reticulum, erythrocytes, freshwater fish, Lysosomes, mutation, mutations, peptides, phenotypes, presenilin, proteolysis, Secretase, signal peptides , aspartic endopeptidase, endosomes, peptidase, Danio rerio, N3 11008 neurochemistry, Q1 01183 taxonomy and morphology.

Krishnan, K., T. Kathiresan, R. Raman, B. Rajini, V.M. Dhople, R.K. Aggrawal, and Y. Sharma (2007). **Ubiquitous lens alpha -, beta -, and gamma -crystallins accumulate in anuran cornea as corneal crystallins**. *Journal of Biological Chemistry* 282(26): 18953-18959. ISSN: 0021-9258.
NAL Call Number: 381J824
Descriptors: zebrafish, *Bufo melanostictus*, *Danio rerio*, *rana tigrina*, amphibia, amphibians, aldehydes, amphibiotic species, animal physiology, dehydrogenases, eyes, freshwater fish.

Kunwar, P.S., D.E. Siekhaus, and R. Lehmann (2006). **In vivo migration: a germ cell perspective**. *Annual Review of Cell and Developmental Biology* 22: 237-265. ISSN: 1081 0706.
NAL Call Number: QH573.A55
Abstract: The basic concepts of the molecular machinery that mediates cell migration have been gleaned from cell culture systems. However, the three-dimensional environment within an organism presents migrating cells with a much greater challenge. They must move between and among other cells while interpreting multiple attractive and repulsive cues to choose their proper path. They must coordinate their cell adhesion with their surroundings and know when to start and stop moving. New insights into the control of these remaining mysteries have emerged from genetic dissection and live imaging of germ cell migration in *Drosophila*, zebrafish, and mouse embryos. In this review, we first describe germ cell migration in cellular and mechanistic detail in these different model systems. We then compare these systems to highlight the emerging principles. Finally, we contrast the migration of germ cells with that of immune and cancer cells to outline the conserved and different mechanisms. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, *Drosophila*, mouse, biochemistry, immune system, reproductive system, metastasis , cell migration, perspective , in vivo migration .

Kurokawa, T., S. Uji, and T. Suzuki (2005). **Identification of pepsinogen gene in the genome of stomachless fish, *Takifugu rubripes***. *Comparative Biochemistry and Physiology B, Biochemistry and Molecular Biology* 140(1): 133-140. ISSN: 0305 0491.
NAL Call Number: QP501.C6
Abstract: Pepsinogen is a precursor of pepsin, a gastric specific protease belonging to the aspartic proteinase family. In teleosts, several species, such as zebrafish and puffer, have independently lost gastric glands. So whether puffer have pepsinogen gene or not is an interesting issue. A search of GSS database for pufferfish, Takifugu rubripes, revealed five different aspartic proteinase genes in its genome. One of them (pPep) has typical pepsinogen structure and belongs to the fish pepsinogen cluster by phylogenic analysis. The pPep antisense probe hybridized to the gastric glands of flounder stomach. Therefore, we concluded that pPep is a pufferfish pepsinogen. The pufferfish pepsinogen mRNA was not expressed in the digestive organs but specifically in the skin. We speculated that while Tetraodontiformes evolutionarily lost gastric glands, pufferfish pepsinogen acquired an alternative function to food digestion. Copyright © 2008 CABI

Descriptors: zebrafish, amino acid sequences, gene expression, genes, genomes, nucleotide sequences, pepsinogen, phylogenetics, Tetraodontidae, Tetraodontiformes.

Lee, K., P. Nallathamby, L. Browning, C. Osgood, and X.-H. Xu (2007). **In vivo imaging of transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos**. *American Chemical Society Nano* 1(2): 133-143. ISSN: 1936-0851.
NAL Call Number: TA418.9.N35
Descriptors: zebrafish, zebrafish embryos, in vivo, imaging, transport, biocompatibility, single silver nanoparticle.

Lee, L.T., F.K.Y. Siu, J.K.V. Tam, I.T.Y. Lau, A.O.L. Wong, M.C.M. Lin, H. Vaudry, and B.K.C. Chow (2007). **Discovery of growth hormone-releasing hormones and receptors in nonmammalian vertebrates**. *Proceedings of the National Academy of Sciences, USA* 104(7): 2133-2138. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, *carassius auratus*, *Danio rerio*, *Fugu*, *Oncorhynchus mykiss*, *Oryzias latipes*, *pleuronectiformes*, *Tetraodon*, flatfishes, flounders, frogs, goldfish, medaka, rainbow trout, soles, swellfishes, toads, amphibiotic species, brain, chromosomes, cyclic AMP, evolution, evolutionary genetics, genomes, growth hormone, growth hormone-releasing hormone, hormones, marine fish, phylogeny, pituitary (anterior), pituitary gland, anura.

Lemeer, S., C. Jopling, F. Naji, R. Ruijtenbeek, M. Slijper, A.J. Heck, and J. den Hertog (2007). **Protein-tyrosine kinase activity profiling in knock down zebrafish embryos.** *Public Library of Science One* 2: E581. ISSN: 1817-101X.
Abstract: BACKGROUND: Protein-tyrosine kinases (PTKs) regulate virtually all biological processes. PTKs phosphorylate substrates in a sequence-specific manner and relatively short peptide sequences determine selectivity. Here, we developed new technology to determine PTK activity profiles using peptide arrays. The zebrafish is an excellent model system to investigate signaling in the whole organism, given its wealth of genetic tools, including morpholino-mediated knock down technology. We used zebrafish embryo lysates to determine PTK activity profiles, thus providing the unique opportunity to directly compare the effect of protein knock downs on PTK activity profiles on the one hand and phenotypic changes on the other. METHODOLOGY: We used multiplex arrays of 144 distinct peptides, spotted on a porous substrate, allowing the sample to be pumped up and down, optimizing reaction kinetics. Kinase reactions were performed using complex zebrafish embryo lysates or purified kinases. Peptide phosphorylation was detected by fluorescent anti-phosphotyrosine antibody binding and the porous chips allowed semi-continuous recording of the signal. We used morpholinos to knock down protein expression in the zebrafish embryos and subsequently, we determined the effects on the PTK activity profiles. RESULTS AND CONCLUSION: Reproducible PTK activity profiles were derived from one-day-old zebrafiish embryos. Morpholino-mediated knock downs of the Src family kinases, Fyn and Yes, induced characteristic phenotypes and distinct changes in the PTK activity profiles. Interestingly, the peptide substrates that were less phosphorylated upon Fyn and Yes knock down were preferential substrates of purified Fyn and Yes. Previously, we demonstrated that Wnt11 knock down phenocopied Fyn/Yes knock down. Interestingly, Wnt11 knock down induced similar changes in the PTK activity profile as Fyn/Yes knock down. The control Nacre/Mitfa knock down did not affect the PTK activity profile significantly. Our results indicate that the novel peptide chip technology can be used to unravel kinase signaling pathways in vivo.
Descriptors: zebrafish embryos, protein-tyrosine kinases, pTKs, Nacre, Mitfa, knock down, activity, peptide chip technology, kinase signaling, in vivo.

Li, L.i., M. Kobayashi, H. Kaneko, Y. Nakajima-Takagi, Y. Nakayama, and M. Yamamoto (2008). **Molecular evolution of Keap1: TWO Keap1 molecules with distinctive intervening region structures are conserved among fish**. *Journal of Biological Chemistry* 283(6): 3248-3255. ISSN: 0021-9258.
NAL Call Number: 381J824
Descriptors: zebrafish, amino acids, cysteine, genomes, mutation, Nrf2 protein, oxidants, transcription activation, transcription factors, ubiquitin-protein ligase, adaptor proteins, molecular evolution, *Danio rerio*.

Lin, Y., X. Zhang, K. Liang, H. Yang, and H. Zhang (2005). **Phylogenetic analysis and developmental expression of brp-like genes in amphioxus and zebrafish.** *Comparative Biochemistry and Physiology. Part B: Biochemistry and Molecular Biology* 141(1): 71-76. ISSN: 0305 0491.
NAL Call Number: QP501.C6
Descriptors: zebrafish, *branchiostoma belcheri tsingTauense, Danio rerio*, nucleic acids, brp like genes, proteins, brp like gene transcript, embryo development, larval development, molecular genetics, transcript distribution in embryo and larval body, possible function and phylogenetic significance, phylogeny.

Liu, J.X. and C.A. Lessman (2007). **Soluble tubulin complexes, gamma -tubulin, and their changing distribution in the zebrafish (*Danio rerio*) ovary, oocyte and embryo**. *Comparative Biochemistry and Physiology. Part B: Biochemistry and Molecular Biology* 147(1): 56-73. ISSN: 0305 0491.
NAL Call Number: QP501.C6
Abstract: Tubulin dynamics, i.e., the interchange of polymeric and soluble forms, is important for microtubule (MTs) cellular functions, and thus plays essential roles in zebrafish oogenesis and embryogenesis. A novel finding in this study revealed that there were soluble pools of tubulins in zebrafish oocytes that were sequestered and maintained in a temporary "oligomeric" state, which retained assembling and disassembling potential (suggested by undetected acetylated tubulin, marker of stable tubulin), but lacked abilities to assemble into MTs spontaneously in vivo. Using differential centrifugation, gel chromatography and DM1A-probed western blot, soluble alpha -tubulin was found to be associated with large molecular weight complexes (MW range to over 2 MDa) which were reduced in amount by the blastula stage, especially in some batches of embryos, with a concomitant decrease in soluble tubulin. Complexes (MW range less than 2 MDa) then increased in the gastrula with an increase in soluble alpha -tubulin. Two different anti- gamma -tubulin monoclonal antibodies, GTU 88 and TU 30, revealed the existence of soluble gamma -tubulin in both zebrafish oocytes and embryos, which also decreased by the blastula stage and increased in the gastrula stage. Soluble alpha -tubulin and gamma -tubulin extracted from zebrafish ovaries, oocytes and embryos co-localized in fractions on three different columns: S-200 Sephacryl, DEAE and Superose-6b. The soluble tubulin complexes were competent to assemble into MTs in vitro induced by taxol, and gamma -tubulin was co-localized with assembled MTs. These soluble tubulin complexes were stable during freeze-thaw cycles and resisted high ionic interaction (up to 1.5 M NaCl). Furthermore, some ovarian soluble alpha -tubulin could be co-immunoprecipitated with gamma -tubulin, and vice versa. Two antibodies specific for Xenopus gamma -tubulin ring complex proteins (Xgrip 109 and Xgrip 195) detected single bands from ovarian extracts in western blots, suggesting the existence of Xgrip 109 and Xgrip 195 homologues in zebrafish. These findings, together with recent work on gamma -tubulin ring complexes in oocytes, eggs and embryos of other species, suggest that soluble gamma -tubulin-associated protein complexes may be involved in regulating tubulin dynamics during zebrafish oogenesis and embryogenesis. Copyright © 2008 CABI

Descriptors: zebrafish, *Danio rerio*, embryogenesis, embryos, microtubules, oocytes, oogenesis, ovaries, tissue distribution, tubulin.

Luo, X., H. Inouye, A.A. Gross, M.M. Hidalgo, D. Sharma, D. Lee, R.L. Avila, M. Salmona, and D.A. Kirschner (2007). **Cytoplasmic domain of zebrafish myelin protein zero: adhesive role depends on beta-conformation.** *Biophysical Journal* 93(10): 3515-28. ISSN: 0006 3495.
NAL Call Number: 442.8B5238
Abstract: Solution spectroscopy studies on the cytoplasmic domain of human myelin protein zero (P0) (hP0-cyt) suggest that H-bonding between beta-strands from apposed molecules is likely responsible for the tight cytoplasmic apposition in compact myelin. As a follow-up to these findings, in the current study we used circular dichroism and x-ray diffraction to analyze the same type of model membranes previously used for hP0-cyt to investigate the molecular mechanism underlying the zebrafish cytoplasmic apposition. This space is significantly narrower in teleosts compared with that in higher vertebrates, and can be accounted for in part by the much shorter cytoplasmic domain in the zebrafish protein (zP0-cyt). Circular dichroism measurements on zP0-cyt showed similar structural characteristics to those of hP0-cyt, i.e., the protein underwent a beta-->alpha structural transition at lipid/protein (L/P) molar ratios >50, and adopted a beta-conformation at lower L/P molar ratios. X-ray diffraction was carried out on lipid vesicle solutions with zP0-cyt before and after dehydration to study the effect of protein on membrane lipid packing. Solution diffraction revealed the electron-density profile of a single membrane bilayer. Diffraction patterns of dried samples suggested a multilamellar structure with the beta-folded P0-cyt located at the intermembrane space. Our findings support the idea that the adhesive role of P0 at the cytoplasmic apposition in compact myelin depends on the cytoplasmic domain of P0 being in the beta-conformation.
Descriptors: zebrafish, myelin p0 protein chemistry, amino acid sequence, cytoplasm metabolism, lipid bilayers chemistry, lipids chemistry, statistical models, molecular sequence data, peptides chemistry, protein binding, protein Conformation, secondary protein structure, tertiary protein structure, X- ray diffraction.

Maack, G., H. Segner, and C.R. Tyler (2003). **Ontogeny of sexual differentiation in different strains of zebrafish (*Danio rerio*)**. *Fish Physiology and Biochemistry* 28(1/4): 125-128. ISSN: 0920 1742.
NAL Call Number: QL639.1.F583
Abstract: The zebrafish (ZF) undergoes juvenile hermaphroditism, where in all fish, the gonad first develops as an ovary-like structure, before the sex is fixed and the gonad then differentiates into either a testis or an ovary. Sexual differentiation is being adopted as an endpoint for assessing endocrine disrupting chemicals, yet there is only very limited information on the timing and variability in sexual differentiation process both within and between strains of ZF. In this study, using gonad histology, the ontogeny of sexual differentiation was studied in two strains of ZF, one with a high level of heterozygocity (WIK strain) and another that had been in-bred for several generations. There was a high variability in the timing of sex differentiation between individuals within a specific strain (with no obvious relationship with body mass), but there were no differences between the two strains. Transformation of the immature gonad into testes in males started in week 5 post fertilization (pf) and completion of sexual differentiation for all fish in both study populations occurred by week 11-12 pf. The size of the fish containing transforming gonads in the WIK strain and in-bred strain were similar (ranging from 12 to 23 mm, and from 13 to 22 mm total length, respectively). Copyright © 2008 CABI
Descriptors: zebrafish, *Danio rerio*, biological development, hermaphroditism, ontogeny, sex differentiation, sexual development, strain differences .

MacGregor, E. and W.H. Kinsey (2004). **Single-cell method for estimation of protein tyrosine kinases in the zebrafish egg.** *Methods in Molecular Biology* 253: 285-91. ISSN: 1064-3745.
Descriptors: zebrafish eggs, biological assay methods, ovum enzymology, protein tyrosine kinases analysis, polyacrylamide gel electrophoresis, phosphorylation.

Madden, P.W., M.J. Babcock, M.E. Vayda, and R.E. Cashon (2004). **Structural and kinetic characterization of myoglobins from eurythermal and stenothermal fish species**. *Comparative Biochemistry and Physiology, Part B: Biochemistry and Molecular Biology* 137(3): 341-350. ISSN: 1096-4959.
NAL Call Number: QP501.C6
Descriptors: zebrafish, *Danio rerio*, *notothenia coriiceps*, *Scomber*, *Thunnus albacares*, dissociation, fish physiology, freshwater fish and marine fish, molecular biology, myoglobins, respiration, serological studies, structural analysis , temperature effects .

Marin, O., V.H. Bustos, L. Cesaro, F. Meggio, M.A. Pagano, M. Antonelli, C.C. Allende, L.A. Pinna, and J.E. Allende (2003). **A noncanonical sequence phosphorylated by casein kinase 1 in beta -catenin may play a role in casein kinase 1 targeting of important signaling proteins**. *Proceedings of the National Academy of Sciences, USA* 100(18): 10193-10200. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, *Danio rerio*, rats, cell division, DNA, enzymatic activity, glycogen, mutations, proteins , recombinants, serine, thyroid, tumors, cK1 protein, beta -catenin, phosphorylation.

Masumoto, J., W. Zhou, F.F. Chen, F. Su, J.Y. Kuwada, E. Hidaka, T. Katsuyama, J. Sagara, S. Taniguchi, P. Ngo Hazelett, J.H. Postlethwait, G. Nunez, and N. Inohara (2003). **Caspy, a zebrafish caspase, activated by ASC oligomerization is required for pharyngeal arch development.** *Journal of Biological Chemistry* 278(6): 4268-76. ISSN: 0021-9258.
NAL Call Number: 381J824
Abstract: The pyrin domain was identified recently in multiple proteins that are associated with apoptosis and/or inflammation, but the physiological and molecular function of these proteins remain poorly understood. We have identified Caspy and Caspy2, two zebrafish caspases containing N-terminal pyrin domains. Expression of Caspy and Caspy2 induced apoptosis in mammalian cells that were inhibited by general caspase inhibitors. Biochemical analysis revealed that both Caspy and Caspy2 are active caspases, but they exhibit different substrate specificity. Caspy, but not Caspy2, interacted with the zebrafish orthologue of ASC (zAsc), a pyrin- and caspase recruitment domain-containing protein identified previously in mammals. The pyrin domains of both Caspy and zAsc were required for their interaction. Furthermore, zAsc and Caspy co-localized to the "speck" when co-transfected into mammalian cells. Enforced oligomerization of zAsc, but not simple interaction with zAsc, induced specific proteolytic activation of Caspy and enhanced Caspy-dependent apoptosis. Injection of zebrafish embryos with a morpholino antisense oligonucleotide corresponding to caspy resulted in an "open mouth" phenotype associated with defective formation of the cartilaginous pharyngeal skeleton. These studies suggest that zAsc mediates the activation of Caspy, a caspase that plays an important role in the morphogenesis of the jaw and gill-bearing arches.
Descriptors: zebrafish, caspases metabolism, cytoskeletal proteins metabolism, pharynx embryology, amino acid sequence, apoptosis, base sequence, biopolymers, caspases chemistry, caspases genetics, DNA primers, complementary DNA, enzyme activation, genome, molecular sequence data, amino acid sequence homology, substrate specificity, proteins chemistry.

Mathew, L.K., S. Sengupta, A. Kawakami, E. Andreasen, C.V. Loehr, C.A. Loynes, S.A. Renshaw, R.T. Peterson, and R.L. Tanguay (2007). **Unraveling tissue regeneration pathways using chemical genetics**. *Journal of Biological Chemistry* 282(48): 35202-35210. ISSN: 0021-9258.
NAL Call Number: 381J824
Descriptors: zebrafish, *Danio rerio*, amputation, antisense, developmental stages, glucocorticoid receptors, glucocorticoids, inflammation, molecular modelling, mutation, mutations, pU.1 protein, receptors, regeneration, signal transduction, wound healing.

Miesbauer, M., T. Bamme, C. Riemer, B. Oidtmann, K.F. Winklhofer, M. Baier, and J. Tatzelt (2006). **Prion protein-related proteins from zebrafish are complex glycosylated and contain a glycosylphosphatidylinositol anchor.** *Biochemical and Biophysical Research Communications* 341(1): 218-24. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: A hallmark of prion diseases in mammals is a conformational transition of the cellular prion protein (PrP(C)) into a pathogenic isoform termed PrP(Sc). PrP(C) is highly conserved in mammals, moreover, genes of PrP-related proteins have been recently identified in fish. While there is only little sequence homology to mammalian PrP, PrP-related fish proteins were predicted to be modified with N-linked glycans and a C-terminal glycosylphosphatidylinositol (GPI) anchor. We biochemically characterized two PrP-related proteins from zebrafish in cultured cells and show that both zePrP1 and zeSho2 are imported into the endoplasmic reticulum and are post-translationally modified with complex glycans and a C-terminal GPI anchor.
Descriptors: zebrafish, mice, endoplasmic reticulum metabolism, glycosylphosphatidylinositols chemistry, glycosylphosphatidylinositols metabolism, prions chemistry and metabolism, amino acid sequence, glycosylation, molecular sequence data.

Mindnich, R., D. Deluca, and J. Adamski (2004). **Identification and characterization of 17 beta-hydroxysteroid dehydrogenases in the zebrafish, *Danio rerio*.** *Molecular and Cellular Endocrinology* 215(1-2): 19-30. ISSN: 0303-7207.
NAL Call Number: QP187.A1M6
Abstract: The 17 beta-hydroxysteroid dehydrogenases (17 beta-HSDs) are key enzymes in the final steps of steroid hormone synthesis. 17beta-HSD type 1 (HSD17B1) catalyzes the reduction of estrone to estradiol, while type 3 (HSD17B3) performs the conversion of androstenedione to testosterone. Here we present a functional genomics study of putative candidates of these enzymes in the zebrafish. By an in silico screen of zebrafish EST databases we identified three candidate homologs for both HSD17B1 and HSD17B3. Phylogenetic analysis, unique expression patterns (RT-PCR) during embryogenesis and adulthood, as well as activity measurements revealed that one of the HSD17B1 candidates is the zebrafish homolog, while the other two are paralogous photoreceptor-associated retinol dehydrogenases. All three HSD17B3 candidate genes showed nearly identical, ubiquitous expressions in embryogenesis and adult tissues and were identified to be paralogs of HSD17B12 and a yet uncharacterized putative steroid dehydrogenase. Phylogenetic analysis shows that HSD17B3 and HSD17B12 are descendants from a common ancestor.
Descriptors: zebrafish, 17 hydroxysteroid dehydrogenases metabolism, developmental gene expression regulation, 17 hydroxysteroid dehydrogenases genetics, 17 hydroxysteroid dehydrogenases isolation and purification, amino acid sequence, databases, factual, exons, gene library, introns, molecular sequence data, phylogeny, messenger RNA genetics and metabolism, recombinant proteins, reverse transcriptase PCR, pcr, amino acid sequence homology.

Mindnich, R., F. Haller, F. Halbach, G. Moeller, M.H.d. Angelis, and J. Adamski (2005). **Androgen metabolism via 17 beta -hydroxysteroid dehydrogenase type 3 in mammalian and non-mammalian vertebrates: comparison of the human and the zebrafish enzyme**. *Journal of Molecular Endocrinology* 35(2): 305-316. ISSN: 0952 5041.

Online: http://dx.doi.org/10.1677/jme.1.01853

NAL Call Number: QP187.3.M64J68

Abstract: Formation and inactivation of testosterone is performed by various members of the 17 beta -hydroxysteroid dehydrogenase (17 beta -HSD) family. The main player in testosterone formation is considered to be 17 beta -HSD type 3, which catalyzes the reduction of androstenedione to testosterone with high efficiency and is almost exclusively expressed in testis. So far, only the mammalian homologs have been characterized but nothing is known about the role of 17 beta -HSD type 3 in other vertebrates. In this study, we describe the identification and characterization of the zebrafish homologue. We found zebrafish 17 beta -HSD type 3 to be expressed in embryogenesis from sphere to 84 h post-fertilization. Expression was also detected in various tissues of both male and female adults, but displayed sexual dimorphism. Interestingly, expression was not highest in male testis but in male liver. In female adults, strongest expression was observed in ovaries. At the subcellular level, both human and zebrafish 17 beta -HSD type 3 localize to the endoplasmic reticulum. The zebrafish enzyme in vitro effectively catalyzed the conversion of androstenedione to testosterone by use of NADPH as cofactor. Among further tested androgens epiandrosterone and dehydroepiandrosterone were accepted as substrates and reduced at C-17 by the human and the zebrafish enzyme. Androsterone and androstanedione though, were only substrates of human 17 beta -HSD type 3, not the zebrafish enzyme. Furthermore, we found that both enzymes can reduce 11-ketoandrostenedione as well as 11 beta -hydroxyandrostenedione at C-17 to the respective testosterone forms. Our results suggest that 17 beta -HSD type 3 might play slightly different roles in zebrafish compared with human although testosterone itself is likely to have similar functions in both organisms. Copyright © 2008 CABI

Descriptors: zebrafish, humans, *Danio rerio*, androgens , androstenedione, cofactors, embryogenesis, endoplasmic reticulum, fertilization, hydroxysteroid dehydrogenase, liver, NAdpH, ovaries, prasterone, sexual dimorphism, steroidogenesis, testes, testosterone.

Mindnich, R., M. Hrabe de Angelis, and J. Adamski (2007). **Functional genome analysis indicates loss of 17beta-hydroxysteroid dehydrogenase type 2 enzyme in the zebrafish.** *Journal of Steroid Biochemistry and Molecular Biology* 103(1): 35-43. ISSN: 0960-0760.

NAL Call Number: QD426.A1J6

Abstract: Among the family of 17beta-hydroxysteroid dehydrogenases, the type 2 (17beta-HSD 2) is the main enzyme responsible for inactivation of estrogens and androgens, catalyzing the oxidation of the C17 hydroxyl group. 17beta-HSD 2 has been studied only in mammals, its occurrence and function in other vertebrates hardly known. We investigated the presence of homologs in non-mammalian species and found sequences of 17beta-HSD 2 and its closest homolog 11beta-HSD 2 in zebrafish (*Danio rerio), Takifugu rubripes, Tetraodon nigroviridis, Xenopus tropicalis* and chicken databases. Furthermore, we cloned zebrafish 17beta-HSD 2 from ovarian tissue and found high expression also in the testis of adult fish and throughout embryogenesis. The enzyme, though, is inactive likely due to a non-sense N-terminal region including a dysfunctional cofactor binding motif. Replacement of the affected part by the corresponding human 17beta-HSD 2 sequence fully restored enzymatic activity. Comparison of all retrieved 17beta-HSD 2 sequences indicates that this functional loss may have occurred only in zebrafish, where steroid inactivation at position C17 seems to pursue without the protein studied. The closely related 11beta-HSD 2 is unlikely to substitute for 17beta-HSD 2 since in our hands it did not catalyze the respective oxidation of testosterone or estradiol.

Descriptors: zebrafish, 17 hydroxysteroid dehydrogenases genetics, genome, 17 hydroxysteroid dehydrogenases metabolism, amino acid sequence, databases, protein, isoenzymes classification, isoenzymes genetics, molecular sequence data, phylogeny, amino acid sequence homology, substrate specificity, tissue distribution, proteins metabolism.

Moise, A.R., A. Isken, M. Dominguez, A.R. de Lera, J. von Lintig, and K. Palczewski (2007). **Specificity of zebrafish retinol saturase: formation of all-trans-13,14-dihydroretinol and all-trans-7,8-dihydroretinol.** *Biochemistry* 46(7): 1811-20. ISSN: 0006 2960.
NAL Call Number: 381 B523
Abstract: Metabolism of vitamin A, all-trans-retinol, leads to the formation of 11-cis-retinaldehyde, the visual chromophore, and all-trans-retinoic acid, which is involved in the regulation of gene expression through the retinoic acid receptor. Enzymes and binding proteins involved in retinoid metabolism are highly conserved across species. We previously described a novel mammalian enzyme that saturates the 13-14 double bond of all-trans-retinol to produce all-trans-13,14-dihydroretinol, which then follows the same metabolic fate as that of all-trans-retinol. Specifically, all-trans-13,14-dihydroretinol is transiently oxidized to all-trans-13,14-dihydroretinoic acid before being oxidized further by Cyp26 enzymes. Here, we report the identification of two putative RetSat homologues in zebrafish, one of which, zebrafish RetSat A (zRetSat A), also had retinol saturase activity, whereas zebrafish RetSat B (zRetSat B) was inactive under similar conditions. Unlike mouse RetSat (mRetSat), zRetSat A had an altered bond specificity saturating either the 13-14 or 7-8 double bonds of all-trans-retinol to produce either all-trans-13,14-dihydroretinol or all-trans-7,8-dihydroretinol, respectively. zRetSat A also saturated the 13-14 or 7-8 double bonds of all-trans-3,4-didehydroretinol (vitamin A2), a second endogenous form of vitamin A in zebrafish. The dual enzymatic activity of zRetSat A displays a newly acquired specificity for the 13-14 double bond retained in higher vertebrates and also the evolutionarily preserved activity of bacterial phytoene desaturases and plant carotenoid isomerases. Expression of zRetSat A was restricted to the liver and intestine of hatchlings and adult zebrafish, whereas zRetSat B was expressed in the same tissues but at earlier developmental stages. Exogenous all-trans-retinol, all-trans-13,14-dihydroretinol, or all-trans-7,8-dihydroretinol led to the strong induction of the expression of the retinoic acid-metabolizing enzyme, Cyp26A1, arguing for an active signaling function of dihydroretinoid metabolites in zebrafish. These findings point to a conserved function but altered specificity of RetSat in vertebrates, leading to the generation of various dihydroretinoid compounds, some of which could have signaling functions.
Descriptors: zebrafish, oxidoreductasesacting on ch ch group donors metabolism, vitamin A analogs and derivatives, amino acid sequence, catalysis, molecular sequence data, organ specificity, oxidoreductasesacting on ch ch group donors biosynthesis, oxidoreductasesacting on ch ch group donors genetics, stereoisomerism, substrate specificity, vitamin A metabolism, proteins biosynthesis.

Molich, A. and N. Heisler (2005). **Determination of pH by microfluorometry: intracellular and interstitial pH regulation in developing early-stage fish embryos (*Danio rerio*).** *Journal of Experimental Biology* 208(Pt 21): 4137-49. ISSN: 0022-0949.
NAL Call Number: 442.8B77
Abstract: Microfluorometric techniques were applied in vivo for continuous monitoring of specific acid-base parameters in zebrafish (Danio rerio) embryos during early stages of ontogeny. Dextran-coupled pH-sensitive single-excitation/dual-emission dye SNARF-1 was pressure-injected into individual cells or the interstitial space of 16- to 256-cell embryos, and pH was continuously recorded during subsequent development for time periods of up to 8 h. A novel calibration technique was developed, essentially characterized by in vitro inorganic buffer calibra-

tion of the optical system and mathematical post-processing according to the effects of in vivo dye modifiers through a correlation established by direct comparison of optical techniques with pH microelectrodes. This approach results in high accuracy of microfluorometry, comparable with that of pH electrodes, and a recovery only limited by the physical stability of the utilized optical system. Intracellular pH (pHi) in Danio rerio embryos between 1k-cells stage and the end of epiboly was found to be well regulated to a mean value of 7.55+/-0.13 (+/-s.d.), a range distinctly more alkaline than typical values for adult fish but in accordance with embryonic pHi of a few non-fish species shortly after fertilization. Also, interstitial pH (pHint) was significantly higher (8.08+/-0.25) than values for extracellular pH in adult fish. Distributions of HCO3- across membranes and between interstitium and ambient fluid compared with respective potentials strongly suggest that pH in these early stages of ontogeny is already adjusted by active transfer processes. Non-respiratory changes in ambient pH between 7.7 and 8.5 did not significantly affect pHi, a result potentially attributable to low membrane leakage rate or to the potency of active transfer mechanisms. In order to assess the pH regulatory systems more quantitatively, embryos were exposed to ambient changes of carbon dioxide partial pressure (PCO2). The direct impact of PCO2 changes on cell pH was alleviated by cell non-bicarbonate buffering and subsequent rapid, almost complete, compensation by changes in cell [HCO3-] as an expression of transmembrane transfer of acid-base relevant ions. On the basis of these results, we conclude that the regulatory potency of embryonic cells is well developed, is active to resist extensive homoiostatic stress and is efficient to maintain critical metabolism in adverse conditions, even at early stages of ontogeny.
Descriptors: cytophotometry methods, intracellular fluid chemistry, benzopyrans, carbon dioxide metabolism, embryos, metabolism, hydrogen ion concentration, naphthols, rhodamines.

Moriguchi, K., T. Takemoto, T. Aoki, S. Nakakita, S. Natsuka, and S. Hase (2007). **Free oligosaccharides with Lewis x structure expressed in the segmentation period of zebrafish embryo.** *Journal of Biochemistry* 142(2): 213-27. ISSN: 0021-924X.
NAL Call Number: 385 J822
Abstract: We previously reported that zebrafishalpha1-3fucosyltrasferase 1 (zFT1) was expressed in embryos at the segmentation period, and was capable of synthesizing the Lewis x epitope [Gal beta1-4(Fuc alpha1-3)GlcNAc] [Kageyama et.al, J. Biochem., 125, 838-845 (1999)]. In the current study, we attempted to detect the enzyme products of zFT1 in zebrafish embryos. Oligosaccharides were prepared from the zebrafish embryos at 12, 18 and 48 h after fertilization and labelled with a fluorophore, 2-aminopyridine, for highly sensitive detections. Pyridylamino (PA)-oligosaccharides that were alpha1-3/4fucosidase sensitive and time-dependently expressed at 18 h after fertilization were identified as candidates for the in vivo products synthesized by zFT1. Structures of these oligosaccharides were determined by a combination of exoglycosidase digestions and two-dimensional HPLC sugar mapping to be the biantennary complex-type structures with two Lewis x epitopes: (Gal beta1-4)(0,1,2)-{Gal beta1-4(Fuc alpha1-3)GlcNAc beta1-2Man alpha1-6[Gal beta1-4(Fuc alpha1-3)GlcNAc beta1-2Man alpha1-3]}Man beta1-4GlcNAc, and (Gal beta1-4)(0,1)-{Gal beta1-4(Fuc alpha1-3)GlcNAc beta1-2Man alpha1-6[Gal beta1-4(Fuc alpha1-3)GlcNAc beta1-2Man alpha1-3]} Man beta1-4GlcNAc beta1-4GlcNAc. The presence of Lewis x structure of these oligosaccharides together with their expression time suggests that they are products of zFT1. Remarkably, most of these oligosaccharides were free form. Furthermore, we detected an endo-beta-N-acetylglucosaminidase activity in the 18 h embryo. These results suggest that the oligosaccharides synthesized by zFT1 are present in the embryo at the segmentation period in free form, owing to the liberation from glycoproteins with endo-beta-N-acetylglucosaminidase(s) and/or glycoamidase(s).
Descriptors: zebrafish embryos, Lewis X structure, free oligosaccharides, zfT1.

Nadauld, L.D., D.N. Shelton, S. Chidester, H.J. Yost, and D.A. Jones (2005). **The zebrafish retinol dehydrogenase, rdh1l, is essential for intestinal development and is regulated by the tumor suppressor adenomatous polyposis coli.** *Journal of Biological Chemistry* 280(34): 30490-5. ISSN: 0021-9258.

NAL Call Number: 381J824

Abstract: Retinoic acid (RA) is a potent signaling molecule that plays important roles in multiple and diverse developmental processes. The contribution of retinoic acid to promoting the development and differentiation of the vertebrate intestine and the factors that regulate RA production in the gut remain poorly defined. Herein, we report that the novel retinol dehydrogenase, rdh1l, is required for proper gut development and differentiation. rdh1l is expressed ubiquitously during early development but becomes restricted to the gut by 3 days postfertilization. Knockdown of rdh1l results in a robust RA-deficient phenotype including lack of intestinal differentiation, which can be rescued by the addition of exogenous retinoic acid. We report that adenomatous polyposis coli (APC) mutant zebrafish harbor an RA-deficient phenotype including aberrant intestinal differentiation and that these mutants can be rescued by treatment with retinoic acid or injection of rdh1l mRNA. Further, we have found that although APC mutants are deficient in rdh1l expression, they harbor increased expression of raldh2 suggesting the control of RA production by APC is via retinol dehydrogenase activity. These results provide genetic evidence that retinoic acid is required for vertebrate gut development and that the tumor suppressor APC controls the production of RA in the gut by regulating the expression of the retinol dehydrogenase, rdh1l.

Descriptors: zebrafish, adenomatous polyposis coli protein metabolism, alcohol oxidoreductases biosynthesis, alcohol oxidoreductases physiology, intestines enzymology, tretinoin metabolism, proteins biosynthesis and physiology, alcohol oxidoreductases chemistry and metabolism, cell differentiation, developmental gene expression regulation, in situ hybridization, intestines embryology, intestines metabolism, mutation, phenotype, messenger RNA metabolism, reverse transcriptase PCR, time factors.

Nomura, R., E. Kamei, Y. Hotta, M. Konishi, A. Miyake, and N. Itoh (2006). **Fgf16 is essential for pectoral fin bud formation in zebrafish.** *Biochemical and Biophysical Research Communications* 347(1): 340-6. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: Zebrafish pectoral fin bud formation is an excellent model for studying morphogenesis. Fibroblast growth factors (Fgfs) and sonic hedgehog (shh) are essential for pectoral fin bud formation. We found that Fgf16 was expressed in the apical ectodermal ridge (AER) of fin buds. A knockdown of Fgf16 function resulted in no fin bud outgrowth. Fgf16 is required for cell proliferation and differentiation in the mesenchyme and the AER of the fin buds, respectively. Fgf16 functions downstream of Fgf10, a mesenchymal factor, signaling to induce the expression of Fgf4 and Fgf8 in the AER. Fgf16 in the AER and shh in the zone of polarizing activity (ZPA) interact to induce and/or maintain each other's expression. These findings have revealed that Fgf16, a newly identified AER factor, plays a crucial role in pectoral fin bud outgrowth by mediating the interactions of AER-mesenchyme and AER-ZPA.

Descriptors: Fgf chemistry and metabolism, limb bud embryology, limb bud physiology, organogenesis physiology, Fgf isolation and purification, developmental gene expression regulation physiology, limb bud anatomy and histology, organ specificity, tissue distribution.

Nunomura, W., Y. Takakuwa, G.N. Cherr, and K. Murata (2007). **Characterization of protein 4.1R in erythrocytes of zebrafish (*Danio rerio*): unique binding properties with transmembrane proteins and calmodulin.** *Comparative Biochemistry and Physiology. Part B, Biochemistry and*

Molecular Biology 148(2): 124-38. ISSN: 0305 0491.
NAL Call Number: QP501.C6
Abstract: Cytoskeletal protein 4.1R is instrumental in regulating erythrocyte plasticity. 4.1R is comprised of four domains identified after chymotryptic digestion: an N-terminal 30 kDa domain responsible for interaction with membrane proteins, a unique domain, a spectrin-actin binding (SAB) domain, and a C-terminal domain (CTD). 4.1R 30 kDa domain interactions with transmembrane proteins are regulated by the Ca(2+)/calmodulin (CaM) complex. Unlike mature mammalian erythrocytes, fish erythrocytes remain nucleated. Comparing their cytoskeleton architecture and functional properties is therefore of great interest. Here we characterized the recently cloned zebrafish (*Danio rerio*, ZF) 4.1R and compared its properties with human 4.1R. We identified three ZF4.1R mRNA transcripts in erythrocytes, all characterized by exclusion of the central domains. The major transcript, referred to as BL31, included a full length 30 kDa domain (ZFR30) and parts of the unique region Ua and of CTD. Two minor transcripts, referred to as BL42 and BL56, expressed parts of ZFR30 and of the unique region Ub and full length SAB and CTD domains. Antibodies to ZFR30, ZF4.1R CTD and ZF glycophorin C (GPC) labeled the ZF erythrocyte plasma membrane. ZFR30 bound to CaM in presence or absence of Ca(2+). Resonant mirror detection binding assays revealed that ZFR30 bound to human Band3 with low K((D)) (approximately 10nM), and to GPC with higher K((D)) (approximately 1nM). The Ca(2+)/CaM complex did not affect ZFR30 binding to Band3 and GPC. Finally, we confirmed ZFR30 binding to erythrocyte plasma membrane proteins by pulling down ZFR30 with human erythrocyte inside-out vesicles (IOV). This study defines unique structural and functional properties for ZF4.1R.
Descriptors: zebrafish, cattle, calmodulin metabolism, cytoskeletal proteins metabolism, erythrocytes metabolism, membrane proteins metabolism, alternative splicing genetics, amino acid sequence, calcium metabolism, cytoskeletal proteins chemistry and genetics, erythrocytes cytology, glycophorin metabolism, immunoblotting, kinetics, membrane proteins chemistry and genetics, molecular sequence data, molecular weight, protein binding, protein isoforms chemistry, genetics and metabolism, messenger RNA genetics and metabolism, proteins chemistry and genetics.

Ochi, H., S. Hans, and M. Westerfield (2008). **Smarcd3 regulates the timing of zebrafish myogenesis onset**. *Journal of Biological Chemistry* 283(6): 3529-3536. ISSN: 0021-9258.
NAL Call Number: 381J824
Descriptors: zebrafish, *Danio rerio*, cardiac muscle, chromatin remodeling, cofactors, fibroblast growth factor, fibroblast growth factor 8, Hedgehog protein, limiting factors, muscles, Myod protein, signal transduction, T-box gene, tails, transcription factors, blastula, myogenesis.

Ohkimoto, K., T. Sugahara, Y. Sakakibara, M. Suiko, M.Y. Liu, G. Carter, and M.C. Liu (2003). **Sulfonation of environmental estrogens by zebrafish cytosolic sulfotransferases.** *Biochemical and Biophysical Research Communications* 309(1): 7-11. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: Environmental estrogen-like chemicals are increasingly recognized as a potential hazardous factor for wildlife as well as humans. We have recently embarked on developing a zebrafish model for investigating the role of sulfonation in the metabolism and adverse functioning of environmental estrogens. Here, we report on a systematic investigation of the sulfonation of representative environmental estrogens (bisphenol A, 4-n-octylphenol, 4-n-nolylphenol, dieth-

ylstilbestrol, and 17 alpha-ethynylestradiol) by zebrafish cytosolic sulfotransferases (STs). Of the seven enzymes tested, four zebrafish STs (designated ZF ST #2, ZF ST #3, ZF ST #4, and ZF DHEA ST) exhibited differential sulfonating activities toward the five environmental estrogens tested, with ZF ST #3 being more highly active than the other three. It was further demonstrated that bisphenol A, 4-n-octylphenol, and 4-n-nonylphenol exerted concentration-dependent inhibition of the sulfonation of 17 beta-estradiol, implying a potential role of these environmental estrogens in interfering with the sulfonation, and possibly homeostasis, of endogenous estrogens. Kinetic studies revealed that the mechanism underlying the inhibition by bisphenol A or 4-n-nonylphenol to be of the competitive type.
Descriptors: zebrafish, cytosol enzymology, estrogens metabolism, sulfotransferases chemistry, cytosol metabolism, complementary DNA metabolism, diethylstilbestrol chemistry, dose response relationship, polyacrylamide gel electrophoresis, environment, estradiol congeners chemistry, estrogen-like chemical, non steroidal chemistry, ethinyl estradiol chemistry, kinetics, phenols chemistry, plasmids metabolism, protein isoforms, recombinant proteins chemistry and metabolism.

Olsson, C., J.D. Holbrook, G. Bompadre, E. Jonsson, C.H. Hoyle, G.J. Sanger, S. Holmgren, and P.L. Andrews (2008). **Identification of genes for the ghrelin and motilin receptors and a novel related gene in fish, and stimulation of intestinal motility in zebrafish (*Danio rerio*) by ghrelin and motilin.** *General and Comparative Endocrinology* 155(1): 217-26. ISSN: 0016-6480.
NAL Call Number: 444.8G28
Abstract: In mammals ghrelin has a diverse range of effects including stimulation of gut motility but although present in teleost fish its effects on motility have not been investigated. The present study used bioinformatics to search for fish paralogues of the ghrelin receptor and the closely related motilin receptor, and investigated the effects of ghrelin and motilin on gut motility in zebrafish, Danio rerio. Fish paralogues of the human ghrelin and motilin receptor genes were identified, including those from the zebrafish. In addition, a third gene was identified in three species of pufferfish (the only fish genome completely sequenced), which is distinct from the ghrelin and motilin receptors but more closely aligned to these receptors relative to other G-protein coupled receptors. Immunohistochemistry demonstrated strong ghrelin receptor-like reactivity in the muscle of the zebrafish intestine. In isolated intestinal bulb and mid/distal intestine preparations, ghrelin, motilin, and the motilin receptor agonist erythromycin all evoked contraction; these responses ranged between 9% and 51% of the contractions evoked by carbachol (10(-6)M). There were some variations in the concentrations found to be active in the different tissues, e.g., whereas motilin and rat ghrelin caused contraction of the intestinal bulb circular muscle at concentrations as low as 10(-8)M, human ghrelin (10(-8) to 10(-6)M) was without activity. Neither ghrelin (10(-7)M) nor erythromycin (10(-5)M) affected the contractions evoked by electrical field stimulation. The results suggest that both ghrelin and motilin can regulate intestinal motility in zebrafish and most likely other teleosts, and are discussed in relation to the evolution of these regulatory peptides.
Descriptors: zebrafish, ghrelin, motilin, receptors, intestinal motility, bioinformatics, immunohistochemistry.

Pelster, B. (2004). **Nitric oxide and vascular reactivity in developing zebrafish**. *Comparative Biochemistry and Physiology Part B Biochemistry and Molecular Biology* 139(1): 141. ISSN: 1096 4959.
NAL Call Number: QP501.C6

Descriptors: zebrafish, development, cardiovascular system, transport and circulation, marine ecology, environmental sciences, development, Austrian Science foundation .

Perlin, J.R. and W.S. Talbot (2007). **Signals on the move: chemokine receptors and organogenesis in zebrafish.** *Science: Signal Transduction Knowledge Environment* 2007(400): 45. ISSN: 1525-8882 .
NAL Call Number: QP517.C45
Abstract: The chemokine SDF1 (stromal cell-derived factor 1) directs cell migration in many different contexts, ranging from embryogenesis to inflammation. SDF1a is the guidance cue for the zebrafish lateral line primordium, a tissue that moves along the flank of the embryo and deposits cells that form mechanosensory organs. The SDF1a receptor CXCR4b acts in cells at the leading edge of the primordium to direct its migration. Two new studies show that a second SDF1 receptor, CXCR7, is required only in the trailing cells of the primordium, and they explore how these two receptors orchestrate migration of the primordium. CXCR4b and CXCR7 are expressed in complementary domains, possibly through mutual repression in which each receptor inhibits expression of the other. These studies illustrate how the entire primordium can respond to a single signal, yet generate cell type-specific responses by using different receptors.
Descriptors: zebrafish, chemokine receptors, organogenesis, stromal cell-derived factor 1, inflammation, Cxcr4B.

Petit, D., A. Maftah, R. Julien, and J.-M. Petit (2006). **En bloc duplications, mutation rates, and densities of amino acid changes clarify the evolution of vertebrate alpha -1,3/4-fucosyltransferases**. *Journal of Molecular Evolution* 63(3): 353-364. ISSN: 0022-2844.
NAL Call Number: QH359.J6
Descriptors: zebrafish, *Bos Taurus*, *gallus gallus*, *Takifugu rubripes*, *Xenopus laevis*, African clawed frog, chicken, domesticated cattle , red junglefowl, amino acid sequence, amino acids, amphibiotic species, developmental stages, epitopes, evolution, evolutionary genetics, freshwater fish, genomes, immune system, mutation rates, mutations, nucleotide sequence, phylogeny, molecular evolution, amniota, chordata .

Pizzo, E., P. Buonanno, A. Di Maro, S. Ponticelli, S. De Falco, N. Quarto, M.V. Cubellis, and G. D'Alessio (2006). **Ribonucleases and angiogenins from fish**. *Journal of Biological Chemistry* 281(37): 27454-27460. ISSN: 0021-9258.
NAL Call Number: 381J824
Descriptors: zebrafish, *Danio rerio*, angiogenesis, digestion, inhibitors, nucleotide sequence, phylogenetics, phylogeny, prototypes, recombinants, ribonuclease, structure-function relationships.

Plata, C., C.R. Sussman, A. Sindic, J.O. Liang, D.B. Mount, Z.M. Josephs, M.H. Chang, and M.F. Romero (2007). **Zebrafish Slc5a12 encodes an electroneutral sodium monocarboxylate transporter (SMCTn): A comparison with the electrogenic SMCT (SMCTe).** *Journal of Biological Chemistry* 282(16): 11996-2009. ISSN: 0021 9258.
NAL Call Number: 381J824
Abstract: We have identified and characterized two different sodium-coupled monocarboxylate cotransporters (SMCT) from zebrafish (Danio rerio), electrogenic (zSMCTe) and electroneutral (zSMCTn). zSMCTn is the 12th member of the zebrafish Slc5 gene family (zSlc5a12). Both zSMCT sequences have ~50% homology to human SLC5A8 (hSMCT). Transport function and kinetics were measured in Xenopus oocytes injected with zSMCT cRNAs by measurement of intracellular Na superscript +(B concentration ([Na superscript +(B]i) and membrane potential. Both zSMCTs oocytes increased [Na superscript +(B]i with addition of monocarboxylates (MC) such as lactate, pyruvate, nicotinate, and butyrate. By using two electrode voltage clamp experi-

ments, we measured currents elicited from zSMCTe after MC addition. MC-elicited currents from zSMCTe were similar to hSMCT currents. In contrast, we found no significant MC-elicited current in either zSMCTn or control oocytes. Kinetic data show that zSMCTe has a higher affinity for lactate, nicotinate, and pyruvate (KmL superscript -(Blactate = 0.17 +/- 0.02 mM, Kmnicotinate = 0.54 +/- 0.12 mM at -150 mV) than zSMCTn (KmL superscript -(Blactate = 1.81 +/- 0.19 mM, Kmnicotinate = 23.68 +/- 4.88 mM). In situ hybridization showed that 1-, 3-, and 5-day-old zebrafish embryos abundantly express both zSMCTs in the brain, eyes, intestine, and kidney. Within the kidney, zSMCTn mRNA is expressed in pronephric tubules, whereas zSMCTe mRNA is more distal in pronephric ducts. zSMCTn is expressed in exocrine pancreas, but zSMCTe is not. Roles for Na superscript +(B-coupled monocarboxylate cotransporters have not been described for the brain or eye. In summary, zSMCTe is the zebrafish SLC5A8 ortholog, and zSMCTn is a novel, electroneutral SMCT (zSlc5a12). Slc5a12 in higher vertebrates is likely responsible for the electroneutral Na superscript +(B/lactate cotransport reported in mammalian and amphibian kidneys.

Descriptors: zebrafish, *Danio rerio*, sodium-coupled monocarboxylate cotransporters, (SMcT) , electrogenic (zSMcTe), electroneutral (zSMcTn), zSMcTn, 12th member, zebrafish Slc5, gene family (zSlc5a12), homology, human, SLc5A8 (hSMcT).

Posner, M. (2003). **A comparative view of alpha crystallins: the contribution of comparative studies to understanding function**. *Integrative and Comparative Biology* 43(4): 481-491. ISSN: 1540-7063.

NAL Call Number: QL.I67

Descriptors: zebrafish, *Danio rerio*, biochemistry, comparative studies, cytochemistry, evolution, freshwater fish, heat shock, human physiology, man-induced effects , molecular biology, transparency .

Pruefert, K., A. Vogel, and G. Krohne (2004). **The lamin CxxM motif promotes nuclear membrane growth**. *Journal of Cell Science* 117(25): 6105-6116. ISSN: 0021 9533.

NAL Call Number: QH301.J6

Abstract: We analyzed the influence of lamins on nuclear envelope growth in cultured Xenopus A6 cells by the overexpression of human lamin A, Xenopus and zebrafish lamins B2 and Drosophila lamins Dm0 and C as GFP fusion proteins. Lamins; containing a CxxM motif in their primary sequence (lamins A, B2, Dm0) induced the formation of lobulated nuclei with multi-membrane-layered, highly folded nuclear membranes and intranuclear membrane assemblies, as observed by electron microscopy. Such morphological alterations were not observed with Drosophila lamin C, a lamin without this motif or with a lamin B2 mutant (B2-SxxM) where the cysteine of the CxxM motif is replaced by a serine. Drosophila lamin C mutants containing a CxxM motif behaved like B-type lamins thus confirming that this tetrapeptide is directly involved in the morphological changes we observed. Nuclear membrane proliferation could also be induced by lamin B2 in COS-7 cells and in zebrafish embryos but not by human lamin A in COS-7 cells. We speculate that the human lamin A is incompletely processed in Xenopus A6 cells and therefore behaves in this cell line like a B-type lamin. Our results indicate that the CxxM motif of B-type lamins has a dual function: it mediates lamin targeting to the inner nuclear membrane thereby promoting nuclear membrane growth. Copyright©Thomson Reuters 2009

Descriptors: zebrafish , biochemistry, membranes, cell biology, electron microscopy, gfp fusion proteins, lobulated nuclei, nuclear membrane growth, zebrafish.

Pullela, P.K., T. Chiku, M.J. Carvan, and D.S. Sem (2006). **Fluorescence-based detection of thiols in vitro and in vivo using dithiol probes**. *Analytical Biochemistry* 352(2): 265-273. ISSN: 0003 2697.

NAL Call Number: 381 An13

Abstract: Thiols play a central role in maintaining biological homeostasis. Their levels can change dramatically in response to oxidative stress associated with toxic insults, bacterial infection, and disease. Therefore, a reagent that call monitor thiol levels both in vitro and in vivo would be useful for assays and as a biomarker. Such a reagent should (i) be selective for thiols, (ii) be able to penetrate cell walls, and (iii) have a low reduction potential so as not to create oxidative stress in a cell. We have developed such a fluorescent reagent (DSSA) based on a dithiol linker: (i) the use of a dithiol linker makes it selective for thiols; (ii) the use of fluorophores that populate neutral states at physiological pH improves cell wall penetration; and (iii) because of the reagent's low reduction potential (-0.60 V), it will not stress cells oxidatively. For example, 5 mu M of reagent is responsive to changes in glutathione levels in the physiologically relevant range of 1 to 10 mM, yet this would oxidize less than 1% of cellular glutathione. In Escherichia coli, decreased thiol levels were detected in cells deficient in glutathione synthesis. In zebrafish embryos, the DSSA reagent permitted detection of unusually high thiol levels in the zebrafish chorion. (c) 2006 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, molecular biophysics, methods and techniques, development, oxidative stress, DSSA.

Reimers, M.J., M.E. Hahn, and R.L. Tanguay (2004). **Two zebrafish alcohol dehydrogenases share common ancestry with mammalian class I, II, IV, and V alcohol dehydrogenase genes but have distinct functional characteristics.** *Journal of Biological Chemistry* 279(37): 38303-12. ISSN: 0021-9258.

NAL Call Number: 381J824

Abstract: Ethanol is teratogenic to many vertebrates. We are utilizing zebrafish as a model system to determine whether there is an association between ethanol metabolism and ethanol-mediated developmental toxicity. Here we report the isolation and characterization of two cDNAs encoding zebrafish alcohol dehydrogenases (ADHs). Phylogenetic analysis of these zebrafish ADHs indicates that they share a common ancestor with mammalian class I, II, IV, and V ADHs. The genes encoding these zebrafish ADHs have been named Adh8a and Adh8b by the nomenclature committee. Both genes were genetically mapped to chromosome 13. The 1450-bp Adh8a is 82, 73, 72, and 72% similar at the amino acid level to the Baltic cod ADH8 (previously named ADH1), the human ADH1B2, the mouse ADH1, and the rat ADH1, respectively. Also, the 1484-bp Adh8b is 77, 68, 67, and 66% similar at the amino acid level to the Baltic cod ADH8, the human ADH1B2, the mouse ADH1, and the rat ADH1, respectively. ADH8A and ADH8B share 86% amino acid similarity. To characterize the functional properties of ADH8A and ADH8B, recombinant proteins were purified from SF-9 insect cells. Kinetic studies demonstrate that ADH8A metabolizes ethanol, with a V(max) of 13.4 nmol/min/mg protein, whereas ADH8B does not metabolize ethanol. The ADH8A K(m) for ethanol as a substrate is 0.7 mm. 4-Methyl pyrazole, a classical competitive inhibitor of class I ADH, failed to inhibit ADH8A. ADH8B has the capacity to efficiently biotransform longer chain primary alcohols (>/=5 carbons) and S-hydroxymethlyglutathione, whereas ADH8A does not efficiently metabolize these substrates. Finally, mRNA expression studies indicate that both ADH8A and ADH8B mRNA are expressed during early development and in the adult brain, fin, gill, heart, kidney, muscle, and liver. Together these results indicate that class I-like ADH is conserved in zebrafish, albeit with mixed functional properties.

Descriptors: zebrafish, rats, mice, insects, alcohol dehydrogenase chemistry, alcohol dehydro-

genase genetics, glutathione analogs and derivatives, amino acid sequence, binding, competitive, cell line, chromosome mapping, conserved sequence, complementary DNA metabolism, ethanol pharmacology, glutathione chemistry, kinetics, molecular sequence data, open reading frames, phylogeny, messenger RNA metabolism, recombinant proteins chemistry, amino acid sequence homology, species specificity, time factors, tissue distribution.

Rico, E.P., M.R. Senger, M.G. Fauth, R.D. Dias, M.R. Bogo, and C.D. Bonan (2003). **ATP and ADP hydrolysis in brain membranes of zebrafish (*Danio rerio*).** *Life Sciences* 73(16): 2071-82. ISSN: 0024-3205.
NAL Call Number: 442.8 L62
Abstract: Nucleotides, e.g. ATP and ADP, are important signaling molecules, which elicit several biological responses. The degradation of nucleotides is catalyzed by a family of enzymes called NTPDases (nucleoside triphosphate diphosphohydrolases). The present study reports the enzymatic properties of a NTPDase (CD39, apyrase, ATP diphosphohydrolase) in brain membranes of zebrafish (Danio rerio). This enzyme was cation-dependent, with a maximal rate for ATP and ADP hydrolysis in a pH range of 7.5-8.0 in the presence of Ca(2+) (5 mM). The enzyme displayed a maximal activity for ATP and ADP hydrolysis at 37 degrees C. It was able to hydrolyze purine and pyrimidine nucleosides 5'-di and triphosphates, being insensitive to classical ATPase inhibitors, such as ouabain (1 mM), N-ethylmaleimide (0.1 mM), orthovanadate (0.1 mM) and sodium azide (0.1 mM). A significant inhibition of ATP and ADP hydrolysis (68% and 34%, respectively) was observed in the presence of 20 mM sodium azide, used as a possible inhibitor of ATP diphosphohydrolase. Levamisole (1 mM) and tetramisole (1 mM), specific inhibitors of alkaline phosphatase and P1, P(5)-di (adenosine 5'-) pentaphosphate, an inhibitor of adenylate kinase did not alter the enzyme activity. The presence of a NTPDase in brain membranes of zebrafish may be important for the modulation of nucleotide and nucleoside levels, controlling their actions on specific purinoceptors in central nervous system of this specie.
Descriptors: zebrafish , acid anhydride hydrolases metabolism, adenosine diphosphate metabolism, adenosine triphosphate metabolism, brain enzymology, intracellular membranes enzymology, hydrolysis, nucleoside triphosphatase, substrate specificity.

Robles, V., E. Cabrita, P.d. Paz, S. Cunado, L. Anel, and M.P. Herraez (2004). **Effect of a vitrification protocol on the lactate dehydrogenase and glucose-6-phosphate dehydrogenase activities and the hatching rates of zebrafish (*Danio rerio*) and turbot (*Scophthalmus maximus*) embryos.** *Theriogenology*: 1367-79. ISSN: 0093 691X.
NAL Call Number: QP251.A1T5
Abstract: Vitrification, is the most promising option for the cryopreservation of fish embryos but requires high concentrations of potentially toxic cryoprotectants. In this study, embryos from Turbot and Zebrafish, each in two developmental stages, were submitted to a four stepwise cryoprotectant incorporation protocol. After incubation in the vitrificant solution (5 M dimethyl sulfoxide, 2 M methanol, 1 M ethylen-glycol and 10% sucrose) embryos were loaded in straws and plunged into liquid nitrogen. The activity of two cytoplasmic enzymes, LDH and G6PDH, and the hatching rates were analyzed in control embryos, those subjected to the cryoprotectant solutions and in frozen/thawed embryos. Results showed that the cryoprotectants incorporation protocol did not have important effects on the analyzed enzymatic activities, which remained at similar levels to that in control embryos but significantly reduced the hatching rates. Turbot was less sensitive than Zebrafish to the toxic effect of the cryoprotectants, achieving hatching rates of 74.8% in comparison with fresh control embryos at G stage, whereas in Zebrafish only 17.7% of hatching was reported with five somites-treated embryos. In Turbot, G stage was more resistant to the cryoprotectants and thus more convenient for further vitrification studies. After vitrification

no survival was recorded and enzymatic activities dropped significantly, particularly in Zebrafish, indicating cell damage and loss of cytoplasmic enzymes. Nevertheless, total cell lysis was not produced, and once again Turbot was more resistant to the effect of vitrification, particularly at the later stage. In that stage, Turbot embryos showed around 50% of G6PDH activity after vitrification, in comparison with the control, indicating the preservation of some cellular activity after freezing!thawing, despite the loss of developmental ability.
Descriptors: zebrafish, *brachyDanio rerio*, laboratory animals, turbot , farmed fish, embryo , cryopreservation , cryoprotectants , toxicity, freezing , hatching , enzyme activity, lactate dehydrogenase, glucose 6 phosphate 1 dehydrogenase.

Runko, A.P. and C.G. Sagerstrom (2004). **Isolation of nlz2 and characterization of essential domains in nlz family proteins.** *Journal of Biological Chemistry* 279(12): 11917-11925. ISSN: 0021 9258.
NAL Call Number: 381J824
Abstract: In this study, we first cloned nlz2, a second zebrafish member of the nlz-related zinc-finger gene family. nlz2 was expressed together with nlz1 in a broad posterior domain during gastrula stages as well as at the midbrain-hindbrain boundary and in the hindbrain caudal to rhombomere 4 during segmentation. nlz2 was also expressed in regions distinct from nlz1, notably in the forebrain, midbrain, and trunk. Misexpression of nlz2 in zebrafish embryos disrupted gene expression in the rostral hindbrain, similar to the effect of misexpressing nlz1. We next compared the nlz1 and nlz2 sequences to identify and characterize domains conserved within this family. We found a C-terminal domain required for nuclear localization and two conserved domains (the Sp motif and a putative C2H2 zinc finger) required for nlz1 function. We also demonstrate that Nlz1 self-associated via its C terminus, interacted with Nlz2, and bound to histone deacetylases. Last, we found two forms of Nlz1 generated from alternative translation initiation sites in vivo. These forms have distinct activities, apparently depending on the function of the N-terminal Sp motif. Our data demonstrate that ntz2 functions similarly to nlz1 and define conserved domains essential for nuclear localization, self-association, and corepressor binding in this novel family of zinc-finger genes. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, enzymology, molecular genetics, cloning, genetic techniques, histone deacetylases.

Salem, M., J.B. Yao, C.E. Rexroad, P.B. Kenney, K. Semmens, J. Killefer, and J. Nath (2005). **Characterization of calpastatin gene in fish: its potential role in muscle growth and fillet quality**. *Comparative Biochemistry and Physiology B, Biochemistry and Molecular Biology* 141(4): 488-497. ISSN: 0305 0491.
NAL Call Number: QP501.C6
Abstract: Calpastatin (CAST), the specific inhibitor of the calpain proteases, plays a role in muscle growth and meat quality. In rainbow trout (RBT), we identified cDNAs coding for two CAST isoforms, a long (CAST-L) and a short isoform (CAST-S), apparently derived from two different genes. Zebrafish and pufferfish CAST cDNA and genomic sequences were retrieved from GenBank and their exon/intron structures were characterized. Fish CASTs are novel in that they have fewer repetitive inhibitory domains as compared to their mammalian counterparts (one or two vs. four). The expressions of CAST mRNAs were measured in three RBT strains with different growth rates and fillet firmness that were fed either high energy or control diets. CAST-L and S expressions were significantly lower ($p<0.01$) in the strain that has the slowest growth rate and yielded the softest fillet. Strain or diet did not affect level of calpain mRNAs. However, the decrease in the CAST/calpain ratio at the mRNA level did not lead to a corresponding change in the calpain catalytic activity. Further investigation should reveal a potential use of the CAST gene as a tool to monitor fish muscle growth and fillet firmness. Copyright © 2008 CABI

Descriptors: zebrafish, rainbow trout, amino acid sequences, complementary DNA, diets, energy intake, firmness, fish feeding, gene expression, genes, growth rate, messenger RNA, muscles, proteinase inhibitors, strains .

Sapede, D., M. Rossel, C. Dambly-Chaudiere, and A. Ghysen (2005). **Role of SDF1 chemokine in the development of lateral line efferent and facial motor neurons**. *Proceedings of the National Academy of Sciences, USA* 102(5): 1714-1718. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, *Danio rerio*, antisense oligonucleotides, axon guidance, brain, cell body, cell migration, chemokines, embryos, hindbrain, innervation, lateral line, motor neurons, motors, nerves, nervous system, neurons, nuclei, receptors, sensory systems, target cells, stromal cell-derived factor 1 alpha.

Schaaf, M.J., D. Champagne, I.v.o.H. van Laanen, D.C. van Wijk, A.H. Meijer, O.C. Meijer, H.P. Spaink, and M.K. Richardson (2008). **Discovery of a functional glucocorticoid receptor beta -isoform in zebrafish**. *Endocrinology* 149(4): 1591-1599. ISSN: 0013-7227.
NAL Call Number: 448.8EN2
Descriptors: zebrafish, glucocorticoid, receptor beta-isoform, metabolic compound.

Schilling, S.H., M.B. Datto, and X.F. Wang (2006). **A phosphatase controls the fate of receptor-regulated smads**. *Cell* 125(5): 838-840. ISSN: 0092 8674.
NAL Call Number: QH573.C42
Descriptors: zebrafish, phosphatase, enzymology, molecular biophysics, Smads.

Schlegel, A. and D.Y.R. Stainier (2006). **Microsomal triglyceride transfer protein is required for yolk lipid utilization and absorption of dietary lipids in zebrafish larvae**. *Biochemistry* 45(51): 15179-15187. ISSN: 0006 2960.
Online: http://dx.doi.org/10.1021/bi0619268
NAL Call Number: 381 B523
Abstract: Although the absorption, transport, and catabolism of dietary lipids have been studied extensively in great detail in mammals and other vertebrates, a tractable genetic system for identifying novel genes involved in these physiologic processes is not available. To establish such a model, we monitored neutral lipid by staining fixed zebrafish larvae with oil red o (ORO). The head structures, heart, vasculature, and swim bladder stained with ORO until the yolk was consumed 6 days after fertilization (6 dpf). Thereafter, the heart and vasculature no longer had stainable neutral lipids. Following a high-fat meal, ORO stained the intestine and vasculature of 6 dpf larvae, and whole-larval triacylglycerol (TAG) and apolipoprotein B levels increased. Levels of microsomal triglyceride transfer protein (Mtp), the protein responsible for packaging TAG and betalipoproteins into lipoprotein particles, were unchanged by feeding. Since the developing zebrafish embryo expresses mtp in the yolk cell layer, liver, and intestine, we determined the effect of targeted knockdown of Mtp expression using an antisense morpholino oligonucleotide approach (Mtp MO) on the transport of yolk and dietary lipids. Mtp MO injection led to loss of Mtp expression and of lipid staining in the vasculature, heart, and head structures. Mtp MO-injected larvae were smaller than age-matched, uninjected larvae, consumed very little yolk, and did not absorb dietary neutral lipids; however, they absorbed a short chain fatty acid that does not require Mtp for transport. Importantly, the vasculature appeared unaffected in Mtp MO-injected larvae. These studies indicate that zebrafish larvae are suitable for genetic studies of lipid transport and metabolism. Copyright © 2008 CABI
Descriptors: zebrafish, *Danio rerio*, absorption, animal models, apolipoproteins, catabolism,

egg yolk, embryos, fish eggs, genes, head, heart, intestines, larvae, lipid metabolism, lipids, liver, microsomes, proteins, swim bladder, triacylglycerols .

Senger, M.R., E.P. Rico, R.D. Dias, M.R. Bogo, and C.D. Bonan (2004). **Ecto-5'-nucleotidase activity in brain membranes of zebrafish (*Danio rerio*).** *Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology* 139(2): 203-7. ISSN: 0305 0491.
NAL Call Number: QP501.C6
Abstract: Adenosine, a well-known neuromodulator, may be formed intracellularly in the CNS from degradation of AMP and then exit via bi-directional nucleoside transporters, or extracellularly by the metabolism of released nucleotides. This study reports the enzymatic properties of an ecto-5'-nucleotidase activity in brain membranes of zebrafish (Danio rerio). This enzyme was cation-dependent, with a maximal rate for AMP hydrolysis in a pH range of 7.0-7.5 in the presence of Mg(2+). The enzyme presented a maximal activity for AMP hydrolysis at 37 degrees C. The apparent K(m) and V(max) values for Mg(2+)-AMP were 135.3+/-16 microM and 29+/-4.2 nmol Pi.min(-1).mg(-1) protein, respectively. The enzyme was able to hydrolyze both purine and pyrimidine monophosphate nucleotides, such as UMP, GMP and CMP. Levamisole and tetramisole (1 mM), specific inhibitors of alkaline phosphatases, did not alter the enzymatic activity. However, a significant inhibition of AMP hydrolysis (42%) was observed in the presence of 100 microM alpha,beta-methylene-ADP, a known inhibitor of ecto-5'-nucleotidase. Since 5'-nucleotidase represents the major enzyme responsible for the formation of extracellular adenosine, the enzymatic characterization is important to understand its role in purinergic systems and the involvement of adenosine in the regulation of neurotransmitter release.
Descriptors: zebrafish, 5' nucleotidase metabolism, brain enzymology, zebrafish, adenosine monophosphate metabolism, divalent cations pharmacology, cell membrane enzymology, enzyme inhibitors pharmacology, hydrogen ion concentration, kinetics, magnesium pharmacology.

Setini, A., F. Pierucci, O. Senatori, and A. Nicotra (2005). **Molecular characterization of monoamine oxidase in zebrafish (*Danio rerio*).** *Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology* 140(1): 153-61. ISSN: 0305 0491.
NAL Call Number: QP501.C6
Abstract: Monoamine oxidase (MAO) is responsible for the degradation of a number of neurotransmitters and other biogenic amines. In terrestrial vertebrates, two forms of the enzyme, named MAO A and B, were found in which mammals are coded by two similar but distinct genes. In teleosts, the biochemical data obtained so far indicate that enzyme activity is due to a single form, whose sequence, obtained for trout, displays 70% identity with mammal MAO A and B. In this paper, we carried out an investigation of zebrafish MAO (Z-MAO) to shed further light on the nature of the MAO form present in aquatic vertebrates. Sequencing studies have revealed an open reading frame 522-amino-acids long with MW 58.7 kDa, displaying 84% identity with trout MAO and about 70% identity with mammal MAO A and MAO B. Analysis of the sequence and of the predicted secondary structure shows that also in Z-MAO principal domains characterizing the MAOs are present. The domain linking the FAD is very well conserved, while the transmembrane domain sequence linking the enzyme to the external mitochondrial membrane does not appear to be conserved even with respect to trout MAO. Comparison with the amino acids which, according to the human MAO B and rat MAO A models, line the substrate-binding site shows that in Z-MAO, several residues (V172, N173, F200, L327) differ from MAO B but are similar or identical to the corresponding ones present in rat MAO A, as well as in trout MAO. A three-dimensional model is reported of the substrate-binding site of Z-MAO obtained by comparative modeling. Our observations support the hypothesis that the MAO form present in aquatic vertebrates is a MAO A-like form. Experiments

performed to test the effect of selective MAO A (clorgyline) and MAO B (deprenyl) inhibitors on the enzyme's activity in liver and brain confirm the presence of a single form of MAO in zebrafish.

Descriptors: zebrafish metabolism, liver enzymology, monoamine oxidase metabolism, amino acid and base sequence, binding sites, molecular cloning, clorgyline pharmacology, complementary DNA, molecular sequence data, monoamine oxidase genetics, monoamine oxidase inhibitors pharmacology, open reading frames, protein binding, protein Conformation, selegiline pharmacology, amino acid sequence homology, substrate specificity.

Sharma, M.K., E.M. Denovan Wright, A. Degrave, C. Thisse, B. Thisse, and J.M. Wright (2004). **Sequence, linkage mapping and early developmental expression of the intestinal-type fatty acid-binding protein gene (fabp2) from zebrafish (*Danio rerio*).** *Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology* 138(4): 391-8. ISSN: 0305 0491.

NAL Call Number: QP501.C6

Abstract: The intestinal fatty acid-binding protein (I-FABP) shows binding specificity for long-chain fatty acids and is proposed to be involved in uptake of dietary fatty acids and their intracellular transport. We have determined the sequence of the gene encoding I-FABP in zebrafish. The zebrafish I-FABP gene contains four exons interrupted by three introns. Radiation hybrid mapping assigned the I-FABP gene to linkage group 1. A 924 bp sequence 5' upstream of the initiation codon in the I-FABP gene contained several putative cis-acting regulatory elements. In adult zebrafish, reverse transcription-polymerase chain reaction (RT-PCR) detected I-FABP mRNA in intestine, brain, liver, muscle and testis. Quantitative RT-PCR demonstrated that I-FABP mRNA was most abundant in intestine, followed by brain. I-FABP mRNA levels were very low in muscle, testis, heart, liver, skin and ovary. RT-PCR using total RNA extracted from zebrafish embryos detected I-FABP mRNA as early as 12 h post-fertilization. Whole-mount in situ hybridization to zebrafish embryos detected I-FABP mRNA in the yolk syncytial layer (YSL) at early somitogenesis. Later during embryonic development the I-FABP mRNA was detected in the intestinal bulb, liver and pancreas primordium. Expression in YSL, liver or pancreas has not been previously reported for fish or mammalian I-FABP genes and may be related to specific physiological differences between fishes and mammals.

Descriptors: zebrafish, carrier proteins biosynthesis, carrier linkage genetics, proteins biosynthesis, amino acid and base sequence, molecular cloning, exons, fatty acid binding proteins, fatty acids chemistry, introns, molecular sequence data, promoter regions genetics, RNA chemistry, messenger RNA metabolism, radiation hybrid mapping, reverse transcriptase PCR, DNA sequence analysis, amino acid sequence homology, time factors, tissue distribution, transcription.

Sibthorpe, D., R.A.N. Sturlaugsdottir, B.K. Kristjansson, H. Thorarensen, S. Skulason, and I.A.N.A. Johnston (2006). **Characterisation and expression of the paired box protein 7 (Pax7) gene in polymorphic Arctic charr (*Salvelinus alpinus*)**. *Comparative Biochemistry and Physiology, Part B: Biochemistry and Molecular Biology* 145(3-4): 371-383. ISSN: 1096-4959.

NAL Call Number: QP501.C6

Descriptors: zebrafish, alternative splicing, body size, casein kinase II, central nervous system, DNA, ecological distribution, embryos, freshwater fish, gene expression, gene polymorphism, growth rate, head, insertion, lakes, life history, mandible, morphometry, phosphorylation, segmentation, serine, skeletal muscle, threonine, *Danio rerio*, *Salvelinus alpinus,* Iceland, Thingvallavatn L., Arctic char.

Smart, E.J., R.A. De Rose, and S.A. Farber (2004). **Annexin 2-caveolin 1 complex is a target of ezetimibe and regulates intestinal cholesterol transport**. *Proceedings of the National Academy of Sciences, USA* 101(10): 3450-3455. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, *Danio rerio*, mice, absorption spectroscopy, antisense, caveolin, cholesterol, freshwater fish, hypercholesterolemia, immunoprecipitation, intestine, intestines, oligonucleotides, annexin 2, sterol.

Song, M., H. Yang, S. Yao, F. Ma, Z. Li, Y. Deng, H. Deng, Q. Zhou, S. Lin, and Y. Wei (2007). **A critical role of vascular endothelial growth factor D in zebrafish embryonic vasculogenesis and angiogenesis.** *Biochemical and Biophysical Research Communications* 357(4): 924-30 . ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: The VEGF family comprises seven members that are designated VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, placental growth factor (PlGF), and VEGF-F. Of these factors, VEGF-D plays important roles for angiogenesis and lymphangiogenesis, and could promote tumor growth and lymphatic metastasis. In this study, we identified a zebrafish VEGF-D homolog that encodes a 272 amino acid protein including a PDGF (platelet-derived growth factor) domain characteristic to VEGF family. Expression profile demonstrated that the VEGF-D began expressed from 13 somite stage. Microinjecting zVEGF-D mRNA into zebrafish 1-cell stage embryos resulted in severe misguidance of intersegmental vessels (ISV) and abnormal connection between dorsal aorta and caudal vein. Microangiography indicated that these abnormal ISVs were not functional. Our studies therefore identified the first non-mammalian VEGF-D and established its in vivo role for vascular system development during vertebrate embryogenesis and provided an alternative animal model to further reveal functions of VEGF-D.
Descriptors: zebrafish embryo, blood vessels growth and development, embryonic development physiology, physiologic neovascularization physiology, vascular endothelial growth factor d metabolism, developmental gene expression regulation physiology.

Souza, A.R., E.O. Kozlowski, V.R. Cerqueira, M.T. Castelo Branco, M.L. Costa, and M.S. Pavao (2007). **Chondroitin sulfate and keratan sulfate are the major glycosaminoglycans present in the adult zebrafish *Danio rerio* (chordata-Cyprinidae).** *Glycoconjugate Journal* 24(9): 521-30. ISSN: 0282-0080.
NAL Call Number: QP752.G56
Abstract: The zebrafish *Danio rerio* (Chordata-Cyprinidae) is a model organism frequently used to study the functions of proteoglycans and their glycosaminoglycan (GAG) chains. Although several studies clearly demonstrate the participation of these polymers in different biological and cellular events that take place during embryonic development, little is known about the GAGs in adult zebrafish. In the present study, the total GAGs were extracted from the whole fish by proteolytic digestion, purified by anion-exchange chromatography and characterized by electrophoresis after degradation with specific enzymes and/or by high-performance liquid chromatography (HPLC) analysis of the disaccharides. Two GAGs were identified: a low-molecular-weight chondroitin sulfate (CS) and keratan sulfate (KS), corresponding to approximately 80% and 20% of the total GAGs, respectively. In the fish eye, KS represents approximately 80% of total GAGs. Surprisingly, no heparinoid was detected, but may be present in the fish at concentrations lower than the limit of the method used. HPLC of the disaccharides formed after chondroitin AC or ABC lyase degradation revealed that the zebrafish CS is composed by DeltaUA-1-->3-GalNAc(4SO(4)) (59.4%), DeltaUA-1-->3-GalNAc(6SO(4)) (23.1%), and DeltaUA-1-->3-GalNAc (17.5%) disaccharide units. No disulfated disaccharides were detected.

Immunolocalization on sections from zebrafish retina using monoclonal antibodies against CS4- or 6-sulfate showed that in the retina these GAGs are restricted to the outer and inner plexiform layers. This is the first report showing the presence of KS in zebrafish eye, and the structural characterization of CS and its localization in the zebrafish retina. Detailed information about the structure and tissue localization of GAGs is important to understand the functions of these polymers in this model organism.

Descriptors: zebrafish adult, research model, chondroitin sulfate, keratan sulfate, glycosaminoglycans, adult.

Stanton, S.E., L.J. McReynolds, T. Evans, and N. Schreiber Agus (2006). **Yaf2 inhibits caspase 8-mediated apoptosis and regulates cell survival during zebrafish embryogenesis.** *Journal of Biological Chemistry* 281(39): 28782-93. ISSN: 0021-9258.

NAL Call Number: 381J824

Abstract: Rybp (DEDAF) is a member of the Rybp/Yaf2 protein family and has been shown to encode pro-apoptotic functions and to be essential for mouse embryogenesis. The related Yaf2 protein has not been studied extensively at the cellular or organismal levels. Here we describe zebrafish yaf2 (zyaf2) and show that it is widely expressed during early embryogenesis, with subsequent enrichment of transcripts in the anterior head region. Depletion of zYaf2 during embryogenesis using specific morpholinos activates a wide-spread program of apoptosis and causes developmental arrest before the one somite stage. Partial depletion of Yaf2, achieved by injecting lower dosages of morpholino, circumvents the early arrest but leads to CNS degeneration associated with excessive apoptosis. These phenotypes can be rescued by co-injection of human YAF2 mRNA with the morpholinos or by treatment with a pan-caspase inhibitor or a caspase 8-specific inhibitor. Finally, the observed activation of caspase 8 in the morphants is in accord with the ability of Yaf2 to inhibit caspase 8-mediated apoptosis in cultured cells. Our findings implicate Yaf2 as a survival factor during early zebrafish development and organogenesis. This may suggest that Yaf2 and Rybp can encode opposing functions in the regulation of apoptosis.

Descriptors: zebrafish , apoptosis, apoptosis regulatory, caspase 8 metabolism, DNA binding, developmental gene expression regulation, amino acid sequence, cell survival, in situ hybridization, molecular sequence data, phenotype, phylogeny, amino acid sequence homology, zebrafish.

Strumbo, B., L. Sangiorgio, S. Ronchi, J.E. Gready, and T. Simonic (2006). **Cloning and analysis of transcripts and genes encoding fish-specific proteins related to PrP**. *Fish Physiology and Biochemistry* 32(4): 339-353. ISSN: 0920 1742.

NAL Call Number: QL639.1.F583

Abstract: We report cloning and tissue expression of transcripts of a fourth fish-specific gene (SPRNB) encoding a prion protein (PrP)-related protein (Shadoo2) from two species, *Cyprinus carpio* (carp) and *Danio rerio* (zebrafish), as well as gene or partial transcript sequences identified by in silico searches of public databases from *Gasterosteus aculeatus* (stickleback), *Oryzias latipes* (medaka fish) and *Salmo salar* (salmon). We also present an up-to-date summary of current knowledge of the other three PrP-related genes and transcripts (PrP-like protein, PrP1 and PrP2) encoded exclusively in fish genomes, including some additional transcripts we identified by in silico searches. We compare levels of protein-sequence conservation among the different genes within given fish species, and among given genes in different fish species, and analyse the gene structures and contexts (flanking genes) where genomic sequence is available. These analyses show remarkably high divergences of sequences and expression patterns for proteins in distant fish, such as Fugu and zebrafish, leaving open the question of how varied their functions may be. Copyright © 2008 CABI

Descriptors: zebrafish, DNA cloning, gene expression, genes, genome analysis, genomes, nucleotide sequences, prion diseases, prion proteins, proteins, transcription, Atlantic salmon, carp, *Danio rerio*, *gasterosteus aculeatus*, *Oryzias latipes*, prions, salmon.

Tan, X., J. Rotllant, H. Li, P. De Deyne, and S.J. Du (2006). **SmyD1, a histone methyltransferase, is required for myofibril organization and muscle contraction in zebrafish embryos.** *Proceedings of the National Academy of Sciences of the United States of America* 103(8): 2713-8. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: Histone modification has emerged as a fundamental mechanism for control of gene expression and cell differentiation. Recent studies suggest that SmyD1, a novo SET domain-containing protein, may play a critical role in cardiac muscle differentiation. However, its role in skeletal muscle development and its mechanism of actions remains elusive. Here we report that SmyD1a and SmyD1b, generated by alternative splicing of SmyD1 gene, are histone methyltransferases that play a key role in skeletal and cardiac muscle contraction. SmyD1a and SmyD1b are specifically expressed in skeletal and cardiac muscles of zebrafish embryos. Knockdown of SmyD1a and SmyD1b expression by morpholino antisense oligos resulted in malfunction of skeletal and cardiac muscles. The SmyD1 morphant embryos (embryos injected with morpholino oligos) could not swim and had no heartbeat. Myofibril organization in the morphant embryos was severely disrupted. The affected myofibers appeared as immature fibers with centrally located nuclei. Together, these data indicate that SmyD1a and SmyD1b are histone methyltransferases and play a critical role in myofibril organization during myofiber maturation.

Descriptors: zebrafish embryo, histone lysine n methyltransferase physiology, muscle contraction genetics, myofibrils ultrastructure, alternative splicing, cell differentiation, chemistry, enzymology, heart embryology, congenital heart defects embryology, congenital heart defects genetics, histone lysine n methyltransferase genetics, molecular sequence data, skeletal muscle abnormalities, skeletal muscle cytology, myocardium cytology, messenger RNA analysis and metabolism.

Tang, J., G. Hu, J.I. Hanai, G. Yadlapalli, Y. Lin, B. Zhang, J. Galloway, N. Bahary, S. Sinha, B. Thisse, C. Thisse, J.P. Jin, L. Zon, and V.P. Sukhatme (2006). **A critical role for calponin 2 in vascular development**. *Journal of Biological Chemistry* 281(10): 6664-6672. ISSN: 0021 9258.

NAL Call Number: 381J824

Abstract: Calponin 2 (h2 calponin, CNN2) is an actin-binding protein implicated in cytoskeletal organization. We have found that the expression of calponin 2 is relatively restricted to vasculature from 16 to 30 h post-fertilization during zebrafish (*Danio rerio*) development. Forty-eight hours after injecting antisense morpholino oligos against calponin 2 into embryos at the 1 - 4-cell stage, zebrafish demonstrated various cardiovascular defects, including sluggish axial and head circulation, absence of circulation in intersegmental vessels and in the dorsal longitudinal anastomotic vessel, enlarged cerebral ventricles, and pericardial edema, in addition to an excess bending, spiraling tail and twisting of the caudal fin. Knockdown of calponin 2 in the Tg(fli1: EGFP)(y1) zebrafish line (in which a fli1 promoter drives vascular-specific enhanced green fluorescent protein expression) indicated that diminished calponin 2 expression blocked the proper migration of endothelial cells during formation of intersegmental vessels. In vitro studies showed that basic fibroblast growth factor-induced human umbilical vein endothelial cell migration was down-regulated by knockdown of calponin 2 expression using an antisense adenovirus, and overexpression of calponin 2 enhanced migration and hastened wound healing. These events were correlated with activation of mitogen-activated protein kinase; moreover, inhibition of this pathway blocked the promigratory effect of calponin 2. Collectively, these data suggest that calponin 2 plays an important role in the migration of endothelial cells both in vivo and in vitro and

that its expression is critical for proper vascular development. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, cardiovascular system, transport and circulation, molecular genetics, marine ecology, environmental sciences, cardiovascular defect, heart disease, vascular disease.

Tarboush, R.A., S.E. MacAvoy, S.A. Macko, and V. Connaughton (2006). **Contribution of catabolic tissue replacement to the turnover of stable isotopes in *Danio rerio***. *Canadian Journal of Zoology/Revue Canadienne De Zoologie* 84(10): 1453-1460. ISSN: 0008-4301.
Descriptors: zebrafish, carbon isotopes, chemical composition, food webs, freshwater fish, growth rate, Nitrogen isotopes, sulphur isotopes, trophic structure, *Danio rerio* .

Tay, T.L., Q. Lin, T.K. Seow, K.H. Tan, C.L. Hew, and Z. Gong (2006). **Proteomic analysis of protein profiles during early development of the zebrafish, *Danio rerio* .** *Proteomics* 6(10): 3176-88. ISSN: 1615-9853.
NAL Call Number: QP752.F3PF76
Abstract: In the present study, profiles of protein expression were examined during early development of zebrafish, an increasingly popular experimental model in vertebrate development and human diseases. By 2-DE, an initial increase in protein spots from 6 h post-fertilization (hpf) to 8-10 hpf was observed. There was no dramatic change in protein profiles up to 18 hpf, but significant changes occurred in subsequent stages. Interestingly, 49% of the proteins detected at 6 hpf remained detectable by 1 week of age. To map the protein expression patterns in 2-D gels, MALDI-TOF/TOF MS was employed to identify selected protein spots from early embryos. 108 protein spots were found to match known proteins and they were derived from 55 distinct genes. Interestingly, 11 (20%) of them produced multiple protein isoforms or distinct cleavage products. Although deyolked embryos were used in the analysis, a large number of vitellogenin derivatives remained prominently present in the embryos. Other than these, most of the identified proteins are cytosolic, cytoskeletal and nuclear proteins, which are involved in diversified functions such as metabolism, cytoskeleton, translation, protein degradation, etc. Some of the proteins with interesting temporal expression profiles during development are further discussed.
Descriptors: zebrafish embryos, metabolism, proteins biosynthesis, two dimensional gel electrophoresis, gastrula metabolism, heat shock proteins biosynthesis, keratins biosynthesis, larva, protein isoforms biosynthesis, proteomics, spectrometry, mass, matrix assisted laser desorption ionization, vitellogenins biosynthesis, zebrafish growth and development.

Thomas, P., Y.F. Pang, Y. Zhu, C. Detweiler, and K. Doughty (2004). **Multiple rapid progestin actions and progestin membrane receptor subtypes in fish**. *Steroids* 69(8/9): 567-573. ISSN: 0039 128X.
NAL Call Number: 381 ST4
Abstract: Progestin hormones exert rapid, nongenomic actions on a variety of target tissues in fish. The induction of oocyte maturation and the progestin membrane receptor (mPR) that mediates this action of progestins have been well characterized in fishes. Progestins also act on Atlantic croaker spermatozoa via an mPR to rapidly increase sperm motility. Preliminary results indicate that progestins can also exert rapid actions in the preoptic anterior hypothalamus (POAH) in this species to down-regulate gonadotropin-releasing hormone (GnRH) secretion. Recently, we reported the cloning, sequencing and characterization of a novel cDNA in a closely related species, spotted seatrout, that has the characteristics of the mPR involved in the progestin induction of oocyte maturation. Three distinct mPR subtypes, named alpha , beta , and gamma , have been identified in both fishes and mammals. The tissue distribution of the mPR alpha protein in seatrout suggests the alpha -subtype mediates progestin actions on GnRH secretion, sperm motility and oocyte maturation. However, mPR beta antisense experiments in zebrafish oocytes suggest

the beta -subtype also participates in the control of oocyte maturation in zebrafish. Copyright © 2008 CABI

Descriptors: zebrafish, biochemical receptors, gnrH, hormones, oocyte maturation, oocytes, spermatozoa, fishes, zebrafish.

Tocher, D.R., M. Agaba, N. Hastings, and A.J. Teale (2003). **Biochemical and molecular studies of the polyunsaturated fatty acid desaturation pathway in fish**. *The big fish bang. Proceedings of the 26th annual larval fish conference,July 22, 2002-July 26, 2002, Os (Norway),* Institute of Marine Research: Bergen Norway, p. 211-227.

Descriptors: zebrafish, animal nutrition, chemical reactions, DNA, diets, enzymes, feed composition, fish culture, fish oils, fish physiology, linoleic acid, nutritional requirements, polyunsaturated fatty acids, rearing, essential fatty acids, alpha -linolenic acid.

Tucker, B. and M. Lardelli (2007). **A rapid apoptosis assay measuring relative acridine orange fluorescence in zebrafish embryos.** *Zebrafish* 4(2): 113-6. ISSN: 1545-8547.

Abstract: The ability to easily analyze apoptosis is important in studies of molecular cell biology and to evaluate the relative toxicity of different treatments or environments. This is particularly the case when substances such as morpholino oligonucleotides are injected into embryos, as such treatments can cause widespread, complex patterns of apoptosis. Zebrafish embryos are well suited for cell biological and environmental toxicity analyses, but the need remains for a simple method that can analyze levels of apoptosis in a statistically significant number of embryos. Here we present a "group fluorescence" method for rapid, large-scale analysis of relative levels of apoptosis based on densitometric techniques.

Descriptors: zebrafish, group fluorescence, apoptosis assay, group fluorescence, morpholino oligonucleotides.

Valencia, C.A., C. Bailey, and R. Liu (2007). **Novel zebrafish caspase-3 substrates.** *Biochemical and Biophysical Research Communications* 361(2): 311-6. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: The zebrafish model has been widely used to investigate numerous signaling pathways in vertebrates, including programmed cell death. Although several zebrafish proteins homologous to mammalian caspases have been identified, our understanding of these zebrafish caspases is still limited. Recently, we identified a large number of natural caspase-3 substrates from the human proteome by using the mRNA-display selection method. Through comparative analysis, we found that the cleavage sites on some of these novel human caspase-3 substrates are highly conserved in their zebrafish orthologs. We report here the identification and characterization of 14 natural zebrafish caspase-3 substrates that have not yet been previously studied. The specific cleavage of these zebrafish proteins was compared with caspases from different species, and the protein fragments that contain the putative cleavage sites were mapped. The work described here could facilitate our understanding of the downstream signaling pathways that are mediated by caspase-3 in zebrafish.

Descriptors: zebrafish, caspase 3 metabolism, binding sites, endopeptidases metabolism, peptide fragments metabolism, protein processing, post translational, amino acid sequence homology, species specificity, substrate specificity.

Van Der Ven, K., M. DeWit, D. Keil, L. Moens, K. Van Leemput, B. Naudts, and W. De Coen (2005). **Development and application of a brain-specific cDNA microarray for effect evaluation of neuro-active pharmaceuticals in zebrafish (*Danio rerio*)**. *Comparative Biochemistry and Physiology B, Biochemistry and Molecular Biology* 141(4): 408-417. ISSN: 0305 0491.

NAL Call Number: QP501.C6

Abstract: The environmental fate and ecotoxicological effect of pharmaceuticals are poorly understood, and standardized tests to detect and evaluate their potential effects in the environment are not available. We developed a zebrafish brain-specific microarray containing 682 neurologically relevant cDNA-fragments. To investigate the applicability of this microarray for studying neurotoxic modes-of-action and impact assessment of neuro-active pharmaceuticals in zebrafish, chlorpromazine was used as a model compound. After exposure to chlorpromazine (75 micro g/L) for 2, 4, 14 and 28 days or control treatment RNA was extracted from brains of males and females. Fluorescently labelled cDNA was prepared and hybridized to the custom microarray. In total, 56 genes were differentially expressed in brains of male and/or female zebrafish, of which most genes were down-regulated. A clear difference in response to chlorpromazine exposure between males and females was observed with exposure time as well as in functional classes of affected genes. The presented study is one of the first reports on molecular effects of human neuro-active pharmaceuticals in aquatic non-target organisms. This new genomic tool successfully detected gene expression effects of exposure to chlorpromazine in the brain of zebrafish. Reported gene expression effects are found to be consistent with literature data for other laboratory animals.
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Descriptors: zebrafish, brain, complementary DNA, drugs, gene expression, neurotoxicity, toxicology, *Danio rerio*.

Wang, L., J. Yao, L. Chen, J. Chen, J. Xue, and W. Jia (2007). **Expression and possible functional roles of cytochromes P450 2J1 (zfCyp 2J1) in zebrafish.** *Biochemical and Biophysical Research Communications* 352(4): 850-5. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: Cytochrome P450 2J (Cyp2J) subfamilies are recognized as catalysts of arachidonic acid metabolism in extrahepatic tissues of many species. However, to date, no P450 2J have been identified in zebrafish. We describe here a zfCyp2J1 cDNA which encodes a putative protein of 496 amino acids and shares 51%, 51%, 50%, 51% and 50% identity with mouse Cyp2J6, rabbit Cyp2J1, human Cyp2J2, cow Cyp2J2, and rat Cyp2J4, respectively. Despite detectable levels of expression by RT-PCR, no expression was shown by in situ hybridization using whole-mount tissues of the embryos. Gene-specific knockdown by antisense morpholino oligonucleotide had no phenotypic effect on embryonic development. However, over-expression of zfCyp2J1 by injection of the embryos with the cDNA resulted in substantial dose-dependent morphological defects. With adult zebrafish, whole-mount in situ hybridization showed that zfCyp2J1 was expressed predominantly in the brain and gonads. A semi-quantitative RT-PCR analysis further revealed that the zfCyp2J1 transcript was also expressed in the ovary, testis, heart, liver, and kidney. High levels of zfCyp2J1 mRNA were evident in primary growth stage (stage I) oocytes and cortical alveolus stage (stage II) oocytes but nearly undetectable in stage III and matured oocytes. These results suggest that zfCyp2J1 may not be involved in zebrafish embryogenesis but may rather play an important role in the functioning of brain and gonads of the adults. In addition, zfCyp2J1 may play a particularly crucial role in early oocyte maturation.

Descriptors: zebrafish embryo, cytochrome P450 enzyme system metabolism, gene expression, metabolism, aging physiology, amino acid sequence, conserved sequence, cytochrome P450 enzyme system chemistry, cytochrome P450 enzyme system genetics, complementary DNA genetics, complementary DNA isolation and purification, embryology, enzymology, developmental gene expression regulation, molecular sequence data, phylogeny, proteins chemistry and genetics.

Wang, Y.H., Y.H. Chen, J.H. Lu, and H.J. Tsai (2005). **A 23-amino acid motif spanning the basic domain targets zebrafish myogenic regulatory factor myf5 into nucleolus.** *DNA and Cell Biology* 24(10): 651-60. ISSN: 1044 5498.

NAL Call Number: QH443.D5

Abstract: Myf5 is a nuclear protein and one of the basic helix-loop-helix (bHLH) myogenic factors that play an important role in muscle specification and differentiation. The motif responsible for the nuclear translocation of Myf5 was unknown. Using on-line monitoring of EGFP (enhanced green fluorescent protein)-tagged zebrafish Myf5 translocation, we demonstrated that Myf5-EGFP protein resided in the nucleoplasm and nucleolus of zebrafish fibroblast cell lines (ZEM2S and ZF4), mammalian nonmuscle cell line (COS1), and muscle cell lines (RD and C2C12). In contrast, zebrafish MyoD-EGFP was localized in the nucleus but did not condense in the nucleolus. Using indirect immunofluorescent staining, we determined that zebrafish Myf5 was colocalized with nucleophosmin/B23, a nucleolus protein. Deletion analysis revealed that amino acid residues 60 to 82 (60KRKASTVDRRRAATMRERRRLKK82) of Myf5 were sufficient and necessary for nucleolus targeting. A GST pulldown assay followed by Western analysis showed that nucleolin/C23 could be pulled down specifically by GST-Myf5, but not by GST-MyoD. Based on these findings, we propose that the distinct functions of Myf5 and MyoD may result from their differential binding affinity to nucleolin/C23.

Descriptors: zebrafish, cell nucleolus metabolism, muscle proteins metabolism, nuclear localization signals metabolism, peptide fragments metabolism, trans activators metabolism, Western blotting , COS cells, cercopithecus aethiops, DNA binding proteins genetics, proteins metabolism andgenetics, fibroblasts cytology, fibroblasts metabolism, fluorescent antibody technique, indirect, glutathione transferase metabolism, green fluorescent green fluorescent proteins metabolism, muscle muscles cytology, muscles metabolism, Myod protein metabolism, myogenic regulatory factor 5, nuclear proteins metabolism, organelles metabolism, peptide fragments genetics, phosphoproteins metabolism, plasmids, protein transport, RNA binding proteins metabolism, trans activators genetics, growth and development.

Wrasidlo, W., A. Mielgo, V.A. Torres, S. Barbero, K. Stoletov, T.L. Suyama, R.L. Klemke, W.H. Gerwick, D.A. Carson, and D.G. Stupack (2008). **The marine lipopeptide somocystinamide A triggers apoptosis via caspase 8**. *Proceedings of the National Academy of Sciences, USA* 105(7): 2313-2318. ISSN: 0027-8424.

NAL Call Number: 500N21P

Descriptors: zebrafish, anticancer drug, marine, organisms, lipopeptide somocystinamide A (ScA), pluripotent inhibitor, antiangiogenic.

Wu, W. and W.H. Kinsey (2004). **Detection and measurement of membrane-bound protein tyrosine kinases in the zebrafish egg.** *Methods in Molecular Biology* 253: 273-83. ISSN: 1064-3745.

Descriptors: zebrafish, cell membrane enzymology, ovum enzymology, protein tyrosine kinases analysis, fertilization in vitro methods, peptides physiology, phosphorylation, protein tyrosine phosphatase analysis, proto oncogene proteins analysis, proto oncogene proteins C fyn.

Xie, Y., Y.Q. Ding, Y. Hong, Z. Feng, S. Navarre , C.X. Xi, X.J. Zhu, C.L. Wang, S.L. Ackerman, D. Kozlowski, L. Mei, and W.C. Xiong (2005). **Phosphatidylinositol transfer protein-alpha in netrin-1-induced plc signalling and neurite outgrowth**. *Nature Cell Biology* 7(11): 1124-1132. ISSN: 1465 7392.

NAL Call Number: QH573

Abstract: Neurite extension is essential for wiring the nervous system during development. Although several factors are known to regulate neurite outgrowth, the underlying mechanisms

remain unclear. Here, we provide evidence for a role of phosphatidylinositol transfer protein-alpha (PITP alpha) in neurite extension in response to netrin-1, an extracellular guidance cue. PITP alpha interacts with the netrin receptor DCC (deleted in colorectal cancer) and neogenin. Netrin-1 stimulates PITP alpha binding to DCC and to phosphatidylinositol (5) phosphate [PI(5) P], increases its lipid-transfer activity and elevates hydrolysis of phosphatidylinositol bis-phosphate (PIP2). In addition, the stimulated PIP2 hydrolysis requires PITP alpha. Furthermore, cortical explants of PITP alpha mutant mice are defective in extending neurites in response to netrin-1. Commissural neurons from chicken embryos expressing a dominant-negative PITP alpha mutant show reduced axon outgrowth. Morpholino-mediated knockdown of PITP alpha expression in zebrafish embryos leads to dose-dependent defects in motor-neuron axons and reduced numbers of spinal-cord neurons. Taken together, these results identify a crucial role for PITP alpha in netrin-1-induced neurite outgrowth, revealing a signalling mechanism for DCC/ neogenin and PITP alpha regulation. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, chicks, development, nervous system, neural coordination, development, neurite outgrowth, axon outgrowth.

Yamashita, M. (2003). **Apoptosis in zebrafish development.** *Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology* 136(4): 731-42. ISSN: 1096 4959.
NAL Call Number: QP501.C6
Abstract: Apoptosis (programmed cell death) is important in normal biological processes and in pathogenesis in vertebrates. This review focuses on some of the prominent features of apoptosis during fish development. Caspases and other apoptosis-regulating genes have been cloned from zebrafish (*Danio rerio*) and other fish species. Elucidation of in vivo functions of apoptosis is focused on development, morphogenesis and sex differentiation. In an attempt to elucidate cause and effect relationships between caspase and development, transgenic zebrafish overexpressing procaspase-3 were generated. Stress-induced apoptosis in zebrafish embryos can be monitored by whole mount TUNEL staining and caspase assay. Thus, zebrafish is a useful experimental model animal for investigation of apoptosis in vivo.
Descriptors: zebrafish embryos, apoptosis physiology, growth and development, genetically modified, caspases genetics, caspases metabolism, sex differentiation, stress pathology, stress physiopathology, embryology, genetics.

Yang, X., D. Kovalenko, R.J. Nadeau, L.K. Harkins, J. Mitchell, O. Zubanova, P.-Y. Chen, and R. Friesel (2004). **Sef Interacts with TAK1 and Mediates JNK Activation and apoptosis**. *Journal of Biological Chemistry* 279(37): 38099-38102. ISSN: 0021-9258.
NAL Call Number: 381J824
Descriptors: zebrafish, apoptosis, cell differentiation, extracellular signal-regulated kinase, fibroblast growth factor, fibroblast growth factor receptors, physiology, freshwater fish, gene expression, growth, MEK kinase, receptors, TAK1 protein, transcription factors, c-Jun amino-terminal kinase, *Danio rerio*, Sef protein.

Yasuda, S., A.P. Kumar, M.Y. Liu, Y. Sakakibara, M. Suiko, L. Chen, and M.C. Liu (2005). **Identification of a novel thyroid hormone-sulfating cytosolic sulfotransferase, SULT1 ST5, from zebrafish.** *Federation of European Biochemical Societies Journal* 272(15): 3828-37. ISSN: 1742 464X.
NAL Call Number: QP501.E8
Abstract: By employing RT-PCR in conjunction with 3'-RACE, a full-length cDNA encoding a novel zebrafish cytosolic sulfotransferase (SULT) was cloned and sequenced. Sequence analysis revealed that this zebrafish SULT (designated SULT1 ST5) is, at the amino acid sequence level, close to 50% identical to human and dog SULT1B1 (thyroid hormone SULT). A recombinant

form of zebrafish SULT1 ST5 was expressed using the pGEX-2TK bacterial expression system and purified from transformed BL21 (DE3) cells. Purified zebrafish SULT1 ST5 migrated as a 34 kDa protein and displayed substrate specificity for thyroid hormones and their metabolites among various endogenous compounds tested. The enzyme also exhibited sulfating activities toward some xenobiotic phenolic compounds. Its pH optima were 6.0 and 9.0 with 3,3',5-tri-iodo-l-thyronine (l-T3) as substrate and 6.0 with beta-naphthol as substrate. Kinetic constants of the enzyme with thyroid hormones and their metabolites as substrates were determined. Quantitative evaluation of the regulatory effects of divalent metal cations on the l-T3-sulfating activity of SULT1 ST5 revealed that Fe2+, Hg2+, Co2+, Zn2+, Cu2+, Cd2+ and Pb2+ exhibited dramatic inhibitory effects, whereas Mn2+ showed a significant stimulation. Developmental stage-dependent expression experiments revealed a significant level of expression of this novel zebrafish thyroid hormone-sulfating SULT at the beginning of the hatching period during embryogenesis, which gradually increased to a high level of expression throughout the larval stage into maturity.
Descriptors: zebrafish, dogs, arylsulfotransferase metabolism, isoenzymes metabolism, thyroid hormones metabolism, amino acid sequence, arylsulfotransferase genetics , arylsulfotransferase isolation and purification, divalent cations metabolism, molecular cloning, hydrogen ion concentration, isoenzymes genetics, isoenzymes isolation and purification, metals metabolism, molecular sequence data, reverse transcriptase PCR, sequence alignment, substrate specificity, embryology, proteins isolation and purification.

Yasuda, S., C.C. Liu, S. Takahashi, M. Suiko, L. Chen, R. Snow, and M.C. Liu (2005). **Identification of a novel estrogen-sulfating cytosolic SULT from zebrafish: molecular cloning, expression, characterization, and ontogeny study.** *Biochemical and Biophysical Research Communications* 330(1): 219-25 . ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: By searching the expressed sequence tag database, a zebrafish cDNA encoding a putative cytosolic sulfotransferase (SULT) was identified. Sequence analysis indicated that this zebrafish SULT belongs to the SULT1 cytosolic SULT gene family. The recombinant form of this novel zebrafish SULT, expressed using the pGEX-2TK expression system and purified from transformed BL21 (DE3) Escherichia coli cells, displayed sulfating activities specifically for estrone and 17beta-estradiol among various endogenous compounds tested as substrates. The enzyme also exhibited sulfating activities toward some xenobiotic phenolic compounds. This new zebrafish SULT showed dual pH optima, at 6.5 and 10-10.5, with estrone or n-propyl gallate as substrate. Kinetic constants of the sulfation of estrone, 17beta-estradiol, and n-propyl gallate were determined. Developmental stage-dependent expression experiments revealed a significant level of expression of this novel zebrafish estrogen-sulfating SULT at the beginning of the hatching period during embryogenesis, which continued throughout the larval stage onto maturity.
Descriptors: zebrafish, estrogens metabolism, sulfates metabolism, sulfotransferases metabolism, base sequence, molecular cloning, cytosol enzymology, DNA primers, polyacrylamide gel electrophoresis, developmental gene expression regulation, hydrogen ion concentration, kinetics, reverse transcriptase PCR.

Yeh, L.-K., C.-Y. Liu, C.-L. Chien, R. Converse, W.W.-Y. Kao, M.-S. Chen, F.-R. Hu, F.-J. Hsieh, and I.-J. Wang (2008). **Molecular analysis and characterization of zebrafish keratocan (zKera) Gene**. *Journal of Biological Chemistry* 283(1): 506-517. ISSN: 0021-9258.
NAL Call Number: 381J824
Descriptors: zebrafish, apoptosis, brain, caspase-3, caspase-8, cornea, development, eggs, embryogenesis, epithelium, exons, eye, fluorescence, forebrain, introns, PCR, promoters, proteoglycans, pseudogenes, reporter gene, stroma, genomics, *Danio rerio*.

Zanotti, G., L. Cendron, I. Ramazzina, C. Folli, R. Percudani, and R. Berni (2006). **Structure of zebra fish HIUase: insights into evolution of an enzyme to a hormone transporter.** *Journal of Molecular Biology* 363(1): 1-9. ISSN: 0022-2836.

NAL Call Number: 442.8 J8224

Abstract: During early vertebrate evolution, a duplication event in the gene encoding 5-hydroxyisourate hydrolase (HIUase), a widely distributed enzyme of purine metabolism, gave rise to transthyretin (TTR), a thyroid hormone transporter. We report here on the crystal structure of zebra fish HIUase in two different crystal forms. Despite the phylogenetic distance, this structure compares well with those of newly characterized bacterial HIUases, especially with regard to catalytic regions, which are highly preserved. Comparison with TTR structure reveals a highly conserved scaffold, harbouring distinct functional sites located in the same regions of the two vertebrate proteins. Residues that are differentially conserved in HIUases compared to TTR map in putative catalytic regions occupying significant portions of the two halves of a central channel that transverses the whole TTR protein. The evolution of TTR has been accompanied by remarkable changes of the HIUase active sites that gave rise to a channel open at both ends, thus allowing free access to hormone molecules.

Descriptors: zebrafish, amidohydrolases chemistry, molecular evolution, prealbumin metabolism, thyroid hormones metabolism, amidohydrolases metabolism, amino acid sequence, biological transport, active, crystallography, x ray, molecular sequence data, phylogeny, protein binding, receptors, albumin chemistry, receptors, albumin metabolism, proteins chemistry and metabolism.

Zetterberg, H., W.A. Campbell, H.W. Yang, and W. Xia (2006). **The cytosolic loop of the gamma-secretase component presenilin enhancer 2 protects zebrafish embryos from apoptosis.** *Journal of Biological Chemistry* 281(17): 11933-9. ISSN: 0021-9258.

NAL Call Number: 381J824

Abstract: The gamma-secretase complex, composed of presenilin, presenilin enhancer 2 (Pen-2), nicastrin, and Aph-1, catalyzes the final cleavage of amyloid precursor protein to generate the toxic amyloid beta protein, the major component of plaques in the brains of Alzheimer disease patients. To understand the in vivo function of Pen-2, we used morphant technology available in zebrafish and transiently knocked down the expression of endogenous Pen-2 by injecting the morpholino (MO) against Pen-2. Two truncated Pen-2 proteins lacking either the cytosolic or the C-terminal domain were expressed in MO-injected embryos. This deletion analysis demonstrated that the Pen-2 cytosolic loop is essential for protecting developing embryos from caspase-dependent apoptosis caused by the reduction of Pen-2. Twelve amino acids in the C terminus of Pen-2 were dispensable and could not rescue the Pen-2 knockdown-induced apoptotic phenotype. Surprisingly, double knockdown of Pen-2 and nuclear factor kappaB component p65 abrogated the single Pen-2 MO-induced caspase activation, indicating that a previously reported pro-apoptotic role of NF-kappaB in some cell types could be manifested in a whole animal and that knockdown of Pen-2 may trigger pro-apoptotic activation of NF-kappaB.

Descriptors: zebrafish embryo, apoptosis genetics, cytosol enzymology, endopeptidases metabolism, membrane, metabolism, amyloid precursor protein secretases, aspartic endopeptidases metabolism, caspases metabolism, cytology, enzyme activation, membrane metabolism, antisense oligonucleotides pharmacology, presenilin 2, transcription factor rela genetics, transcription factor rela physiology, proteins genetics.

Zhang, C., Q. Li, and Y.J. Jiang (2007). **Zebrafish Mib and Mib2 are mutual E3 ubiquitin ligases with common and specific delta substrates.** *Journal of Molecular Biology* 366(4): 1115-28. ISSN: 0022-2836.

NAL Call Number: 442.8 J8224

Abstract: It was already known that both mind bomb (mib) and mind bomb-2 (mib2) encode E3 ubiquitin ligases that target Delta in Notch activation. Here we further demonstrated that zebrafish Mib and Mib2, similar to their mouse orthologs, have a C-terminal-most RING finger-dependent E3 ubiquitin ligase activity. Mib and Mib2 are reciprocal E3 ubiquitin ligases and substrates. They function similarly in Notch signaling by using DeltaC as a common substrate. However, Mib2 behaves differently from Mib in DeltaD internalization. In addition, Mib and Mib2 bind differently to extracellular and intracellular parts of DeltaA and DeltaC. Finally, mutant Mibs, Mib(ta52b) with a missense mutation in the C-terminal-most RING finger (M1013R) and Mib(m132) with a premature stop codon that leads to a deletion of three RING fingers (C785stop), act dominant-negatively and compete with Mib2 in DeltaC ubiquitylation and internalization, suggesting a molecular basis for the antimorphic phenotypes (stronger than the null phenotypes) observed in zebrafish mib(ta52b) and mib(m132) alleles.

Descriptors: zebrafish, membrane proteins metabolism, ubiquitin metabolism, ubiquitin protein ligases metabolism, amino acid sequence, COS cells, *cercopithecus aethiops*, molecular sequence data, protein binding, amino acid sequence homology, signal transduction , substrate specificity, transfection, ubiquitin protein ligases genetics, proteins genetics.

Zhou Li and D.M. Irwin (2004). **Fish proglucagon genes have differing coding potential**. *Comparative Biochemistry and Physiology. Part B: Biochemistry and Molecular Biology* 137(2): 255-264. ISSN: 1096 4959.

NAL Call Number: QP501.C6

Abstract: Regulation of tissue-specific hormone production involves transcription, post-translational and physiological regulation. The proglucagon gene has been an ideal molecule for understanding many aspects of regulation of hormone production. Gene duplications often allow the evolution of new gene function, and the proglucagon gene has been duplicated on the lineage leading to teleost fish, while most other vertebrates have only a single proglucagon gene. The recent characterization of near complete pufferfish and zebrafish genomes allowed us to determine the structure of the duplicated genes. We searched the pufferfish and zebrafish genomes for proglucagon-like sequences. Our searches identified two proglucagon-like genes in both the pufferfish and zebrafish genomes. In pufferfish the proglucagon-like genes are surrounded by genes that are similar to genes that flank the proglucagon gene in mammalian genomes, indicating that gene order near the proglucagon gene has been maintained since the fish-mammal divergence approximately 450 million years ago. Characterization of the duplicated fish proglucagon genes indicates that one of the two genes is predicted to encode glucagon, GLP-1 and GLP-2. In contrast, the second proglucagon gene is predicted to encode only glucagon and GLP-1, but not GLP-2. Thus the two fish proglucagon genes have different coding potential, and, therefore likely differ in function. Regulation of gene expression potentially has a role in the regulation of production of proglucagon derived peptides in fish. Copyright © 2008 CABI

Descriptors: zebrafish, genes, genomes, hormonal control, nucleotide sequences, regulatory genes, *Danio rerio*.

Cancer Research

Anonymous (2007). **Development of the model of cell cycle synchronization in early embryos of *Danio rerio* (Teleostei)**. *Ontogene* 38(5): 372-9. ISSN: 0950-9232.

Abstract: An attempt was undertaken to develop a model system based on artificial cell cycle synchronization by means of reversible mitosis blocking in zebrafish embryos for studying the role of cell cycle synchrony in embryogenesis. Dechorionized and intact embryos at the stages of 512-cell blastula and 75% epiboly were treated with nocodazole and then washed within several times of exposure. When working on dechorionized embryos, we succeeded to obtain complete block mitosis in the presence of low nocodazole concentrations: 0.5-1.0 microg/ml. Block of mitosis was relieved in all experimental series within a certain time after the beginning of washing. This inertia depended on both nocodazole concentration and duration of treatment. The nocodazole elimination was significantly accelerated only after five (or more) changes of washing medium containing DMSO. As a result, the conditions were established for obtaining a parasynchronous cell population in the zebrafish gastrulas with a peak of mitosis up to 17.2%.

Descriptors: zebrafish, cell cycle synchronization, embryogenesis, nocodazole, blastula.

Language of Text: Russian.

Azuma, M., L.J. Embree, H. Sabaawy, and D.D. Hickstein (2007). **Ewing sarcoma protein EWSR1 maintains mitotic integrity and proneural cell survival in the zebrafish embryo.** *Public Library of Science One* 2(10): E979. ISSN: 1817-101X.

Abstract: BACKGROUND: The Ewing sarcoma breakpoint region 1 gene (EWSR1), also known as EWS, is fused to a number of different partner genes as a result of chromosomal translocation in diverse sarcomas. Despite the involvement of EWSR1 in these diverse sarcomas, the in vivo function of wild type EWSR1 remains unclear. PRINCIPAL FINDINGS: We identified two zebrafish EWSR1 orthologues, ewsr1a and ewsr1b, and demonstrate that both genes are expressed maternally, and are expressed ubiquitously throughout zebrafish embryonic development. Morpholino induced knockdown of both zebrafish ewsr1 genes led to mitotic defects with multipolar or otherwise abnormal mitotic spindles starting from the bud stage (10 hour post-fertilization (hpf)). The abnormalities in mitotic spindles were followed by p53-mediated apoptosis in the developing central nervous system (CNS) leading to a reduction in the number of proneural cells, disorganization of neuronal networks, and embryonic lethality by 5 days post-fertilization. siRNA silencing of EWSR1 in Hela cells resulted in mitotic defects accompanied by apoptotic cell death, indicating that the role of EWSR1 is conserved between zebrafish and human. CONCLUSIONS: Ewsr1 maintains mitotic integrity and proneural cell survival in early zebrafish development.

Descriptors: zebrafish, Ewing sarcoma, protein, EWSR1, mitosis, proneural, cell survival, embryo.

Beckman, M. (2007). **Zebrafish take the stage in cancer research.** *Journal of the National Cancer Institute* 99(7): 500-501. ISSN: 0027-8874.

Descriptors: zebrafish, disease models, animal, genes, p53, mice, neoplasms, reproduction, research methods.

Belleri, M., D. Ribatti, t. Nicoli, F. Cotelli, L. Forti, V. Vannini, L.A. Stivala, and M. Presta (2005). **Anti-angiogenic and vascular-targeting activity of the microtubule-destabilizing trans-resveratrol derivative 3,5,4 '-trimethoxystilbene**. *Molecular Pharmacology* 67(5): 1451-1459. ISSN: 0026 895X.

NAL Call Number: QP901.M6

Abstract: Neovascularization plays an important role in neoplasia and angioproliferative diseases. Two major modalities have been developed so far to affect neovascularization: its prevention by antiangiogenic compounds, and immature vessel disruption by vascular-targeting agents. trans-Resveratrol, found in grapes and wine, exerts antioxidant, antineoplastic, and antiangiogenic activities. Here, among various synthetic trans-resveratrol derivatives tested, 3,5,4'-trimethoxystilbene was an antiangiogenic agent 30 to 100 times more potent than parent compound in inhibiting endothelial cell proliferation, sprouting, collagen gel invasion, and morphogenesis (ID50 = 0.3-3.0 mu M). In addition, 3,5,4'-trimethoxystilbene acts as a vascular-targeting agent by causing microtubule disassembling and tubulin depolymerization and by impairing the repositioning of the microtubule organization center and the formation of membrane ruffles in migrating endothelial cells. In keeping with a vascular-targeting ability, 3,5,4'-trimethoxystilbene induced apoptosis only in subconfluent endothelial cells and apoptotic regression of immature vessels in the ex vivo rat aorta ring assay. In vivo, 3,5,4'-trimethoxystilbene caused the rapid stasis of blood flow and regression of intersegmental vessels in the trunk of zebrafish embryos. In addition, it inhibited blood vessel growth and caused the disappearance of pre-existing blood vessels in the area vasculosa of the chick embryo. In conclusion, 3,5,4'-trimethoxystilbene associates an antiangiogenic profile to a significant vascular-targeting activity. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, pharmacology, cardiovascular system, tumor biology, neoplasia, neoplastic disease, drug therapy, rat aorta ring assay, apoptosis, blood flow, neovascularization, morphogenesis, vascular targeting, microtubule destabilization.

Berghmans, S., C. Jette, D. Langenau, K. Hsu, R. Stewart, T. Look, and J.P. Kanki (2005). **Making waves in cancer research: new models in the zebrafish.** *BioTechniques* 39(2): 227-37. ISSN: 0736 6205.

NAL Call Number: RB37.1B56

Abstract: The zebrafish (*Danio rerio*) has proven to be a powerful vertebrate model system for the genetic analysis of developmental pathways and is only beginning to be exploited as a model for human disease and clinical research. The attributes that have led to the emergence of the zebrafish as a preeminent embryological model, including its capacity for forward and reverse genetic analyses, provides a unique opportunity to uncover novel insights into the molecular genetics of cancer. Some of the advantages of the zebrafish animal model system include fecundity, with each female capable of laying 200-300 eggs per week, external fertilization that permits manipulation of embryos ex utero, and rapid development of optically clear embryos, which allows the direct observation of developing internal organs and tissues in vivo. The zebrafish is amenable to transgenic and both forward and reverse genetic strategies that can be used to identify or generate zebrafish models of different types of cancer and may also present significant advantages for the discovery of tumor suppressor genes that promote tumorigenesis when mutationally inactivated. Importantly, the transparency and accessibility of the zebrafish embryo allows the unprecedented direct analysis of pathologic processes in vivo, including neoplastic cell transformation and tumorigenic progression. Ultimately, high-throughput modifier screens based on zebrafish cancer models can lead to the identification of chemicals or genes involved in the suppression or prevention of the malignant phenotype. The identification of small molecules or gene products through such screens will serve as ideal entry points for novel drug development for the treatment of cancer. This review focuses on the current technology that takes advantage of the zebrafish model system to further our understanding of the genetic basis of cancer and its treatment.
Descriptors: zebrafish, disease models, neoplasm metabolism, neoplasms genetics, neoplasms metabolism, gene expression regulation genetic predisposition to disease embryology, genetic predisposition to disease genetics, neoplasms embryology, tumor markers.

Capurro, M.I., W. Shi, S. Sandal, and J. Filmus (2005). **Processing by convertases is not required for glypican-3-induced stimulation of hepatocellular carcinoma growth**. *Journal of Biological Chemistry* 280(50): 41201-41206. ISSN: 0021 9258.
NAL Call Number: 381J824
Abstract: Glypicans are a family of heparan sulfate proteoglycans that are bound to the cell surface by a lipid anchor. Six members of this family have been identified in mammals (GPC1-GPC6). Glypicans act as regulators of the activity of various cytokines, including Wnts, Hedgehogs, and bone morphogenetic proteins. It has been reported that processing by a convertase is required for GPC3 activity during convergent extension in zebrafish embryos, for GPC3-induced regulation of Wnt signaling, and for the binding of GPC3 to Wnt5a. In our laboratory, we have recently demonstrated that GPC3 promotes the growth of hepatocellular carcinomas (HCCs) by stimulating canonical Wnt signaling. Because there is increasing evidence indicating that the structural requirements for GPC3 activity are cell type specific, we decided to investigate whether GPC3 needs to be processed by convertases to stimulate cell proliferation and Wnt signaling in HCC cells. We report here that a mutant GPC3 that cannot be processed by convertases is still able to play its stimulatory role in Wnt activity and HCC growth. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, tumor biology, biochemistry, hepatocellular carcinoma, neoplastic disease.

Cinar, B., P.K. Fang, M. Lutchman, N. Pavlova, P.C. Yelick, and M.R. Freeman (2006). **The STE 20-like kinase MST1 is an inhibitor of AKT 1/protein kinase B alpha**. *Proceedings of the American Association for Cancer Research Annual Meeting* 47: 542. ISSN: 0197 016X.
NAL Call Number: RC261.A4
Descriptors: zebrafish, RNA interference, genetic techniques, maldi tof mass spectrometry, matrix assisted laser desorption ionization, time of flight mass spectrometry, spectrum analysis techniques.

Cucina, A., P.M. Biava, F. D'Anselmi, P. Coluccia, F. Conti, R. di Clemente, A. Miccheli, L. Frati, A. Gulino, and M. Bizzarri (2006). **Zebrafish embryo proteins induce apoptosis in human colon cancer cells (Caco2).** *Apoptosis* 11(9): 1617-28. ISSN: 1360 8185.
NAL Call Number: QH671
Abstract: Previous studies have shown that proteins extracted from Zebrafish embryo share some cytostatic characteristics in cancer cells. Our study was conducted to ascertain the biological properties of this protein network. Cancer cell growth and apoptosis were studied in Caco2 cells treated with embryonic extracts. Cell proliferation was significantly inhibited in a dose-dependent manner. Cell-cycle analysis in treated cells revealed a marked accumulation in the G(2)/M phase preceding induction of apoptosis. Embryo proteins induced a significant reduction in FLIP levels, and increased caspase-3 and caspase-8 activity as well as the apoptotic rate. Increased phosphorylated pRb values were obtained in treated Caco2 cells: the modified balance in pRb phosphorylation was associated with an increase in E2F1 values and c-Myc over-expression. Our data support previous reports of an apoptotic enhancing effect displayed by embryo extracts, mainly through the pRb/E2F1 apoptotic pathway, which thus suggests that Zebrafish embryo proteins have complex anti-cancer properties.
Descriptors: zebrafish, antineoplastic agents pharmacology, apoptosis drug effects, caco 2 cells drug effects, proteins pharmacology, apoptosis regulatory, casp8 and fadd like apoptosis regulatinG protein physiology, cell division drug effects, cell proliferation drug effects, e2f1 transcription factor physiology, g2 phase drug effects, genes, myc physiology, retinoblastoma protein physiology, transfection.

Daroczi, B., G. Kari, M.F. McAleer, J.C. Wolf, U. Rodeck, and A.P. Dicker (2006). **In vivo radioprotection by the fullerene nanoparticle DF-1 as assessed in a zebrafish model.** *Clinical Cancer Research* 12(23): 7086-91. ISSN: 1078 0432.

Abstract: PURPOSE: We have previously shown that zebrafish (*Danio rerio*) embryos can be used as an in vivo model to validate modifiers of the radiation response. Here, we evaluated the radioprotective effect of the nanoparticle DF-1, a fullerene with antioxidant properties, in zebrafish embryos. EXPERIMENTAL DESIGN: Zebrafish embryos were exposed to different doses of ionizing radiation ranging from 20 to 80 Gy in the presence and absence of DF-1. Toxicity and radioprotective effects were assessed by monitoring overall survival and morphology as well as organ functions by employing assays to measure kidney excretory function and development of sensory nerve cells (neuromasts). Antioxidant properties of DF-1 were assessed in whole fish. RESULTS: DF-1 had no apparent adverse effects on normal zebrafish morphology or viability throughout the concentration range tested (1-1,000 micromol/L). Ionizing radiation (10-40 Gy) caused time-dependent and dose-dependent perturbations of normal zebrafish morphology and physiology, notably defective midline development resulting in dorsal curvature of the body axis ("curly-up"), neurotoxicity, impaired excretory function, and decreased survival of the exposed embryos. DF-1 (100 micromol/L) markedly attenuated overall and organ-specific radiation-induced toxicity when given within 3 hours before or up to 15 minutes after radiation exposure. By contrast, DF-1 afforded no protection when given 30 minutes after ionizing radiation. The degree of radioprotection provided by DF-1 was comparable with that provided by the Food and Drug Administration-approved radioprotector amifostine (4 mmol/L). Protection against radiation-associated toxicity using DF-1 in zebrafish embryos was associated with marked reduction of radiation-induced reactive oxygen species. CONCLUSION: The fullerene DF-1 protects zebrafish embryos against deleterious effects of ionizing radiation due, in part, to its antioxidant properties.

Descriptors: zebrafish, disease models, embryo drug effects, fullerenes pharmacology, nanoparticles chemistry, radiation protective agents pharmacology, drug dose response relationship, radiation dose response relationship, preclinical drug evaluation, nonmammalian radiation effects, fullerenes adverse effects, kidney drug effects, kidney embryology, kidney radiation effects, kidney function tests, nanoparticles adverse effects, radiation protective agents adverse effects, time factors.

Daroczi, B., G. Kati, M.F. Mcaleer, U. Rodeck, and A.P. Dicker (2006). **The fullerene df-1 demonstrates general and organ specific radioprotective effects in a zebrafish model.** *Proceedings of the American Association for Cancer Research Annual Meeting* 47: 1350. ISSN: 0197 016X.

NAL Call Number: RC261.A4

Descriptors: zebrafish, pharmacology, toxicology, radiation biology, microplate reader, laboratory equipment, ionizing radiation.

Davidson, A.J. and L.I. Zon (2004). **The 'definitive' (and 'primitive') guide to zebrafish hematopoiesis.** *Oncogene* 23(43): 7233-46. ISSN: 0950-9232.

Abstract: Progressive advances using zebrafish as a model organism have provided hematologists with an additional genetic system to study blood cell formation and hematological malignancies. Despite extensive evolutionary divergence between bony fish (teleosts) and mammals, the molecular pathways governing hematopoiesis have been highly conserved. As a result, most (if not all) of the critical hematopoietic transcription factor genes identified in mammals have orthologues in zebrafish. As in other vertebrates, all of the teleost blood lineages are believed to originate from a pool of pluripotent, self-renewing hematopoietic stem cells. Here, we provide a detailed review of the timing, anatomical location, and transcriptional regulation of zebrafish 'primitive' and 'defini-

tive' hematopoiesis as well as discuss a model of T-cell leukemia and recent advances in blood cell transplantation. Given that many of the regulatory genes that control embryonic hematopoiesis have been implicated in oncogenic pathways in adults, an understanding of blood cell ontogeny is likely to provide insights into the pathophysiology of human leukemias.
Descriptors: zebrafish, hematopoiesis, blood cells cytology, cell lineage, embryo, physiology, forecasting, gastrula cytology, developmental gene expression regulation, genetic complementation test, growth substances physiology, hematopoietic stem cell transplantation, hematopoietic stem cells cytology, leukemia genetics, leukemia pathology, mesoderm cytology, mutagenesis, pluripotent stem cells cytology, radiation chimera, transcription factors physiology, genetic transcription, proteins genetics.

Davidson, W.R., G. Kari, M.F. Mcaleer, C. Kari, A.P. Dicker, and U. Rodeck (2006). **Off-target effects of the p53 inhibitor pifithrin-a related to inhibition of p73 in zebrafish embryos.** *Proceedings of the American Association for Cancer Research Annual Meeting* 47: 1215. ISSN: 0197 016X.
NAL Call Number: RC261.A4
Descriptors: zebrafish, biochemistry, molecular biophysics, radiation biology, ionizing radiation, pharmacological inhibition, off target effect.

Geiger, G.A., A.P. Beothy, and G.D. Kao (2006). **Rapid bioluminescent detection of caspase activation induced after radiation and radioprotection in zebrafish embryos.** *Proceedings of the American Association for Cancer Research Annual Meeting* 47 : 1245. ISSN: 0197 016X.
NAL Call Number: RC261.A4
Descriptors: zebrafish, pharmaceuticals, pharmacology, enzymology, radiation biology, high throughput screening assay, genetic techniques, bioluminescence imaging, imaging, radioprotection , microplate based assay, ionizing radiation, gamma radiation.

Goessling, W., T.E. North, and L.I. Zon (2007). **New waves of discovery: modeling cancer in zebrafish.** *Journal of Clinical Oncology* 25(17): 2473-79. ISSN: 0732-183X.
Descriptors: zebrafish, disease models, neoplasms, cancer model.

Goessling, W., T.E. North, and L.I. Zon (2007). **Ultrasound biomicroscopy permits in vivo characterization of zebrafish liver tumors.** *Nature Methods* 4(7): 551-3. ISSN: 1548-7091.
NAL Call Number: QH315
Abstract: Zebrafish are a valuable vertebrate model to study carcinogenesis, but noninvasive imaging is challenging because adult fish are not transparent. Here we show that tumors could be readily detected in vivo using high-resolution microscopic ultrasound in zebrafish. We successfully obtained tissue perfusion calculations and cellular aspirates, and analyzed tumor progression and response to treatment. Ultrasound biomicroscopy allows longitudinal studies of tumor development and real-time assessment of therapeutic effects in zebrafish.
Descriptors: zebrafish, disease models, liver neoplasms, experimental ultrasonography, acoustic microscopy methods, liver neoplasms, experimental pathology.

Gordon, S., G. Akopyan, H. Garban, and B. Bonavida (2006). **Transcription factor YY1: structure, function, and therapeutic implications in cancer biology**. *Oncogene* 25(8): 1125-1142. ISSN: 0950 9232.
Abstract: The ubiquitous transcription factor Yin Yang 1 (YY1) is known to have a fundamental role in normal biologic processes such as embryogenesis, differentiation, replication, and cellular proliferation. YY1 exerts its effects on genes involved in these processes via its ability to initiate, activate, or repress transcription depending upon the context in which it binds. Mechanisms

of action include direct activation or repression, indirect activation or repression via cofactor recruitment, or activation or repression by disruption of binding sites or conformational DNA changes. YY1 activity is regulated by transcription factors and cytoplasmic proteins that have been shown to abrogate or completely inhibit YY1-mediated activation or repression; however, these mechanisms have not yet been fully elucidated. Since expression and function of YY1 are known to be intimately associated with progression through phases of the cell cycle, the physiologic significance of YY1 activity has recently been applied to models of tumor biology. The majority of the data are consistent with the hypothesis that YY1 overexpression and/or activation is associated with unchecked cellular proliferation, resistance to apoptotic stimuli, tumorigenesis and metastatic potential. Studies involving hemato-poetic tumors, epithelial-based tumors, endocrine organ malignancies, hepatocellular carcinoma, and retinoblastoma support this hypothesis. Molecular mechanisms that have been investigated include YY1-mediated downregulation of p53 activity, interference with poly-ADP-ribose polymerase, alteration in c-myc and nuclear factor-kappa B (NF-kappa B) expression, regulation of death genes and gene products, and differential YY1 binding in the presence of inflammatory mediators. Further, recent findings implicate YY1 in the regulation of tumor cell resistance to chemotherapeutics and immune-mediated apoptotic stimuli. Taken together, these findings provide strong support of the hypothesis that YY1, in addition to its regulatory roles in normal biologic processes, may possess the potential to act as an initiator of tumorigenesis and may thus serve as both a diagnostic and prognostic tumor marker; furthermore, it may provide an effective target for antitumor chemotherapy and/or immunotherapy. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, tumor biology, molecular genetics, epithelial tumor, neoplastic disease, pathology, hepatocellular carcinoma, neoplastic disease, pathology, genetics, basal cell carcinoma, genetics, retinoblastoma, genetics, endocrine organ malignancy, pathology, apoptosis, epigenetic regulation.

Haldi, M., C. Ton, W.L. Seng, and P. McGrath (2006). **Human melanoma cells transplanted into zebrafish proliferate, migrate, produce melanin, form masses and stimulate angiogenesis in zebrafish.** *Angiogenesis* 9(3): 139-51. ISSN: 0969 6970.

NAL Call Number: QP106.6.A54

Abstract: In this research, we optimized parameters for xenotransplanting WM-266-4, a metastatic melanoma cell line, including zebrafish site and stage for transplantation, number of cells, injection method, and zebrafish incubation temperature. Melanoma cells proliferated, migrated and formed masses in vivo. We transplanted two additional cancer cell lines, SW620, a colorectal cancer cell line, and FG CAS/Crk, a pancreatic cancer cell line and these human cancers also formed masses in zebrafish. We also transplanted CCD-1092Sk, a human fibroblast cell line established from normal foreskin and this cell line migrated, but did not proliferate or form masses. We quantified the number of proliferating melanoma and normal skin fibroblasts by dissociating xenotransplant zebrafish, dispensing an aliquot of CM-DiI labeled human cells from each zebrafish onto a hemocytometer slide and then visually counting the number of fluorescently labeled cancer cells. Since zebrafish are transparent until approximately 30 dpf, the interaction of labeled melanoma cells and zebrafish endothelial cells (EC) can be visualized by whole-mount immunochemical staining. After staining with Phy-V, a mouse anti-zebrafish monoclonal antibody (mAb) that specifically labels activated EC and angioblasts, using immunohistology and 2-photon microscopy, we observed activated zebrafish EC embedded in human melanoma cell masses. The zebrafish model offers a rapid efficient approach for assessing human cancer cells at various stages of tumorigenesis.

Descriptors: zebrafish, melanoma pathology, pathologic neovascularization pathology, cell

movement, cell proliferation, melanins metabolism, melanoma blood supply, neoplasm transplantation, skin neoplasms blood supply, cultured tumor cells .

Haramis, A.P., A. Hurlstone, Y. van der Velden, H. Begthel, M. van den Born, G.J. Offerhaus, and H.C. Clevers (2006). **Adenomatous polyposis coli-deficient zebrafish are susceptible to digestive tract neoplasia.** *European Molecular Biology Organization Journal Reports* 7(4): 444-9. ISSN: 1469 221X.

NAL Call Number: QH506 .E463

Abstract: Truncation of the tumour suppressor adenomatous polyposis coli (APC) constitutively activates the Wnt/beta-catenin signalling pathway. This event constitutes the primary transforming event in sporadic colorectal cancer in humans. Moreover, humans or mice carrying germline truncating mutations in APC develop large numbers of intestinal adenomas. Here, we report that zebrafish that are heterozygous for a truncating APC mutation spontaneously develop intestinal, hepatic and pancreatic neoplasias that are highly proliferative, accumulate beta-catenin and express Wnt target genes. Treatment with the chemical carcinogen 7,12-dimethylbenz[a]anthracene accelerates the induction of these lesions. These observations establish apc-mutant zebrafish as a bona fide model for the study of digestive tract cancer.

Descriptors: zebrafish, adenoma metabolism, adenoma pathology, adenomatous polyposis coli protein deficiency, adenomatous polyposis coli protein metabolism, digestive system neoplasms metabolism, digestive system neoplasms pathology, metabolism, adenoma genetics, adenomatous polyposis coli protein genetics, digestive system neoplasms genetics, gene expression regulation.

Karinos, T., K. Vougas, A. Vlahou, M. Fountoulakis, G. Koumoundouros, and C.N. Flytzanis (2005). **Towards the characterization of the zebrafish proteome**. *Cancer Genomics and Proteomics* 2(3): 187. ISSN: 1109 6535.

Descriptors: zebrafish , nervous system, muscular system, development , reproduction, matrix assisted laser desorption, ionization time of flight mass spectrometry, maldi tof mass spectrometry, spectrum analysis techniques, 2 d electrophoresis, 2 d gel electrophoresis, electrophoretic techniques, reproductive potential, cost effective breeding, temperature sex determination.

Kiser, K. (2005). **Fishing for a cure. Does the tiny zebrafish hold a key to stopping tumor growth?** *Minnesota Medicine* 88(4): 8-9. ISSN: 0026-556X.

Descriptors: zebrafish, genetic research, neoplasms blood supply, pathologic neovascularization genetics, angiogenesis inhibitors therapeutic use, monoclonal antibodies therapeutic use, gene expression regulation drug effects, gene therapy, membrane glycomice , minnesota, neoplasms therapy, pathologic neovascularization therapy, proteoglycans genetics, syndecan 2, vascular endothelialgrowth factor A antagonists and inhibitors, proteins.

Lam, S.H. and Z. Gong (2006). **Modeling liver cancer using zebrafish: a comparative oncogenomics approach.** *Cell Cycle* 5(6): 573-7. ISSN: 1538 4101.

Abstract: Although the zebrafish has many attributes of a promising cancer model, one outstanding question is how similar zebrafish and human tumors are at the molecular level. To date, supporting data from histology and 'gene-to-gene' comparisons with human data offer limited insights. Using comparative microarray analyses, we found striking molecular similarities between zebrafish and human liver neoplasia. Our data indicate that zebrafish liver tumors possess the general molecular hallmarks of human liver cancer and some of the molecular similarities extend to the progression of liver tumors. The molecular conservation between fish and human liver tumors underscored the strong association and fundamental importance of these genes in liver neoplasia as well as their clinical potentials as diagnostic markers and/or therapeutic targets. In

addition, our comparative oncogenomic work provides a general framework for comparing and validating microarray data of zebrafish model with human cancer, thus adding confidence of using the zebrafish to model human cancers.

Descriptors: zebrafish, gene expression regulation, neoplastic genetics, genomics methods, liver neoplasms, oncocell transformation, neoplastic genetics, neoplastic metabolism, disease models, gene expression profiling methods and trends, experimental metabolism, oncogene proteins analysis, oncogene oncogene proteins metabolism, tumor markers, tumor markers, metabolism.

Langenau, D.M., H. Feng, S. Berghmans, J.P. Kanki, J.L. Kutok, and A.T. Look (2005). **Cre/lox-regulated transgenic zebrafish model with conditional myc-induced T cell acute lymphoblastic leukemia.** *Proceedings of the National Academy of Sciences of the United States of America* 102(17): 6068-73. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: We have created a stable transgenic rag2-EGFP-mMyc zebrafish line that develops GFP-labeled T cell acute lymphoblastic leukemia (T-ALL), allowing visualization of the onset and spread of this disease. Here, we show that leukemias from this transgenic line are highly penetrant and render animals moribund by 80.7 +/- 17.6 days of life (+/-1 SD, range = 50-158 days). These T cell leukemias are clonally aneuploid, can be transplanted into irradiated recipient fish, and express the zebrafish orthologues of the human T-ALL oncogenes tal1/scl and lmo2, thus providing an animal model for the most prevalent molecular subgroup of human T-ALL. Because T-ALL develops very rapidly in rag2-EGFP-mMyc transgenic fish (in which "mMyc" represents mouse c-Myc), this line can only be maintained by in vitro fertilization. Thus, we have created a conditional transgene in which the EGFP-mMyc oncogene is preceded by a loxed dsRED2 gene and have generated stable rag2-loxP-dsRED2-loxP-EGFP-mMyc transgenic zebrafish lines, which have red fluorescent thymocytes and do not develop leukemia. Transgenic progeny from one of these lines can be induced to develop T-ALL by injecting Cre RNA into one-cell-stage embryos, demonstrating the utility of the Cre/lox system in the zebrafish and providing an essential step in preparing this model for chemical and genetic screens designed to identify modifiers of Myc-induced T-ALL.

Descriptors: genes, myc, green fluorescent integrases metabolism, leukemia,T cell, acute genetics, genetically modified, DNA binding proteins genetics, extracellular matrix proteins metabolism, genetic markers, mice, molecular sequence data, nuclear proteins, protein lysine 6 oxidase metabolism.

Langenau, D.M., D. Traver, A.A. Ferrando, J.L. Kutok, J.C. Aster, J.P. Kanki, S. Lin, E. Prochownik, N.S. Trede, L.I. Zon, and A.T. Look (2003). **Myc-induced T cell leukemia in transgenic zebrafish.** *Science* 299(5608): 887-90. ISSN: 0036-8075.

NAL Call Number: 470Sci2

Abstract: The zebrafish is an attractive model organism for studying cancer development because of its genetic accessibility. Here we describe the induction of clonally derived T cell acute lymphoblastic leukemia in transgenic zebrafish expressing mouse c-myc under control of the zebrafish Rag2 promoter. Visualization of leukemic cells expressing a chimeric transgene encoding Myc fused to green fluorescent protein (GFP) revealed that leukemias arose in the thymus, spread locally into gill arches and retro-orbital soft tissue, and then disseminated into skeletal muscle and abdominal organs. Leukemic cells homed back to the thymus in irradiated fish transplanted with GFP-labeled leukemic lymphoblasts. This transgenic model provides a platform for drug screens and for genetic screens aimed at identifying mutations that suppress or enhance c-myc- induced carcinogenesis.

Descriptors: zebrafish, cell transformation, neoplastic, disease models, genes, myc, leukemia,T

cell, acute genetics, leukemia,T cell, acute pathology, T lymphocytes pathology, genetically modified, cell lineage, clone cells, DNA binding proteins genetics, fertilization in vitro, gene expression profiling, green fluorescent proteins, kidney pathology , leukemic infiltration, luminescent proteins metabolism, mice, mutation, neoplasm transplantation, olfactory bulb pathology, promoter regions genetics, recombinant fusion proteins metabolism, spleen pathology, T lymphocytes immunology, T lymphocytes physiology, thymus gland pathology, transgenes.

Le, X., D.M. Langenau, M.D. Keefe, J.L. Kutok, D.S. Neuberg, and L.I. Zon (2007). **Heat shock-inducible Cre/Lox approaches to induce diverse types of tumors and hyperplasia in transgenic zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 104(22): 9410-5. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: RAS family members are among the most frequently mutated oncogenes in human cancers. Given the utility of zebrafish in both chemical and genetic screens, developing RAS-induced cancer models will make large-scale screens possible to understand further the molecular mechanisms underlying malignancy. We developed a heat shock-inducible Cre/Lox-mediated transgenic approach in which activated human kRASG12D can be conditionally induced within transgenic animals by heat shock treatment. Specifically, double transgenic fish Tg(B-actin-LoxP-EGFP-LoxP-kRASG12D; hsp70-Cre) developed four types of tumors and hyperplasia after heat shock of whole zebrafish embryos, including rhabdomyosarcoma, myeloproliferative disorder, intestinal hyperplasia, and malignant peripheral nerve sheath tumor. Using ex vivo heat shock and transplantation of whole kidney marrow cells from double transgenic animals, we were able to generate specifically kRASG12D-induced myeloproliferative disorder in recipient fish. This heat shock-inducible recombination approach allowed for the generation of multiple types of RAS-induced tumors and hyperplasia without characterizing tissue-specific promoters. Moreover, these tumors and hyperplasia closely resemble human diseases at both the morphologic and molecular levels.

Descriptors: transgenic zebrafish, gene expression regulation, heat shock response physiology, hyperplasia genetics, hyperplasia pathology, neoplasms genetics, neoplasms pathology, aging physiology, attachment sites, microbiological genetics, integrases genetics, integrases metabolism, kidney metabolism, survival rate, time factors, RAS ras proteins metabolism.

Lee, J.W., D.S. Na, J.Y. Kang, S.H. Lee, and B.K. Ju (2006). **Differentiation of mouse p19 embryonic carcinoma stem cells injected into an empty zebrafish egg chorion in a microfluidic device.** *Bioscience, Biotechnology, and Biochemistry* 70(6): 1325-30. ISSN: 0916 8451.

NAL Call Number: QH301.B564

Abstract: Mouse P19 embryonic carcinoma (EC) stem cells were xenotransplanted into the emptied chorion, the transparent envelope of a fertilized zebrafish egg (rather than mouse native zona pellucida) combined with a microfluidic device to study P19 EC cell differentiation in the chorion biomaterial. A distilled-water jet was used to remove the innate yolk and perivitelline inner mass from the chorion. P19 EC cells were injected into the emptied chorion using a micropipette, and they were subsequently cultured until the inner space of the chorion became completely occupied by cells. A simple microfluidic device was used for handling convenience and effective experiment. At d15, we found neural cells in the outer layer of the cell mass and beating cardiomyocytes in the inner layer of the large embryoid body. We propose that even though the species are different, the external innate membranes developed for embryo protection represent a useful type of ECM.

Descriptors: zebrafish, carcinoma, embryonal pathology, cell differentiation, chorion cytol-

ogy, microfluidic analytical techniques instrumentation, oocytes cytology, stem cells pathology, zebrafish, cell lineage, cultured cells, immunohistochemistry, mice, stem cell transplantation.

Lee, L.M., E.A. Seftor, G. Bonde, R.A. Cornell, and M.J. Hendrix (2005). **The fate of human malignant melanoma cells transplanted into zebrafish embryos: assessment of migration and cell division in the absence of tumor formation.** *Developmental Dynamics* 233(4): 1560-70. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, cell division physiology, cell movement physiology, melanoma pathology, tumor cell line, fibroblasts physiology, fibroblasts transplantation, melanocytes physiology, melanocytes transplantation, confocal microscopy, neoplasm metastasis, neoplasm transplantation, heterologous transplantation.

Maiorana, A., X. Tu, G. Cheng, and R. Baserga (2004). **Role of pescadillo in the transformation and immortalization of mammalian cells**. *Oncogene* 23(42): 7116-7124. ISSN: 0950 9232.
Abstract: The murine and human homologs of the zebrafish pescadillo protein (Pes1 and PES1, respectively) play important roles in ribosome biogenesis and DNA replication. We investigated the effect of Pes1 on the growth of mouse embryo (3T3-like) fibroblasts and conditionally immortalized human fibroblasts expressing the SV40 T antigen (AR5 cells). Increased expression of Pes1 causes transformation of mouse and human fibroblasts in culture (colony formation in soft agar). Although Pes1 can replace the SV40 T antigen in inducing colony formation in soft agar, it cannot substitute the T antigen in the immortalization of human fibroblasts, indicating that it distinguishes between the two functions. As the biological effects of Pes1 are similar to those of the insulin receptor substrate- 1 (IRS-1), we investigated the interactions of Pes1 with IRS-1 itself and with the SV40 T antigen. The Pes1 protein (which localizes to the nuclei and nucleoli of cells) interacts with both IRS-1 and the SV40 T antigen, and markedly decreases the interaction of T antigen with p53. Taken together, these results suggest mechanisms for the ability of Pes1 to transform cells, and its failure to immortalize them. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, cell biology, infection, tumor biology, northern blot, confocal microscopy, imaging and microscopy techniques, laboratory techniques; immunoprecipitation, immunologic techniques.

McAleer, M.F., C. Davidson, W.R. Davidson, B. Yentzer, S.A. Farber, U. Rodeck, and A.P. Dicker (2005). **Novel use of zebrafish as a vertebrate model to screen radiation protectors and sensitizers.** *International Journal of Radiation Oncology, Biology, Physics* 61(1): 10-13. ISSN: 0306-3016.
NAL Call Number: RC271.R3
Abstract: PURPOSE: Zebrafish (*Danio rerio*) embryos provide a unique vertebrate model to screen therapeutic agents easily and rapidly because of their relatively close genetic relationship to humans, ready abundance and accessibility, short embryonal development, and optical clarity. To validate zebrafish embryos as a screen for radiation modifiers, we evaluated the effects of ionizing radiation in combination with a known radioprotector (free radical scavenger Amifostine) or radiosensitizing agent (tyrosine kinase inhibitor AG1478). METHODS AND MATERIALS: Viable zebrafish embryos were exposed to 0-10 Gy single-fraction 250 kVp X-rays with or without either Amifostine (0-4 mM) or AG1478 (0-10 microM) at defined developmental stages from 1-24 h postfertilization (hpf). Embryos were examined for morphologic abnormalities and viability until 144 hpf. RESULTS: Radiation alone produced a time- and dose-dependent perturbation of normal embryonic development and survival with maximal sensitivity at doses > or =4 Gy delivered before 4 hpf. Amifostine markedly attenuated this effect, whereas AG1478 enhanced

teratogenicity and lethality, particularly at therapeutically relevant (2-6 Gy) radiation doses. CONCLUSIONS: Collectively, these data validate the use of zebrafish as a vertebrate model to assess the effect of radiation alone or with radiation response modulators. Zebrafish embryos may thus provide a rapid, facile system to screen novel agents ultimately intended for human use in the context of therapeutic or accidental radiation exposure.

Descriptors: zebrafish embryolgy, amifostine therapeutic use, models, radiation protective agents therapeutic use, radiation sensitizing agents toxicity, tyrphostins toxicity, radiation dose response relationship, preclinical drug evaluation, radiation effects, radiation injuries, experimental prevention and control, survival analysis.

McAleer, M.F., K.T. Duffy, W.R. Davidson, G. Kari, A.P. Dicker, U. Rodeck, and E. Wickstrom (2006). **Antisense inhibition of cyclin D1 expression is equivalent to flavopiridol for radiosensitization of zebrafish embryos.** *International Journal of Radiation Oncology, Biology, Physics* 66(2): 546-51. ISSN: 0306 3016.

NAL Call Number: RC271.R3

Abstract: PURPOSE: Flavopiridol, a small molecule pan-cyclin inhibitor, has been shown to enhance the radiation response of tumor cells both in vitro and in vivo. The clinical utility of flavopiridol, however, is limited by toxicity, previously attributed to pleiotropic inhibitory effects on several targets affecting multiple signal transduction pathways. Here we used zebrafish embryos to investigate radiosensitizing effects of flavopiridol in normal tissues. METHODS AND MATERIALS: Zebrafish embryos at the 1- to 4-cell stage were treated with 500 nM flavopiridol or injected with 0.5 pmol antisense hydroxylprolyl-phosphono nucleic acid oligomers to reduce cyclin D1 expression, then subjected to ionizing radiation (IR) or no radiation. RESULTS: Flavopiridol-treated embryos demonstrated a twofold increase in mortality after exposure to 40 Gy by 96 hpf and developed distinct radiation-induced defects in midline development (designated as the "curly up" phenotype) at higher rates when compared with embryos receiving IR only. Cyclin D1-deficient embryos had virtually identical IR sensitivity profiles when compared with embryos treated with flavopiridol. This was particularly evident for the IR-induced curly up phenotype, which was greatly exacerbated by both flavopriridol and cyclin D1 downregulation. CONCLUSIONS: Treatment of zebrafish embryos with flavopiridol enhanced radiation sensitivity of zebrafish embryos to a degree that was very similar to that associated with downregulation of cyclin D1 expression. These results are consistent with the hypothesis that inhibition of cyclin D1 is sufficient to account for the radiosensitizing action of flavopiridol in the zebrafish embryo vertebrate model.

Descriptors: zebrafish embryos, cyclin D1 antagonists and inhibitors, radiation effects, flavonoids pharmacology, oligodeoxyribonucleotides pharmacology, piperidines pharmacology, radiation sensitizing agents pharmacology, cyclin D1 genetics, dose response relationship, drug effects, models, phenotype, survival analysis.

McAleer, M.F., S.A. Farber, U. Rodeck, and A.P. Dicker (2004). **Differential sensitivity of zebrafish embryos to distinct classes of DNA-damaging agents associated with differential expression of DNA repair enzymes**. *International Journal of Radiation Oncology Biology Physics* 60(1, Suppl. S): S349. ISSN: 0360 3016.

NAL Call Number: RC271.R3

Descriptors: zebrafish, development, enzymology, radiation biology, microarray analysis, genetic techniques, gene expression, ionizing radiation, differential sensitivity, DNA damage repair, midblastula transition, UV radiation, DNA intrastrand damaging agent.

McAleer, M.F., C. Kati, A.P. Dicker, and U. Rodeck (2006). **Differential response to ultraviolet versus ionizing radiation in zebrafish embryos pretreated with caffeine**. *Proceedings of the American Association for Cancer Research Annual Meeting* 47: 1215. ISSN: 0197 016X.
NAL Call Number: RC261.A4
Descriptors: zebrafish, radiation biology, molecular mechanism, ionizing radiation, ultraviolet irradiation, differential response.

Mizgireuv, I.V., I.G. Majorova, V.M. Gorodinskaya, V.V. Khudoley, and S.Y. Revskoy (2004). **Carcinogenic effect of N-nitrosodimethylamine on diploid and triploid zebrafish (*Danio rerio*).** *Toxicologic Pathology* 32(5): 514-8. ISSN: 0192-6233.
Abstract: Viability of polyploid organisms in lower vertebrates including fish provides an additional tool to investigate genetic mechanisms of neoplastic transformation caused by carcinogens. Here we present data on differential sensitivity of diploid and triploid zebrafish *(Danio rerio*) to N-nitrosodimethylamine (NDMA) induced hepatocarcinogenesis. The effect of the carcinogen was studied in 100 diploid and 120 triploid zebrafish. Zebrafish, age 5-6 weeks, were exposed to 50 ppm NDMA for 8 weeks and then were transferred into fresh carcinogen-free water until necropsy. At the necropsy performed 24 weeks after beginning the treatment, cholangiolar tumors (cholangiocarcinomas and cholangiomas) were essentially observed in diploid zebrafish only, while the incidence of hepatocellular tumors (hepatocellular carcinomas and adenomas) was similar in diploid and triploid zebrafish, 7.7% and 9.5%, respectively. By contrast, 36 weeks after beginning the treatment, the incidence of hepatocellular tumors was significantly lower in diploid animals as compared to triploid ones, 10.3% and 33.8%, respectively. The incidence of cholangiolar tumors in diploid and triploid zebrafish was not significantly different, 10.3% and 14.9%, respectively. Therefore, the increase of ploidy appeared to have a differential effect on the induction of these 2 types of liver tumors in zebrafish. This finding suggests a difference in genetic mechanisms of the tumor development revealed by utilization of triploid animals in this study. However, triploid zebrafish demonstrated an overall increase in latency period in the development of both types of hepatic tumors, a finding that can be interpreted as an increased resistance of triploid animals to the carcinogenic effect of NDMA.
Descriptors: zebrafish, carcinogens toxicity, diploidy, liver drug effects, experimental chemically induced liver neoplasms, , nitrosamines toxicity, polyploidy, adenoma, genetics, adenoma, liver cell pathology, carcinoma, hepatocellular chemically induced, carcinoma, hepatocellular genetics, carcinoma, hepatocellular pathology, genetic predisposition to disease, experimental genetics and pathology.

Nicoli, S. and M. Presta (2007). **The zebrafish/tumor xenograft angiogenesis assay.** *Nature Protocals* 2(11): 2918-23. ISSN: 1754-2189.
Abstract: Here we describe a method to study tumor angiogenesis in zebrafish (*Danio rerio*) based on the injection of proangiogenic mammalian tumor cells into the perivitelline space of zebrafish embryos at 48 h post-fertilization. Within 24-48 h, proangiogenic tumor grafts induce a neovascular response originating from the developing subintestinal vessels. This can be observed at macroscopic and microscopic levels after whole-mount alkaline phosphatase staining of wild-type zebrafish embryos, or by fluorescence microscopy in transgenic VEGFR2:G-RCFP embryos in which endothelial cells express the green fluorescent protein under the control of the VEGFR2/KDR promoter. Angiogenesis inhibitors added to the injected cell suspension or to the fish water prevent tumor-induced neovascularization. The assay is rapid and inexpensive, representing a novel tool for investigating tumor angiogenesis and for antiangiogenic drug discovery. Also, gene inactivation by antisense morpholino oligonucleotides injection in zebrafish embryos may allow the identification of genes involved in tumor angiogenesis.

Descriptors: zebrafish embryo model, xenograft, angiogenesis, tumor, neovascularization, drug discovery .

Sapede, D. (2003). **Migrations cellulaires: etudier le poisson-zebre pour comprendre les metastases? [Zebrafish, a model for migration of cancer cells?]**. *Medecine Sciences* 19(8-9): 780-82. ISSN: 0767-0974.
Descriptors: zebrafish, cell movement, models, neoplasm metastasis, chemokines physiology.
Language of Text: French.

Scata, K.A. and W.S. El Deiry (2004). **Zebrafish: swimming towards a role for fanconi genes in DNA repair.** *Cancer Biology and Therapy* 3(6): 501-2. ISSN: 1538-4047.
Abstract: The zebrafish, *Danio rerio*, has become a favorite model organism for geneticists and developmental biologists. Recently cancer biologists have turned to this tiny fish to help them unravel the mysteries of conserved pathways such as the Fanconi Anemia (FA) pathway. Although a relatively rare disease, the genes involved in FA are part of a large network of DNA damage response/repair genes. Liu and colleagues have recapitulated some of the clinical manifestations of human FA by knocking down the zebrafish FANC-D2 gene thereby providing a new model for probing the underlying causes of these phenotypes.
Descriptors: zebrafish, cell cycle proteins metabolism, DNA repair and binding proteins metabolism, Fanconi anemia genetics, nuclear proteins metabolism, DNA damage, Fanconi anemia metabolism, Fanconi anemia pathology, Fanconi anemia complementation group proteins.

Stern, H.M. and L.I. Zon (2003). **Cancer genetics and drug discovery in the zebrafish.** *Nature Reviews. Cancer* 3(7): 533-9. ISSN: 1474-175X.
NAL Call Number: RC267
Abstract: Fish have a long history of use in cancer toxicology studies, because they develop neoplasms that are histologically similar to human cancers. Because of considerable progress in zebrafish genetics and genomics over the past few years, the zebrafish system has provided many useful tools for studying basic biological processes. These tools include forward genetic screens, transgenic models, specific gene disruptions and small-molecule screens. By combining carcinogenesis assays, genetic analyses and small-molecule screening techniques, the zebrafish is emerging as a powerful system for identifying novel cancer genes and for cancer drug discovery.
Descriptors: zebrafish, antineoplastic agents therapeutic use, neoplasms drug therapy, neoplasms genetics, drug evaluation methods.

Stoletov, K., V. Montel, R.D. Lester, S.L. Gonias, and R. Klemke (2007). **High-resolution imaging of the dynamic tumor cell vascular interface in transparent zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 104(44): 17406-11. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: Cell metastasis is a highly dynamic process that occurs in multiple steps. Understanding this process has been limited by the inability to visualize tumor cell behavior in real time by using animal models. Here, we employ translucent zebrafish and high-resolution confocal microscopy to study how human cancer cells invade in tissues, induce angiogenesis, and interact with newly formed vessels. We use this system to study how the human metastatic gene RhoC promotes the initial steps of metastasis. We find that RhoC expression induces a primitive amoeboid-like cell invasion characterized by the formation of dynamic membrane protrusions and blebs. Surprisingly, these structures penetrate the blood vessel wall exclusively at sites of vascular remodeling and not at regions of existing intact vessels. This process requires tumor cells to secrete VEGF, which induces vascular openings, which in turn, serve as portholes allowing access

of RhoC-expressing cells to the blood system. Our results support a model in which the early steps in intravasation and metastasis require two independent events: (i) dynamic regulation of the actin/myosin cytoskeleton within the tumor cell to form protrusive structures and (ii) vascular permeablization and vessel remodeling. The integration of zebrafish transgenic technology with human cancer biology may aid in the development of cancer models that target specific organs, tissues, or cell types within the tumors. Zebrafish could also provide a cost-effective means for the rapid development of therapeutic agents directed at blocking human cancer progression and tumor-induced angiogenesis.

Descriptors: zebrafish , three dimensional imaging methods, neoplasms blood supply, neoplasms pathology, metabolism, tumor cell line, cell movement, disease progression, neoplasm invasiveness, neoplasms metabolism, phenotype, vascular endothelialgrowth factor A metabolism, xenograft model antitumor assays, Rho GTP binding proteins metabolism.

Taylor, K.M., S. Hiscox, and R.I. Nicholson (2004). **Zinc transporter liv-1: a link between cellular development and cancer progression**. *Trends in Endocrinology and Metabolism* 15(10): 461-463. ISSN: 1043 2760.

NAL Call Number: RC648.A1

Abstract: Genes involved in normal developmental processes often have a role in tumour progression. Epithelial-mesenchymal transition (EMT) is a general phenomenon that is an essential event in embryonic development, tissue remodelling and wound repair. Genes that regulate EMT are attracting significant attention as mediators of tumour progression. Indeed, EMT is considered an important event during the evolution of malignant tumours and their progression to metastasis, with several reports of molecules involved in this process facilitating migration and invasion of epithelial tumour cells. Recent work linking the zinc transporter LIV-1 with the transcription factors STAT3 and Snail in zebrafish embryogenesis, throws new light on this transition and has important implications for the development of cancer. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, cell biology, development, reproductive system, tumor biology, cell development, embryogenesis, epithelial mesenchymal transition, tumor progression.

Topczewska, J.M., L.M. Postovit, N.V. Margaryan, A. Sam, A.R. Hess, W.W. Wheaton, B.J. Nickoloff, J. Topczewski, and M.J.C. Hendrix (2006). **Embryonic and tumorigenic pathways converge via nodal signaling: role in melanoma aggressiveness**. *Nature Medicine* 12(8): 925-932. ISSN: 1078 8956.

NAL Call Number: RB113

Abstract: Bidirectional cellular communication is integral to both cancer progression and embryological development. In addition, aggressive tumor cells are phenotypically plastic, sharing many properties with embryonic cells. Owing to the similarities between these two types of cells, the developing zebrafish can be used as a biosensor for tumor-derived signals. Using this system, we show that aggressive melanoma cells secrete Nodal (a potent embryonic morphogen) and consequently can induce ectopic formation of the embryonic axis. We further show that Nodal is present in human metastatic tumors, but not in normal skin, and thus may be involved in melanoma pathogenesis. Inhibition of Nodal signaling reduces melanoma cell invasiveness, colony formation and tumorigenicity. Nodal inhibition also promotes the reversion of melanoma cells toward a melanocytic phenotype. These data suggest that Nodal signaling has a key role in melanoma cell plasticity and tumorigenicity, thereby providing a previously unknown molecular target for regulating tumor progression. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, development, integumentary system, chemical coordination and homeostasis, tumor biology, melanoma, neoplastic skin disease, cell signaling, tumorigenic pathways.

VanRooijen, E., C. VanRooijen, E.E. Voest, R.H. Giles, and F. VanEeden (2006). **Proper vascular development and heart morphogenesis require tumor suppressor vhl in zebrafish development.** *Proceedings of the American Association for Cancer Research Annual Meeting* 47: 1202. ISSN: 0197 016X.

NAL Call Number: RC261.A4

Descriptors: zebrafish, blood and lymphatics, transport and circulation, development, cardiovascular system, transport and circulation, von Hippel Lindau disease, vascular disease, congenital disease, polycythemia, blood and lymphatic disease, in situ hybridization, histology and cytology techniques, genetic techniques, microarray gene expression analysis, transplantation experiment, heart morphogenesis.

Yee, N.S. and M. Pack (2005). **Zebrafish as a model for pancreatic cancer research.** *Methods in Molecular Medicine* 103: 273-98. ISSN: 1543-1894.

Abstract: Elucidation of basic mechanisms that regulate pancreatic organogenesis may help define molecular pathways involved in the development of exocrine pancreas cancer. The zebrafish has emerged as a powerful model for genetic dissection of the mechanisms underlying vertebrate organogenesis including formation of the pancreas. Unique properties of zebrafish enable genetic and embryological analyses not feasible using other vertebrate model organisms. The optical clarity of the zebrafish embryos allows visual detection of markers for pancreatic morphogenesis and cytodifferentiation by whole mount immunohistochemistry and RNA in situ hybridization. This feature, coupled with the accessibility of the externally fertilized zebrafish embryo and the small size and fecundity of adult zebrafish, facilitates large-scale forward genetic screens using chemical or insertional mutagenesis techniques. Furthermore, these properties allow high throughput studies that target functions of known genes via antisense or enforced expression studies. Together, such studies are predicted to identify novel genes, or known genes essential for pancreas development. Work in zebrafish is predicted to complement research performed using other vertebrate model organisms, and may help identify markers that define early stages of pancreatic tumorigenesis as well as potential targets for therapy.

Descriptors: zebrafish, pancreatic neoplasms pathology, animal husbandry methods, antibodies, neoplasm, disease models, genetic markers , immunohistochemistry methods, in situ hybridization methods, pancreatic neoplasms genetics, transcription.

Development

Abbate, F., G.P. Germana, F. De Carlos, G. Montalbano, R. Laura, M.B. Levanti, and A. Germana (2006). **The oral cavity of the adult zebrafish (*Danio rerio*).** *Anatomia Histologia Embryologia* 35(5): 299-304. ISSN: 0340 2096.
NAL Call Number: SF761.Z4
Abstract: The zebrafish is a common model for developmental studies including those regarding tooth, palate and tongue. Nevertheless, little information is available about the morphology of the oral cavity in this teleost, especially in adult animals. In this study we used light, scanning and transmission electron microscopy to describe in detail the morphology of the oral cavity of adult zebrafish. The oral cavity could be divided into three different zones: the outer containing the lips, the intermediate corresponding to the internal valves and the internal that corresponds to the tongue. In the upper and lower intermediate zones, there were semilunar shaped valves, more prominent in the upper part. The internal lower zones correspond to the palate and the tongue, which is an individualized structure filled with numerous transversal ridges. Both the intermediate and internal zones were covered by a stratified epithelium containing numerous mucous and rodlet cells. Present data provide the first description of the morphology and structure of the oral cavity in the adult zebrafish and might serve as a baseline for developmental studies of the oral cavity using this teleost as a model
Descriptors: zebrafish, *Danio rerio*, lips, mouth , oral cavity, morphology, salivary glands, buccal region, tongue.

Abe, G., H. Ide, and K. Tamura (2007). **Function of FGF signaling in the developmental process of the median fin fold in zebrafish.** *Developmental Biology* 304(1): 355-66. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Median fins, unpaired appendages in fish, are fundamental locomotory organs that are believed to have evolved before paired lateral appendages in vertebrates. However, the early process of median fin development remains largely unknown. We investigated the early development of the median fin fold, a rudiment of median fins, and report here the process in zebrafish embryos and the function of FGF signaling in the process. Using expressions of three genes, dlx5a, sp9 and fgf24, as markers of different phases of fold development, our findings suggest that the early process of median fin fold development can be divided into two steps, specification of the median fin fold territory and construction of the fold structure. Both loss-of-function and gain-of-function assays revealed that FGF signaling plays roles in each step, suggesting a common mechanism for the development of median appendages and paired lateral appendages.
Descriptors: zebrafish, structures embryology, median finfold development, Fgf metabolism, developmental gene expression regulation, morphogenesis physiology, signal transduction physiology, homeodomain proteins metabolism, in situ hybridization, microspheres, pyrroles, transcription factors metabolism, proteins metabolism.

Ablooglu, A.J., J. Kang, R.I. Handin, D. Traver, and S.J. Shattil (2007). **The zebrafish vitronectin receptor: characterization of integrin alphaV and beta3 expression patterns in early vertebrate development.** *Developmental Dynamics* 236(8): 2268-76. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, embryonic development, developmental gene expression regulation, integrin alphav genetics, integrin alphavbeta3 genetics , integrin beta3 genetics, molecular cloning, complementary DNA genetics, gene expression profiling, integrin alphaV analysis, integrin alphavbeta3 analysis, integrin beta3 analysis, tissue distribution, vertebrates.

Adolf, B., P. Chapouton, C.S. Lam, S. Topp, B. Tannhauser, U. Strahle, M. Gotz, and L. Bally Cuif (2006). **Conserved and acquired features of adult neurogenesis in the zebrafish telencephalon.** *Developmental Biology* 295(1): 278-93. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Our understanding of the cellular and molecular mechanisms underlying the adult neural stem cell state remains fragmentary. To provide new models on this issue, we searched for stem cells in the adult brain of the zebrafish. Using BrdU tracing and immunodetection of cell-type-specific markers, we demonstrate that the adult zebrafish telencephalon contains self-renewing progenitors, which show features of adult mammalian neural stem cells but distribute along the entire dorso-ventral extent of the telencephalic ventricular zone. These progenitors give rise to newborn neurons settling close to the ventricular zone within the telencephalon proper. They have no equivalent in mammals and therefore constitute a new model of adult telencephalic neural stem cells. In addition, progenitors from the ventral subpallium generate rapidly dividing progenitors and neuroblasts that reach the olfactory bulb (OB) via a rostral migratory stream and differentiate into GABAergic and TH-positive neurons. These ventral progenitors are comparable to the mammalian neural stem cells of the subependymal zone. Interestingly, dorsal and ventral progenitors in the adult telencephalon express a different combination of transcription factors than their embryonic counterparts. In the case of neurogenin1, this is due to the usage of different enhancer elements. Together, our results highlight the conserved and unique phylogenic and ontogenic features of adult neurogenesis in the zebrafish telencephalon and open the way to the identification of adult neural stem cell characters in cross-species comparative studies.
Descriptors: zebrafish, neurons cytology, telencephalon cytology, age factors, cell movement, cell proliferation, neurons physiology, olfactory bulb cytology, stem cells cytology, stem cells physiology, telencephalon metabolism, telencephalon physiology, tyrosine 3 monooxygenase metabolism, gamma aminobutyric acid metabolism.

Aerne, B. and D. Ishhorowicz (2004). **Receptor tyrosine phosphatase psi is required for delta/notch signalling and cyclic gene expression in the presomitic mesoderm**. *Development* 131(14): 3391-3399. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Segmentation in vertebrate embryos is controlled by a biochemical oscillator ('segmentation clock') intrinsic to the cells in the unsegmented presomitic mesoderm, and is manifested in cyclic transcription of genes involved in establishing somite polarity and boundaries. We show that the receptor protein tyrosine phosphatase psi (RPTPpsi) gene is essential for normal functioning of the somitogenesis clock in zebrafish. We show that reduction of RPTPpsi activity using morpholino, antisense oligonucleotides results in severe disruption of the segmental pattern of the embryo, and loss of cyclic gene expression in the presomitic mesoderm. Analysis of cyclic genes in RPTPpsi morphant embryos indicates an important requirement for RPTPpsi in the control of the somitogenesis clock upstream of or in parallel with Delta/Notch signalling. Impairing RPTPpsi activity also interferes with convergent extension during gastrulation. We discuss this dual requirement for RPTPpsi in terms of potential functions in Notch and Wnt signalling. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, enzymology, molecular genetics, molecular biophysics, convergent extension, segmentation control, somitogenesis clock, RPTPpsi activity.

Ahlgren, S. and T. Schwend (2006). **Zebrafish lacking a functional dispatched 1 display variable craniofacial anomalies in part due to defects in neural crest cell morphogenesis**. *Developmental Biology* 295(1): 332. ISSN: 0012 1606.
NAL Call Number: 442.8D49

Descriptors: zebrafish, molecular biophysics, development, skeletal system, holoprocencephaly, congenital disease, bone disease, etiology, morphogenesis, neural crest.

Ahmed, H., S.J. Du, R. Alvarez, and G.R. Vasta (2004). **Differential expression of the galectin repertoire from zebrafish (*Danio rerio*) during early embryogenesis: biochemical and molecular characterization**. *Glycobiology* 14(11): 1104. ISSN: 0959 6658.
NAL Call Number: QP552.G59G593
Descriptors: zebrafish embryo, development, molecular genetics, molecular biophysics.

Aizawa, H., M. Goto, T. Sato, and H. Okamoto (2007). **Temporally regulated asymmetric neurogenesis causes left-right difference in the zebrafish habenular structures.** *Developmental Cell* 12(1): 87-98. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: The habenular neurons on both sides of the zebrafish diencephalon show an asymmetric (laterotopic) axonal projection pattern into the interpeduncular nucleus. We previously revealed that the habenula could be subdivided into medial and lateral subnuclei, and a prominent left-right difference in the size ratio of these subnuclei accounts for the asymmetry in its neural connectivity. In the present study, birth date analysis showed that neural precursors for the lateral subnuclei were born at earlier stages than those for the medial subnuclei. More neurons for the early-born lateral subnuclei were generated on the left side, while more neurons for the late-born medial subnuclei were generated on the right side. Genetic hyperactivation and repression of Notch signaling revealed that differential timing determines both specificity and asymmetry in the neurogenesis of neural precursors for the habenular subnuclei.
Descriptors: zebrafish embryos, body patterning physiology, cell differentiation, habenula cytology, habenula embryology, neurons cytology, anatomy and histology, cytology, developmental gene expression regulation, habenula anatomy and histology, neurological models, nerve tissue nerve tissue proteins metabolism, messenger RNA genetics and metabolism, receptor protein tyrosine kinases genetics, receptor protein tyrosine kinases metabolism, Notch receptors metabolism, signal transduction, time factors and metabolism.
Notes: Comments: Comment In:Dev Cell. 2007 Jan;12(1):1-2.

Albert, S., F. Muller, N. Fischer, D. Biellmann, C. Neumann, P. Blader, and U. Strahle (2003). **Cyclops-independent floor plate differentiation in zebrafish embryos.** *Developmental Dynamics* 226(1): 59-66. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, transforming growth factor beta genetics and physiology, alleles, cell differentiation, developmental gene expression regulation, intracellular signaling peptides and proteins, video microscopy, mutation, neural crest cytology, rhombencephalon embryology, signal transduction, spinal cord embryology, time factors, transfection, transforming growth factor beta biosynthesis, transgenes, proteins.

Albertson, R.C., T.L. Payne Ferreira, J. Postlethwait, and P.C. Yelick (2005). **Zebrafish acvr2a and acvr2b exhibit distinct roles in craniofacial development.** *Developmental Dynamics* 233(4): 1405-18. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, Type I activin receptors physiology, facial bones embryology, skull embryology, embryology, Type I activin receptors genetics, apoptosis physiology, branchial region abnormalities, branchial region embryology, cartilage abnormalities, chromosome mapping, facial bones abnormalities, tooth abnormalities, genetics.

Albertson, R.C. and P.C. Yelick (2007). **Fgf8 haploinsufficiency results in distinct craniofacial defects in adult zebrafish.** *Developmental Biology* 306(2): 505-15. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Significant progress has been made toward understanding the role of fgf8 in directing early embryonic patterning of the pharyngeal skeleton. Considerably less is known about the role this growth factor plays in the coordinated development, growth, and remodeling of the craniofacial skeleton beyond embryonic stages. To better understand the contributions of fgf8 in the formation of adult craniofacial architecture, we analyzed the skeletal anatomy of adult ace(ti282a)/fgf8 heterozygous zebrafish. Our results revealed distinct skeletal defects including facial asymmetries, aberrant craniofacial geometry, irregular patterns of cranial suturing, and ectopic bone formation. These defects are similar in presentation to several human craniofacial disorders (e.g., craniosynostosis, hemifacial microsomia), and may be related to increased levels of bone metabolism observed in ace(ti282a)/fgf8 heterozygotes. Moreover, skeletal defects observed in ace(ti282a)/fgf8 heterozygotes are consistent with expression patterns of fgf8 in the mature craniofacial skeleton. These data reveal previously unrecognized roles for fgf8 during skeletogenesis, and provide a basis for future investigations into the mechanisms that regulate craniofacial development beyond the embryo.
Descriptors: zebrafish, Fgf physiology, gene expression regulation, body patterning, bone and bones embryology, craniofacial abnormalities genetics, molecular evolution, heterozygote, in situ hybridization, messenger RNA metabolism, signal transduction.

Albertson, R.C. and P.C. Yelick (2004). **Morphogenesis of the jaw: development beyond the embryo**. *The Zebrafish: Cellular and Developmental Biology,* 2 edition, Vol. 76, ELSEVIER ACADEMIC PRESS INC.: SAN DIEGO, CA 92101-4495 USA, p. 437-454. ISBN: 0125641710.
NAL Call Number: 442P92 v76
Descriptors: zebrafish, skeletal system, development, ingestion and assimilation, skeletal defect, bone disease, morphogenesis .

Albertson, R.C. and P.C. Yelick (2005). **Roles for fgf8 signaling in left-right patterning of the visceral organs and craniofacial skeleton**. *Developmental Biology* 283(2): 310-321. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Laterality is fundamental to the vertebrate body plan. Here, we investigate the roles of fgf8 signaling in LR patterning of the zebrafish embryo. We find that fgf8 is required for proper asymmetric development of the brain, heart and gut. When fgf8 is absent, nodal signaling is randomized in the lateral plate mesoderm, leading to aberrant LR orientation of the brain and visceral organs. We also show that fgf8 is necessary for proper symmetric development of the pharyngeal skeleton. Attenuated fgf8 signaling results in consistently biased LR asymmetric development of the pharyngeal arches and craniofacial skeleton. Approximately 1/3 of zebrafish ace/fgf8 mutants are missing Kupffer's vesicle (KV), a ciliated structure similar to Hensen's node. We correlate fgf8 deficient laterality defects in the brain and viscera with the absence of KV, supporting a role for KV in proper LR patterning of these structures. Strikingly, we also correlate asymmetric craniofacial development in ace/fgf8 mutants with the presence of KV, suggesting roles for KV in lateralization of the pharyngeal skeleton when fgf8 is absent. These data provide new insights into vertebrate laterality and offer the zebrafish ace/fgf8 mutant as a novel molecular tool to investigate tissue-specific molecular laterality mechanisms. (c) 2005 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, molecular genetics, skeletal system, Nodal signaling, asymmetric development.

Alexa, K.M., P. Dilorio, L. Etheridge, and C. Sagerstrom (2005). **Endodermal patterning in zebrafish: pancreas development.** *Developmental Biology* 283(2): 661. ISSN: 0012 1606.
NAL Call Number: 442.8 D49
Descriptors: zebrafish, pancreas development, biochemistry, phenotype, signaling pathway, endodermal patterning.

Allende, M.L., P. Hernandez, F. Olivari, A. Sarrazin, V. Nunez, P. Sandoval, and Gallardo (2007). **Development and regeneration in the zebrafish lateral line**. *Developmental Biology* 306(1): 316. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, developmental stages, freshwater fish, lateral line, *Danio rerio*.

Alsop, D., J. Matsumoto, S. Brown, and G. Van Der Kraak (2008). **Retinoid requirements in the reproduction of zebrafish**. *General and Comparative Endocrinology* 156(1): 51-62. ISSN: 0016-6480.
NAL Call Number: 444.8G28
Descriptors: zebrafish, retinoid, reproduction, zebrafish, RT-PCR, ovaries, testes, enzymes, retinoic acid (RA), raldh2, cyp26a, receptors, raraa, rarga, rxrba, rxrbb, rxrga, isoforms, tissues, diethylaminobenzaldehyde (DEAB), inhibitor, diet.

Alt, B., S. Reibe, N.M. Feitosa, O.A. Elsalini, T. Wendl, and K.B. Rohr (2006). **Analysis of origin and growth of the thyroid gland in zebrafish.** *Developmental Dynamics* 235(7): 1872-83. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, thyroid gland growth and development, growth and development, calcitonin metabolism, cell differentiation, cell lineage physiology, endoderm cytology, endoderm metabolism, larva growth and development, larva metabolism, morphogenesis, thyroid gland embryology, thyroid gland metabolism, proteins biosynthesis.

Alt, B., O.A. Elsalini, P. Schrumpf, N. Haufs, N.D. Lawson, G.C. Schwabe, S. Mundlos, A. Gruters, H. Krude, and K.B. Rohr (2006). **Arteries define the position of the thyroid gland during its developmental relocalisation**. *Development* 133(19): 3797-3804. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: During vertebrate development, the thyroid gland undergoes a unique relocalisation from its site of induction to a distant species-specific position in the cervical mesenchyme. We have analysed thyroid morphogenesis in wild-type and mutant zebrafish and mice, and find that localisation of growing thyroid tissue along the anteroposterior axis in zebrafish is linked to the development of the ventral aorta. In grafting experiments, ectopic vascular cells influence the localisation of thyroid tissue cell non-autonomously, showing that vessels provide guidance cues in zebrafish thyroid morphogenesis. In mouse thyroid development, the midline primordium bifurcates and two lobes relocalise cranially along the bilateral pair of carotid arteries. In hedgehog-deficient mice, thyroid tissue always develops along the ectopically and asymmetrically positioned carotid arteries, suggesting that, in mice (as in zebrafish), co-developing major arteries de. ne the position of the thyroid. The similarity between zebrafish and mouse mutant phenotypes further indicates that thyroid relocalisation involves two morphogenetic phases, and that variation in the second phase accounts for species-specific. c differences in thyroid morphology. Moreover, the involvement of vessels in thyroid relocalisation sheds new light on the interpretation of congenital thyroid defects in humans. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, cardiovascular system, development, endocrine system, developmental relocalization, *Danio rerio*.

Alvarez, Y., M.T. Alonso, V. Vendrell, L.C. Zelarayan, P. Chamero, T. Theil, M.R. Boesl, S. Kato , M. Maconochie, D. Riethmacher, and T. Schimmang (2003). **Requirements for fgf3 and fgf10 during inner ear formation.** *Development* 130(25): 6329-6338. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Descriptors: zebrafish, development, molecular genetics, sense organs, embryogenesis, organogenesis .

Amack, J.D., X. Wang, and H.J. Yost (2007). **Two T-box genes play independent and cooperative roles to regulate morphogenesis of ciliated Kupffer's vesicle in zebrafish.** *Developmental Biology* 310(2): 196-210. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The brain, heart and gastro-intestinal tract develop distinct left-right (LR) asymmetries. Asymmetric cilia-dependent fluid flow in the embryonic node in mouse, Kupffer's vesicle in zebrafish, notochordal plate in rabbit and gastrocoel roof plate in frog appears to be a conserved mechanism that directs LR asymmetric gene expression and establishes the orientation of organ asymmetry. However, the cellular processes and genetic pathways that control the formation of these essential ciliated structures are unknown. In zebrafish, migratory dorsal forerunner cells (DFCs) give rise to Kupffer's vesicle (KV), a ciliated epithelial sheet that forms a lumen and generates fluid flow. Using the epithelial marker atypical Protein Kinase C (aPKC) and other markers to analyze DFCs and KV cells, we describe a multi-step process by which DFCs form a functional KV. Using mutants and morpholinos, we show that two T-box transcription factors-No tail (Ntl)/Brachyury and Tbx16/Spadetail-cooperatively regulate an early step of DFC mesenchyme to epithelial transition (MET) and KV cell specification. Subsequently, each transcription factor independently controls a distinct step in KV formation: Tbx16 regulates apical clustering of KV cells and Ntl is necessary for KV lumen formation. By targeting morpholinos to DFCs, we show that these cell autonomous functions in KV morphogenesis are necessary for LR patterning throughout the embryo.
Descriptors: zebrafish embryos, mesoderm cytology, T-box domain, body patterning physiology, cell differentiation physiology, cilia physiology, physiology, epithelium physiology, mesoderm physiology, protein kinase C metabolism, T-box domain proteins genetics.

Amali, A.A., C.J. Lin, Y.H. Chen, W.L. Wang, H.Y. Gong, C.Y. Lee, Y.L. Ko, J.K. Lu, G.M. Her, T.T. Chen, and J.L. Wu (2004). **Up-regulation of muscle-specific transcription factors during embryonic somitogenesis of zebrafish (*Danio rerio*) by knock-down of myostatin-1.** *Developmental Dynamics* 229(4): 847-56. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, skeletal muscle embryology, myogenic regulatory factors biosynthesis, somites metabolism, transforming growth factor beta physiology, metabolism, gene expression regulation, gene silencing, skeletal muscle metabolism, Myod protein genetics, myogenic regulatory factors genetics, myogenin genetics, messenger RNA analysis, somites cytology, transforming growth factor beta genetics, up regulation.

Amoyel, M., Y.C. Cheng, Y.J. Jiang, and D.G. Wilkinson (2005). **Wnt1 regulates neurogenesis and mediates lateral inhibition of boundary cell specification in the zebrafish hindbrain.** *Development* 132(4): 775-85. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The formation of localised signalling centres is essential for patterning of a number of tissues during development. Previous work has revealed that a distinct population of bound-

ary cells forms at the interface of segments in the vertebrate hindbrain, but the role of these cells is not known. We have investigated the function of the Wnt1 signalling molecule that is expressed by boundary and roof plate cells in the zebrafish hindbrain. Knockdown of wnt1 or of tcf3b, a mediator of Wnt signalling, leads to ectopic expression of boundary cell markers, rfng and foxb1.2, in non-boundary regions of the hindbrain. Ectopic boundary marker expression also occurs following knockdown of rfng, a modulator of Notch signalling required for wnt1 expression at hindbrain boundaries. We show that the boundary and roof plate expression of wnt1 each contribute to upregulation of proneural and delta gene expression and neurogenesis in non-boundary regions, which in turn blocks ectopic boundary marker expression. Boundary cells therefore play a key role in the regulation of cell differentiation in the zebrafish hindbrain. The network of genes underlying the regulation of neurogenesis and lateral inhibition of boundary cell formation by Wnt1 has a striking similarity to mechanisms at the dorsoventral boundary in the *Drosophila* wing imaginal disc.
Descriptors: zebrafish embryo hindbrain, cell differentiation physiology, developmental gene expression regulation physiology, intercellular signaling peptides and proteins metabolism, rhombencephalon embryology, developmental gene expression regulation genetics, in situ hybridization, intercellular signaling peptides and membrane proteins metabolism, morphogenesis physiology, Notch receptors, rhombencephalon metabolism, Wnt proteins, Wnt1 protein.

Amsterdam, A. (2003). **Insertional mutagenesis in zebrafish**. *Developmental Dynamics* 228(3): 523-534. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Insertional mutagenesis is a method for identifying genes essential for a given biological process by using the integration of DNA as the mutagen, thereby facilitating the cloning of the mutated gene. The use of retrovirus-mediated insertional mutagenesis in zebrafish has led to the mutation and rapid identification of hundreds of genes required for embryonic development and cell viability and growth, revealing the diversity of gene products required for the development of this vertebrate. Here, I will review the methodology of this approach and the results to date, as well as other potential ways to use insertional mutagenesis for genetic screens. Copyright © 2008 CABI
Descriptors: zebrafish, DNA, genes, genetic analysis, insertional mutagenesis, mutagens, mutations, transposable elements, *Danio rerio*.

Amsterdam, A. and T.S. Becker (2005). **Transgenes as screening tools to probe and manipulate the zebrafish genome**. *Developmental Dynamics* 234(2): 255-268. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: The zebrafish, originally an object of study as an inexpensive and prolific vertebrate embryological model with a plethora of genetic tricks, has over the past decade moved to large-scale chemical mutagenesis and recently came of age as a high throughput transgenic model with a sequenced genome nearing completion. Insertional mutagenesis, gene trapping and enhancer detection are all contributing to the increasing speed with which research in this biomedical model is progressing. We review here some of the recent developments in the emerging field of zebrafish developmental genomics and transgenesis. Copyright © 2008 CABI
Descriptors: zebrafish, genetic models, genome analysis, genomes, mutagenesis, *Danio rerio*.

Ando, H., M. Kobayashi, T. Tsubokawa, K. Uyemura, T. Furuta, and H. Okamoto (2005). **Lhx2 mediates the activity of Six3 in zebrafish forebrain growth.** *Developmental Biology* 287(2): 456-68 . ISSN: 0012 1606.
NAL Call Number: 442.8D49

Abstract: The telencephalon shows the greatest degree of size variation in the vertebrate brain. Understanding the genetic cascade that regulates telencephalon growth is crucial to our understanding of how evolution of the normal human brain has supported such a variation in size. Here, we present a simple and quick approach to analyze this cascade that combines caged-mRNA technology and the use of antisense morpholino oligonucleotides in zebrafish embryos. Lhx2, a LIM-homeodomain protein, and Six3s (Six3b and Six3a), another homeodomain proteins, show very similar expression patterns early in forebrain development, and these are known to be involved in the growth of this part of the brain. The telencephalon of six3b and six3a double morphant (six3 morphant) embryos is markedly reduced in size due to impaired cellular proliferation. Head-specific overexpression of Lhx2 by photoactivation of a caged-lhx2 mRNA completely rescued this size reduction, whereas similar head-specific activation of Six3b could not rescue the knockdown effect of lhx2. In the forebrain of medaka embryos, Six3 facilitates cellular proliferation by sequestration of Geminin from Cdt1, a key component in the assembly of the prereplication complex. Our results suggest that Lhx2 may mediate an alternative or parallel pathway for control of cellular proliferation in the developing forebrain via Six3.
Descriptors: zebrafish embryo forebrain, eye proteins metabolism, homeodomain proteins metabolism, nerve tissue proteins metabolism, prosencephalon physiology, cell proliferation, prosencephalon embryology, signal transduction, telencephalon embryology, telencephalon physiology.

Anzenberger, U., N. Bit Avragim, S. Rohr, F. Rudolph, B. Dehmel, T.E. Willnow, and S. Abdelilah Seyfried (2006). **Elucidation of megalin/LRP2-dependent endocytic transport processes in the larval zebrafish pronephros.** *Journal of Cell Science* 119(Pt 10): 2127-37. ISSN: 0021-9533.
NAL Call Number: QH301.J6
Abstract: Megalin/LRP2 is an endocytic receptor in the proximal tubules of the mammalian kidney that plays a central role in the clearance of metabolites from the glomerular filtrate. To establish a genetic model system for elucidation of molecular components of this retrieval pathway, we characterized orthologous transport processes in the zebrafish. We show that expression of megalin/LRP2 and its co-receptor cubilin is conserved in the larval zebrafish pronephros and demarcates a segment of the pronephric duct that is active in clearance of tracer from the ultrafiltrate. Knock-down of megalin/LRP2 causes lack of Rab4-positive endosomes in the proximal pronephric duct epithelium and abrogates apical endocytosis. Similarly, knock-down of the megalin/LRP2 adaptor Disabled 2 also blocks renal clearance processes. These results demonstrate the conservation of the megalin/LRP2 retrieval pathway between the larval zebrafish pronephros and the mammalian kidney and set the stage for dissection of the renal endocytic machinery in a simple model organism. Using this model system, we provide first genetic evidence that renal tubular endocytosis and formation of endosomes is a ligand-induced process that crucially depends on megalin/LRP2 activity.
Descriptors: kidney embryology, kidney metabolism, ldl receptor related protein 2 metabolism, adaptor proteins, vesicular transport metabolism, amino acid sequence, genetically modified, endocytosis, kidney blood supply, larva, molecular sequence data.

Appel, B., P. Marasco, L.E. McClung, and A.J. Latimer (2003). **Lunatic fringe regulates Delta-Notch induction of hypochord in zebrafish.** *Developmental Dynamics* 228(2): 281-6. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: developmental gene expression regulation drug effects, glycosyltransferases metabolism, membrane proteins metabolism, notochord physiology, genetics, body patterning, molecular

cloning, gastrula, glycosyltransferases genetics, in situ hybridization, ligands , membrane antisense oligonucleotides pharmacology, Notch receptors, signal transduction.

Arduini, B.L. and P.D. Henion (2004). **Melanophore sublineage-specific requirement for zebrafish touchtone during neural crest development.** *Mechanisms of Development* 121(11): 1353-64. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: The specification, differentiation and maintenance of diverse cell types are of central importance to the development of multicellular organisms. The neural crest of vertebrate animals gives rise to many derivatives, including pigment cells, peripheral neurons, glia and elements of the craniofacial skeleton. The development of neural crest-derived pigment cells has been studied extensively to elucidate mechanisms involved in cell fate specification, differentiation, migration and survival. This analysis has been advanced considerably by the availability of large numbers of mouse and, more recently, zebrafish mutants with defects in pigment cell development. We have identified the zebrafish mutant touchtone (tct), which is characterized by the selective absence of most neural crest-derived melanophores. We find that although wild-type numbers of melanophore precursors are generated in the first day of development and migrate normally in tct mutants, most differentiated melanophores subsequently fail to appear. We demonstrate that the failure in melanophore differentiation in tct mutant embryos is due at least in part to the death of melanoblasts and that tct function is required cell autonomously by melanoblasts. The tct locus is located on chromosome 18 in a genomic region apparently devoid of genes known to be involved in melanophore development. Thus, zebrafish tct may represent a novel as well as selective regulator of melanoblast development within the neural crest lineage. Further, our results suggest that, like other neural crest-derived sublineages, melanogenic precursors constitute a heterogeneous population with respect to genetic requirements for development.
Descriptors: zebrafish, melanophores cytology, neural crest growth and development, and, alleles, apoptosis, caspase 3, caspases analysis , cell differentiation genetics, cell differentiation physiology, cell lineage genetics, cell lineage physiology, chromosome mapping, embryo, cytology, physiology, melanophores chemistry, melanophores physiology, mutation genetics, genetics.

Argenton, F., S. Giudici, G. Deflorian, S. Cimbro, F. Cotelli, and M. Beltrame (2004). **Ectopic expression and knockdown of a zebrafish sox21 reveal its role as a transcriptional repressor in early development.** *Mechanisms of Development* 121(2): 131-42. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: Sox proteins are DNA-binding proteins belonging to the HMG box superfamily and they play key roles in animal embryonic development. Zebrafish Sox21a is part of group B Sox proteins and its chicken and mouse orthologs have been described as transcriptional repressor and activator, respectively, in two different target gene contexts. Zebrafish sox21a is present as a maternal transcript in the oocyte and is mainly expressed at the developing midbrain-hindbrain boundary from the onset of neurulation. In order to understand its role in vivo, we ectopically expressed sox21a by microinjection. Ectopic expression of full length sox21a leads to dorsalization of the embryos. A subset of the dorsalized embryos shows a partial axis splitting, and hence an ectopic neural tube, as an additional phenotype. At gastrulation, injected embryos show expansion of the expression domains of organizer-specific genes, such as chordin and goosecoid. Molecular markers used in somitogenesis highlight that sox21a-injected embryos have shortened AP axis, undulating axial structures, enlarged or even radialized paraxial territory. The developmental abnormalities caused by ectopic expression of sox21a are suggestive of defects in convergence-extension morphogenetic movements. Antisense morpholino oligonucleotides, designed to functionally knockdown sox21a, cause ventralization of the embryos. Moreover, gain-

of-function experiments with chimeric constructs, where Sox21a DNA-binding domain is fused to a transcriptional activator (VP16) or repressor (EnR) domain, suggests that zebrafish Sox21a acts as a repressor in dorso-ventral patterning.
Descriptors: zebrafish, DNA binding proteins genetics, high mobility group embryology, genetics, genetically modified, base sequence, body patterning genetics, body patterning physiology, DNA binding, gastrula, gene expression, gene targeting, high mobility group, oligodeoxyribonucleotides, antisense genetics, oligodeoxyribonucleotides, antisense pharmacology, repressor repressor.

Avaron, F., L. Hoffman, D. Guay, and M.A. Akimenko (2006). **Characterization of two new zebrafish members of the Hedgehog family: atypical expression of a zebrafish Indian Hedgehog gene in skeletal elements of both endochondral and dermal origins.** *Developmental Dynamics* 235(2): 478-89. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, developmental gene expression regulation genetics, musculoskeletal system embryology, musculoskeletal system metabolism, trans activators classification, trans activators metabolism, metabolism, amino acid sequence, collagen type X metabolism, Hedgehog proteins, larva genetics, larva metabolism, molecular sequence data, musculoskeletal system chemistry, phylogeny, sequence alignment, trans activators chemistry, trans activators genetics, classification and genetics.

Avaron, F., L. Hoffman, and M.A. Akimenko (2004). **Characterization of two new Hedgehog genes in zebrafish.** *Developmental Biology* 271(2): 608. ISSN: 0012 1606.
NAL Call Number: 442.8 D49
Descriptors: zebrafish, development, molecular genetics, biochemistry, molecular biophysics, Hedgehog.

Azodi, E. and Q. Liu (2004). **Analysis of N-cadherin function in developing zebrafish (*Danio rerio*) retinal ganglion cells**. *Ohio Journal of Science* 104(1): A13. ISSN: 0030-0950.
Descriptors: zebrafish, adhesion, antibodies, developmental stages, embryonic development, freshwater fish, ganglia, PCR, retinas, *Danio rerio*.

Babb Clendenon, S., Y.C. Shen, Q. Liu, K.E. Turner, M.S. Mills, G.W. Cook, C.A. Miller, V.H.2. Gattone, K.F. Barald, and J.A. Marrs (2006). **Cadherin-2 participates in the morphogenesis of the zebrafish inner ear.** *Journal of Cell Science* 119(Pt 24): 5169-77. ISSN: 0021 9533.
NAL Call Number: QH301.J6
Abstract: Molecular mechanisms that control inner ear morphogenesis from the placode to the three-dimensional functional organ are not well understood. We hypothesize that cell-cell adhesion, mediated by cadherin molecules, contributes significantly to various stages of inner ear formation. Cadherin-2 (Cdh2) function during otic vesicle morphogenesis was investigated by examining morpholino antisense oligonucleotide knockdown and glass onion (glo) (Cdh2 mutant) zebrafish embryos. Placode formation, vesicle cavitation and specification occurred normally, but morphogenesis of the otic vesicle was affected by Cdh2 deficiency: semicircular canals were reduced or absent. Phalloidin staining of the hair cell stereocillia demonstrated that cadherin-2 (cdh2) loss-of-function did not affect hair cell number, but acetylated tubulin labeling showed that hair cell kinocilia were shorter and irregularly shaped. Statoacoustic ganglion size was significantly reduced, which suggested that neuron differentiation or maturation was affected. Furthermore, cdh2 loss-of-function did not cause a general developmental delay, since differentiation of other tissues, including eye, proceeded normally. These findings demonstrate that Cdh2

selectively affects epithelial morphogenetic cell movements, particularly semicircular canal formation, during normal ear mophogenesis.

Descriptors: zebrafish embryo, cadherins physiology, inner ear metabolism, morphogenesis physiology, cadherins genetics, cadherins metabolism, cell adhesion genetics, cell adhesion physiology, inner ear cytology, inner ear growth and development, developmental gene expression regulation, hair cells cytology, hair cells metabolism, hair cells ultrastructure, in situ hybridization, electron transmission microscopy, morphogenesis genetics.

Babb, S.G., S.M. Kotradi, B. Shah, C. Chiappini Williamson, L.N. Bell, G. Schmeiser, E. Chen, Q. Liu, and J.A. Marrs (2005). **Zebrafish R-cadherin (Cdh4) controls visual system development and differentiation.** *Developmental Dynamics* 233(3): 930-45. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: In zebrafish, R-cadherin (cadherin-4 or Cdh4) is expressed in the retina and in retino-recipient brain regions, suggesting that Cdh4 functions during visual system development. Cdh4 function was examined during retinogenesis and retinal axon outgrowth using antisense morpholino oligonucleotides and mutant Cdh4 construct expression. In knockdowns, Cdh4 was reduced or absent, eyes were small, and retinae lacked discrete laminae. Increased cell death produced the small eye phenotype. Zn5-, Pax6-, and zpr-1-positive cells were reduced or absent in knockdown retinas but, when present, were in the correct laminae. Cdh4 knockdowns had sparse or absent retinal ganglion cell axons. When present, axons projected contralaterally but lacked fine branching and failed to reach the tectum or arborize the entire tectum. Mutant Cdh4 construct expression during retinal ganglion cell differentiation reduced or ablated neurite formation. Cdh4 is necessary for neural retina survival and differentiation, and required for normal retinotectal projection formation and tectal arborization.

Descriptors: zebrafish embryo, cadherins metabolism, cell differentiation, retina cytology, retina embryology, vision physiology, genetically modified fish, apoptosis, cadherins genetics, cytology, metabolism, developmental gene expression regulation, mutation genetics, neurons cytology, neurons metabolism, phenotype, retina metabolism.

Babb, S.G. and J.A. Marrs (2004). **E-cadherin regulates cell movements and tissue formation in early zebrafish embryos.** *Developmental Dynamics* 230(2): 263-77. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryos, cadherins metabolism, cell movement, metabolism, cadherins genetics, cell division, cell survival, gastrula cytology, gastrula metabolism, developmental gene expression regulation, antisense oligoribonucleotides genetics, antisense oligoribonucleotides metabolism, RNA genetics and messenger metabolism, somites cytology, somites metabolism.

Bae, Y.K., T. Shimizu, and M. Hibi (2005). **Patterning of proneuronal and inter-proneuronal domains by hairy- and enhancer of split-related genes in zebrafish neuroectoderm.** *Development* 132(6): 1375-85. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: In teleosts and amphibians, the proneuronal domains, which give rise to primary-motor, primary-inter and Rohon-Beard (RB) neurons, are established at the beginning of neurogenesis as three longitudinal stripes along the anteroposterior axis in the dorsal ectoderm. The proneuronal domains are prefigured by the expression of basic helix-loop-helix (BHLH) proneural genes, and separated by domains (inter-proneuronal domains) that do not express the proneural genes. Little is known about how the formation of these domains is spatially regulated. We have found that the zebrafish hairy- and enhancer of split-related (Her) genes her3 and her9 are expressed in the inter-proneuronal domains, and are required for their formation. her3 and

her9 expression was not regulated by Notch signaling, but rather controlled by positional cues, in which Bmp signaling is involved. Inhibition of Her3 or Her9 by antisense morpholino oligonucleotides led to ectopic expression of the proneural genes in part of the inter-proneuronal domains. Combined inhibition of Her3 and Her9 induced ubiquitous expression of proneural and neuronal genes in the neural plate, and abolished the formation of the inter-proneuronal domains. Furthermore, inhibition of Her3/Her9 and Notch signaling led to ubiquitous and homogeneous expression of proneural and neuronal genes in the neural plate, revealing that Her3/Her9 and Notch signaling have distinct roles in neurogenesis. These data indicate that her3 and her9 function as prepattern genes that link the positional dorsoventral polarity information in the posterior neuroectoderm to the spatial regulation of neurogenesis.
Descriptors: zebrafish, ectoderm metabolism, nervous system metabolism, repressor proteins metabolism , and proteins metabolism, basic helix loop helix transcription factors, body patterning physiology, bone morphogenetic proteins metabolism, membrane proteins metabolism, neurons metabolism, Notch receptors, repressor proteins genetics.

Bae, Y.K., T. Shimizu, T. Yabe, C.H. Kim, T. Hirata , H. Nojima, O. Muraoka, T. Hirano, and M. Hibi (2003). **A homeobox gene, pnx, is involved in the formation of posterior neurons in zebrafish.** *Development* 130(9): 1853-65. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: A homeobox gene, pnx, is expressed in prospective posterior neurogenic regions and later in primary neurons. pnx expression was regulated by a signal from the non-axial mesendoderm and by Notch signaling. Pnx contains an Eh1 repressor domain, which interacted with Groucho and acted as a transcriptional repressor. Misexpression of pnx increased neural precursor cells and postmitotic neurons, which express neurogenin1 and elavl3/HuC, respectively. Expression of an antimorphic Pnx (VP16Pnx) or inhibition of Pnx by antisense morpholino oligonucleotide led to the reduction in the number of a subset of primary neurons. Misexpression of pnx promoted neurogenesis independent of Notch signaling. Epistatic analyses showed that Pnx also functions downstream of the Notch signal. These data indicate that pnx is a novel repressor-type homeobox gene that regulates posterior neurogenesis.
Descriptors: zebrafish embryos, homeobox genes, homeodomain nervous system embryology, nuclear proteins, transcription factors, amino acid sequence, developmental gene expression regulation physiology, homeodomain proteins metabolism, membrane proteins metabolism, molecular sequence data, nerve tissue phylogeny, Notch receptors, signal transduction physiology, genetic transcription physiology.

Bagatto, B., J. Francl, B. Liu, and Q. Liu (2006). **Cadherin2 (N-cadherin) plays an essential role in zebrafish cardiovascular development.** *BioMed Central Developmental Biology* 6:(1) ISSN: 1471 213X.
NAL Call Number: QH491.B63
Abstract: BACKGROUND: Cadherins are cell surface adhesion molecules that play important roles in development of vertebrate tissues and organs. We studied cadherin2 expression in developing zebrafish heart using in situ hybridization and immunocytochemical methods, and we found that cadherin2 was strongly expressed by the myocardium of the embryonic zebrafish. To gain insight into cadherin2 role in the formation and function of the heart, we analyzed cardiac differentiation and performance in a cadherin2 mutant, glass onion (glo). RESULTS: We found that the cadherin2 mutant had enlarged pericardial cavity, disorganized atrium and ventricle, and reduced expression of a ventricular specific marker vmhc. Individual myocardiocytes in the glo mutant embryos became round shaped and loosely aggregated. In vivo measurements of cardiac performance revealed that the mutant heart had significantly reduced heart rate, stroke volume

and cardiac output compared to control embryos. Formation of the embryonic vascular system in the glo mutants was also affected. CONCLUSION: Our results suggest that cadherin2 plays an essential role in zebrafish cardiovascular development. Although the exact mechanisms remain unknown as to the formation of the enlarged pericardium and reduced peripheral blood flow, it is clear that myocardiocyte differentiation and physiological cardiovascular performance is impaired when cadherin2 function is disrupted.
Descriptors: zebrafish embryos, cadherins physiology, cardiovascular system growth and development, cadherins genetics, developmental gene expression regulation, heart growth and development, mutation, myocardium metabolism.
Notes: Artcle Number 23.

Bakkers, J., C. Kramer, J. Pothof, N.E. Quaedvlieg, H.P. Spaink, and M. Hammerschmidt (2004). **Has2 is required upstream of Rac1 to govern dorsal migration of lateral cells during zebrafish gastrulation.** *Development* 131(3): 525-37. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The large extracellular polysaccharide Hyaluronan (HA) and its synthesizing enzymes (Has) have been implicated in regulating the migratory potential of metastatic cancer cells. Here, we analyze the roles of zebrafish Has2 in normal development. Antisense morpholino oligonucleotide (MO)-mediated knockdown of zebrafish Has2 leads to the loss of HA, and severe migratory defects during gastrulation, somite morphogenesis and primordial germ cell migration. During gastrulation, ventrolateral cells of has2 morphant embryos fail to develop lamellipodia and to migrate dorsally, resulting in a blockage of dorsal convergence, whereas extension of the dorsal axis is normal. The effect is cell autonomous, suggesting that HA acts as an autocrine signal to stimulate the migration of HA-generating cells. Upon ectopic expression in axial cells, has2 causes the formation of supernumerary lamellipodia and a blockage of axis extension. Epistasis analyses with constitutively active and dominant-negative versions of the small GTPase Rac1 suggest that HA acts by Rac1 activation, rather than as an essential structural component of the extracellular matrix. Together, our data provide evidence that convergence and extension are separate morphogenetic movements of gastrulation. In addition, they suggest that the same HA pathways are active to auto-stimulate cell migration during tumor invasion and vertebrate embryogenesis.
Descriptors: zebrafish, gastrula metabolism, glucuronosyltransferase metabolism, glycosyltransferases, membrane proteins, transferases, *Xenopus* proteins, proteins, Rac1 GTP binding protein metabolism, genetics, physiology, cell movement physiology, glucuronosyltransferase genetics, phylogeny, Rac1 GTP binding protein genetics.

Bardet, P.L., M. Schubert, B. Horard, L.Z. Holland, V. Laudet, N.D. Holland, and J.M. Vanacker (2005). **Expression of estrogen-receptor related receptors in amphioxus and zebrafish: implications for the evolution of posterior brain segmentation at the invertebrate-to-vertebrate transition.** *Evolution and Development* 7(3): 223-33. ISSN: 1520 541X.
NAL Call Number: QH359
Abstract: Summary The evolutionary origin of vertebrate hindbrain segmentation is unclear since the amphioxus, the closest living invertebrate relative to the vertebrates, possesses a hindbrain homolog that displays no gross morphological segmentation. Three of the estrogen-receptor related (ERR) receptors are segmentally expressed in the zebrafish hindbrain, suggesting that their common ancestor was expressed in a similar, reiterated manner. We have also cloned and determined the developmental expression of the single homolog of the vertebrate ERR genes in the amphioxus (AmphiERR). This gene is also expressed in a segmented manner in a region considered homologous to the vertebrate hindbrain. In contrast to the expression of amphioxus islet (a LIM-homeobox gene that also labels motoneurons), AmphiERR expression persists longer in the

hindbrain homolog and does not later extend to additional posterior cells. In addition, Amphi-ERR and one of its vertebrate homologs (ERRalpha) are expressed in the developing somitic musculature of amphioxus and zebrafish, respectively. Altogether, our results are consistent with fine structural evidence suggesting that the amphioxus hindbrain is segmented, and indicate that chordate ERR gene expression is a marker for both hindbrain and muscle segmentation. Furthermore, our data support an evolution model of chordate brain segmentation: originally, the program for anterior segmentation in the protochordate ancestors of the vertebrates resided in the developing axial mesoderm which imposed reiterated patterning on the adjacent neural tube; during early vertebrate evolution, this segmentation program was transferred to and controlled by the neural tube.

Descriptors: zebrafish embryos, chordata, nonvertebrate genetics, evolution, developmental gene expression regulation, estrogen receptorschemistry, estrogen receptors genetics, rhombencephalon embryology, amino acid sequence, chordata, nonvertebrate embryology, molecular sequence data, phylogeny, cytoplasmic and nuclear chemistry and genetics, rhombencephalon metabolism, sequence alignment, proteins chemistry.

Barembaum, M. and M. Bronner-Fraser (2005). **Early steps in neural crest specification**. *Seminars in Cell & Developmental Biology* 16(6): 642-646. ISSN: 1084-9521.

NAL Call Number: QH573

Descriptors: zebrafish, toads, chickens, mice, frogs, mollusks, aquatic organisms, bone morphogenetic proteins, ectoderm, fibroblast growth factor, freshwater fish, gene families, molecular modelling, neural crest and plate development, neurophysiology, reviews, signal transduction, specificity, transcription, transcription factors , Wnt protein, c-Myc protein, anura, *Danio rerio*, mollusca, foxD3 protein, snail protein, SoxE protein .

Barrallo Gimeno, A., J. Holzschuh, W. Driever, and E.W. Knapik (2004). **Neural crest survival and differentiation in zebrafish depends on mont blanc/tfap2a gene function.** *Development* 131(7): 1463-77. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Neural crest progenitor cells are the main contributors to craniofacial cartilage and connective tissue of the vertebrate head. These progenitor cells also give rise to the pigment, neuronal and glial cell lineages. To study the molecular basis of neural crest differentiation, we have cloned the gene disrupted in the mont blanc (mob(m610)) mutation, which affects all neural crest derivatives. Using a positional candidate cloning approach we identified an A to G transition within the 3' splice site of the sixth intron of the tfap2a gene that abolishes the last exon encoding the crucial protein dimerization and DNA-binding domains. Neural crest induction and specification are not hindered in mob(m610) mutant embryos, as revealed by normal expression of early neural crest specific genes such as snail2, foxd3 and sox10. In addition, the initial stages of cranial neural crest migration appear undisturbed, while at a later phase the craniofacial primordia in pharyngeal arches two to seven fail to express their typical set of genes (sox9a, wnt5a, dlx2, hoxa2/b2). In mob(m610) mutant embryos, the cell number of neuronal and glial derivatives of neural crest is greatly reduced, suggesting that tfap2a is required for their normal development. By tracing the fate of neural crest progenitors in live mont blanc (mob(m610)) embryos, we found that at 24 hpf neural crest cells migrate normally in the first pharyngeal arch while the preotic and postotic neural crest cells begin migration but fail to descend to the pharyngeal region of the head. TUNEL assay and Acridine Orange staining revealed that in the absence of tfap2a a subset of neural crest cells are unable to undergo terminal differentiation and die by apoptosis. Furthermore, surviving neural crest cells in tfap2a/mob(m610) mutant embryos proliferate normally and later differentiate to individual derivatives. Our results indicate that tfap2a is essential

to turn on the normal developmental program in arches 2-7 and in trunk neural crest. Thus, tfap2a does not appear to be involved in early specification and cell proliferation of neural crest, but it is a key regulator of an early differentiation phase and is required for cell survival in neural crest derived cell lineages.

Descriptors: zebrafish embryos, cell differentiation physiology, cell survival physiology, DNA binding proteins genetics, proteins metabolism, morphogenesis physiology, neural crest physiology, transcription factors metabolism, genetics, amino acid sequence, apoptosis physiology, body patterning, brain cytology, brain embryology, brain metabolism, branchial region cytology, branchial region embryology, cell lineage, DNA binding proteins genetics, developmental gene expression regulation, in situ hybridization, jaw anatomy and histology, jaw embryology, kidney embryology, kidney metabolism, molecular sequence data , neural crest cytology, neurons cytology, neurons metabolism, neurons physiology, pigmentation physiology, sequence alignment, transcription factor ap 2, transcription factors genetics, proteins.

Barresi, M.J., L.D. Hutson, C.B. Chien, and R.O. Karlstrom (2005). **Hedgehog regulated slit expression determines commissure and glial cell position in the zebrafish forebrain.** *Development* 132(16): 3643-56. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Three major axon pathways cross the midline of the vertebrate forebrain early in embryonic development: the postoptic commissure (POC), the anterior commissure (AC) and the optic nerve. We show that a small population of Gfap+ astroglia spans the midline of the zebrafish forebrain in the position of, and prior to, commissural and retinal axon crossing. These glial ;bridges' form in regions devoid of the guidance molecules slit2 and slit3, although a subset of these glial cells express slit1a. We show that Hh signaling is required for commissure formation, glial bridge formation, and the restricted expression of the guidance molecules slit1a, slit2, slit3 and sema3d, but that Hh does not appear to play a direct role in commissural and retinal axon guidance. Reducing Slit2 and/or Slit3 function expanded the glial bridges and caused defasciculation of the POC, consistent with a ;channeling' role for these repellent molecules. By contrast, reducing Slit1a function led to reduced midline axon crossing, suggesting a distinct role for Slit1a in midline axon guidance. Blocking Slit2 and Slit3, but not Slit1a, function in the Hh pathway mutant yot (gli2DR) dramatically rescued POC axon crossing and glial bridge formation at the midline, indicating that expanded Slit2 and Slit3 repellent function is largely responsible for the lack of midline crossing in these mutants. This analysis shows that Hh signaling helps to pattern the expression of Slit guidance molecules that then help to regulate glial cell position and axon guidance across the midline of the forebrain.

Descriptors: zebrafish embryos, intracellular signaling peptides and proteins metabolism, nerve tissue proteins metabolism, neuroglia metabolism, prosencephalon embryology, trans activators metabolism, developmental gene expression regulation, Hedgehog proteins, in situ hybridization, intercellular signaling peptides and proteins, intracellular signaling peptides and morphogenesis, nerve growth factors genetics, nerve growth factors metabolism, nerve tissue neurons cytology , neurons metabolism, antisense oligonucleotides genetics, antisense oligonucleotides metabolism, prosencephalon cytology, prosencephalon metabolism, semaphorins genetics, semaphorins metabolism, signal transduction, trans activators genetics, metabolism, proteins genetics.

Bart, A.N. and H.A. Kyaw (2003). **Survival of zebrafish, *Brachydanio rerio* (Hamilton-Buchanan), embryo after immersion in methanol and exposure to ultrasound with implications to cryopreservation.** *Aquaculture Research.* 34(8): 609-15. ISSN: 1355 557X.

NAL Call Number: SH1.F8

Abstract: This study examined the viability of embryos after immersion in highly concentrated methanol solutions (40-60%) and exposing embryos to ultrasound to enhance efficient transport of the cryoprotectant. The exposure to ultrasound, methanol concentrations, duration of treatment and the stages of embryonic development was found to have measurable effects on embryo viability. The effect of ultrasound was more evident at high voltage (>440 V) settings and at early developmental stages (30 and 60% epiboly stage). Older embryos were more resistant to cryoprotectant toxicity and ultrasound-induced mortality. The high concentration of methanol (60%) was more toxic to embryos than the low concentration (40%). When methanol treatment and ultrasound were applied simultaneously the optimum concentration was found to be 45% methanol (45% survival; $P<0.05$) in a 3 min treatment. Although there was no significant difference between the 2 and 3 min treatments, embryos treated for 4 min had a significantly lower survival rate ($P<0.05$). These findings provide initial results to select the developmental stage of the embryo, the concentration of methanol for the preparation of a vitrification solution and duration of ultrasound treatment for cryopreservation. Furthermore, it indicates the potential use of ultrasound to enhance the transport of methanol intracellularly with minimum mortality of the developing embryos.
Descriptors: zebrafish, *Brachydanio rerio*, embryo, embryogenesis, cryoprotectants, methanol, ultrasonic treatment, cryopreservation.

Bassett, D.I., R.J. Bryson Richardson, D.F. Daggett, P. Gautier, D.G. Keenan, and P.D. Currie (2003). **Dystrophin is required for the formation of stable muscle attachments in the zebrafish embryo.** *Development* 130(23): 5851-60. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: A class of recessive lethal zebrafish mutations has been identified in which normal skeletal muscle differentiation is followed by a tissue-specific degeneration that is reminiscent of the human muscular dystrophies. Here, we show that one of these mutations, sapje, disrupts the zebrafish orthologue of the X-linked human Duchenne muscular dystrophy (DMD) gene. Mutations in this locus cause Duchenne or Becker muscular dystrophies in human patients and are thought to result in a dystrophic pathology through disconnecting the cytoskeleton from the extracellular matrix in skeletal muscle by reducing the level of dystrophin protein at the sarcolemma. This is thought to allow tearing of this membrane, which in turn leads to cell death. Surprisingly, we have found that the progressive muscle degeneration phenotype of sapje mutant zebrafish embryos is caused by the failure of embryonic muscle end attachments. Although a role for dystrophin in maintaining vertebrate myotendinous junctions (MTJs) has been postulated previously and MTJ structural abnormalities have been identified in the Dystrophin-deficient mdx mouse model, in vivo evidence of pathology based on muscle attachment failure has thus far been lacking. This zebrafish mutation may therefore provide a model for a novel pathological mechanism of Duchenne muscular dystrophy and other muscle diseases.
Descriptors: zebrafish embryos, membrane proteins metabolism, muscle fibers metabolism, muscle proteins metabolism, proteins metabolism, amino acid sequence, macromolecular substances, membrane proteins classification, membrane molecular sequence data, muscle fibers pathology, muscle fibers ultrastructure, muscle proteins classification, muscle Duchenne muscular dystrophy genetics, Duchenne muscular dystrophy metabolism, phenotype, phylogeny, recombinant fusion proteins metabolism, sarcolemma metabolism, sequence alignment, transgenes, anatomy and histology, genetics, proteins classification, lethal mutations.

Bates, J.M., E. Mittge, J. Kuhlman, K.N. Baden, S.E. Cheesman, and K. Guillemin (2006). **Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation.** *Developmental Biology* 297(2): 374-86. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: All animals exist in intimate associations with microorganisms that play important roles in the hosts' normal development and tissue physiology. In vertebrates, the most populous and complex community of microbes resides in the digestive tract. Here, we describe the establishment of the gut microbiota and its role in digestive tract differentiation in the zebrafish model vertebrate, *Danio rerio*. We find that in the absence of the microbiota, the gut epithelium is arrested in aspects of its differentiation, as revealed by the lack of brush border intestinal alkaline phosphatase activity, the maintenance of immature patterns of glycan expression and a paucity of goblet and enteroendocrine cells. In addition, germ-free intestines fail to take up protein macromolecules in the distal intestine and exhibit faster motility. Reintroduction of a complex microbiota at later stages of development or monoassociation of germ-free larvae with individual constituents of the microbiota reverses all of these germ-free phenotypes. Exposure of germ-free zebrafish to heat-killed preparations of the microbiota or bacterial lipopolysaccharide is sufficient to restore alkaline phosphatase activity but not mature patterns of Gal alpha1,3Gal containing glycans, indicating that the host perceives and responds to its associated microbiota by at least two distinct pathways.

Descriptors: zebrafish embryos, intestines embryology, alkaline phosphatase metabolism, bacteria metabolism, cell differentiation, cell lineage, digestive system pathology, epithelium metabolism, homeostasis, intestinal mucosa embryology, intestines metabolism, intestines microbiology, lipopolysaccharides metabolism.

Baye, L.M. and B.A. Link (2007). **The disarrayed mutation results in cell cycle and neurogenesis defects during retinal development in zebrafish.** *BioMed Central Developmental Biology* 7: 28. ISSN: 1471 213X.

NAL Call Number: QH491.B63

Abstract: BACKGROUND: The vertebrate retina is derived from proliferative neuroepithelial cells of the optic cup. During retinal development, cell proliferation and the processes of cell cycle exit and neurogenesis are coordinated in neuroepithelial progenitor cells. Previous studies have demonstrated reciprocal influences between the cell cycle and neurogenesis. However the specific mechanisms and exact relationships of cell cycle regulation and neurogenesis in the vertebrate retina remain largely unknown. RESULTS: We have isolated and characterized a zebrafish mutant, disarrayed (drya64), which exhibits retinal defects in cell cycle regulation and neurogenesis. By 42 hours post fertilization, disarrayed mutants show small eyes and a reduced forebrain. Other aspects of development appear normal. Although retinogenesis is delayed, mutant retinal cells eventually differentiate to all major cell types. Examination of the disarrayed mitotic cycle using BrdU and direct imaging techniques revealed that retinal neuroepithelial cells have an extended cell cycle period and reduced rate of cell cycle exit and neurogenesis, despite the fact that neurogenesis initiates at the appropriate time of development. Genetic mosaic analyses indicate that the cell cycle phenotype of disarrayed is cell-non-autonomous. CONCLUSION: The disarrayed mutant shows defects in both cell cycle regulation and neurogenesis and provides insights into the coordinated regulation of these processes during retinal development.

Descriptors: zebrafish embryos, mutation, retina embryology, genetics, cell cycle, cell proliferation, lethal, recessive genes, genetic markers, genotype, immunohistochemistry, in situ hybridization, organogenesis, retina cytology.

Begemann, G., M. Marx, K. Mebus, A. Meyer, and M. Bastmeyer (2004). **Beyond the neckless phenotype: influence of reduced retinoic acid signaling on motor neuron development in the zebrafish hindbrain.** *Developmental Biology* 271(1): 119-29. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Retinoic acid (RA) has been identified as a key signal involved in the posteriorization of vertebrate neural ectoderm. The main biosynthetic enzyme responsible for RA signaling in the hindbrain and spinal cord is Raldh2. However, neckless/raldh2-mutant (nls) zebrafish exhibit only mild degrees of anteriorization in the neural ectoderm, compared to full vitamin A deficiency in amniotes and the Raldh2-/- mouse. Here we investigated the role of RA during neuronal development in the zebrafish hindbrain and anterior spinal cord using DEAB, an inhibitor of retinaldehyde dehydrogenases. We show that the nls hindbrain and spinal cord are not fully devoid of RA, since blocking Raldh-mediated RA signaling leads to a more severe hindbrain phenotype than in nls. The anteroposterior distribution of branchiomotor neurons in the facial and more posterior nuclei depends on full RA signaling throughout early and late gastrula stages. In contrast, inhibition of RA synthesis after gastrulation reduces the number of branchiomotor neurons in the vagal nucleus, but has no effect on anteroposterior cell fates. In addition, blockage of RA-mediated signaling not only interferes with the differentiation of branchiomotor neurons and their axons in the hindbrain, but also affects the development of the posterior lateral line nerve.

Descriptors: zebrafish embryos, aldehyde oxidoreductases physiology, motor neurons physiology, phenotype, rhombencephalon embryology, signal transduction physiology, P aminoazobenzene analogs and derivatives, aldehyde oxidoreductases genetics, immunohistochemistry, in situ hybridization, morphogenesis, retinal dehydrogenase, tretinoin physiology, retinoic acid.

Beis, D., T. Bartman, S.W. Jin, I.C. Scott, L.A. D'Amico, E.A. Ober, H. Verkade, J. Frantsve, H.A. Field, A. Wehman, H. Baier, A. Tallafuss, L. Bally Cuif, J.N. Chen, D.Y. Stainier, and B. Jungblut (2005). **Genetic and cellular analyses of zebrafish atrioventricular cushion and valve development.** *Development* 132(18): 4193-204. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Defects in cardiac valve morphogenesis and septation of the heart chambers constitute some of the most common human congenital abnormalities. Some of these defects originate from errors in atrioventricular (AV) endocardial cushion development. Although this process is being extensively studied in mouse and chick, the zebrafish system presents several advantages over these models, including the ability to carry out forward genetic screens and study vertebrate gene function at the single cell level. In this paper, we analyze the cellular and subcellular architecture of the zebrafish heart during stages of AV cushion and valve development and gain an unprecedented level of resolution into this process. We find that endocardial cells in the AV canal differentiate morphologically before the onset of epithelial to mesenchymal transformation, thereby defining a previously unappreciated step during AV valve formation. We use a combination of novel transgenic lines and fluorescent immunohistochemistry to analyze further the role of various genetic (Notch and Calcineurin signaling) and epigenetic (heart function) pathways in this process. In addition, from a large-scale forward genetic screen we identified 55 mutants, defining 48 different genes, that exhibit defects in discrete stages of AV cushion development. This collection of mutants provides a unique set of tools to further our understanding of the genetic basis of cell behavior and differentiation during AV valve development.

Descriptors: zebrafish embryos, cell differentiation physiology, endocardium embryology, heart valves embryology, morphogenesis physiology, signal transduction physiology, genetically modified, calcineurin metabolism, cell differentiation genetics, DNA mutational analysis, endocardium cytology, fluorescence, immunohistochemistry, confocal microscopy, Notch receptors metabolism.

Bellipanni, G., M. Varga, S. Maegawa, Y. Imai, C. Kelly, A.P. Myers, F. Chu, W.S. Talbot , and E.S. Weinberg (2006). **Essential and opposing roles of zebrafish beta-catenins in the formation of dorsal axial structures and neurectoderm.** *Development* 133(7): 1299-309. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: In *Xenopus*, Wnt signals and their transcriptional effector beta-catenin are required for the development of dorsal axial structures. In zebrafish, previous loss-of-function studies have not identified an essential role for beta-catenin in dorsal axis formation, but the maternal-effect mutation ichabod disrupts beta-catenin accumulation in dorsal nuclei and leads to a reduction of dorsoanterior derivatives. We have identified and characterized a second zebrafish beta-catenin gene, beta-catenin-2, located on a different linkage group from the previously studied beta-catenin-1, but situated close to the ichabod mutation on LG19. Although the ichabod mutation does not functionally alter the beta-catenin-2 reading frame, the level of maternal beta-catenin-2, but not beta-catenin-1, transcript is substantially lower in ichabod, compared with wild-type, embryos. Reduction of beta-catenin-2 function in wild-type embryos by injection of morpholino antisense oligonucleotides (MOs) specific for this gene (MO2) results in the same ventralized phenotypes as seen in ichabod embryos, and administration of MO2 to ichabod embryos increases the extent of ventralization. MOs directed against beta-catenin-1 (MO1), by contrast, had no ventralizing effect on wild-type embryos. beta-catenin-2 is thus specifically required for organizer formation and this function is apparently required maternally, because the ichabod mutation causes a reduction in maternal transcription of the gene and a reduced level of beta-catenin-2 protein in the early embryo. A redundant role of beta-catenins in suppressing formation of neurectoderm is revealed when both beta-catenin genes are inhibited. Using a combination of MO1 and MO2 in wild-type embryos, or by injecting solely MO1 in ichabod embryos, we obtain expression of a wide spectrum of neural markers in apparently appropriate anteroposterior pattern. We propose that the early, dorsal-promoting function of beta-catenin-2 is essential to counteract a later, dorsal- and neurectoderm-repressing function that is shared by both beta-catenin genes.

Descriptors: zebrafish embryos, body patterning genetics, nervous system embryology, proteins, beta catenin metabolism, amino acid sequence, chromosome mapping, chromosomes, computer simulation, developmental gene expression regulation, genetic markers , genome, microinjections, molecular sequence data, antisense oligonucleotides pharmacology, organizers, embryonic metabolism, phenotype, phylogeny, messenger RNA metabolism, reverse transcriptase PCR, amino acid sequence homology, beta catenin chemistry, beta catenin genetics.

Bennett, J.T., K. Joubin, S. Cheng, P. Aanstad, R. Herwig, M. Clark, H. Lehrach, and A.F. Schier (2007). **Nodal signaling activates differentiation genes during zebrafish gastrulation.** *Developmental Biology* 304(2): 525-40. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Nodal signals induce mesodermal and endodermal progenitors during vertebrate development. To determine the role of Nodal signaling at a genomic level, we isolated Nodal-regulated genes by expression profiling using macroarrays and gene expression databases. Putative Nodal-regulated genes were validated by in situ hybridization screening in wild type and Nodal signaling mutants. 46 genes were identified, raising the currently known number of Nodal-regulated genes to 72. Based on their expression patterns along the dorsoventral axis, most of these genes can be classified into two groups. One group is expressed in the dorsal margin, whereas the other group is expressed throughout the margin. In addition to

transcription factors and signaling components, the screens identified several new functional classes of Nodal-regulated genes, including cytoskeletal components and molecules involved in protein secretion or endoplasmic reticulum stress. We found that x-box binding protein-1 (xbp1) is a direct target of Nodal signaling and required for the terminal differentiation of the hatching gland, a specialized secretory organ whose specification is also dependent on Nodal signaling. These results indicate that Nodal signaling regulates not only specification genes but also differentiation genes.

Descriptors: zebrafish embryo, gastrula metabolism, transforming growth factor beta physiology, cell differentiation, DNA binding proteins genetics, proteins metabolism, developmental gene expression regulation, oligonucleotide array sequence analysis, signal transduction, transcription factors metabolism, proteins biosynthesis.

Berdougo, E., H. Coleman, D.H. Lee, D.Y. Stainier, and D. Yelon (2003). **Mutation of weak atrium/atrial myosin heavy chain disrupts atrial function and influences ventricular morphogenesis in zebrafish.** *Development* 130(24): 6121-9. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: The embryonic vertebrate heart is composed of two major chambers, a ventricle and an atrium, each of which has a characteristic size, shape and functional capacity that contributes to efficient circulation. Chamber-specific gene expression programs are likely to regulate key aspects of chamber formation. Here, we demonstrate that epigenetic factors also have a significant influence on chamber morphogenesis. Specifically, we show that an atrium-specific contractility defect has a profound impact on ventricular development. We find that the zebrafish locus weak atrium encodes an atrium-specific myosin heavy chain that is required for atrial myofibrillar organization and contraction. Despite their atrial defects, weak atrium mutants can maintain circulation through ventricular contraction. However, the weak atrium mutant ventricle becomes unusually compact, exhibiting a thickened myocardial wall, a narrow lumen and changes in myocardial gene expression. As weak atrium/atrial myosin heavy chain is expressed only in the atrium, the ventricular phenotypes in weak atrium mutants represent a secondary response to atrial dysfunction. Thus, not only is cardiac form essential for cardiac function, but there also exists a reciprocal relationship in which function can influence form. These findings are relevant to our understanding of congenital defects in cardiac chamber morphogenesis.

Descriptors: zebrafish embryos, atrial function physiology, atrial myosins metabolism, heart atria embryology, heart ventricles embryology, myocardial contraction physiology, myosin heavy chains metabolism, atrial myosins genetics, atrial natriuretic factor genetics, atrial natriuretic factor metabolism, epigenesis, genetic, heart physiology, metabolism, heart atria ultrastructure, metabolism, ultrastructure, morphogenesis, mutation, myocardium cytology, myocardium metabolism, myosin heavy chains genetics.

Bernardos, R.L., S.I. Lentz, M.S. Wolfe, and P.A. Raymond (2005). **Notch-Delta signaling is required for spatial patterning and Muller glia differentiation in the zebrafish retina.** *Developmental Biology* 278(2): 381-95. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Notch-Delta signaling has been implicated in several alternative modes of function in the vertebrate retina. To further investigate these functions, we examined retinas from zebrafish embryos in which bidirectional Notch-Delta signaling was inactivated either by the mind bomb (mib) mutation, which disrupts E3 ubiquitin ligase activity, or by treatment with gamma-secretase inhibitors, which prevent intramembrane proteolysis of Notch and Delta. We found that inactivating Notch-Delta signaling did not prevent differentia-

tion of retinal neurons, but it did disrupt spatial patterning in both the apical-basal and planar dimensions of the retinal epithelium. Retinal neurons differentiated, but their laminar arrangement was disrupted. Photoreceptor differentiation was initiated normally, but its progression was slowed. Although confined to the apical retinal surface as in normal retinas, the planar organization of cone photoreceptors was disrupted: cones of the same spectral subtype were clumped rather than regularly spaced. In contrast to neurons, Muller glia failed to differentiate suggesting an instructive role for Notch-Delta signaling in gliogenesis.
Descriptors: zebrafish embryos, body patterning physiology, membrane, neuroglia physiology, retina embryology, cell death, retina cones embryology, cytology, physiology, fluorescence microscopy, mutation, Notch receptors, retina cytology, retinal rodsembryology, signal transduction physiology, ubiquitin protein ligases deficiency, ubiquitin protein ligases genetics.

Berndt, J.D. and M.C. Halloran (2006). **Semaphorin 3d promotes cell proliferation and neural crest cell development downstream of TCF in the zebrafish hindbrain.** *Development* 133(20): 3983-92. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Neural crest cells (NCCs) are pluripotent migratory cells that are crucial to the development of the peripheral nervous system, pigment cells and craniofacial cartilage and bone. NCCs are specified within the dorsal ectoderm and undergo an epithelial to mesenchymal transition (EMT) in order to migrate to target destinations where they differentiate. Here we report a role for a member of the semaphorin family of cell guidance molecules in NCC development. Morpholino-mediated knockdown of Sema3d inhibits the proliferation of hindbrain neuroepithelial cells. In addition, Sema3d knockdown reduces markers of migratory NCCs and disrupts NCC-derived tissues. Similarly, expression of a dominant-repressor form of TCF (DeltaTCF) reduces hindbrain cell proliferation and leads to a disruption of migratory NCC markers. Moreover, expression of DeltaTCF downregulates sema3d RNA expression. Finally, Sema3d overexpression rescues reduced proliferation caused by DeltaTCF expression, suggesting that Sema3d lies downstream of Wnt/TCF signaling in the molecular pathway thought to control cell cycle in NCC precursors.
Descriptors: zebrafish embryo, nerve growth factors physiology, neural crest growth and development, pluripotent stem cells physiology, rhombencephalon growth and development, semaphorins physiology, body patterning, cell movement genetics, cell proliferation, cyclins genetics, gene expression, mutation, nerve growth factors antagonists and inhibitors, nerve growth factors genetics, neural crest chemistry, neural crest cytology, pluripotent stem cells chemistry, pluripotent stem cells cytology, messenger RNA analysis and metabolism, rhombencephalon chemistry, rhombencephalon cytology, semaphorins antagonists and inhibitors, semaphorins genetics, Wnt proteins metabolism, proteins antagonists and inhibitors, proteins genetics.

Bertrand, J.Y., A.D. Kim, E.P. Violette, D.L. Stachura, J.L. Cisson, and D. Traver (2007). **Definitive hematopoiesis initiates through a committed erythromyeloid progenitor in the zebrafish embryo.** *Development* 134(23): 4147-56. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Shifting sites of blood cell production during development is common across widely divergent phyla. In zebrafish, like other vertebrates, hematopoietic development has been roughly divided into two waves, termed primitive and definitive. Primitive hematopoiesis is characterized by the generation of embryonic erythrocytes in the intermediate cell mass and a distinct population of macrophages that arises from cephalic mesoderm. Based on

previous gene expression studies, definitive hematopoiesis has been suggested to begin with the generation of presumptive hematopoietic stem cells (HSCs) along the dorsal aorta that express c-myb and runx1. Here we show, using a combination of gene expression analyses, prospective isolation approaches, transplantation, and in vivo lineage-tracing experiments, that definitive hematopoiesis initiates through committed erythromyeloid progenitors (EMPs) in the posterior blood island (PBI) that arise independently of HSCs. EMPs isolated by coexpression of fluorescent transgenes driven by the lmo2 and gata1 promoters exhibit an immature, blastic morphology and express only erythroid and myeloid genes. Transplanted EMPs home to the PBI, show limited proliferative potential, and do not seed subsequent hematopoietic sites such as the thymus or pronephros. In vivo fate-mapping studies similarly demonstrate that EMPs possess only transient proliferative potential, with differentiated progeny remaining largely within caudal hematopoietic tissue. Additional fate mapping of mesodermal derivatives in mid-somitogenesis embryos suggests that EMPs are born directly in the PBI. These studies provide phenotypic and functional analyses of the first hematopoietic progenitors in the zebrafish embryo and demonstrate that definitive hematopoiesis proceeds through two distinct waves during embryonic development.
Descriptors: zebrafish embryo, hematopoiesis, erythromyeloid, progenitor.

Bessarab, D.A., S.W. Chong, and V. Korzh (2004). **Expression of zebrafish six1 during sensory organ development and myogenesis.** *Developmental Dynamics* 230(4): 781-6. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: *Drosophila sine oculis* homologous genes in vertebrates are homeobox-containing transcription factors functioning within the Pax-Six-Eya-Dach regulatory network during development. In this study, we describe the cloning and expression of a zebrafish homolog of sine oculis, six1. The reverse transcription-polymerase chain reaction demonstrated accumulation of six1 transcripts at mid-gastrula, and in situ hybridization showed their subsequent expression in the cranial placode and later in the olfactory, otic, and lateral line placodes, inner ear, and neuromasts. In addition, six1 is expressed in the pituitary, branchial arches, somites, pectoral fin, ventral abdomen muscle, and the cranial muscles of the eye and lower jaw. An increase of six1 expression was observed in the lateral line, muscles, and inner ear of the mind bomb mutant, illustrating a regulatory effect of the Notch pathway on expression of Six genes.
Descriptors: zebrafish, homeodomain proteins biosynthesis, homeodomain, proteins biosynthesis, amino acid sequence, molecular cloning, *Drosophila* proteins, ear embryology, expressed sequence tags, gastrula metabolism, in situ hybridization, membrane, molecular sequence data, muscle development, muscles embryology, mutation, phylogeny, messenger RNA metabolism, Notch receptors, reverse transcriptase PCR, amino acid sequence homology, time factors, tissue distribution.

Bessarab, D., S.W. Chong, M. Richardson, and V. Korzh (2004). **Analysis of regulation and function of six genes in embryonic zebrafish.** *Developmental Biology* 271(2): 619. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, molecular genetics, development, gene regulation.

Besser, J., J.T. Leito, D.L. van der Meer, and C.P. Bagowski (2007). **Tip-1 induces filopodia growth and is important for gastrulation movements during zebrafish development.** *Development Growth and Differentiation* 49(3): 205-14. ISSN: 0012 1592.

NAL Call Number: QL951.E4
Abstract: Wnt signaling is essential during animal development and also plays important roles in pathological conditions. Two mayor pathways have been described: the beta-catenin-dependent canonical (or classical) pathway and the beta-catenin-independent non-canonical Wnt pathway. Recent binding studies suggest links between the small PDZ protein TIP-1 (Tax-1 interacting protein) to components of both Wnt pathways. We have cloned and characterized the zebrafish tip-1 gene. Whole mount in situ hybridization and semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) indicated that zebrafish tip-1 is present as a maternal RNA and is ubiquitously expressed during early development. After 24 h of development, tip-1 expression was high in the central nervous system (CNS) whereas only weak expression was detected in the caudal regions of the zebrafish embryo. Tip-1 knockdown using antisense morpholino oligonucleotides, as well as ectopic tip-1 expression, led to elongation defects in zebrafish embryos and larvae. Both knockdown and overexpression of tip-1 resulted in a widened goosecoid (gsc) expression domain in shield stage embryos, led to an abbreviated prechordal plate, and to reduced convergent extension movements during gastrulation. We constructed a green fluorescence protein (GFP)/TIP-1 fusion protein which, when expressed in cultured fibroblasts (ZF4-cells), induced filopodia growth. Our observations indicate a role for TIP-1 in gastrulation movements and in filopodia growth induction.
Descriptors: zebrafish, gastrula physiology, homeodomain, pseudopodia physiology, amino acid sequence, molecular cloning, chemistry , metabolism, green fluorescent proteins analysis, green fluorescent homeodomain proteins antagonists and inhibitors, homeodomain molecular sequence data, pseudopodia genetics, messenger RNA analysis, messenger RNA metabolism, recombinant fusion proteins analysis, recombinant fusion proteins antagonists and inhibitors, proteins genetics.

Bickenbach, J.R. and M. Stern (2004). **Developmental environments after the fate of aged epidermal stem cells**. *Journal of Investigative Dermatology* 122(3): A77. ISSN: 0022 202X.
Descriptors: zebrafish, development, integumentary system, chemical coordination and homeostasis, cell division.

Bingham, S., S. Chaudhari, G. Vanderlaan, M. Itoh, A. Chitnis, and A. Chandrasekhar (2003). **Neurogenic phenotype of mind bomb mutants leads to severe patterning defects in the zebrafish hindbrain.** *Developmental Dynamics* 228(3): 451-63. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, body patterning genetics, rhombencephalon embryology, ubiquitin protein ligases genetics, genetics, cell differentiation genetics, in situ hybridization, motor neurons physiology, site directed mutagenesis phenotype.

Bird, N.C. and P.M. Mabee (2003). **Developmental morphology of the axial skeleton of the zebrafish, *Danio rerio* (*Ostariophysi: Cyprinidae*).** *Developmental Dynamics* 228(3): 337-57. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, bone and bones embryology, morphogenesis physiology, zebrafish classification, physiology, phylogeny, spine embryology.

Bischof, J. and W. Driever (2004). **Regulation of hhex expression in the yolk syncytial layer, the potential Nieuwkoop center homolog in zebrafish.** *Developmental Biology* 276(2): 552-62. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The Nieuwkoop center is the earliest signaling center during dorsal-ventral pattern formation in amphibian embryos and has been implied to function in induction of the Spemann-Mangold organizer. In zebrafish, Nieuwkoop-center-like activity resides in the dorsal yolk syncytial layer (YSL) at the interface of the vegetal yolk cell and the blastoderm. hex homologs are expressed in the anterior endomesoderm in frogs (Xhex), the anterior visceral endoderm in mice, and the dorsal YSL in zebrafish (hhex). Here, we investigate the control of hhex expression in the YSL. We demonstrate that bozozok (boz) is absolutely required for early hhex expression, while overexpression of boz causes ectopic hhex expression. Activation of Wnt/beta-catenin signaling by LiCl induces hhex expression in wild-type YSL but not in boz mutant embryos, revealing that boz activity is required downstream of Wnt/beta-catenin signaling for hhex expression. Further, we show that the boz-mediated induction of hhex is independent of the Boz-mediated repression of bmp2b. Our data reveal that repressive effects of both Vega1 and Vega2 may be responsible for the exclusion of hhex expression from the ventral and lateral parts of the YSL. In summary, zebrafish hhex appears to be activated by Wnt/beta-catenin in the dorsal YSL, where Boz acts in a permissive way to limit repression of hhex by Vega1 and Vega2.

Descriptors: zebrafish, body patterning, egg yolk cytology, egg yolk metabolism, developmental gene expression regulation, homeodomain proteins metabolism, organizers, embryonic , proteins metabolism, bone morphogenetic bone morphogenetic proteins metabolism, cytoskeletal proteins metabolism, homeodomain in situ hybridization, repressor repressor proteins metabolism, signal transduction physiology, trans activators metabolism, transforming growth factor beta genetics, transforming growth factor beta metabolism, Wnt proteins, beta catenin.

Bischof, J.M., M. Ekker, and R. Wevrick (2003). **A MAGE/NDN-like gene in zebrafish.** *Developmental Dynamics* 228(3): 475-9. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish, brain embryology, central nervous system embryology, nerve tissue nuclear genetics, amino acid sequence, body patterning genetics, conserved sequence, developmental gene expression regulation genetics , in situ hybridization, mammals, molecular sequence data, multigene family, RNA genetics, sequence alignment, amino acid sequence homology.

Bisgrove, B.W., B.S. Snarr, A. Emrazian, and H.J. Yost (2005). **Polaris and Polycystin-2 in dorsal forerunner cells and Kupffer's vesicle are required for specification of the zebrafish left-right axis.** *Developmental Biology* 287(2): 274-88. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Recently, it has become clear that motile cilia play a central role in initiating a left-sided signaling cascade important in establishing the LR axis during mouse and zebrafish embryogenesis. Two genes proposed to be important in this cilia-mediated signaling cascade are polaris and polycystin-2 (pkd2). Polaris is involved in ciliary assembly, while Pkd2 is proposed to function as a Ca(2+)-permeable cation channel. We have cloned zebrafish homologues of polaris and pkd2. Both genes are expressed in dorsal forerunner cells (DFCs) from gastrulation to early somite stages when these cells form a ciliated Kupffer's vesicle (KV). Morpholino-mediated knockdown of Polaris or Pkd2 in zebrafish results in misexpression of left-side-specific genes, including southpaw, lefty1 and lefty2, and randomization of heart and gut looping. By targeting morpholinos to DFCs/KV, we show that polaris and pkd2 are required in DFCs/KV for normal LR development. Polaris morphants have defects in KV

cilia, suggesting that the laterality phenotype is due to problems in cilia function per se. We further show that expression of polaris and pkd2 is dependent on the T-box transcription factors no tail and spadetail, respectively, suggesting that these genes have a previously unrecognized role in regulating ciliary structure and function. Our data suggest that the functions of polaris and pkd2 in LR patterning are conserved between zebrafish and mice and that Kupffer's vesicle functions as a ciliated organ of asymmetry.
Descriptors: zebrafish embryos, membrane proteins metabolism, body patterning, cilia metabolism, developmental gene expression regulation, membrane mice, mutation, trpp cation channels, transforming growth factor beta metabolism, tumor suppressor genetics.

Bjornson, C.R., K.J. Griffin, G.H.3. Farr, A. Terashima, C. Himeda, Y. Kikuchi, and D. Kimelman (2005). **Eomesodermin is a localized maternal determinant required for endoderm induction in zebrafish.** *Developmental Cell* 9(4): 523-33. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: In zebrafish, endoderm induction occurs in marginal blastomeres and requires Casanova (Cas), the first endoderm-specific factor expressed in the embryo. Whereas the transcription factors Gata5 and Bon are necessary and sufficient for cas expression in marginal blastomeres, Bon and Gata5 are unable to induce cas in animal pole cells, suggesting that cas expression requires an additional, unidentified factor(s). Here, we show that cas expression depends upon the T box transcription factor Eomesodermin (Eomes), a maternal determinant that is localized to marginal blastomeres. Eomes synergizes potently with Bon and Gata5 to induce cas, even in animal pole blastomeres. We show that Eomes is required for endogenous endoderm induction, acting via an essential binding site in the cas promoter. Direct physical interactions between Eomes, Bon, and Gata5 suggest that Eomes promotes endoderm induction in marginal blastomeres by facilitating the assembly of a transcriptional activating complex on the cas promoter.
Descriptors: zebrafish embryos, embryonic induction, endoderm physiology, high mobility group proteins metabolism, T-box domain proteins metabolism, transcription factors metabolism, proteins metabolism, endoderm cytology, GATA5 transcription factor genetics, GATA5 transcription factor metabolism, developmental gene expression regulation, high mobility group homeodomain proteins metabolism, in situ hybridization, antisense oligoribonucleotides genetics, antisense oligoribonucleotides metabolism, promoter regions genetics, protein binding, T-box domain transcription factors genetics, genetic transcription, transforming growth factor beta genetics, transforming growth factor beta metabolism, anatomy and histology.

Blader, P., C. Plessy, and U. Strahle (2003). **Multiple regulatory elements with spatially and temporally distinct activities control neurogenin1 expression in primary neurons of the zebrafish embryo.** *Mechanisms of Development* 120(2): 211-8. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: The basic Helix-Loop-Helix gene neurogenin1 (ngn1) is expressed in a complex pattern in the neural plate of zebrafish embryos, demarcating the sites of primary neurogenesis. We have dissected the ngn1 locus to identify cis-regulatory regions that control this expression. We have isolated two upstream elements that drive expression in precursors of Rohon-Beard sensory neurons and hindbrain interneurons and in clusters of neuronal precursors in the anterior neural plate, respectively. A third regulatory region mediates later expression. Thus, regulatory sequences with temporally and spatially distinct activities control ngn1 expression in primary neurons of the zebrafish embryo. These regions are highly similar to 5' sequences in the mouse and human ngn1 gene, suggesting that amniote embryos,

despite lacking primary neurons, utilize related mechanism to control ngn1 expression.
Descriptors: zebrafish embryos, nerve tissue neurons physiology, regulatory sequences, nucleic acid, transcription factors genetics, amino acid sequence, basic helix loop helix transcription factors , bone morphogenetic bone morphogenetic proteins metabolism, conserved sequence, developmental gene expression regulation, molecular sequence data, nerve tissue proteins metabolism, nervous system embryology, neurons metabolism, signal transduction, transcription factors metabolism, transgenes, genetics.

Blader, P., C.S. Lam, S. Rastegar, R. Scardigli, J.C. Nicod, N. Simplicio, C. Plessy, N. Fischer, C. Schuurmans, F. Guillemot, and U. Straehle (2004). **Conserved and acquired features of neurogenin 1 regulation**. *Development* 131(22): 5627-5637. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The telencephalon shows vast morphological variations among different vertebrate groups. The transcription factor neurogenin1 (ngn1) controls neurogenesis in the mouse pallium and is also expressed in the dorsal telencephalon of the evolutionary distant zebrafish. The upstream regions of the zebrafish and mammalian ngn1 loci harbour several stretches of conserved sequences. Here, we show that the upstream region of zebrafish ngn1 is capable of faithfully recapitulating endogenous expression in the zebrafish and mouse telencephalon. A single conserved regulatory region is essential for dorsal telencephalic expression in the zebrafish, and for expression in the dorsal pallium of the mouse. However, a second conserved region that is inactive in the fish telencephalon is necessary for expression in the lateral pallium of mouse embryos. This regulatory region, which drives expression in the zebrafish diencephalon and hindbrain, is dependent on Pax6 activity and binds recombinant Pax6 in vitro. Thus, the regulatory elements of ngn1 appear to be conserved among vertebrates, with certain differences being incorporated in the utilisation of these enhancers, for the acquisition of more advanced features in amniotes. Our data provide evidence for the co-option of regulatory regions as a mechanism of evolutionary diversification of expression patterns, and suggest that an alteration in Pax6 expression was crucial in neocortex evolution.
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Descriptors: zebrafish, molecular genetics, nervous system, neural coordination, neurogenesis, neurgenin 1, endogenous expression, telencephalon.

Blair, S.S. (2004). **Developmental Biology: Notching the hindbrain**. *Current Biology* 14(14): R570-R572. ISSN: 0960-9822.
NAL Call Number: QH301.C85
Descriptors: zebrafish, biological development, brain, cell lineage, cell migration, cells, freshwater fish, hindbrain, Notch protein, pattern formation, signal transduction, *Danio rerio*.

Blanco, M.J., A. Barrallo Gimeno, H. Acloque, A.E. Reyes, M. Tada, M.L. Allende, R. Mayor, and M.A. Nieto (2007). **Snail1a and Snail1b cooperate in the anterior migration of the axial mesendoderm in the zebrafish embryo.** *Development* 134(22): 4073-81. ISSN: 0950-1991.
NAL Call Number: QL951.D38
Abstract: The Snail genes are implicated in processes that involve cell movement, both during embryonic development and tumour progression. In teleosts, the vertebrate Snail1 gene is represented by two distinct genes, snail1a and snail1b (previously snail1 and snail2). These genes are expressed in complementary mesodermal domains and their combined expression matches that of their mammalian counterpart. By analysing their loss and gain of function, we found that the most-anterior axial mesendodermal cells, the precursors of the

polster, move in a cohesive manner directed by the activity of snail1a- and snail1b-expressing cells surrounding these precursors. The cell-autonomous function of Snail1 proteins regulates cell motility and influences the behaviour of Snail-negative neighbouring cells. Snail1a is required by the prechordal plate for it to reach its normal position, whereas Snail1b controls the acquisition of its normal shape. These non-redundant functions of Snail1a and Snail1b in controlling axial mesendoderm migration comply with the duplication-degeneration-complementation model, and indicate that Snail genes not only act as inducers of epithelial-to-mesenchymal transition, but also as more general regulators of cell adhesion and movement.

Descriptors: zebrafish embryo, Snail1a , Snail1b, anterior migration, axial, mesendoderm, embryo.

Blaser, H., S. Eisenbeiss, M. Neumann, M. Reichman Fried, B. Thisse, C. Thisse, and E. Raz (2005). **Transition from non-motile behaviour to directed migration during early PGC development in zebrafish.** *Journal of Cell Science* 118(Pt 17): 4027-38. ISSN: 0021-9533.

NAL Call Number: QH301.J6

Abstract: The migration of zebrafish primordial germ cells (PGCs) is directed by SDF-1a and serves as a model for long-range chemokine-guided cell migration. Whereas the development and migration of zebrafish PGCs have been studied in great detail starting at mid-gastrulation stages when the cells exhibit guided active migration [7-8 hours post fertilization (hpf)], earlier stages have not yet been examined. Here we show that the PGCs acquire competence to respond to the chemokine following discrete maturation steps. Using the promoter of the novel gene askopos and RNA elements of nanos1 to drive GFP expression in PGCs, we found that immediately after their specification (about 3 hpf) PGCs exhibit simple cell shape. This stage is followed by a phase at which the cells assume complex morphology yet they neither change their position nor do they respond to SDF-1a. During the third phase, a transition into a ;migratory stage' occurs as PGCs become responsive to directional cues provided by somatic cells secreting the chemokine SDF-1a. This transition depends on zygotic transcription and on the function of the RNA-binding protein Dead end and is correlated with down regulation of the cell adhesion molecule E-cadherin. These distinctive morphological and molecular alterations could represent a general occurrence in similar processes critical for development and disease.

Descriptors: zebrafish, cell movement physiology, Cxc chemokines metabolism, germ cells cytology, germ cells physiology, *Xenopus* proteins metabolism, embryology, proteins metabolism, genetically modified, cadherins genetics, cadherins metabolism, caspases metabolism, cell shape, developmental gene expression regulation, in situ hybridization, site directed mutagenesis antisense oligonucleotides genetics, antisense oligonucleotides metabolism, promoter regions genetics, recombinant fusion proteins metabolism, genetic transcription, anatomy and histology, physiology, proteins genetics.

Blaser, H., M. Reichman Fried, I. Castanon, K. Dumstrei, F.L. Marlow, K. Kawakami, L. Solnica Krezel, C.P. Heisenberg, and E. Raz (2006). **Migration of zebrafish primordial germ cells: a role for myosin contraction and cytoplasmic flow.** *Developmental Cell* 11(5): 613-27. ISSN: 1534 5807.

NAL Call Number: QH573.C42

Abstract: The molecular and cellular mechanisms governing cell motility and directed migration in response to the chemokine SDF-1 are largely unknown. Here, we demonstrate that zebrafish primordial germ cells whose migration is guided by SDF-1 generate bleb-like protrusions that are powered by cytoplasmic flow. Protrusions are formed at sites of higher

levels of free calcium where activation of myosin contraction occurs. Separation of the acto-myosin cortex from the plasma membrane at these sites is followed by a flow of cytoplasm into the forming bleb. We propose that polarized activation of the receptor CXCR4 leads to a rise in free calcium that in turn activates myosin contraction in the part of the cell responding to higher levels of the ligand SDF-1. The biased formation of new protrusions in a particular region of the cell in response to SDF-1 defines the leading edge and the direction of cell migration.

Descriptors: zebrafish, chemotaxis, cytoplasm physiology, germ cells physiology, myosins physiology, physiology, actins physiology, cell membrane physiology, cell polarity, Cxc chemokines physiology, cytoskeleton physiology, pseudopodia physiology, Cxcr4 receptors physiology.

Blasiole, B., V.A. Canfield, M.A. Vollrath, D. Huss, M.A. Mohideen, J.D. Dickman, K.C. Cheng, D.M. Fekete, and R. Levenson (2006). **Separate Na,K-ATPase genes are required for otolith formation and semicircular canal development in zebrafish.** *Developmental Biology* 294(1): 148-60. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: We have investigated the role of Na,K-ATPase genes in zebrafish ear development. Six Na,K-ATPase genes are differentially expressed in the developing zebrafish inner ear. Antisense morpholino knockdown of Na,K-ATPase alpha1a.1 expression blocked formation of otoliths. This effect was phenocopied by treatment of embryos with ouabain, an inhibitor of Na,K-ATPase activity. The otolith defect produced by morpholinos was rescued by microinjection of zebrafish alpha1a.1 or rat alpha1 mRNA, while the ouabain-induced defect was rescued by expression of ouabain-resistant zebrafish alpha1a.1 or rat alpha1 mRNA. Knockdown of a second zebrafish alpha subunit, alpha1a.2, disrupted development of the semicircular canals. Knockdown of Na,K-ATPase beta2b expression also caused an otolith defect, suggesting that the beta2b subunit partners with the alpha1a.1 subunit to form a Na,K-ATPase required for otolith formation. These results reveal novel roles for Na,K-ATPase genes in vestibular system development and indicate that different isoforms play distinct functional roles in formation of inner ear structures. Our results highlight zebrafish gene knockdown-mRNA rescue as an approach that can be used to dissect the functional properties of zebrafish and mammalian Na,K-ATPase genes.

Descriptors: zebrafish, Na+ K+ exchanging ATPase genetics, otolithic membrane enzymology, semicircular canals enzymology, ear growth and development isoenzymes, morphogenesis , Na+ K+ exchanging ATPase physiology, protein subunits, rats.

Blasiole, B., A. Degrave, V. Canfield, W. Boehmler, C. Thisse, B. Thisse, M.A. Mohideen, and R. Levenson (2003). **Differential expression of Na,K-ATPase alpha and beta subunit genes in the developing zebrafish inner ear.** *Developmental Dynamics* 228(3): 386-92. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish, inner ear embryology, Na+ K+ exchanging ATPase genetics, embryology, body patterning, inner ear enzymology, morphogenesis, protein subunits genetics.

Blitz, I.L., K. Cho, and C. Chang (2003). **Twisted gastrulation loss-of-function analyses support its role as a BMP inhibitor during early *Xenopus* embryogenesis**. *Development* 130(20): 4975-4988. ISSN: 0950-1991.

NAL Call Number: QL951.D38

Descriptors: zebrafish, amphibiotic species, animal morphology, biological development,

bone morphogenetic proteins, defects, developmental stages, embryogenesis, embryonic development, gastrulation, inhibitors, pattern formation, amniota, anura, *Danio rerio*, *Drosophila*, *Xenopus laevis*, African clawed frog, Spemann's organizer, twisted gastrulation protein.

Boehmler, W., S. Obrecht Pflumio, V. Canfield, C. Thisse, B. Thisse, and R. Levenson (2004). **Evolution and expression of D2 and D3 dopamine receptor genes in zebrafish.** *Developmental Dynamics* 230(3): 481-93. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, molecular evolution, dopamine receptors D2 genetics, dopamine receptors D2 metabolism, genetics, amino acid sequence, central nervous system metabolism, chromosome mapping, conserved sequence, exons, introns, linkage genetics, molecular sequence data, phylogeny, tertiary protein structure, dopamine receptors D2 chemistry, dopamine receptors D3, amino acid sequence homology, somites metabolism, synteny, tissue distribution.

Bollig, F., R. Mehringer, B. Perner, C. Hartung, M. Schafer, M. Schartl, J.N. Volff, C. Winkler, and C. Englert (2006). **Identification and comparative expression analysis of a second wt1 gene in zebrafish.** *Developmental Dynamics* 235(2): 554-61. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, developmental gene expression regulation genetics, Wt1 proteins genetics, amino acid sequence, chemistry, molecular sequence data, phylogeny, sequence alignment, amino acid sequence homology, Wt1 proteins chemistry, Wt1 proteins metabolism, growth and development.

Bonkowsky, J.L. and C.B. Chien (2005). **Molecular cloning and developmental expression of FOXP2 in zebrafish.** *Developmental Dynamics* 234(3): 740-6. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, forkhead transcription factors genetics, developmental gene expression regulation genetics, amino acid sequence, molecular cloning, forkhead transcription factors chemistry and metabolism, molecular sequence data, phylogeny, messenger RNA genetics, sequence alignment, amino acid sequence homology, telencephalon chemistry and metabolism, telencephalon embryology.

Borday Birraux, V., C. Van der Heyden, M. Debiais Thibaud, L. Verreijdt, D. Stock, A. Huysseune, and J.Y. Sire (2006). **Expression of Dlx genes during the development of the zebrafish pharyngeal dentition: evolutionary implications.** *Evolution and Development* 8(2): 130-141. ISSN: 1520 541X.
NAL Call Number: QH359
Abstract: In order to investigate similarities and differences in genetic control of development among teeth within and between species, we determined the expression pattern of all eight Dlx genes of the zebrafish during development of the pharyngeal dentition and compared these data with that reported for mouse molar tooth development. We found that (i) dlx1a and dlx6a are not expressed in teeth, in contrast to their murine orthologs, Dlx1 and Dlx6; (ii) the expression of the six other zebrafish Dlx genes overlaps in time and space, particularly during early morphogenesis; (iii) teeth in different locations and generations within the zebrafish dentition differ in the number of genes expressed; (iv) expression similarities and differences between zebrafish Dlx genes do not clearly follow phylogenetic and linkage relationships; and (v) similarities and differences exist in the expression of zebrafish and mouse Dlx orthologs. Taken together, these results indicate that the Dlx gene family, despite

having been involved in vertebrate tooth development for over 400 million years, has undergone extensive diversification of expression of individual genes both within and between dentitions. The latter type of difference may reflect the highly specialized dentition of the mouse relative to that of the zebrafish, and/or genome duplication in the zebrafish lineage facilitating a redistribution of Dlx gene function during odontogenesis.
Descriptors: zebrafish, *Danio rerio*, teeth , pharyngeal teeth, gene expression during development, evolutionary implications, gill skeleton, pharynx, gene expression, odontogenesis.

Bradfield, J.Y., S.E. Maier, and J.R. West (2004). **Teratogenesis in zebrafish induced by alcohol.** *Alcoholism Clinical and Experimental Research* 28(5, Suppl. S): 166A. ISSN: 0145 6008.
Descriptors: zebrafish, behavior, toxicology, development, gas chromatography, chromatographic techniques, vertebrate development, teratogenesis, behavioral dysfunction, somatic derangement, developmental retardation, abnormal curvature.

Brown, K.S. and S.L. Amacher (2005). **A cell cycle regulatory gene, gadd45 beta is involved in zebrafish mesoderm patterning.** *Developmental Biology* 283(2): 690. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, development, molecular genetics, molecular biophysics, cell cycle, regulatory gene.

Brownlie, A., C. Hersey, A.C. Oates, B.H. Paw, A.M. Falick, H.E. Witkowska, J. Flint, D. Higgs, J. Jessen, N. Bahary, H. Zhu, S. Lin, and L. Zon (2003). **Characterization of embryonic globin genes of the zebrafish.** *Developmental Biology* 255(1): 48-61. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Hemoglobin switching is a complex process by which distinct globin chains are produced during stages of development. In an effort to characterize the process of hemoglobin switching in the zebrafish model system, we have isolated and characterized several embryonic globin genes. The embryonic and adult globin genes are found in clusters in a head-to-head configuration. One cluster of embryonic and adult genes is localized to linkage group 3, whereas another embryonic cluster is localized on linkage group 12. Several embryonic globin genes demonstrate an erythroid-specific pattern of expression early during embryogenesis and later are downregulated as definitive hematopoiesis occurs. We utilized electrospray mass spectroscopy to correlate globin genes and protein expression in developing embryonic red cells. The mutation, zinfandel, has a hypochromic microcytic anemia as an embryo, but later recovers in adulthood. The zinfandel gene maps to linkage group 3 near the major globin gene locus, strongly suggesting that zinfandel represents an embryonic globin defect. Our studies are the first to systematically evaluate the embryonic globins in the zebrafish and will ultimately be useful in evaluating zebrafish mutants with defects in hemoglobin production and switching.
Descriptors: zebrafish embryo, blood supply, globins genetics, embryology and genetics, amino acid sequence, developmental gene expression regulation, genes switch, globins metabolism, hematopoiesis genetics, linkage genetics, molecular weight, mutation, phenotype, messenger RNA genetics, sequence alignment.

Bruce, A.E., C. Howley, M. Dixon Fox, and R.K. Ho (2005). **T-box gene eomesodermin and the homeobox-containing Mix/Bix gene mtx2 regulate epiboly movements in the zebrafish.** *Developmental Dynamics* 233(1): 105-14. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryo, developmental gene expression regulation physiology,

membrane proteins metabolism, T-box domain proteins metabolism, blastula and gastrula metabolism, membrane T-box domain genetics, metabolism, proteins genetics.

Bruce, A.E., C. Howley, Y. Zhou, S.L. Vickers, L.M. Silver, M.L. King, and R.K. Ho (2003). **The maternally expressed zebrafish T-box gene eomesodermin regulates organizer formation.** *Development* 130(22): 5503-17. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Early embryonic development in many organisms relies upon maternal molecules deposited into the egg prior to fertilization. We have cloned and characterized a maternal T-box gene in the zebrafish, eomesodermin (eomes). During oogenesis, the eomes transcript becomes localized to the cortex of the oocyte. After fertilization during early cleavage stages, eomes is expressed in a vegetal to animal gradient in the embryo, whereas Eomesodermin protein (Eom) is distributed cytoplasmically throughout the blastoderm. Strikingly, following midblastula transition, nuclear-localized Eomesodermin is detected on the dorsal side of the embryo only. Overexpression of eomes results in Nodal-dependent and nieuwkoid/dharma (nwk/dhm) independent ectopic expression of the organizer markers goosecoid (gsc), chordin (chd) and floating head (flh) and in the formation of secondary axes. The same phenotypes are observed when a VP16-activator construct is injected into early embryos, indicating that eomes acts as a transcriptional activator. In addition, a dominant-negative construct and antisense morpholino oligonucleotides led to a reduction in gsc and flh expression. Together these data indicate that eomes plays a role in specifying the organizer.
Descriptors: zebrafish, organizers, embryonic metabolism, T-box domain Xenopus proteins, proteins, cell nucleus metabolism, messenger RNA metabolism, signal transduction physiology, T-box domain proteins deficiency, T-box domain proteins metabolism, transforming growth factor beta metabolism, *Xenopus*.

Bryson Richardson, R.J., S. Berger, T.F. Schilling, T.E. Hall, N.J. Cole, A.J. Gibson, J. Sharpe, and P.D. Currie (2007). **FishNet: an online database of zebrafish anatomy.** *BioMed Central Biology* 5: 34. ISSN: 1741 7007.
NAL Call Number: QH1.B565
Abstract: BACKGROUND: Over the last two decades, zebrafish have been established as a genetically versatile model system for investigating many different aspects of vertebrate developmental biology. With the credentials of zebrafish as a developmental model now well recognized, the emerging new opportunity is the wider application of zebrafish biology to aspects of human disease modelling. This rapidly increasing use of zebrafish as a model for human disease has necessarily generated interest in the anatomy of later developmental phases such as the larval, juvenile, and adult stages, during which many of the key aspects of organ morphogenesis and maturation take place. Anatomical resources and references that encompass these stages are non-existent in zebrafish and there is therefore an urgent need to understand how different organ systems and anatomical structures develop throughout the life of the fish. RESULTS: To overcome this deficit we have utilized the technique of optical projection tomography to produce three-dimensional (3D) models of larval fish. In order to view and display these models we have created FishNet http://www.fishnet.org.au, an interactive reference of zebrafish anatomy spanning the range of zebrafish development from 24 h until adulthood. CONCLUSION: FishNet contains more than 36,000 images of larval zebrafish, with more than 1,500 of these being annotated. The 3D models can be manipulated on screen or virtually sectioned. This resource represents the first complete embryo to adult atlas for any species in 3D.
Descriptors: zebrafish embryo to adult atlas, databases, factual, online systems, anatomy

and histology, embryo anatomy and histology, three dimensional imaging, larva anatomy and histology.

Bryson Richardson, R.J., D.F. Daggett, F. Cortes, C. Neyt, D.G. Keenan, and P.D. Currie (2005). **Myosin heavy chain expression in zebrafish and slow muscle composition.** *Developmental Dynamics* 233(3): 1018-22. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: In the zebrafish embryo, two distinct classes of muscle fibers have been described in the forming myotome that arise from topographically separable precursor populations. Based entirely on cross-reactivity with antibodies raised against mammalian and chick myosin heavy chain isoforms slow twitch muscle has been shown to arise exclusively from "adaxial" myoblasts, which migrate from their origin flanking the notochord to form a single layer of subcutaneous differentiated muscle cells. The remainder of the myotome differentiates behind this migration as muscle fibers recognized by anti-fast myosin heavy chain (MyHC) antibodies. To identify unambiguous molecular markers of cell fate in the myotome, we have characterized genes encoding zebrafish fast and slow MyHC. Using phylogenetic and expression analysis, we demonstrate that these genes are definitive molecular markers of slow and fast twitch fates. We also demonstrate that zebrafish embryonic slow twitch muscle co-expresses both slow and fast twitch MyHC isoforms, a property that they share with primary fibers of the amniote myotome.

Descriptors: zebrafish, developmental gene expression regulation, muscle fibers, slow twitch metabolism, myosin heavy chains genetics and metabolism, immunohistochemistry, in situ hybridization, phylogeny, protein isoforms genetics and metabolism, proteins genetics and metabolism.

Buckles, G.R., C.J. Thorpe, M.C. Ramel, and A.C. Lekven (2004). **Combinatorial Wnt control of zebrafish midbrain-hindbrain boundary formation.** *Mechanisms of Development* 121(5): 437-47. ISSN: 0925-4773.

NAL Call Number: QH607.A1C4

Abstract: Wnt signaling is known to be required for the normal development of the vertebrate midbrain and hindbrain, but genetic loss of function analyses in the mouse and zebrafish yield differing results regarding the relative importance of specific Wnt loci. In the zebrafish, Wnt1 and Wnt10b functionally overlap in their control of gene expression in the ventral midbrain-hindbrain boundary (MHB), but they are not required for the formation of the MHB constriction. Whether other wnt loci are involved in zebrafish MHB development is unclear, although the expression of at least two wnts, wnt3a and wnt8b, is maintained in wnt1/wnt10b mutants. In order to address the role of wnt3a in zebrafish, we have isolated a full length cDNA and examined its expression and function via knockdown by morpholino antisense oligonucleotide (MO)-mediated knockdown. The expression pattern of wnt3a appears to be evolutionarily conserved between zebrafish and mouse, and MO knockdown shows that Wnt3a, while not uniquely required for MHB development, is required in the absence of Wnt1 and Wnt10b for the formation of the MHB constriction. In zebrafish embryos lacking Wnt3a, Wnt1 and Wnt10b, the expression of engrailed orthologs, pax2a and fgf8 is not maintained after mid-somitogenesis. In contrast to acerebellar and no isthmus mutants, in which midbrain and hindbrain cells acquire new fates but cell number is not significantly affected until late in embryogenesis, zebrafish embryos lacking Wnt3a, Wnt1 and Wnt10b undergo extensive apoptosis in the midbrain and cerebellum anlagen beginning in mid-somitogenesis, which results in the absence of a significant portion of the midbrain and cerebellum. Thus, the requirement for Wnt signaling in forming the MHB constriction

is evolutionarily conserved in vertebrates and it is possible in zebrafish to dissect the relative impact of multiple Wnt loci in midbrain and hindbrain development.

Descriptors: zebrafish embryo, midbrain and hindbrain metabolism, intercellular signaling peptides and proteins metabolism, metabolism, amino acid sequence, genetically modified, apoptosis, brain cytology, embryo cytology, nonmammalian metabolism, gene expression regulation, in situ hybridization, intercellular signaling peptides and proteins chemistry and genetics, intercellular signaling peptides and proteins isolation and purification, molecular sequence data, phylogeny, proteins chemistry and proteins metabolism, messenger RNA genetics and metabolism, sequence alignment, signal transduction, somites metabolism, Wnt proteins, Wnt1 protein.

Burgess, S.M., Z. Wang, L. Garrett-Beals, A. Elkahloun, and Y. Chen (2005). **Evolutionary filtering for genes in the oct4 (pou5f1) pathway.** *Developmental Biology* 283(2): 641-642. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish, molecular genetics, microarray analysis, genetic techniques, phenotype, transcriptional profile, pluripotency, core regulatory pathway.

Busch Nentwich, E., C. Sollner, H. Roehl, and T. Nicolson (2004). **The deafness gene DFNA5 is crucial for ugdh expression and HA production in the developing ear in zebrafish.** *Development* 131(4): 943-51. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Over 30 genes responsible for human hereditary hearing loss have been identified during the last 10 years. The proteins encoded by these genes play roles in a diverse set of cellular functions ranging from transcriptional regulation to K(+) recycling. In a few cases, the genes are novel and do not give much insight into the cellular or molecular cause for the hearing loss. Among these poorly understood deafness genes is DFNA5. How the truncation of the encoded protein DFNA5 leads to an autosomal dominant form of hearing loss is not clear. In order to understand the biological role of DFNA5, we took a reversegenetic approach in zebrafish. Here we show that morpholino antisense nucleotide knock-down of dfna5 function in zebrafish leads to disorganization of the developing semicircular canals and reduction of pharyngeal cartilage. This phenotype closely resembles previously isolated zebrafish craniofacial mutants including the mutant jekyll. jekyll encodes Ugdh [uridine 5'-diphosphate (UDP)-glucose dehydrogenase], an enzyme that is crucial for production of the extracellular matrix component hyaluronic acid (HA). In DFNA5 morphants, expression of ugdh is absent in the developing ear and pharyngeal arches, and HA levels are strongly reduced in the outgrowing protrusions of the developing semicircular canals. Previous studies suggest that HA is essential for differentiating cartilage and directed outgrowth of the epithelial protrusions in the developing ear. We hypothesize that the reduction of HA production leads to uncoordinated outgrowth of the canal columns and impaired facial cartilage differentiation.

Descriptors: zebrafish embryos, carrier deafness genetics, ear embryology, hyaluronic acid metabolism, receptors, estrogen, uridine diphosphate glucose dehydrogenase genetics, proteins, amino acid sequence, carrier proteins metabolism, inner ear embryology, mandible abnormalities, mandible embryology, molecular sequence data, mutation, RNA splice sites, sequence alignment, protein sequence analysis, uridine diphosphate glucose dehydrogenase biosynthesis.

Cadwallader, A.B. and H.J. Yost (2006). **Combinatorial expression patterns of heparan sulfate sulfotransferases in zebrafish: I. the 3-O-sulfotransferase family.** *Developmental Dynamics* 235(12): 3423-31. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, mice, sulfotransferases genetics, embryology, genetics, amino acid sequence, catalytic domain genetics, molecular cloning, developmental gene expression regulation, enzymologic gene expression regulation, in situ hybridization, molecular sequence data, multigene family, phylogeny, tertiary protein structure, amino acid sequence homology, species specificity, sulfotransferases chemistry, sulfotransferases metabolism, proteins chemistry and metabolism.

Cadwallader, A.B. and H.J. Yost (2006). **Combinatorial expression patterns of heparan sulfate sulfotransferases in zebrafish: II. the 6-O-sulfotransferase family.** *Developmental Dynamics* 235(12): 3432-7. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryo, sulfotransferases genetics, embryology, genetics, amino acid sequence, molecular cloning, developmental gene expression regulation, enzymologic gene expression regulation, in situ hybridization, molecular sequence data, multigene family, phylogeny, messenger RNA genetics and metabolism, amino acid sequence homology, sulfotransferases metabolism.

Cadwallader, A.B. and H.J. Yost (2007). **Combinatorial expression patterns of heparan sulfate sulfotransferases in zebrafish: III. 2-O-sulfotransferase and C5-epimerases.** *Developmental Dynamics* 236(2): 581-6. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Heparan sulfate (HS) is an unbranched chain of repetitive disaccharides, which specifically binds ligands when attached to the cell surface or secreted extracellularly. HS chains contain sulfated domains, termed the HS fine structure, which give HS specific binding affinities for extracellular ligands. HS 2-O-sulfotransferase (2-OST) catalyzes the transfer of sulfate groups to the 2-O position of uronic acid residues of HS. We report here the characterization and developmental expression patterns of 2-OST in several tissues/organs throughout early zebrafish development, including early cleavage stages, eyes, somites, brain, internal organ primordial, and pectoral fin. The 2-OST gene has spatially and temporally distinct expression, which is a surprise given the essential role of 2-OST in HS fine structure formation. Furthermore, although 2-OST and C5-epimerase are predicted to be interdependent for protein translocation from the endoplasmic reticulum to the Golgi, their expression is not coordinately regulated during zebrafish development.
Descriptors: zebrafish embryos, developmental gene expression regulation, phylogeny, racemases and epimerases metabolism, sulfotransferases metabolism, amino acid and base sequence, cloning, cluster analysis, in situ hybridization, molecular sequence data, DNA sequence analysis, species specificity, sulfotransferases genetics.

Callander, D.C., R.E. Lamont, S.J. Childs, and S. McFarlane (2007). **Expression of multiple class three semaphorins in the retina and along the path of zebrafish retinal axons.** *Developmental Dynamics* 236(10): 2918-24. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, semaphorins, Sema3s, retina, retinal axons, retinal ganglion cells (RGC).

Camarata, T., J. Topczewski, and H.G. Simon (2006). **LMP4 regulates TBX5 during zebrafish heart development**. *Developmental Biology* 295(1): 460-461. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, cardiovascular system, transport and circulation, molecular genetics, Holt Oram syndrome, congenital disease, subcellular localization.

Cao, Y., J. Zhao, Y. Wang, and A. Meng (2003). **Expression of zebrafish Lc3 synthase gene in embryonic lens requires Hedgehog signaling.** *Developmental Dynamics* 228(3): 308-12. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish Embryo, developmental gene expression regulation genetics, crystalline lens embryology, N acetylglucosaminyltransferases genetics, genetics, enzymology, in situ hybridization, crystalline lens enzymology, N acetylglucosaminyltransferases isolation and purification, organ specificity, proteins isolation and purification.

Cao, Y., J. Zhao, Z. Sun, J. Postlethwait, and A. Meng (2004). **Fgf17b, a novel member of Fgf family, helps patterning zebrafish embryos**. *Developmental Biology* 271(1): 130-143. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Fibroblast growth factors (Fgfs) play important roles in the pattern formation of early vertebrate embryos. We have identified a zebrafish ortholog of human FGF17, named fgf17b. The first phase of fgf17b expression occurs in the blastodermal margin of late blastulae and in the embryonic shield of early gastrulae. The second phase starts after the onset of segmentation, mainly in the presomitic mesoderm and newly formed somites. Injection of fgf17b mRNA into one-cell embryos induces expression of the mesodermal marker no tail (ntl) and rescues ntl expression suppressed by overexpression of lefty1 (lft1). Overexpression of fgf17b dorsalizes zebrafish gastrulae by enhancing expression of chordin (chd), which is an antagonist of the ventralizing signals BMPs. In addition, overexpression of fgf17b posteriorizes the neuroectoderm. Simultaneous knockdown of fgf17b and fgf8 with antisense morpholinos results in reduction of chd and ntl. Knockdown of fgf17b can alleviate inhibitory effect of ectopic expression of fgf3 on otx1. These data together suggest that Fgf17b plays a role in early embryonic patterning. We also demonstrate that fgf17b and fgf8 have stronger mesoderm inducting activity than fgf3, whereas fgf17b and fgf3 have stronger activity in posteriorizing the neuroectoderm than fgf8. Like fgf8, activation of fgf17b expression depends on Nodal signaling. Copyright 2004 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, development, molecular genetics, dorsalization, Fgf, mesoderm, blastula, gastrula.

Carnevali, O., C. Cionna, L. Tosti, E. Lubzens, and F. Maradonna (2006). **Role of cathepsins in ovarian follicle growth and maturation**. *General and Comparative Endocrinology* 146(3): 195-203. ISSN: 0016 6480.
NAL Call Number: 444.8G28
Abstract: Several complex processes are involved in the production of viable eggs. The aim of this review is to provide an overview on the role played by lysosomal enzymes, especially cathepsins B, D, and L, during ovarian follicle growth and maturation. Specific attention is focused on the relationship between the second proteolytic cleavage of yolk proteins (YP) and the resumption of the meiosis during germinal vesicle break down (GVBD). Maturation represents the final stage of oocytes development prior to ovulation. Oocytes in this phase

appear translucent. In many teleosts GVBD is accompanied by water uptake and among marine teleosts with pelagic eggs, most of the final volume is reached by this process. The last phase of maturation in benthonic eggs also occurs concomitant to a second proteolytic cleavage and is related with a slight hydration process. In vitro maturation by 17 alpha ,20 beta -dihydroxy-4-pregnen-3one in class III Danio rerio oocytes, induced 80% of GVBD. The maturation of these oocytes is known to be associated with proteolysis of their major yolk components. In the present study, we show that inhibition of specific enzymes (cathepsins) involved in the second YP processing, did not affect the occurrence of GVBD as the oocytes become translucent and display a slight increase in size. More specifically, in vitro incubation of the maturing oocytes with a cathepsin B inhibitor suppressed both cathepsin B and L activities and the proteolysis of YP. On the contrary, the addition of cathepsin L inhibitor, only affected cathepsin L activity, indicating that cathepsin B is probably involved in Cathepsin L activation, and this enzyme is probably responsible for the second YP processing. These results, together with previous studies, indicate that the GVBD process is independent of the occurrence of the second proteolytic process. It supports the hypothesis that the maturation process is under K+ ion flux control, while yolk proteolysis is related to the temporal and specific activation of cathepsins by acidification of yolk spheres. Copyright © 2008 CABI
Descriptors: zebrafish ovaries, cathepsins, eggs, enzyme activity, enzyme inhibitors, oocyte maturation, oocytes, ovarian development, ovarian follicles, proteolysis, sexual development, sexual maturity, *Danio rerio*.
Notes: Review Article.

Carney, T.J., S. von der Hardt, C. Sonntag, A. Amsterdam, J. Topczewski, N. Hopkins, and M. Hammerschmidt (2007). **Inactivation of serine protease Matriptase1a by its inhibitor Hai1 is required for epithelial integrity of the zebrafish epidermis.** *Development* 134(19): 3461-71. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Epithelial integrity requires the adhesion of cells to each other as well as to an underlying basement membrane. The modulation of adherence properties is crucial to morphogenesis and wound healing, and deregulated adhesion has been implicated in skin diseases and cancer metastasis. Here, we describe zebrafish that are mutant in the serine protease inhibitor Hai1a (Spint1la), which display disrupted epidermal integrity. These defects are further enhanced upon combined loss of hai1a and its paralog hai1b. By applying in vivo imaging, we demonstrate that Hai1-deficient keratinocytes acquire mesenchymal-like characteristics, lose contact with each other, and become mobile and more susceptible to apoptosis. In addition, inflammation of the mutant skin is evident, although not causative of the epidermal defects. Only later, the epidermis exhibits enhanced cell proliferation. The defects of hai1 mutants can be phenocopied by overexpression and can be fully rescued by simultaneous inactivation of the serine protease Matriptase1a (St14a), indicating that Hai1 promotes epithelial integrity by inhibiting Matriptase1a. By contrast, Hepatocyte growth factor (Hgf), a well-known promoter of epithelial-mesenchymal transitions and a prime target of Matriptase1 activity, plays no major role. Our work provides direct genetic evidence for antagonistic in vivo roles of Hai1 and Matriptase1a to regulate skin homeostasis and remodeling.
Descriptors: zebrafish embryos, proteinase inhibitory proteins, secretory metabolism, serine endopeptidases metabolism, serine proteinase inhibitors metabolism, embryology, metabolism, proteins antagonists and inhibitors, genetically modified, apoptosis, epidermis embryology, epidermis metabolism , keratinocytes cytology, keratinocytes metabolism, insertional mutagenesis, phenotype, proteinase inhibitory proteins, secretory genetics,

serine endopeptidases genetics, serine proteinase inhibitors genetics, signal transduction and metabolism.

Carney, T.J., K. Dutton, E. Greenhill, M. Delfino-Machin, P. Dufourcq, P. Blader, and R.N. Kelsh (2006). **A direct role for sox10 in specification of neural crest-derived sensory neurons**. *Development* 133(23): 4619-4630. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: sox10 is necessary for development of neural and pigment cell derivatives of the neural crest (NC). However, whereas a direct role for Sox10 activity has been established in pigment and glial lineages, this is more controversial in NC-derived sensory neurons of the dorsal root ganglia (DRGs). We proposed that sox10 functioned in specification of sensory neurons, whereas others suggested that sensory neuronal defects were merely secondary to absence of glia. Here we provide evidence that in zebrafish, early DRG sensory neuron survival is independent of differentiated glia. Critically, we demonstrate that Sox10 is expressed transiently in the sensory neuron lineage, and species sensory neuron precursors by regulating the proneural gene neurogenin1. Consistent with this, we have isolated a novel sox10 mutant that lacks glia and yet displays a neurogenic DRG phenotype. In conjunction with previous findings, these data establish the generality of our model of Sox10 function in NC fate specification. Copyright©Thomson Reuters 2009
Descriptors: zebrafish , development, nervous system, neural coordination, phenotype.

Carreira Barbosa, F., M.L. Concha, M. Takeuchi, N. Ueno, S.W. Wilson, and M. Tada (2003). **Prickle 1 regulates cell movements during gastrulation and neuronal migration in zebrafish.** *Development* 130(17): 4037-46. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: During vertebrate gastrulation, mesodermal and ectodermal cells undergo convergent extension, a process characterised by prominent cellular rearrangements in which polarised cells intercalate along the medio-lateral axis leading to elongation of the antero-posterior axis. Recently, it has become evident that a noncanonical Wnt/Frizzled (Fz)/Dishevelled (Dsh) signalling pathway, which is related to the planar-cell-polarity (PCP) pathway in flies, regulates convergent extension during vertebrate gastrulation. Here we isolate and functionally characterise a zebrafish homologue of *Drosophila* prickle (pk), a gene that is implicated in the regulation of PCP. Zebrafish pk1 is expressed maternally and in moving mesodermal precursors. Abrogation of Pk1 function by morpholino oligonucleotides leads to defective convergent extension movements, enhances the silberblick (slb)/wnt11 and pipetail (Ppt)/wnt5 phenotypes and suppresses the ability of Wnt11 to rescue the slb phenotype. Gain-of-function of Pk1 also inhibits convergent extension movements and enhances the slb phenotype, most likely caused by the ability of Pk1 to block the Fz7-dependent membrane localisation of Dsh by downregulating levels of Dsh protein. Furthermore, we show that pk1 interacts genetically with trilobite (tri)/strabismus to mediate the caudally directed migration of cranial motor neurons and convergent extension. These results indicate that, during zebrafish gastrulation Pk1 acts, in part, through interaction with the noncanonical Wnt11/Wnt5 pathway to regulate convergent extension cell movements, but is unlikely to simply be a linear component of this pathway. In addition, Pk1 interacts with Tri to mediate posterior migration of branchiomotor neurons, probably independent of the noncanonical Wnt pathway.
Descriptors: zebrafish embryos, cell movement physiology, gastrula metabolism, neurons metabolism, embryology, proteins, DNA binding proteins genetics, proteins genetics and proteins metabolism, *Drosophila* proteins genetics and metabolism, membrane proteins

metabolism, mesoderm metabolism, proto oncogene proteins metabolism, signal transduction physiology, Wnt proteins.

Catalano, A.E., P.A. Raymond, D. Goldman, and X. Wei (2007). **Zebrafish dou yan mutation causes patterning defects and extensive cell death in the retina.** *Developmental Dynamics* 236(5): 1295-306. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, mutation, retina abnormalities and pathology, embryology, genetics, brain embryology, cell death, cell proliferation, eye abnormalities abnormalities genetics and pathology, organ size genetics, phenotype.

Cau, E. and S.W. Wilson (2003). **Ash1a and Neurogenin1 function downstream of Floating head to regulate epiphysial neurogenesis**. *Development* 130(11): 2455-2466. ISSN: 0950-1991.
NAL Call Number: QL951.D38
Descriptors: zebrafish, cell fate, defects, developmental genetics, freshwater fish, helix-loop-helix proteins, neurogenesis, neurons, pattern formation, phenotypes, photoreceptors, transcription, transcription factors, *Danio rerio*, Ash1a protein, floating head protein, neurogenin1.

Cha, Y.I., S.H. Kim, L. Solnica-Krezel, and R.N. DuBois (2005). **Cyclooxygenase-1 signaling is required for vascular tube formation during development**. *Developmental Biology* 282(1): 274-283. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, antisense oligonucleotides, arachidonic acid, circulatory system, cyclooxygenase-1, cyclooxygenase-2, defects, embryogenesis, embryonic development, physiology, gastrulation, gene expression, kidneys, mesoderm, oligonucleotides, prostaglandin E2, prostaglandins, signal transduction, translation, uterus, vascular system, prostaglandin H2, prostaglandin endoperoxide synthase, somitogenesis, *Danio rerio*.

Cha, Y.I., L. Solnica-Krezel, and R.N. Dubois (2006). **Fishing for prostanoids: deciphering the developmental functions of cyclooxygenase-derived prostaglandins**. *Developmental Biology* 289(2): 263-272. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Prostaglandin G/H synthases (PGHS), commonly referred to as cyclooxygenases (COX-1 and COX-2), catalyze a key step in the synthesis of biologically active prostaglandins (PGs), the conversion of arachidonic acid (AA) into prostaglandin H-2 (PGH(2)). PGs have important functions in a variety of physiologic and pathologic settings, including inflammation, cardiovascular homeostasis, reproduction, and carcinogenesis. However, an evaluation of prostaglandin function in early development has been difficult due to the maternal contribution of prostaglandins from the uterus. The emergence of zebrafish as a model system has begun to provide some insights into the roles of this signaling cascade during vertebrate development. In zebrafish, COX-1 derived prostaglandins are required for two distinct stages of development, namely during gastrulation and segmentation. During gastrulation, PGE(2) Signaling promotes cell motility, without altering the cell shape or directional migration of gastrulating cells. During segmentation, COX-1 signaling is also required for posterior mesoderm development, including the formation of vascular tube structures, angiogenesis of intersomitic vessels, and pronephros morphogenesis. We propose that deciphering the role for prostaglandin signaling in zebrafish development could yield insight and ultimately address the mechanistic details underlying various disease processes that result from perturba-

tion of this pathway. (c) 2005 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, cardiovascular system, circulation, reproductive system.

Chai, C., Y.W. Liu, and W.K. Chan (2003). **Ff1b is required for the development of steroidogenic component of the zebrafish interrenal organ.** *Developmental Biology* 260(1): 226-44. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The zebrafish ftz-f1 gene, ff1b, is activated in two cell clusters lateral to the midline in the trunk during late embryogenesis. These cell clusters coalesce to form a discrete organ at around 30 hpf, which then begins to acquire a steroidogenic identity as evidenced by the expression of the steroidogenic enzyme genes, cyp11a and 3beta-hsd. The migration of the cell clusters to the midline is impaired in zebrafish midline signaling mutants. Knockdown of Ff1b activity by antisense ff1b morpholino oligonucleotide (ff1bMO) leads to phenotypes that are consistent with impaired osmoregulation. Injection of ff1bMO was also shown to downregulate the expression of cyp11a and 3beta-hsd. Histological comparison of wild-type and ff1b morphants at various embryonic and juvenile stages revealed the absence of interrenal tissue development in ff1b morphants. The morphological defects of ff1b morphants could be mimicked by treatment with aminoglutethimide, an inhibitor of de novo steroid synthesis. Based on these data, we propose that ff1b is required for the development of the steroidogenic tissue of the interrenal organ.

Descriptors: zebrafish embryos, interrenal gland growth and development, embryology, aminoglutethimide pharmacology, body patterning, cell movement, cholesterol side chain cleavage enzyme genetics and metabolism, enzyme inhibitors pharmacology, gene expression regulation interrenal gland drug effects, interrenal gland physiology, mutation, antisense oligonucleotides pharmacology, recombinant fusion proteins metabolism, trans-activation genetics and metabolism, water electrolyte balance drug effects, proteins drug effects.

Challa, A.K., M.L. McWhorter, C. Wang, M.A. Seeger, and C.E. Beattie (2005). **Robo3 isoforms have distinct roles during zebrafish development.** *Mechanisms of Development* 122(10): 1073-86. ISSN: 0925-4773.

NAL Call Number: QH607.A1C4

Abstract: Roundabout (Robo) receptors and their secreted ligand Slits have been shown to function in a number of developmental events both inside and outside of the nervous system. We previously cloned zebrafish robo orthologs to gain a better understanding of Robo function in vertebrates. Further characterization of one of these orthologs, robo3, has unveiled the presence of two distinct isoforms, robo3 variant 1 (robo3var1) and robo3 variant 2 (robo3var2). These two isoforms differ only in their 5'-ends with robo3var1, but not robo3var2, containing a canonical signal sequence. Despite this difference, both forms accumulate on the cell surface. Both isoforms are contributed maternally and exhibit unique and dynamic gene expression patterns during development. Functional analysis of robo3 isoforms using an antisense gene knockdown strategy suggests that Robo3var1 functions in motor axon pathfinding, whereas Robo3var2 appears to function in dorsoventral cell fate specification. This study reveals a novel function for Robo receptors in specifying ventral cell fates during vertebrate development.

Descriptors: zebrafish embryos, *Drosophila*, nervous system embryology, immunologic receptors physiology, embryology, Drosophila embryonic development genetics, mutation, nervous system chemistry, protein isoforms genetics, protein isoforms physiology, messenger RNA analysis and metabolism, immunologic receptors genetics.

Chan, B., S. Sinha, D. Cho, R. Ramchandran, and V.P. Sukhatme (2005). **Critical roles of CD146 in zebrafish vascular development.** *Developmental Dynamics* 232(1): 232-44. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: In this report, we use zebrafish as a model system to understand the importance of CD146 in vascular development. Endothelial-specific expression of CD146 was verified by whole-mount in situ hybridization. Suppression of CD146 protein expression by antisense morpholino oligonucleotides (MO) resulted in poorly developed intersomitic vessels (ISVs). In CD146 morphants, we observed a lack of blood flow through the ISV region, despite that fluorescence microangiography showed that the ISVs were present. This finding suggests that the lumens of the developing ISVs may be too narrow for proper circulation. Additionally, remodeling of the caudal vein plexus into functional vascular tubes appeared to be affected. Suppression of CD146 protein expression resulted in a circulation shunt that caused the circulation to by-pass part of the caudal artery/vein system. The same vascular defects were recapitulated by using a second morpholino oligonucleotide. This morphant expressed a truncated CD146 protein with amino acids V32 to T57 at the N terminus deleted in an in-frame manner. This region, therefore, is likely to contain elements critical for CD146 function. This study provides the first in vivo functional assessment of CD146 in embryonic development by showing that knockdown of CD146 protein expression severely hinders vascular development in zebrafish.

Descriptors: zebrafish embryo, antigens, CD physiology, blood vessels embryology, vascular endothelium embryology, developmental gene expression regulation, neural cell adhesion molecules physiology, amino acid sequence, angiography, antigens, cd metabolism, antigens, CD146, base sequence, molecular cloning, complementary DNA metabolism, in situ hybridization, fluorescence microscopy, molecular sequence data, neural cell adhesion molecules metabolism, oligonucleotides chemistry, tertiary protein structure, messenger RNA metabolism, reverse transcriptase PCR, amino acid sequence homology, time factors.

Chandrasekhar, A. (2004). **Turning heads: development of vertebrate branchiomotor neurons.** *Developmental Dynamics* 229(1): 143-161. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: The cranial motor neurons innervate muscles that control eye, jaw, and facial movements of the vertebrate head and parasympathetic neurons that innervate certain glands and organs. These efferent neurons develop at characteristic locations in the brainstem, and their axons exit the neural tube in well-defined trajectories to innervate target tissues. This review is focused on a subset of cranial motor neurons called the branchiomotor neurons, which innervate muscles derived from the branchial (pharyngeal) arches. First, the organization of the branchiomotor pathways in zebrafish, chick, and mouse embryos will be compared, and the underlying axon guidance mechanisms will be addressed. Next, the molecular mechanisms that generate branchiomotor neurons and specify their identities will be discussed. Finally, the caudally directed or tangential migration of facial branchiomotor neurons will be examined. Given the advances in the characterization and analysis of vertebrate genomes, we can expect rapid progress in elucidating the cellular and molecular mechanisms underlying the development of these vital neuronal networks.
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Descriptors: zebrafish, development, muscular system, nervous system, facial movement, genome, neural networks, zebrafish.

Chang, L.L. and D.S. Kessler (2005). **Foxd3 regulation of mesoderm induction in the zebrafish embryo.** *Developmental Biology* 283(2): 619-620. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, development, somitogenesis, gastrulation, mesoderm, zebrafish embryo .

Chapouton, P., B. Adolf, C. Leucht, B. Tannhauser, S. Ryu, W. Driever, and L. Bally Cuif (2006). **Her5 expression reveals a pool of neural stem cells in the adult zebrafish midbrain.** *Development* 133(21): 4293-303. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Current models of vertebrate adult neural stem cells are largely restricted to the rodent forebrain. To extract the general mechanisms of neural stem cell biology, we sought to identify new adult stem cell populations, in other model systems and/or brain areas. The teleost zebrafish appears to be an ideal system, as cell proliferation in the adult zebrafish brain is found in many more niches than in the mammalian brain. As a starting point towards identifying stem cell populations in this system, we used an embryonic neural stem cell marker, the E(spl) bHLH transcription factor Her5. We demonstrate that her5 expression is not restricted to embryonic neural progenitors, but also defines in the adult zebrafish brain a new proliferation zone at the junction between the mid- and hindbrain. We show that adult her5-expressing cells proliferate slowly, self-renew and express neural stem cell markers. Finally, using in vivo lineage tracing in her5:gfp transgenic animals, we demonstrate that the her5-positive population is multipotent, giving rise in situ to differentiated neurons and glia that populate the basal midbrain. Our findings conclusively identify a new population of adult neural stem cells, as well as their fate and their endogenous environment, in the intact vertebrate brain. This cell population, located outside the forebrain, provides a powerful model to assess the general mechanisms of vertebrate neural stem cell biology. In addition, the first transcription factor characteristic of this cell population, Her5, points to the E(Spl) as a promising family of candidate adult neural stem cell regulators.
Descriptors: zebrafish, basic helix loop helix transcription factors metabolism, mesencephalon cytology, neurons physiology, stem cells physiology, metabolism, genetically modified, basic helix loop helix transcription factors genetics, biological markers metabolism, cell proliferation, mesencephalon metabolism, neurons cytology, recombinant fusion proteins metabolism, stem cells cytology.

Chatterjee, B., A.J. Chin, G. Valdimarsson, C. Finis, J.M. Sonntag, B.Y. Choi, L. Tao, K. Balasubramanian, C. Bell, A. Krufka, D.J. Kozlowski, R.G. Johnson, and C.W. Lo (2005). **Developmental regulation and expression of the zebrafish connexin43 gene.** *Developmental Dynamics* 233(3): 890-906. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: We cloned and sequenced the zebrafish (*Danio rerio*) connexin43 (Cx43alpha1) gene. The predicted protein sequence shows a high degree of sequence conservation. Transcript analyses revealed multiple transcription start sites and a potential alternative transcript encoding a N-terminally truncated Cx43alpha1 protein. Maternal Cx43alpha1 transcripts were detected, with zygotic expression initiated before gastrulation. In situ hybridization revealed many Cx43alpha1 expression domains, including the notochord and brain, heart and vasculature, many resembling patterns seen in mammalian embryos. Of interest, a reporter construct under control of the mouse Cx43alpha1 promoter was observed to drive green fluorescent protein expression in zebrafish embryos in domains mimicking the native Cx43alpha1 expression pattern in fish and mice. Sequence comparison between the mouse

and zebrafish Cx43alpha1 promoter sequences showed the conservation of several transcription factor motifs, which otherwise shared little overall sequence homology. The conservation of protein sequence and developmental gene regulation would suggest that Cx43alpha1 gap junctions are likely to have conserved roles in vertebrate embryonic development.
Descriptors: zebrafish, mice, connexin 43 genetics, connexin 43 metabolism, developmental gene expression regulation, amino acid and base sequence, molecular cloning, connexin 43 chemistry, conserved sequence genetics, complementary DNA genetics, gene expression profiling, genomics, in situ hybridization, molecular sequence data, phylogeny, promoter regions genetics, messenger RNA genetics, messenger RNA metabolism, sequence alignment, transcription initiation site, metabolism.

Cheah, F.S., E.W. Jabs, and S.S. Chong (2005). **Genomic, cDNA, and embryonic expression analysis of zebrafish transforming growth factor beta 3 (tgfbeta3).** *Developmental Dynamics* 232(4): 1021-30. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, developmental gene expression regulation physiology, transforming growth factor beta biosynthesis, proteins biosynthesis, base sequence, complementary DNA genetics, genome, molecular sequence data, transforming growth factor beta genetics, transforming growth factor beta3, proteins genetics.

Cheesman, S.E., M.J. Layden, T. Von Ohlen, C.Q. Doe, and J.S. Eisen (2004). **Zebrafish and fly Nkx6 proteins have similar CNS expression patterns and regulate motoneuron formation.** *Development* 131(21): 5221-32. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Genes belonging to the Nkx, Gsh and Msx families are expressed in similar dorsovental spatial domains of the insect and vertebrate central nervous system (CNS), suggesting the bilaterian ancestor used this genetic program during CNS development. We have investigated the significance of these similar expression patterns by testing whether Nkx6 proteins expressed in ventral CNS of zebrafish and flies have similar functions. In zebrafish, Nkx6.1 is expressed in early-born primary and later-born secondary motoneurons. In the absence of Nkx6.1, there are fewer secondary motoneurons and supernumerary ventral interneurons, suggesting Nkx6.1 promotes motoneuron and suppresses interneuron formation. Overexpression of fish or fly Nkx6 is sufficient to generate supernumerary motoneurons in both zebrafish and flies. These results suggest that one ancestral function of Nkx6 proteins was to promote motoneuron development.
Descriptors: zebrafish, central nervous system metabolism, Drosophila proteins metabolism, Drosophila melanogaster metabolism, developmental gene expression regulation, motor neurons metabolism, transcription factors metabolism, amino acid sequence, body patterning, central nervous system cytology, conserved sequence, Drosophila proteins chemistry, Drosophila Drosophila melanogaster cytology, Drosophila melanogaster embryology, Drosophila melanogaster genetics, Hedgehog proteins, interneurons cytology, interneurons metabolism, molecular sequence data, motor neurons cytology, phylogeny, sequence alignment, signal transduction, trans activators metabolism, transcription factors chemistry, transcription factors genetics, proteins chemistry.

Chen, E., S.E. Stringer, M.A. Rusch, S.B. Selleck, and S.C. Ekker (2005). **A unique role for 6-O sulfation modification in zebrafish vascular development.** *Developmental Biology* 284(2): 364-76. ISSN: 0012 1606.
NAL Call Number: 442.8D49

Abstract: Heparan sulfate proteoglycans are important modulators of growth factor signaling in a variety of patterning processes. Secreted growth factors that play critical roles in angiogenesis bind to heparan sulfate, and this association is affected by 6-O-sulfation of the heparan sulfate chains. Addition of 6-O-sulfate is catalyzed by a family of sulfotransferases (HS6STs), and genetic manipulation of their function permits an assessment of their contribution to vascular assembly. We report on the biochemical activity and expression patterns of two zebrafish HS6ST genes. In situ hybridization reveals dynamic and distinct expression patterns of these two genes during development. Structural analysis of heparan sulfate from wild-type and morpholino antisense 'knockdown' embryos suggests that HS6ST-1 and HS6ST-2 have similar biochemical activity. HS6ST-2, but not HS6ST-1, morphants exhibit abnormalities in the branching morphogenesis of the caudal vein during embryonic development of the zebrafish. Our finding that HS6ST-2 is required for the branching morphogenesis of the caudal vein is the first in vivo evidence for an essential role of a gene encoding a heparan sulfate modifying enzyme in vertebrate angiogenesis. Our analysis of two zebrafish HS6ST genes suggests that a wide range of biological processes may be regulated by an array of sulfation-modifying enzymes in the vertebrate genome.
Descriptors: zebrafish, blood vessels growth and development, developmental gene expression regulation, sulfotransferases metabolism, amino acid sequence, genetically modified, blood vessels embryology, molecular cloning embryonic development, expressed sequence tags, green fluorescent proteins metabolism, heparitin sulfate metabolism, in situ hybridization, microinjections, molecular sequence data, antisense oligonucleotides pharmacology, phylogeny, DNA sequence analysis, amino acid sequence homology, somites metabolism, sulfotransferases antagonists and inhibitors, sulfotransferases chemistry, sulfotransferases genetics, sulfotransferases isolation and purification.

Chen, W. and D. Casey Corliss (2004). **Three modules of zebrafish Mind bomb work cooperatively to promote Delta ubiquitination and endocytosis.** *Developmental Biology* 267(2): 361-73. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Precise regulation of Notch signaling activity is critical for development of many different tissues. Here, we show that the zebrafish insertional mutation Hi904 attenuates Notch signaling, and is allelic to mind bomb. We show that Mind bomb protein displays E3 ubiquitin ligase activity in vitro and that it is associated with Delta and enhances its ubiquitination and internalization in transfected cells. Furthermore, by functional analysis of three conserved regions of Mind bomb, we show that the N-terminal half is required for Delta association, the ankyrin repeats are important for Delta internalization, and the ring fingers are required for Delta ubiquitination. Thus, the three functionally distinct modules of Mind bomb work cooperatively to regulate Notch signaling by associating with, ubiquitinating, and internalizing Delta.
Descriptors: zebrafish, endocytosis, membrane proteins metabolism, ubiquitin protein ligases metabolism, ubiquitins metabolism, proteins metabolism, amino acid sequence, DNA primers , complementary DNA genetics, fluorescent antibody technique, immunoblotting, immunohistochemistry, in situ hybridization, molecular sequence data, plasmids, precipitin tests, Notch receptors, sequence alignment, signal transduction physiology, ubiquitin protein ligases genetics.

Chen, Y.H., H.C. Lee, C.F. Liu, C.Y. Lin, and H.J. Tsai (2003). **Novel regulatory sequence -82/-62 functions as a key element to drive the somite-specificity of zebrafish myf-5.** *Developmental Dynamics* 228(1): 41-50. ISSN: 1058 8388.

NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, DNA binding proteins genetics, proteins, muscle proteins metabolism, promoter regions genetics, somites metabolism, trans-activators, transcription factors metabolism, embryology, genetically modified, base sequence, cytomegalovirus genetics, developmental gene expression regulation, regulator genes, reporter genes, green fluorescent proteins, luminescent proteins metabolism, muscle proteins chemistry, skeletal muscle growth and development, site directed mutagenesis myogenic regulatory factor 5, recombinant fusion proteins metabolism, sequence deletion, time factors, transcription factors chemistry.

Chen Yau, H., H. Wang Yun, Chang MinYen, Y. Lin Cheng, W. Weng Chih, M. Westerfield, and J. Tsai Huai (2007). **Multiple upstream modules regulate zebrafish myf5 expression**. *Bio Med Central Developmental Biology* 7(1) ISSN: 1471 213X.
Online: http://www.biomedcentral.com/content/pdf/1471-213X-7-1.pdf
NAL Call Number: QH491.B63
Abstract: Background: Myf5 is one member of the basic helix-loop-helix family of transcription factors, and it functions as a myogenic factor that is important for the specification and differentiation of muscle cells. The expression of myf5 is somite- and stage-dependent during embryogenesis through a delicate regulation. However, this complex regulatory mechanism of myf5 is not clearly understood. Results: We isolated a 156-kb bacterial artificial chromosome clone that includes an upstream 80-kb region and a downstream 70-kb region of zebrafish myf5 and generated a transgenic line carrying this 156-kb segment fused to a green fluorescent protein (GFP) reporter gene. We find strong GFP expression in the most rostral somite and in the presomitic mesoderm during segmentation stages, similar to endogenous myf5 expression. Later, the GFP signals persist in caudal somites near the tail bud but are down-regulated in the older, rostral somites. During the pharyngula period, we detect GFP signals in pectoral fin buds, dorsal rostral myotomes, hypaxial myotomes, and inferior oblique and superior oblique muscles, a pattern that also corresponds well with endogenous myf5 transcripts. To characterize the specific upstream cis-elements that regulate this complex and dynamic expression pattern, we also generated several transgenic lines that harbor various lengths within the upstream 80-kb segment. We find that (1) the -80 kb/-9977 segment contains a fin and cranial muscle element and a notochord repressor; (2) the -9977/-6213 segment contains a strong repressive element that does not include the notochord-specific repressor; (3) the -6212/-2938 segment contains tissue-specific elements for bone and spinal cord; (4) the -2937/-291 segment contains an eye enhancer, and the -2937/-2457 segment is required for notochord and myocyte expression; and (5) the -290/-1 segment is responsible for basal transcription in somites and the presomitic mesoderm. Conclusion: We suggest that the cell lineage-specific expression of myf5 is delicately orchestrated by multiple modules within the distal upstream region. This study provides an insight to understand the molecular control of myf5 and myogenesis in the zebrafish. Copyright © 2008 CABI
Descriptors: zebrafish, bacterial artificial chromosomes, cell lines, embryonic development, embryos, gene expression, genes, tissue distribution, transcription, transcription factors, *Danio rerio* .

Cheng, C.W., C.H. Yan, S.W. Choy, M.N. Hui, C.C. Hui, and S.H. Cheng (2007). **Zebrafish homologue irx1a is required for the differentiation of serotonergic neurons.** *Developmental Dynamics* 236(9): 2661-7. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, developmental gene expression regulation, homeodomain, transcrip-

tion factors physiology, acridine orange pharmacology, apoptosis, developmental biology methods, fluorescent dyes pharmacology, homeodomain proteins metabolism, in situ hybridization, neurons metabolism, neurotransmitter agents metabolism, serotonin metabolism, transcription factors metabolism.

Cheng, C.W., C.H. Yan, C.C. Hui, U. Strahle, and S.H. Cheng (2006). **The homeobox gene irx1a is required for the propagation of the neurogenic waves in the zebrafish retina.** *Mechanisms of Development* 123(3): 252-63. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: Neurogenesis in the compound eyes of *Drosophila* and the camera eyes of vertebrates spreads in a wave-like fashion. In both phyla, waves of hedgehog expression are known to drive the wave of neuronal differentiation. The mechanism controlling the propagation of hedgehog expression during retinogenesis of the vertebrate eye is poorly understood. The Iroquois homeobox genes play important roles in Drosophila eye development; they are required for the up-regulation of hedgehog expression during propagation of the morphogenetic furrow. Here, we show that the zebrafish Iroquois homolog irx1a is expressed during retinogenesis and knockdown of irx1a results in a retinal phenotype strikingly similar to those of sonic hedgehog (shh) mutants. Analysis of shh-GFP transgene expression in irx1a knockdown retinas revealed that irx1a is required for the propagation of shh expression through the retina. Transplantation experiments illustrated that the effects of irx1a on shh expression are both cell-autonomous and non-cell-autonomous. Our results reveal a role for Iroquois genes in controlling hedgehog expression during vertebrate retinogenesis.
Descriptors: zebrafish embryos, homeodomain retina embryology, retinal ganglion cells metabolism, transcription factors genetics, proteins and genetics, genetically modified, cell differentiation, eye diseases genetics, metabolism, Fgf metabolism, developmental gene expression regulation, Hedgehogs genetics and metabolism, homeodomain proteins metabolism, retina cytology, retina metabolism, signal transduction, transcription factors metabolism.

Cheng, J.C., A.L. Miller, and S.E. Webb (2004). **Organization and function of microfilaments during late epiboly in zebrafish embryos.** *Developmental Dynamics* 231(2): 313-23. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryo, nonmammalian anatomy and histology, drug effects, physiology, microfilaments metabolism, morphogenesis, actins metabolism, cytochalasin B pharmacology, cytoskeleton metabolism, egtazic acid analogs and derivatives, egtazic acid chemistry and pharmacology.

Cheng, K.C., R. Levenson, and J.D. Robishaw (2003). **Functional genomic dissection of multimeric protein families in zebrafish.** *Developmental Dynamics* 228(3): 555-67. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, mice, genomics, genetics, heterotrimeric GTP binding transgenic, genetic models, Na+ K+ exchanging ATPase enzyme genetics, protein subunits genetics, signal transduction, proteins classification.

Cheng, L., X.F. Guo, X.Y. Yang, M. Chong, J. Cheng, G. Li, Y.H. Gui, and D.R. Lu (2006). **Delta-sarcoglycan is necessary for early heart and muscle development in zebrafish**. *Biochemical and Biophysical Research Communications* 344(4): 1290-1299. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: delta-sarcoglycan, one member of the sarcoglycan complex, is a very conservative muscle-specific protein exclusively expressed in the skeletal and cardiac muscles of vertebrates. Mutations in sarcoglycans are known to be involved in limb-girdle muscular dystrophy (LGMD) and dilated cardiomyopathy (DCM) in humans. To address the role of delta-sarcoglycan gene in zebrafish development, we have studied expression pattern of delta-sarcoglyean in zebrafish embryos and examined the role of delta-sarcoglyean in zebrafish embryonic development by morpholino. Strong expression of delta-sarcoglycan was observed in various muscles including those of the segment, heart, eye, jaw, pectoral fin, branchial arches, and swim bladder in zebrafish embryo. delta-sarcoglycan was also expressed in midbrain and retina. Knockdown of delta-sarcoglyean resulted in severe abnormality in both the cardiac and skeletal muscles. Some severe ones displayed serious morphological abnormality such as hypoplastic head, linear heart, very weak heartbeats, and runtish trunk, all dead within 5 dpf. Whole-mount in situ hybridization analysis showed that adaxial cells and muscle pioneers were affected in delta-sarcoglycan knockdown embryos. In addition, absence of delta-sarcoglycan protein severely delayed the cardiac development and influenced the differentiation of cardiac muscle, and the cardiac left-right asymmetry was dramatically changed in morpholino-treated embryos. These data together suggest that delta-sarcoglycan plays an important role in early heart and muscle development. (c) 2006 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryo, freshwater ecology, environmental sciences, molecular genetics, biochemistry and molecular biophysics, cell development, heart and muscle development.

Cheng, S., T. Christie, and G. Valdimarsson (2003). **Expression of connexin48.5, connexin44.1, and connexin43 during zebrafish (*Danio rerio*) lens development.** *Developmental Dynamics* 228(4): 709-15. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish, connexin 43 biosynthesis, connexins biosynthesis, developmental gene expression regulation, crystalline lens metabolism, proteins biosynthesis, chickens, DNA primers pharmacology, gap junctions, in situ hybridization, mice, phylogeny, messenger RNA metabolism, reverse transcriptase PCR, time factors, tissue distribution.

Cheng, W., L. Guo, Z. Zhang, H.M. Soo, C. Wen, W. Wu, and J. Peng (2006). **HNF factors form a network to regulate liver-enriched genes in zebrafish.** *Developmental Biology* 294(2): 482-96. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Defects in some of liver-enriched genes in mammals will cause liver- and/or blood-related diseases. However, due to the fact that embryogenesis happens intrauterinally in the mammals, the function of these liver-enriched genes during liver organogenesis is poorly studied. We report here the identification of 129 genuine liver-enriched genes in adult zebrafish and show that, through in situ hybridization, 69 of these genes are also enriched in the embryonic liver. External embryogenesis coupled with the well-established morpholino-mediated gene knock-down technique in zebrafish offers us a unique opportunity to study if this group of genes plays any role during liver organogenesis in the future. As an example, preliminary study using morpholino-mediated gene knock-down method revealed that a novel liver-enriched gene leg1 is crucial for the liver expansion growth. We also report the analysis of promoter regions of 51 liver-enriched genes by searching putative binding sites for Hnf1, Hnf3, Hnf4 and Hnf6, four key transcription factors enriched in the liver. We found

that promoter regions of majority of liver-enriched genes contain putative binding sites for more than one HNF factors, suggesting that most of liver-enriched genes are likely co-regulated by different combination of HNF factors. This observation supports the hypothesis that these four liver-enriched transcription factors form a network in controlling the expression of liver-specific or -enriched genes in the liver.

Descriptors: zebrafish embryos, developmental gene expression regulation, hepatocyte nuclear factors metabolism, liver physiology, embryology, genetics, proteins metabolism, amino acid sequence, anatomy and histology, physiology, gene expression profiling, hepatocyte nuclear factors genetics, in situ hybridization, liver embryology, molecular sequence data, oligonucleotide array sequence analysis, antisense oligonucleotides genetics, antisense oligonucleotides metabolism, promoter regions genetics, sequence alignment.

Cheng, Y.C., M. Amoyel, X. Qiu, Y.J. Jiang, Q. Xu, and D.G. Wilkinson (2004). **Notch activation regulates the segregation and differentiation of rhombomere boundary cells in the zebrafish hindbrain.** *Developmental Cell* 6(4): 539-50. ISSN: 1534 5807.

NAL Call Number: QH573.C42

Abstract: During segmentation of the vertebrate hindbrain, a distinct population of boundary cells forms at the interface between each segment. Little is known regarding mechanisms that regulate the formation or functions of these cells. We have investigated a potential role of Notch signaling and find that in the zebrafish hindbrain, radical fringe is expressed in boundary cells and delta genes are expressed adjacent to boundaries, consistent with a sustained activation of Notch in boundary cells. Mosaic expression experiments reveal that activation of the Notch/Su(H) pathway regulates cell affinity properties that segregate cells to boundaries. In addition, Notch signaling correlates with a delayed neurogenesis at hindbrain boundaries and is required to inhibit premature neuronal differentiation of boundary cells. These findings reveal that Notch activation couples the regulation of location and differentiation in hindbrain boundary cells. Such coupling may be important for these cells to act as a stable signaling center.

Descriptors: zebrafish embryo, cell surface receptors metabolism, rhombencephalon embryology, stem cells metabolism, transcription factors, proteins, body patterning genetics, cell differentiation genetics, cell movement genetics, cytology, membrane membrane proteins metabolism, molecular sequence data, neurons cytology, neurons metabolism, Notch1 receptor, cell surface receptors genetics, rhombencephalon cytology, rhombencephalon metabolism, signal transduction genetics, stem cells cytology.

Cherdantseva, E.M. and V.G. Cherdantsev (2006). **Geometry and mechanics of teleost gastrulation and the formation of primary embryonic axes**. *International Journal of Developmental Biology* 50(2-3): 157-168. ISSN: 0214 6282.

Abstract: Examination of normal shaping dynamics and immediate and long-term responses to blastoderm cutting in zebrafish and loach embryos prior to the onset of gastrulation and during the course of epiboly revealed that anteroposterior (AP) and dorsoventral (DV) polarity formation is connected with shaping of the blastoderm circumferential region, which stretches along and shrinks across its movement axes and originates the non-isotropic fields of tensile stresses. Based on data from cutting experiments and quantitative morphology, we reconstructed the movement-shaping patterns of epiboly and embryonic shield formation. We revealed that AP and DV axes originate as a mass cell movement subject to the movement-shaping equivalence principle, which means the spatial series of differently shaped areas corresponding to the time succession of the same area shaping. Maintenance of the main body axes in orthogonal orientation depends on the mechanical equilibrium principle

allowing for converting shape asymmetry into that of tensile stresses and vice versa. The causal relationship between the main movement-shaping axes and that of embryonic polarity was proved in cutting experiments in which the DV axis direction was subject to rearrangement so as to adjust to the new direction of mass cell movement axes induced by healing the wound in the blastoderm circumferential region. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, membranes, cell biology, morphogenesis, gastrulation, tensile stress, embryonic axis formation.

Chittenden, T.W., F. Claes, A. Lanahan, M. Autiero, R.T. Palac, E.V. Tkachenko, A. Elfenbein, C.R. DeAlmodovar, E. Dedkov, R. Tomanek, W. Li, M. Westmore, J. Singh, A. Horowitz, M.J. Mulligan-Kehoe, K.L. Moodie, Z.W. Zhuang, P. Carmeliet, and M. Simons (2006). **Selective regulation of arterial branching morphogenesis by synectin**. *Developmental Cell* 10(6): 783-795. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Branching morphogenesis is a key process in the formation of vascular networks. To date, little is known regarding the molecular events regulating this process. We investigated the involvement of synectin in this process. In zebrafish embryos, synectin knockdown resulted in a hypoplastic dorsal aorta and hypobranched, stunted, and thin intersomitic vessels due to impaired migration and proliferation of angioblasts and arterial endothelial cells while not affecting venous development. Synectin(-/-) mice demonstrated decreased body and organ size, reduced numbers of arteries, and an altered pattern of arterial branching in multiple vascular beds while the venous system remained normal. Murine synectin(-/-) primary arterial, but not venous, endothelial cells showed decreased in vitro tube formation, migration, and proliferation and impaired polarization due to abnormal localization of activated Rac1. We conclude that synectin is involved in selective regulation of arterial, but not venous, growth and and that Rac1 plays an important role in this process. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, cell biology, cell proliferation, branching morphogenesis, murine synectin, branching morphogenesis, Rac1.

Chocron, S., M.C. Verhoeven, F. Rentzsch, M. Hammerschmidt, and J. Bakkers (2007). **Zebrafish Bmp4 regulates left-right asymmetry at two distinct developmental time points.** *Developmental Biology* 305(2): 577-88. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Left-right (LR) asymmetry is regulated by early asymmetric signals within the embryo. Even though the role of the bone morphogenetic protein (BMP) pathway in this process has been reported extensively in various model organisms, opposing models for the mechanism by which BMP signaling operates still prevail. Here we show that in zebrafish embryos there are two distinct phases during LR patterning in which BMP signaling is required. Using transgenic lines that ectopically express either noggin3 or bmp2b, we show a requirement for BMP signaling during early segmentation to repress southpaw expression in the right lateral plate mesoderm and regulate both visceral and heart laterality. A second phase was identified during late segmentation, when BMP signaling is required in the left lateral plate mesoderm to regulate left-sided gene expression and heart laterality. Using morpholino knock down experiments, we identified Bmp4 as the ligand responsible for both phases of BMP signaling. In addition, we detected bmp4 expression in Kupffer's vesicle and show that restricted knock down of bmp4 in this structure results in LR patterning defects. The identification of these two distinct and opposing activities of BMP signaling provides new insight into how BMP signaling can regulate LR patterning.

Descriptors: zebrafish embryos, body patterning physiology, bone morphogenetic, Type I activin receptors physiology, genetically modified, bone morphogenetic proteins deficiency, bone morphogenetic functional laterality genetics, heart embryology, signal transduction genetics, proteins deficiency.

Choi, J., L. Dong, J. Ahn, D. Dao, M. Hammerschmidt, and J.N. Chen (2007). **FoxH1 negatively modulates flk1 gene expression and vascular formation in zebrafish.** *Developmental Biology* 304(2): 735-44. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Flk1 is the major receptor for VEGF on endothelial cells. During embryogenesis, flk1 is required for both vasculogenesis and angiogenesis and abnormally elevated flk1 expression is often associated with pathological conditions in adults. While the biological function of flk1 has been studied extensively, very little is known about how the flk1 gene is regulated at the transcriptional level. Our transgenic study led to the identification of a flk1 endothelial enhancer positioned approximately 5 kb upstream of the flk1 translation initiation site. Binding sites for FoxH1, scl, ets and gata factors are found in the zebrafish flk1 endothelial enhancer, as well as in upstream sequences of mouse flk1 and human kdr genes, suggesting that the regulatory machinery for flk1/kdr is conserved from fish to mammals. The roles of scl, ets and gata factors in hemangioblasts have been well defined, but the significance of FoxH1 in vessel formation has not been explored previously. Here we show that FoxH1 binds to the flk1 endothelial enhancer in vitro and functions as a repressor for flk1 transcription in cultured cells. Consistent with these findings, the expression level of flk1 is elevated in embryos lacking both maternal and zygotic FoxH1. We further show that overexpression of FoxH1 has a negative effect on vascular formation that can be counteracted by the down-regulation of smad2 activity in zebrafish embryos. Taken together, our data provide the first evidence that flk1 is a direct target of FoxH1 and that FoxH1 is involved in vessel formation in zebrafish.
Descriptors: zebrafish embryos, blood vessels embryology, forkhead transcription factors biosynthesis, developmental gene expression regulation, vascular endothelial growth factor receptor 2 metabolism, proteins biosynthesis, genetically modified, base sequence, cultured cells, enhancer elements genetics, molecular sequence data, signal transduction, Smad2 protein metabolism, transforming growth factor beta metabolism, vascular endothelial growth factor receptor 2 genetics.

Chong, S.W., T.T. Nguyen, L.T. Chu, Y.J. Jiang, and V. Korzh (2005). **Zebrafish id2 developmental expression pattern contains evolutionary conserved and species-specific characteristics.** *Developmental Dynamics* 234(4): 1055-63. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, brain metabolism, developmental gene expression regulation, inhibitor of differentiation protein 2 metabolism, signal transduction physiology, amino acid and base sequence, cluster analysis, cryoultramicrotomy, DNA primers, complementary DNA genetics, inhibitor of differentiation protein 2 genetics, molecular sequence data, Notch receptors metabolism, reverse transcriptase PCR, DNA sequence analysis, signal transduction genetics, species specificity.

Chong, S.W., L.M. Nguyet, Y.J. Jiang, and V. Korzh (2007). **The chemokine Sdf-1 and its receptor Cxcr4 are required for formation of muscle in zebrafish.** *BioMed Central Developmental Biology* 7: 54. ISSN: 1471 213X.
NAL Call Number: QH491.B63

Abstract: BACKGROUND: During development cell migration takes place prior to differentiation of many cell types. The chemokine receptor Cxcr4 and its ligand Sdf1 are implicated in migration of several cell lineages, including appendicular muscles. RESULTS: We dissected the role of sdf1-cxcr4 during skeletal myogenesis. We demonstrated that the receptor cxcr4a is expressed in the medial-anterior part of somites, suggesting that chemokine signaling plays a role in this region of the somite. Previous reports emphasized co-operation of Sdf1a and Cxcr4b. We found that during early myogenesis Sdf1a co-operates with the second Cxcr4 of zebrafish - Cxcr4a resulting in the commitment of myoblast to form fast muscle. Disrupting this chemokine signal caused a reduction in myoD and myf5 expression and fast fiber formation. In addition, we showed that a dimerization partner of MyoD and Myf5, E12, positively regulates transcription of cxcr4a and sdf1a in contrast to that of Sonic hedgehog, which inhibited these genes through induction of expression of id2. CONCLUSION: We revealed a regulatory feedback mechanism between cxcr4a-sdf1a and genes encoding myogenic regulatory factors, which is involved in differentiation of fast myofibers. This demonstrated a role of chemokine signaling during development of skeletal muscles.
Descriptors: zebrafish embryos, Cxc chemokines genetics, developmental gene expression regulation, Cxcr4 receptors genetics, signal transduction, genetics, cell movement, immunohistochemistry, in situ hybridization, muscle development genetics, fast twitch muscle fibers physiology, reverse transcriptase PCR, transcription, growth and development.

Choo, B.G., I. Kondrichin, S. Parinov, A. Emelyanov, W. Go, W.C. Toh, and V. Korzh (2006). **Zebrafish transgenic enhancer TRAP line database (ZETRAP).** *BioMed Central Developmental Biology* 6: 5. ISSN: 1471 213X.
NAL Call Number: QH491.B63
Abstract: BACKGROUND: The zebrafish, *Danio rerio*, is used as a model organism to study vertebrate genetics and development. An effective enhancer trap (ET) in zebrafish using the Tol2 transposon has been demonstrated. This approach could be used to study embryogenesis of a vertebrate species in real time and with high resolution. DESCRIPTION: The information gathered during the course of systematic investigation of many ET transgenic lines have been collected and compiled in the form of an online database--the Zebrafish Enhancer TRAP lines database (ZETRAP). CONCLUSION: ZETRAP is a web-based system that provides data and information to the scientific community about the developmental, genetic and genomic aspects of transgenic zebrafish lines obtained using Tol2 transposon-mediated transgenesis. The current version (version 1.0) contains description of 27 ET lines that express EGFP in various organs and tissues, for example, heart, brain, notochord, gut, etc. It also includes information on insertion sites of the Tol2 transposon in these lines.
Descriptors: zebrafish enbryos, genetic databases, genetics, genetically modified embryology, genetically modified genetics, growth and development, DNA transposable elements, gene expression, green fluorescent proteins analysis, internet, embryology.

Chopra, S.S., H. Watanabe, T.P. Zhong, and D.M. Roden (2007). **Molecular cloning and analysis of zebrafish voltage-gated sodium channel beta subunit genes: implications for the evolution of electrical signaling in vertebrates.** *BioMed Central Evolutionary Biology* 7: 113. ISSN: 1471 2148.
NAL Call Number: QH359.B63
Abstract: BACKGROUND: Action potential generation in excitable cells such as myocytes and neurons critically depends on voltage-gated sodium channels. In mammals, sodium channels exist as macromolecular complexes that include a pore-forming alpha subunit and

1 or more modulatory beta subunits. Although alpha subunit genes have been cloned from diverse metazoans including flies, jellyfish, and humans, beta subunits have not previously been identified in any non-mammalian species. To gain further insight into the evolution of electrical signaling in vertebrates, we investigated beta subunit genes in the teleost *Danio rerio* (zebrafish). RESULTS: We identified and cloned single zebrafish gene homologs for beta1-beta3 (zbeta1-zbeta3) and duplicate genes for beta4 (zbeta4.1, zbeta4.2). Sodium channel beta subunit loci are similarly organized in fish and mammalian genomes. Unlike their mammalian counterparts, zbeta1 and zbeta2 subunit genes display extensive alternative splicing. Zebrafish beta subunit genes and their splice variants are differentially-expressed in excitable tissues, indicating tissue-specific regulation of zbeta1-4 expression and splicing. Co-expression of the genes encoding zbeta1 and the zebrafish sodium channel alpha subunit Nav1.5 in Chinese Hamster Ovary cells increased sodium current and altered channel gating, demonstrating functional interactions between zebrafish alpha and beta subunits. Analysis of the synteny and phylogeny of mammalian, teleost, amphibian, and avian beta subunit and related genes indicated that all extant vertebrate beta subunits are orthologous, that beta2/beta4 and beta1/beta3 share common ancestry, and that beta subunits are closely related to other proteins sharing the V-type immunoglobulin domain structure. Vertebrate sodium channel beta subunit genes were not identified in the genomes of invertebrate chordates and are unrelated to known subunits of the para sodium channel in *Drosophila*. CONCLUSION: The identification of conserved orthologs to all 4 voltage-gated sodium channel beta subunit genes in zebrafish and the lack of evidence for beta subunit genes in invertebrate chordates together indicate that this gene family emerged early in vertebrate evolution, prior to the divergence of teleosts and tetrapods. The evolutionary history of sodium channel beta subunits suggests that these genes may have played a key role in the diversification and specialization of electrical signaling in early vertebrates.
Descriptors: zebrafish, molecular evolution , sodium channels genetics, alternative splicing, molecular cloning, gene expression regulation.

Christie, T.L. and S. Childs (2007). **PlexinD1: A dual role in zebrafish angiogenesis and lymphangiogenesis?** *Developmental Biology* 306(1): 292. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, *Danio rerio*, PlexinD1, embryo developmnet.

Ciruna, B., A. Jenny, D. Lee, M. Mlodzik, and A.F. Schier (2006). **Planar cell polarity signalling couples cell division and morphogenesis during neurulation**. *Nature* 439(7073): 220-224. ISSN: 0028-0836.
NAL Call Number: 472 N21
Descriptors: zebrafish, abnormalities, cell division, central nervous system, congenital defects, convergence, embryonic development, freshwater fish, gene expression, morphogenesis, nervous system, neural stem cells, neural tube, polarity, polarization, signal transduction, Wnt protein, *Danio rerio*, neurulation, planar cell polarity signalling, Vangl2 protein.

Clelland, E.S., Q. Tan, A. Balofsky, R. Lacivita, and C. Peng (2007). **Inhibition of premature oocyte maturation: a role for bone morphogenetic protein 15 in zebrafish ovarian follicles.** *Endocrinology* 148(11): 5451-8. ISSN: 0013 7227.
NAL Call Number: 448.8EN2
Abstract: Bone morphogenetic protein-15 (BMP-15) is a member of the TGF-beta superfamily known to regulate ovarian functions in mammals. Recently, we cloned zebrafish BMP-15 (zfBMP-15) cDNA and demonstrated that it may play a role in oocyte maturation.

In this study, we further investigated the role of BMP-15 in zebrafish follicular development and oocyte maturation using an antiserum developed for zfBMP-15 and by microinjection of follicles with antisense zfBMP-15 N-morpholino oligonucleotides or an expression construct containing zfBMP-15 cDNA. Injection with antiserum caused a significant decrease in maturation-incompetent [insensitive to maturation-inducing hormone (MIH)] early growth phase follicles and a concomitant increase in mature follicles in vivo. In vitro maturation assays showed that incubation with antiserum resulted in a significant increase in oocyte maturation as compared with follicles incubated in preimmune serum or media control. Next, early growth phase follicles were collected and preincubated with either antiserum, preimmune serum, or medium control before treatment with MIH or human chorionic gonadotropin (hCG). Antiserum significantly increased oocyte maturation in response to MIH, but not to hCG, and enhanced basal maturation rate in longer-term incubations. Knockdown of BMP-15 in early growth stage follicles with a BMP-15 antisense oligonucleotide resulted in increased oocyte maturation, whereas microinjection of BMP-15 cDNA into oocytes significantly reduced MIH- and hCG-induced oocyte maturation in normally competent, mid-growth-phase follicles. Collectively, these findings suggest that BMP-15 modulates follicular growth and prevents premature oocyte maturation in zebrafish, in part, by suppressing the sensitivity of follicles to MIH.

Descriptors: zebrafish, intercellular signaling peptides and, oocytes cytology, oogenesis genetics, ovarian follicle cytology, genetically modified, cell size drug effects, gonadotropins pharmacology, hydroxyprogesterones pharmacology, immune sera pharmacology, intercellular signaling peptides and intercellular signaling peptides and proteins immunology, oocytes drug effects, oogenesis drug effects, ovarian follicle drug effects, growth and development, transfection , proteins pharmacology.

Clements, W.K. and D. Kimelman (2005). **LZIC regulates neuronal survival during zebrafish development.** *Developmental Biology* 283(2): 322-34. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Development of the brain and central nervous system is a complex process involving localized gene expression and regulated cell death and proliferation. Here, we describe a gene involved in neuronal survival, the zebrafish ortholog of the human lzic gene. Zebrafish lzic is expressed ubiquitously during early development and later becomes enriched in the developing brain. Using antisense morpholino oligonucleotides, we demonstrate that zebrafish lzic is required zygotically for the survival of distinct neuronal populations. LZIC is closely related to ICAT, a physiological inhibitor of the Wnt pathway that interacts physically and functionally with beta-catenin to prevent the transcription of Wnt target genes. LZIC's ICAT-homologous region is highly similar to ICAT with particular conservation of residues that are used by ICAT for beta-catenin-binding. Surprisingly, despite this high similarity, LZIC does not interact with beta-catenin in vitro or in vivo. Our results reveal that LZIC, a protein conserved in vertebrates, is required for neuronal survival in zebrafish.

Descriptors: zebrafish embryo, brain embryology, DNA binding proteins genetics, proteins metabolism, neurons physiology, embryology, proteins metabolism, amino acid sequence, brain cytology, brain metabolism, cell survival, conserved sequence, cytoskeletal proteins metabolism, DNA binding proteins genetics , intracellular signaling peptides and proteins, mice, molecular sequence data, antisense oligonucleotides genetics, protein binding, amino acid sequence homology, trans activators metabolism, *Xenopus* proteins, *Xenopus laevis* embryology, metabolism, beta catenin.

Codina, M., N. Montserrat, D. Garcia de la Serrana, C. Ralliere, J.C. Gabillard, I. Navarro , P.Y. Rescan, and J. Gutierrez (2006). **Role of insulin and IGFs in fish muscle development and quality**. *Archiv Fur Tierzucht* 49(Special Issue): 33-38. ISSN: 0003 9438.
NAL Call Number: 49 AR23
Abstract: In vivo and in vitro experiments were conducted to identify the possible relationships between insulin, insulin-like growth factors (IGFs) and the various transcription factors involved in cell proliferation and muscle growth (such as MyoD, myogenin and myostatin). Changes in the feeding regimen of rainbow trout (*Onchorhynchus mykiss*) altered the IGF system at the peptide and receptor level. The expression of myogenin and myostatin was also altered. IGF expression and muscle growth were partially restored by refeeding. In preliminary experiments in zebrafish (Danio rerio) muscle, ectopic overexpression of IGF-I increased MyoD and myogenin expression. By Western Blot, we studied the effects of insulin, IGF-I and IGF-II on the two main signalling pathways in muscle using primary cultures of rainbow trout and seabream (Sparus aurata) muscle cells. IGFs activated both pathways and their efficiency depended on the culture stage, and human and fish peptides had similar effect. Incubation with specific inhibitors showed that wortmannin decreased Akt phosphorylation stimulated by hIGF-II, while treatment with PD98059 reduced the activation of MAPK. The role of insulin and IGFs in metabolic processes was studied in seabream myocytes and compared with the results in trout. Both IGFs showed a similar effect on glucose uptake stimulation, which decreased when the cells were incubated with wortmannin, confirming that the PI3K/Akt pathway is important for this process in muscle. Insulin and IGFs also stimulated alanine uptake. To find the best markers for fish growth and quality, preliminary experiments were done using c-met. Its expression was high during the first stages of development and decreased as differentiation progressed. Copyright © 2008 CABI
Descriptors: zebrafish, biochemical pathways, biological development, cell culture, in vitro, insulin, insulin like growth factor, muscles, plane of nutrition, transcription factors, Pagrus aurata, rainbow trout, sea bream, trout.
Language of Text: German.

Cohen, S.M. and J. Brennecke (2006). **Mixed Messages in early development**. *Science* 312(5770): 65-66. ISSN: 0036-8075.
NAL Call Number: 470Sci2
Descriptors: zebrafish, development, embryonic development, fertilization, zebrafish eggs, freshwater fish, genomes, oogenesis, RNA, reviews, mRNA turnover, miRNA, *Danio rerio*.
Notes: A review article.

Connolly, M.H. and B.K. Hall (2008). **Embryonic heat shock reveals latent hsp90 translation in zebrafish (*Danio rerio*).** *International Journal of Developmental Biology, The* 52(1): 71-9. ISSN: 0214-6282.
Abstract: There is increasing evidence that more genetic variation is present among metazoans than is normally expressed in the phenotype, due in part to the canalization of development. Among teleosts (as in other vertebrates), this genetic variation is often expressed as phenotypic change in response to environmental cues. Using embryonic zebrafish (*Danio rerio*), this 'hidden' variation is explored in the context of environmental stress by investigating the activity of heat shock protein-90 (HSP90), a cytosolic chaperone that interacts with transcription factors to mediate multiple developmental pathways. Following a 37C heat shock during early somitogenesis (1 hour heat shock, targeting 2-14 somite stages), phenotypic variability was expressed in the lower trunk and tail bud regions, where somite development was reduced or ceased prematurely. In situ hybridization showed

that hsp90 was localized to this caudal region 16 hours after heat shock, indicating its potential to coordinate somitic fate. By following transcription and translation of this chaperone, we show that 24 hours following heat shock zebrafish embryos express a protein signature which reflects the RNA message. However, by 48 hours, message and protein are uncoupled; while endogenous gene expression is downregulated, heat-shocked embryos express a discrete segmented protein pattern within the trunk, suggesting regulation of transcription and of translation in response to environmental stress.
Descriptors: zebrafish, HSP90 translation, heat shock , metazoans, transcription, translation, stress.

Conway, G. (2006). **Stat3-dependent pathfinding and control of axonal branching and target selection**. *Developmental Biology* 296(1): 119-136. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Signal transducers and transcription factors are used in common for developmental cell migration, vasculogenesis, branching morphogenesis, as well as neuronal pathfinding. STAT3, a transcription factor, has been shown to function in all of these processes except neuronal pathfinding. Here, it is shown that STAT3 also facilitates this process. Elimination of STAT3 signaling results in half of zebrafish Cap motoneurons stalling along their ventral pathfinding trajectory. Conversely, constitutive activation leads to precocious branching and redefines CaP axons as a responding population to dorsal guidance cues, resulting in bifurcated axons innervating normal ventral targets as well as additional dorsal muscle groups. These results are consistent with and highlight a fundamental role for STAT3 as a factor promoting cellular responses to guidance cues, not only in nonneural cells but also in pathfinding neurons. (c) 2006 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, molecular genetics, nervous system, immunochemistry, immunologic techniques, morphogenesis, vasculogenesis, cell migration, target selection, axonal branching control, neuronal pathfinding.

Cooper, K.L., J. Armstrong, and C.B. Moens (2005). **Zebrafish foggy/spt 5 is required for migration of facial branchiomotor neurons but not for their survival.** *Developmental Dynamics* 234(3): 651-8. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, cell movement, motor neurons cytology, motor neurons metabolism, nuclear proteins metabolism, transcription factors metabolism, proteins metabolism, base sequence, cell survival, face embryology, developmental gene expression regulation, molecular sequence data, nuclear proteins deficiency, nuclear transcription factors deficiency, transcription factors genetics, proteins deficiency, proteins genetics.

Cooper, M.S., D.P. Szeto, G. Sommers Herivel, J. Topczewski, L. Solnica Krezel, H.C. Kang, I. Johnson, and D. Kimelman (2005). **Visualizing morphogenesis in transgenic zebrafish embryos using BODIPY TR methyl ester dye as a vital counterstain for GFP.** *Developmental Dynamics* 232(2): 359-68. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, genetically modified, boron compounds pharmacology, genetic techniques, confocal microscopy methods, coloring agents pharmacology, esters pharmacology, green fluorescent proteins chemistry, green fluorescent proteins pharmacology, luminescent proteins chemistry, fluorescence microscopy, software, spectrophotometry, time factors, transgenes.

Cooper, M.S. (2006). **Imaging and visualization of zebrafish morphogenesis**. *Federation of American Societies for Experimental Biology Journal* 20(4, Part 1): A4. ISSN: 0892 6638.

NAL Call Number: QH301.F3

Abstract: Among vertebrates, the zebrafish embryo represents one of the most ideal experimental systems to visualize the cellular dynamics of early morphogenesis. Owing to their optical transparency and exceeding rapid rate of development, detailed morphogenetic behaviors of fluorescently labeled cells can be readily visualized within both wildtype and mutant zebrafish embryos, as well as in "mosaic embryos" in which wild-type or mutant cells have been transplanted. For this reason, morphogenetic cell behaviors have become increasingly used as "bioassays" for examining gene function during zebrafish embryogenesis, pathogenesis, and tissue regeneration. In this presentation, we examine how fluorescent vital stains and targeted GFP expression can be used in tandem to bulk label tissue cells within living embryos. These fluorescent labeling methods allow the social behavior of hundreds of embryonic cells to be imaged en masse, revealing detailed relationships between tissue morphogenesis and the cytological activities of the zebrafish's embryonic cells. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, development, freshwater ecology, environmental sciences, imaging techniques, embryogenesis, zebrafish morphogenesis.

Cornell, R.A., E. Yemm, G. Bonde, W. Li, C. d'Alencon, L. Wegman, J. Eisen, and A. Zahs (2004). **Touchtone promotes survival of embryonic melanophores in zebrafish.** *Mechanisms of Development* 121(11): 1365-76. ISSN: 0925-4773.

NAL Call Number: QH607.A1C4

Abstract: An outstanding problem in the study of vertebrate development is the identification of the genes that direct neural crest precursor cells to adopt and maintain specific differentiated cell fates. In an effort to identify such genes, we have carried out a mutagenesis screen in zebrafish and isolated mutants that lack neural crest-derived melanophores. In this manuscript we describe the phenotype of one such mutant, touchtone(b722) (tct), and the map position of the gene it defines. Analysis of expression of dopachrome tautomerase (dct) and microphthalmia (mitfa) suggests that melanophore precursors are specified normally in homozygous tct mutants. However, differentiated melanophores are pale, small, and about half of them have disappeared by 48 h of development, apparently by cell death. We show that melanophores require Tct function cell autonomously. Signals from the receptor tyrosine kinase receptor C-kit are essential for survival of melanophores in zebrafish and mammals. However, differences in the phenotypes of tct and c-kit homozygous mutants, and an absence of interaction between c-kit and tct heterozygotes, suggest that Tct functions independently of the C-kit pathway. Other neural crest derivatives, including other pigment cell types, appear normal in tct mutants. Interestingly, tct mutant embryos undergo a temporary period of near complete paralyzis during the second day of development, although markers of axons of motor and sensory neurons look normal in this period. A fraction of tct(b722) mutants survive to adulthood, but mutant adults are small, indicating a role for Tct in post-larval growth. The tct gene maps to a small interval near a telomere of chromosome 18. Thus, we have identified a zebrafish gene that when mutated produces semi-viable offspring and that may serve as a model of human diseases that have both pigmentation and neurological symptoms.

Descriptors: zebrafish embryos, melanophores cytology, embryology, cell death physiology, cell differentiation physiology, cell survival, chromosome mapping, cytology, physiology, developmental gene expression regulation, melanophores chemistry, melanophores physi-

ology, mutation genetics, phenotype, proto oncogene proteins C kit physiology, proteins analysis and genetics.

Cornell, R., J. Paulsen, M. Elizondo, B. Arduini, E. Burninght, P.D. Henion, D. Parichy, and M.Y. Hsu (2005). **Cation channel TRPM7 is necessary for survival of embryonic melanophores in zebrafish and is expressed in human melanoma cell lines.** *Developmental Biology* 283(2): 707. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, nervous system, integumentary system, molecular genetics, mutagenesis screen, genetic techniques, metastatic melanoma cells.

Corredor Adamez, M., M.C. Welten, H.P. Spaink, J.E. Jeffery, R.T. Schoon, M.A. de Bakker, C.P. Bagowski, A.H. Meijer, F.J. Verbeek, and M.K. Richardson (2005). **Genomic annotation and transcriptome analysis of the zebrafish (*Danio rerio*) hox complex with description of a novel member, hox b 13a.** *Evolution and Development* 7(5): 362-75. ISSN: 1520 541X.
NAL Call Number: QH359
Abstract: The zebrafish *(Danio rerio*) is an important model in evolutionary developmental biology, and its study is being revolutionized by the zebrafish genome project. Sequencing is at an advanced stage, but annotation is largely the result of in silico analyses. We have performed genomic annotation, comparative genomics, and transcriptional analysis using microarrays of the hox homeobox-containing transcription factors. These genes have important roles in specifying the body plan. Candidate sequences were located in version Z v 4 of the Ensembl genome database by TBLASTN searching with Danio and other vertebrate published Hox protein sequences. Homologies were confirmed by alignment with reference sequences, and by the relative position of genes along each cluster. RT-PCR using adult Tubingen cDNA was used to confirm annotations, to check the genomic sequence and to confirm expression in vivo. Our RT-PCR and microarray data show that all 49 hox genes are expressed in adult zebrafish. Significant expression for all known hox genes could be detected in our microarray analysis. We also find significant expression of hox 8 paralogs and hox b 7 a in the anti-sense direction. A novel gene, D. rerio hox b 13 a, was identified, and a preliminary characterization by in situ hybridization showed expression at 24 hpf at the tip of the developing tail. We are currently characterizing this gene at the functional level. We argue that the oligo design for microarrays can be greatly enhanced by the availability of genomic sequences.
Descriptors: zebrafish embryos, genome, homeodomain proteins biosynthesis, proteins biosynthesis, nucleic acid databases, developmental gene expression regulation, homeodomain phylogeny , reverse transcriptase PCR, DNA sequence analysis methods, nucleic acid sequence homology, proteins genetics.

Cortes, F., D. Daggett, R.J. Bryson Richardson, C. Neyt, J. Maule, P. Gautier, G.E. Hollway, D. Keenan, and P.D. Currie (2003). **Cadherin-mediated differential cell adhesion controls slow muscle cell migration in the developing zebrafish myotome.** *Developmental Cell* 5(6): 865-76. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Slow-twitch muscle fibers of the zebrafish myotome undergo a unique set of morphogenetic cell movements. During embryogenesis, slow-twitch muscle derives from the adaxial cells, a layer of paraxial mesoderm that differentiates medially within the myotome, immediately adjacent to the notochord. Subsequently, slow-twitch muscle cells migrate through the entire myotome, coming to lie at its most lateral surface. Here we examine the

cellular and molecular basis for slow-twitch muscle cell migration. We show that slow-twitch muscle cell morphogenesis is marked by behaviors typical of cells influenced by differential cell adhesion. Dynamic and reciprocal waves of N-cadherin and M-cadherin expression within the myotome, which correlate precisely with cell migration, generate differential adhesive environments that drive slow-twitch muscle cell migration through the myotome. Removing or altering the expression of either protein within the myotome perturbs migration. These results provide a definitive example of homophilic cell adhesion shaping cellular behavior during vertebrate development.
Descriptors: zebrafish embryos, cadherins genetics, cell movement physiology, muscle fibers, slow twitch cytology, muscle fibers, slow twitch physiology, skeletal muscle cytology, skeletal muscle embryology, cadherins metabolism, cell adhesion physiology, developmental gene expression regulation, skeletal muscle physiology, mutagenesis, site directed.

Cortez, C.K. and K.B. Artinger (2006). **Lineage analysis of rohon-beard sensory neurons and neural crest cells**. *Developmental Biology* 295(1): 411. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, nervous system, neural coordination, development.

Coutinho, P., M.J. Parsons, K.A. Thomas, E.M.A. Hirst, L. Saude, I. Campos, P.H. Williams, and D.L. Stemple (2004). **Differential requirements for copi transport during vertebrate early development**. *Developmental Cell* 7(4): 547-558. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: The coatomer vesicular coat complex is essential for normal Golgi and secretory activities in eukaryotic cells. Through positional cloning of genes controlling zebrafish notochord development, we found that the sneezy, happy, and dopey loci encode the alpha, beta, and beta' subunits of the coatomer complex. Export from mutant endoplasmic reticulum is blocked, Golgi structure is disrupted, and mutant embryos eventually degenerate due to widespread apoptosis. The early embryonic phenotype, however, demonstrates that despite its "housekeeping" functions, coatomer activity is specifically and cell autonomously required for normal chordamesoderm differentiation, perinotochordal basement membrane formation, and melanophore pigmentation. Hence, differential requirements for coatomer activity among embryonic tissues lead to tissue-specific developmental defects. Moreover, we note that the mRNA encoding alpha coatomer is strikingly upregulated in notochord progenitors, and we present data suggesting that a coatomer transcription is tuned to activity- and cell type-specific secretory loads. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, notochord development, membranes, cell biology, molecular genetics, cloning, genetic techniques, molecular analysis, differentiation, phenotype , transcription.

Covassin, L., J.D. Amigo, K. Suzuki, V. Teplyuk, J. Straubhaar, and N.D. Lawson (2006). **Global analysis of hematopoietic and vascular endothelial gene expression by tissue specific microarray profiling in zebrafish.** *Developmental Biology* 299(2): 551-62. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: In this study, we utilize fluorescent activated cell sorting (FACS) of cells from transgenic zebrafish coupled with microarray analysis to globally analyze expression of cell type specific genes. We find that it is possible to isolate cell populations from Tg(fli1:egfp)(y1) zebrafish embryos that are enriched in vascular, hematopoietic and pharyngeal arch cell types. Microarray analysis of GFP+ versus GFP- cells isolated from Tg(fli1:egfp)(y1) embryos identifies genes expressed in hematopoietic, vascular and pharyngeal arch tissue, consistent

with the expression of the fli1:egfp transgene in these cell types. Comparison of expression profiles from GFP+ cells isolated from embryos at two different time points reveals that genes expressed in different fli1+ cell types display distinct temporal expression profiles. We also demonstrate the utility of this approach for gene discovery by identifying numerous previously uncharacterized genes that we find are expressed in fli1:egfp-positive cells, including new markers of blood, endothelial and pharyngeal arch cell types. In parallel, we have developed a database to allow easy access to both our microarray and in situ results. Our results demonstrate that this is a robust approach for identification of cell type specific genes as well as for global analysis of cell type specific gene expression in zebrafish embryos.
Descriptors: zebrafish embryos, vascular endothelium metabolism, hematopoietic system metabolism, genetically modified, branchial region embryology, branchial region metabolism, cell separation, vascular endothelium cytology, vascular endothelium embryology, gene expression, green fluorescent proteins metabolism, hematopoietic system embryology, oligonucleotide array sequence analysis.

Coverdale, L.E. and C.C. Martin (2004). **Fish n' chips: using DNA microarrays to analyze gene expression changes in zebrafish embryos deficient in histone deacetylase-1 (hdac-1).** *Developmental Biology* 271(2): 564. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, molecular genetics, DNA microarray analysis, genetic techniques, histone deacetylase-1.

Cox, J.A., S. Kucenas, and M.M. Voigt (2005). **Molecular characterization and embryonic expression of the family of N-methyl-D-aspartate receptor subunit genes in the zebrafish.** *Developmental Dynamics* 234(3): 756-66. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, developmental gene expression regulation, N methyl D aspartate receptors genetics, alternative splicing genetics, amino acid sequence, chromosomes genetics, molecular cloning, conserved sequence, complementary DNA genetics, genome genetics, in situ hybridization, molecular sequence data, nervous system embryology, nervous system metabolism, phylogeny, protein subunits chemistry, protein subunits genetics, radiation hybrid mapping, N methyl D aspartate receptorschemistry, sequence alignment, amino acid sequence homology, embryology, metabolism.

Crawford, B.D. and D.B. Pilgrim (2005). **Ontogeny and regulation of matrix metalloproteinase activity in the zebrafish embryo by in vitro and in vivo zymography.** *Developmental Biology* 286(2): 405-14. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Remodeling of the extracellular matrix (ECM) during development, angiogenesis, wound healing, tumor metastasis, and other morphogenetic processes depends on the exquisitely regulated activities of matrix metalloproteinases (MMPs). Yet very little is known about the activity patterns of these proteases in vivo. We have employed fluorescent MMP-substrates, both in vitro and in vivo, to characterize patterns of MMP activity in the zebrafish embryo. Qualitatively similar patterns of degradation are detected using native Type I or Type IV collagen substrates, suggesting that multiple MMPs are being regulated concomitantly. MMP activity is observed primarily in ECM-rich structures predicted to be undergoing active remodeling, such as the perichordal sheath and somite boundaries. Patterns of Type I and Type IV collagen hydrolysis are similar, but not identical in embryos of any given stage. Conventional gelatin zymography shows MMPs present in embryos as early

as 3-somites (11 h) and our in vivo assays detect Type IV collagen degradation at somite boundaries as early as 4-somites (11.5 h). However, we are unable to detect significant in vitro activity using homogenates made from embryos prior to Prim-16 (31 h). Mixed lysate assays demonstrate that this is the result of endogenous inhibitors present in early embryos, suggesting a model of matrix remodeling regulated by spatially heterogeneous MMP inhibition.

Descriptors: zebrafish , enzymology, matrix metalloproteinases metabolism, collagen Type I metabolism, collagen Type IV metabolism, electrophoresis, agar gel, extracellular matrix metabolism, matrix metalloproteinases analysis, matrix metalloproteinases antagonists and inhibitors, morphogenesis, protease inhibitors, somites enzymology, somites metabolism.

Crawford, B.D., M.D. Po, and D.B. Pilgrim (2004). **Ontogeny of MMP expression and activity during zebrafish development.** *Developmental Biology* 271(2): 608. ISSN: 0012 1606.

NAL Call Number: 442.8 D49

Descriptors: zebrafish, enzymology, biochemistry and molecular biophysics, embryo development, MMP expression.

Cresko, W.A., Y.l. Yan, D.A. Baltrus, A. Amores, A. Singer, A. Rodriguez Mari, and J.H. Postlethwait (2003). **Genome duplication, subfunction partitioning, and lineage divergence: Sox9 in stickleback and zebrafish.** *Developmental Dynamics* 228(3): 480-489. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: Teleosts are the most species-rich group of vertebrates, and a genome duplication (tetraploidization) event in ray-fin fish appears to have preceded this remarkable explosion of biodiversity. What is the relationship of the ray-fin genome duplication to the teleost radiation? Genome duplication may have facilitated lineage divergence by partitioning different ancestral gene subfunctions among co-orthologs of tetrapod genes in different teleost lineages. To test this hypothesis, we investigated gene expression patterns for Sox9 gene duplicates in stickleback and zebrafish, teleosts whose lineages diverged early in Euteleost evolution. Most expression domains appear to have been partitioned between Sox9a and Sox9b before the divergence of stickleback and zebrafish lineages, but some ancestral expression domains were distributed differentially in each lineage. We conclude that some gene subfunctions, as represented by lineage-specific expression domains, may have assorted differently in separate lineages and that these may have contributed to lineage diversification during teleost evolution.

Descriptors: zebrafish, stickleback fish, teleostei , molecular genetics, Sox9 genes, mutation, genome duplication, evolution , explosive radiation, role of genome duplication, test of hypothesis using Sox9 gene comparisons between lineages.

Creton, R. (2004). **The calcium pump of The endoplasmic reticulum plays a role in midline signaling during early zebrafish development.** *Brain Research* 151(1-2): 33-41. ISSN: 0006 8993.

NAL Call Number: QP376

Abstract: During early vertebrate development, a signaling network is activated along the midline of the embryo. This signaling network induces the neural tube floor plate and ventral brain regions. In turn, induction of the ventral brain region is important for bilateral division of the forebrain and bilateral separation of the eyes. The present study provides direct evidence for a role of the endoplasmic reticulum Ca(2+) pump in zebrafish midline signaling. The endoplasmic reticulum Ca(2+) pump was inhibited in zebrafish embryos using thapsigargin or cyclopiazonic acid. Inhibition of the endoplasmic reticulum Ca(2+) pump during

early gastrulation induces cyclopia, mimicking defects observed in cyclops, squint, one-eyed pinhead, and silberblick mutant embryos. In contrast, inhibition of the endoplasmic reticulum Ca(2+) pump during mid-gastrulation does not induce cyclopia, but does induce tail defects, mimicking defects observed in no-tail mutant embryos. This study is the first to relate thapsigargin and cyclopiazonic acid with induction of cyclopia. In addition, obtained results provide new information on the roles of Ca(2+) in embryonic development and may lead to new insights on the mechanisms underlying holoprosencephaly, a relatively common brain defect in human development.

Descriptors: zebrafish embryos, body patterning physiology, calcium transporting ATPases physiology, endoplasmic reticulum physiology, body patterning drug effects, calcium metabolism, calcium pharmacology, drug effects, ultrastructure, enzyme inhibitors pharmacology, indoles pharmacology, scanning electron microscopy methods, thapsigargin pharmacology, time factors.

Crosnier, C., N. Vargesson, S. Gschmeissner, L. Ariza McNaughton, A. Morrison, and J. Lewis (2005). **Delta-Notch signalling controls commitment to a secretory fate in the zebrafish intestine.** *Development* 132(5): 1093-104. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: The transparency of the juvenile zebrafish and its genetic advantages make it an attractive model for study of cell turnover in the gut. BrdU labelling shows that the gut epithelium is renewed in essentially the same way as in mammals: the villi are lined with non-dividing differentiated cells, while cell division is confined to the intervillus pockets. New cells produced in the pockets take about 4 days to migrate out to the tips of the villi, where they die. We have generated monoclonal antibodies to identify the absorptive and secretory cells in the epithelium, and we have used these antibodies to examine the part that Delta-Notch signalling plays in producing the diversity of intestinal cell types. Several Notch receptors and ligands are expressed in the gut. In particular, the Notch ligand DeltaD (Delta1 in the mouse) is expressed in cells of the secretory lineage. In an aei mutant, where DeltaD is defective, secretory cells are overproduced. In mind bomb (mib), where all Delta-Notch signalling is believed to be blocked, almost all the cells in the 3-day gut epithelium adopt a secretory character. Thus, secretory differentiation appears to be the default in the absence of Notch activation, and lateral inhibition mediated by Delta-Notch signalling is required to generate a balanced mixture of absorptive and secretory cells. These findings demonstrate the central role of Notch signalling in the gut stem-cell system and establish the zebrafish as a model for study of the mechanisms controlling renewal of gut epithelium.

Descriptors: zebrafish, developmental gene expression regulation, intestines embryology, intestines metabolism, membrane proteins metabolism, signal transduction, monoclonal antibodies metabolism, Western blotting , cell differentiation, cell lineage, cell movement, cell proliferation, in situ nick end labeling, ligands, mutation, phenotype, RNA metabolism, Notch receptors, reverse transcriptase PCR.

Crotwell, P.L. and P.M. Mabee (2007). **Gene expression patterns underlying proximal-distal skeletal segmentation in late-stage zebrafish, *Danio rerio*.** *Developmental Dynamics* 236(11): 3111-28. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish, tetrapods, bmp2b, bmp4, chordin, gdf5, interradial mesenchyme, ZS, bapx1, col2a1, noggin3, Sox9a, chondrocytes, Wnt9a .

Croushore, J.A., B. Blasiole, R.C. Riddle, C. Thisse, B. Thisse, V.A. Canfield, G.P. Robertson, K.C. Cheng, and R. Levenson (2005). **Ptena and ptenb genes play distinct roles in zebrafish embryogenesis.** *Developmental Dynamics* 234(4): 911-21. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: gene expression, PTEN phosphohydrolase metabolism, phosphoprotein phosphatase metabolism, signal transduction physiology, proteins metabolism, amino acid sequence, computational biology, DNA primers, complementary DNA genetics, in situ hybridization, molecular sequence data, antisense oligonucleotides, PTEN phosphohydrolase genetics, phosphoprotein phosphatase genetics, reverse transcriptase PCR, DNA sequence analysis, signal transduction genetics.

Crump, J.G., L. Maves, N.D. Lawson, B.M. Weinstein, and C.B. Kimmel (2004). **An essential role for Fgfs in endodermal pouch formation influences later craniofacial skeletal patterning**. *Development* 131(22): 5703-5716. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Fibroblast growth factor (Fgf) proteins are important regulators of pharyngeal arch development. Analyses of Fgf8 function in chick and mouse and Fgf3 function in zebrafish have demonstrated a role for Fgfs in the differentiation and survival of postmigratory neural crest cells (NCC) that give rise to the pharyngeal skeleton. Here we describe, in zebrafish, an earlier, essential function for Fgf8 and Fgf3 in regulating the segmentation of the pharyngeal endoderm into pouches. Using time-lapse microscopy, we show that pharyngeal pouches form by the directed lateral migration of discrete clusters of endodermal cells. In animals doubly reduced for Fgf8 and Fgf3, the migration of pharyngeal endodermal cells is disorganized and pouches fail to form. Transplantation and pharmacological experiments show that Fgf8 and Fgf3 are required in the neural keel and cranial mesoderm during early somite stages to promote first pouch formation. In addition, we show that animals doubly reduced for Fgf8 and Fgf3 have severe reductions in hyoid cartilages and the more posterior branchial cartilages. By examining early pouch and later cartilage phenotypes in individual animals hypomorphic for Fgf function, we find that alterations in pouch structure correlate with later cartilage defects. We present a model in which Fgf signaling in the mesoderm and segmented hindbrain organizes the segmentation of the pharyngeal endoderm into pouches. Moreover, we argue that the Fgf-dependent morphogenesis of the pharyngeal endoderm into pouches is critical for the later patterning of pharyngeal cartilages. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, biochemistry and molecular biophysics, development, skeletal system, time lapse microscopy, imaging and microscopy techniques, phenotype.

Crump, J.G., M.E. Swartz, J.K. Eberhart, and C.B. Kimmel (2006). **Moz-dependent Hox expression controls segment-specific fate maps of skeletal precursors in the face**. *Development* 133(14): 2661-2669. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Development of the facial skeleton depends on interactions between intrinsic factors in the skeletal precursors and extrinsic signals in the facial environment. Hox genes have been proposed to act cell-intrinsically in skeletogenic cranial neural crest cells (CNC) for skeletal pattern. However, Hox genes are also expressed in other facial tissues, such as the ectoderm and endoderm, suggesting that Hox genes could also regulate extrinsic signalling from non-CNC tissues. Here we study moz mutant zebrafish in which hoxa2b and hoxb2a expression is lost and the support skeleton of the second pharyngeal segment is transformed into a duplicate of the first-segment-derived jaw skeleton. By performing tissue mosaic

experiments between moz(-) and wild-type embryos, we show that Moz and Hox genes function in CNC, but not in the ectoderm or endoderm, to specify the support skeleton. How then does Hox expression within CNC specify a support skeleton at the cellular level? Our fate map analysis of skeletal precursors reveals that Moz specifies a second-segment fate map in part by regulating the interaction of CNC with the first endodermal pouch (p1). Removal of p1, either by laser ablation or in the itga5(b926) mutant, reveals that p1 epithelium is required for development of the wild-type support but not the moz(-) duplicate jaw-like skeleton. We present a model in which Moz-dependent Hox expression in CNC shapes the normal support skeleton by instructing second-segment CNC to undergo skeletogenesis in response to local extrinsic signals. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics, skeletal system, segment specific fate map, Moz and Hox genes , cranial neural crest cells.

Cunliffe, V.T. (2004). **Histone deacetylase 1 is required to repress Notch target gene expression during zebrafish neurogenesis and to maintain the production of motoneurones in response to Hedgehog signalling.** *Development* 131(12): 2983-95. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Histone deacetylases (Hdacs) are widely implicated as key components of transcriptional silencing mechanisms. Here, I show that hdac1 is specifically required in the zebrafish embryonic CNS to maintain neurogenesis. In hdac1 mutant embryos, the Notch-responsive E(spl)-related neurogenic gene her6 is ectopically expressed at distinct sites within the developing CNS and proneural gene expression is correspondingly reduced or eliminated. Using an hdac1-specific morpholino, I show that this requirement for hdac1 is epistatic to the requirement for Notch signalling. Consequently, hdac1-deficient embryos exhibit several defects of neuronal specification and patterning, including a dramatic deficit of hedgehog-dependent branchiomotor neurones that is refractory to elevated levels of hedgehog signalling. Thus, in the zebrafish embryo, hdac1 is an essential component of the transcriptional silencing machinery that supports the formation and subsequent differentiation of neuronal precursors.
Descriptors: zebrafish embryos, developmental gene expression regulation genetics , histone deacetylases genetics, histone deacetylases metabolism, membrane morphogenesis genetics, motor neurons physiology, nervous system embryology, trans activators physiology, cell division, molecular cloning, physiology, gene deletion, Hedgehog proteins, histone deacetylases deficiency, in situ hybridization, motor neurons cytology, antisense oligonucleotides pharmacology, messenger RNA genetics, Notch receptors, signal transduction.

Cunliffe, V.T. and P. Casaccia Bonnefil (2006). **Histone deacetylase 1 is essential for oligodendrocyte specification in the zebrafish CNS.** *Mechanisms of Development* 123(1): 24-30. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: Histone deacetylases are critical components of transcriptional silencing mechanisms that regulate embryonic development. Recent work has shown that histone deacetylase 1 (hdac1) is required for neuronal specification during zebrafish CNS development. We show here that specification of oligodendrocytes, the myelinating cells of the CNS, also fails to occur in the hdac1 mutant hindbrain, but persistence of neural progenitors in the hindbrain ventricular zone, which express pax6a and sox2, is independent of hdac1 activity. Commitment of ventral neural progenitors to the oligodendrocyte fate is thought to require co-ordinate, hedgehog-dependent expression of olig2 and nkx2.2a in these cells, leading to expression of sox10 and subsequent differentiation of oligodendrocytes. Remarkably,

transcription of olig2 is extinguished in ventral neural progenitors of the hdac1 mutant hindbrain, whereas expression of nkx2.2a is up-regulated in these cells, and sox10 expression is suppressed. Our results identify hdac1 as a novel, essential component of the mechanism that allocates neural progenitors to the oligodendrocyte fate, by attenuating expression of a subset of neural progenitor genes and rendering olig2 expression responsive to Hedgehog signalling.
Descriptors: zebrafish, central nervous system embryology, central nervous system enzymology, histone deacetylases metabolism, oligodendroglia enzymology, metabolism, basic helix loop helix transcription factors genetics, cell differentiation, central nervous system cytology, developmental gene expression regulation, histone deacetylases genetics, homeodomain in situ hybridization, nerve tissue oligodendroglia cytology, rhombencephalon cytology, rhombencephalon embryology, rhombencephalon enzymology, stem cells cytology, stem cells enzymology, transcription factors genetics, proteins genetics.

Curado, S., R.M. Anderson, B. Jungblut, J. Mumm, E. Schroeter, and D.Y. Stainier (2007). **Conditional targeted cell ablation in zebrafish: A new tool for regeneration studies.** *Developmental Dynamics* 236(4): 1025-35. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Conditional targeted cell ablation in zebrafish would greatly expand the utility of this genetic model system in developmental and regeneration studies, given its extensive regenerative capabilities. Here, we show that, by combining chemical and genetic tools, one can ablate cells in a temporal- and spatial-specific manner in zebrafish larvae. For this purpose, we used the bacterial Nitroreductase (NTR) enzyme to convert the prodrug Metronidazole (Mtz) into a cytotoxic DNA cross-linking agent. To investigate the efficiency of this system, we targeted three different cell lineages in the heart, pancreas, and liver. Expression of the fusion protein Cyan Fluorescent Protein-NTR (CFP-NTR) under control of tissue-specific promoters allowed us to induce the death of cardiomyocytes, pancreatic beta-cells, and hepatocytes at specific times. Moreover, we have observed that Mtz can be efficiently washed away and that, upon Mtz withdrawal, the profoundly affected tissue can quickly recover. These findings show that the NTR/Mtz system is effective for temporally and spatially controlled cell ablation in zebrafish, thereby constituting a most promising genetic tool to analyze tissue interactions as well as the mechanisms underlying regeneration.
Descriptors: zebrafish, genes, transgenic, suicide, genetic engineering methods, regeneration physiology, physiology, genetically modified, embryo developmental gene expression regulation, green fluorescent green fluorescent proteins metabolism, heart physiology, hepatocytes physiology, insulin secreting cells physiology, myocardium cytology, cardiac myocytes cytology, nitroreductases genetics, nitroreductases metabolism, organ specificity genetics, phenotype, promoter regions genetics, embryology, genetics.

Daggett, D.F., C.R. Domingo, P.D. Currie, and S.L. Amacher (2007). **Control of morphogenetic cell movements in the early zebrafish myotome.** *Developmental Biology* 309(2): 169-79. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: As the vertebrate myotome is generated, myogenic precursor cells undergo extensive and coordinated movements as they differentiate into properly positioned embryonic muscle fibers. In the zebrafish, the "adaxial" cells adjacent to the notochord are the first muscle precursors to be specified. After initially differentiating into slow-twitch myosin-expressing muscle fibers, these cells have been shown to undergo a remarkable radial migration through the lateral somite, to populate the superficial layer of slow-twitch muscle of the mature myotome. Here we characterize an earlier set of adaxial cell behaviors; the tran-

sition from a roughly 4x5 array of cuboidal cells to a 1x20 stack of elongated cells, prior to the migration event. We find that adaxial cells display a highly stereotypical series of behaviors as they undergo this rearrangement. Furthermore, we show that the actin regulatory molecule, Cap1, is specifically expressed in adaxial cells and is required for the progression of these behaviors. The requirement of Cap1 for a cellular apical constriction step is reminiscent of similar requirements of Cap during apical constriction in *Drosophila* development, suggesting a conservation of gene function for a cell biological event critical to many developmental processes.
Descriptors: zebrafish embryo, morphogenesis, myotome, "adaxial" cells, Cap1, *Drosophila*.

Davidson, A.E., D. Balciunas, D. Mohn, J. Shaffer, S. Hermanson, S. Sivasubbu, M.P. Cliff, P.B. Hackett, and S.C. Ekker (2003). **Efficient gene delivery and gene expression in zebrafish using the Sleeping Beauty transposon.** *Developmental Biology* 263(2): 191-202. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: We used the Tc1/mariner family transposable element Sleeping Beauty (SB) for transgenesis and long-term expression studies in the zebrafish (Danio rerio), a popular organism for clinical disease, vertebrate patterning, and cell biology applications. SB transposase enhanced the transgenesis and expression rate sixfold (from 5 to 31%) and more than doubled the total number of tagged chromosomes over standard, plasmid injection-based transgenesis methods. Molecular analysis of these loci demonstrated a precise integration of these elements into recipient chromosomes with genetic footprints diagnostic of transposition. GFP expression from transposase-mediated integrants was Mendelian through the eighth generation. A blue-shifted GFP variant (BFP) and a red fluorescent protein (DsRed) were also useful transgenesis markers, indicating that multiple reporters are practical for use with SB in zebrafish. We showed that SB is suitable for tissue-specific transgene applications using an abbreviated gamma-crystallin GFP cassette. Finally, we describe a general utility transposon vector for chromosomal engineering and molecular genetics experiments in zebrafish. Together, these data indicate that SB is an efficient tool for transgenesis and expression in zebrafish, and that the transposon will be useful for gene expression in cell biology applications as well as an insertional mutagen for gene discovery during development.
Descriptors: DNA transposable elements, gene transfer techniques, insertional mutagenesisgenetics, gene dosage, gene expression, organ specificity, promoter regions genetics, transposases physiology.

Davidson, A.J. and L.I. Zon (2006). **The caudal-related homeobox genes cdx1a and cdx4 act redundantly to regulate hox gene expression and The formation of putative hematopoietic stem cells during zebrafish embryogenesis.** *Developmental Biology* 292(2): 506-18. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The hox genes play a central role in organogenesis and are implicated in the formation of hematopoietic stem cells (HSCs). The cdx genes encode homeodomain transcription factors that act as master regulators of the hox genes. In zebrafish, mutations in cdx4 cause a severe, but not complete, deficit in embryonic blood cells. Here, we report the expression and function of cdx1a, a zebrafish Cdx1 paralogue. Using morpholino-mediated knockdown of cdx1a in a cdx4 mutant background, we show that a deficiency in both cdx genes causes a severe perturbation of hox gene expression and a complete failure to specify blood. The hematopoietic defect in cdx-deficient embryos does not result from a general block in posterior mesoderm differentiation as endothelial cells and kidney progenitors are

still formed in the doubly deficient embryos. In addition, cdx-deficient embryos display a significant reduction in runx1a(+) putative HSCs in the zebrafish equivalent to the aorta-gonad-mesonephros (AGM) region. Overexpressing hoxa9a in cdx-deficient embryos rescues embryonic erythropoiesis in the posterior mesoderm as well as the formation of HSCs in the AGM region. Taken together, these results suggest that the cdx-hox pathway plays an essential role in the formation of both embryonic erythroid cells and definitive HSCs during vertebrate embryogenesis.

Descriptors: zebrafish embryos, embryonic development, developmental gene expression regulation, homeobox genes, hematopoietic stem cells metabolism, embryology, proteins metabolism, amino acid sequence, chromosomes, erythropoiesis, hematopoietic stem cells cytology, homeodomain proteins chemistry, homeodomain proteins isolation and purification, homeodomain proteins metabolism, in situ hybridization, molecular sequence data, antisense oligonucleotides pharmacology, tertiary protein structure, radiation hybrid mapping, amino acid sequence homology, synteny, genetics.

Davison, J.M., C.M. Akitake, M.G. Goll, J.M. Rhee, N. Gosse, H. Baier, M.E. Halpern, S.D. Leach, and M.J. Parsons (2007). **Transactivation from Gal4-VP16 transgenic insertions for tissue-specific cell labeling and ablation in zebrafish.** *Developmental Biology* 304(2): 811-24. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Prior studies with transgenic zebrafish confirmed the functionality of the transcription factor Gal4 to drive expression of other genes under the regulation of upstream activator sequences (UAS). However, widespread application of this powerful binary system has been limited, in part, by relatively inefficient techniques for establishing transgenic zebrafish and by the inadequacy of Gal4 to effect high levels of expression from UAS-regulated genes. We have used the Tol2 transposition system to distribute a self-reporting gene/enhancer trap vector efficiently throughout the zebrafish genome. The vector uses the potent, hybrid transcription factor Gal4-VP16 to activate expression from a UAS:eGFP reporter cassette. In a pilot screen, stable transgenic lines were established that express eGFP in reproducible patterns encompassing a wide variety of tissues, including the brain, spinal cord, retina, notochord, cranial skeleton and muscle, and can transactivate other UAS-regulated genes. We demonstrate the utility of this approach to track Gal4-VP16 expressing migratory cells in UAS:Kaede transgenic fish, and to induce tissue-specific cell death using a bacterial nitroreductase gene under UAS control. The Tol2-mediated gene/enhancer trapping system together with UAS transgenic lines provides valuable tools for regulated gene expression and for targeted labeling and ablation of specific cell types and tissues during early zebrafish development.

Descriptors: zebrafish, enhancer elements genetics, trans activators metabolism, metabolism, genetically modified , cell death, DNA transposable elements, *Escherichia coli* developmental gene expression regulation, reporter genes, green fluorescent proteins biosynthesis, green fluorescent nitroreductases genetics, organ specificity, trans activation genetics, trans activators biosynthesis, trans activators genetics.

Dawid, I.B. (2004). **Developmental biology of zebrafish.** *Annals of the New York Academy of Sciences* 1038: 88-93. ISSN: 0077 8923.

NAL Call Number: 500 N484

Abstract: The biology and genetics of zebrafish has become a very popular system in developmental biology especially because of their suitability for genetics and the transparent embryo of this small tropical fish. Studies reported briefly here demonstrate that one can

use gene expression in the zebrafish embryo to find new components of important and well-studied developmental mechanisms and signal transduction pathways. The study of zebrafish developmental genetics has proven invaluable in determining many aspects of vertebrate development. Further use of this model organism promises to generate many interesting and useful data.

Descriptors: zebrafish embryo, developmental biology , developmental gene expression regulation , embryology, growth and development, Fgf metabolism, green fluorescent proteins metabolism, signal transduction physiology, proteins metabolism.

Dawid, I.B. (2004). **Understanding and Optimizing human development: From cells to Patients to Populations** . *Developmental Biology of Zebrafish* , 1038, NEW YORK ACADEMY OF SCIENCES: NEW YORK, NY 10021 USA, p. 88-93. ISBN: 1573315206.

Abstract: The biology and genetics of zebrafish has become a very popular system in developmental biology especially because of their suitability for genetics and the transparent embryo of this small tropical fish. Studies reported briefly here demonstrate that one can use gene expression in the zebrafish embryo to find new components of important and well-studied developmental mechanisms and signal transduction pathways. The study of zebrafish developmental genetics has proven invaluable in determining many aspects of vertebrate development. Further use of this model organism promises to generate many interesting and useful data. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, molecular genetics, development, signal transduction, gene expression, developmental biology.

DeCarvalho, A.C., S.L. Cappendijk, and J.M. Fadool (2004). **Developmental expression of the POU domain transcription factor Brn-3b (Pou4f2) in the lateral line and visual system of zebrafish.** *Developmental Dynamics* 229(4): 869-76. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish, brain embryology, DNA binding proteins genetics , DNA binding proteins genetics, proteins metabolism, eye embryology, transcription factors genetics and metabolism, amino acid sequence, brain metabolism, molecular cloning, DNA binding proteins genetics, proteins isolation and purification, metabolism, eye metabolism, developmental gene expression regulation, molecular sequence data, messenger RNA analysis, retina embryology, retina metabolism, sequence alignment, superior colliculus embryology, superior colliculus metabolism, transcription factor Brn 3, transcription factor Brn 3b, transcription factor Brn 3c, transcription factors isolation and purification.

Dekens, M.P., F.J. Pelegri, H.M. Maischein, and C. Nusslein Volhard (2003). **The maternal-effect gene futile cycle is essential for pronuclear congression and mitotic spindle assembly in the zebrafish zygote.** *Development* 130(17): 3907-16. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Embryos have been successfully used for the general study of the cell cycle. Although there are significant differences between the early embryonic and the somatic cell cycle in vertebrates, the existence of specialised factors that play a role during the early cell cycles has remained elusive. We analysed a lethal recessive maternal-effect mutant, futile cycle (fue), isolated in a maternal-effect screen for nuclear division defects in the zebrafish (*Danio rerio*). The pronuclei fail to congress in zygotes derived from homozygous fue mothers. In addition, a defect in the formation of chromosomal microtubules prevents mitotic spindle assembly and thus chromosome segregation in fue zygotes. However, centrosomal functions

do not appear to be affected in fue embryos, suggesting this mutant blocks a subset of microtubule functions. Cleavage occurs normally for several divisions resulting in many anucleate cells, thus showing that nuclear- and cell division can be uncoupled genetically. Therefore, we propose that in mitotic spindle assembly chromosome-dependent microtubule nucleation is essential for the coupling of nuclear and cell division.

Descriptors: zebrafish, cell division physiology, cleavage stage, ovum physiology, CDC genes, mitotic spindle apparatus physiology, zygote physiology, centrosome physiology, cleavage stage, ovum cytology, meiosis physiology, mitosis physiology, genetic transcription physiology, zygote cytology.

Del Giacco, L., P. Sordino, A. Pistocchi, N. Andreakis, R. Tarallo, B. Di Benedetto, and F. Cotelli (2006). **Differential regulation of the zebrafish orthopedia 1 gene during fate determination of diencephalic neurons.** *BioMed Central Developmental Biology* 6: 50. ISSN: 1471 213X.

NAL Call Number: QH491.B63

Abstract: BACKGROUND: The homeodomain transcription factor Orthopedia (Otp) is essential in restricting the fate of multiple classes of secreting neurons in the neuroendocrine hypothalamus of vertebrates. However, there is little information on the intercellular factors that regulate Otp expression during development. RESULTS: Here, we identified two otp orthologues in zebrafish (otp1 and otp2) and explored otp1 in the context of the morphogenetic pathways that specify neuroectodermal regions. During forebrain development, otp1 is expressed in anterior groups of diencephalic cells, positioned in the preoptic area (PO) (anterior alar plate) and the posterior tuberculum (PT) (posterior basal plate). The latter structure is characterized by Tyrosine Hydroxylase (TH)-positive cells, suggesting a role for otp1 in the lineage restriction of catecholaminergic (CA) neurons. Disruptions of Hedgehog (HH) and Fibroblast Growth Factor (FGF) pathways point to the ability of SHH protein to trigger otp1 expression in PO presumptive neuroblasts, with the attenuating effect of Dzip1 and FGF8. In addition, our data disclose otp1 as a determinant of CA neurons in the PT, where otp1 activity is strictly dependent on Nodal signaling and it is not responsive to SHH and FGF. CONCLUSION: In this study, we pinpoint the evolutionary importance of otp1 transcription factor in cell states of the diencephalon anlage and early neuronal progenitors. Furthermore, our data indicate that morphogenetic mechanisms differentially regulate otp1 expression in alar and basal plates.

Descriptors: zebrafish, diencephalon growth and development , diencephalon metabolism, developmental gene expression regulation physiology, neurons metabolism, transcription factors biosynthesis, growth and development, proteins biosynthesis, amino acid sequence, developmental gene expression regulation genetics , homeodomain proteins biosynthesis, homeodomain molecular sequence data, nerve tissue proteins biosynthesis, nerve tissue neurons cytology, neurons physiology, phylogeny, protein isoforms biosynthesis , protein isoforms genetics, transcription factors genetics, proteins genetics.

Derenzo, C. and G. Seydoux (2004). **A clean start: degradation of maternal proteins at the oocyte-to-embryo transition**. *Trends in Cell Biology* 14(8): 420-426. ISSN: 0962 8924.

NAL Call Number: QH573.T73

Abstract: In many organisms, the transition from oocyte to embryo occurs in the absence of mRNA transcription. Therefore, early developmental programs rely on maternal mRNAs and proteins that are synthesized during oogenesis. The regulated translation of maternal RNAs is essential for the proper deployment of regulatory factors during early embryogenesis. Recent studies suggest that the degradation of maternal proteins by the ubiquitin-pro-

teasome pathway is also crucial for the oocyte-to-embryo transition. In this article, we explore the hypothesis that the coordinated degradation of germline proteins is essential for remodeling the oocyte into a totipotent zygote that is capable of somatic development. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, cell biology, development, reproductive system, early embryogenesis, oocyte, totipotent zygote.

Detrich, H.W., M. Westerfield and L.I. Zon (2004). ***The zebrafish. cellular and developmental Biology***. 2nd edition, Vol. 76, ISBN: 0125641710.
Descriptors: zebrafish, developmental biology, cells.

Detrich, H.W.I.E., L.I.E. Zon, and M.E. Westerfield (2004). ***The zebrafish: 2nd edition - cellular and development biology.*** *Methods in Cell Biology* 76: 1-631. ISSN: 0091 679X.
NAL Call Number: 442P92
Descriptors: zebrafish, *Danio rerio*, comprehensive works, cellular and developmental biology, collected papers, cytological techniques, laboratory protocols, cytology, development, genetics.

DiBiase, A., R.A. Harte, Y. Zhou, L. Zon, and W.J. Kent (2006). **Piloting the zebrafish genome browser**. *Developmental Dynamics* 235(3): 747-753. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: This correspondence is a primer for the zebrafish research community on zebrafish tracks available in the UCSC Genome Browser at http://genome.ucsc.edu based on Sanger's Zv4 assembly. A primary capability of this facility is comparative informatics between humans (as well as many other model organisms) and zebrafish. The zebrafish genome sequencing project has played important roles in mutant mapping and cloning, and comparative genomic research projects. This easy-to-use genome browser aims to display and download useful genome sequence information for zebrafish mutant mapping and cloning projects. Its user-friendly interface expedites annotation of the zebrafish genome sequence. Copyright © 2008 CABI
Descriptors: zebrafish, DNA cloning, gene mapping, genetic mapping, genomes, nucleotide sequences, *Danio rerio*.

DiIorio, P.J., A. Runko, C.A. Farrell, and N. Roy (2005). **Sid4: A secreted vertebrate immunoglobulin protein with roles in zebrafish embryogenesis.** *Developmental Biology* 282(1): 55-69 . ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The small members of the immunoglobulin superfamily (IGSF) are a molecularly diverse group of proteins composed solely of immunoglobulin domains. They may be secreted or tethered to the cell mebrane via GPI linkages and are proposed to have important functions in vivo. However, very few small IGSFs have been functionally characterized. During an ongoing in situ hybridization analysis of expressed sequence tags in zebrafish we identified secreted immunoglobulin domain 4 (sid4), a gene encoding a soluble vertebrate protein composed solely of four immunoglobulin domains. Throughout development, sid4 is expressed in regions of the embryo undergoing active cell division and migration. Functional analysis using morpholino antisense oligonucleotides demonstrates that timing of gene expression is normal in morphants, but these embryos are smaller and exhibit defects in epiboly and patterning of axial and prechordal mesoderm. Analyses of chordin, pax2, krox20, and dlx2 expression in morphants demonstrate that early brain patterning is normal but later

organization of hindbrain neurons and development of cranial neural crest are perturbed. Levels of apoptosis in morphants were normal prior to 90% epiboly, but were elevated after 10 h post-fertilization (hpf). Apoptosis does not account for early patterning defects of axial mesoderm, but likely contributes to overall reduction in embryo size. Phylogenetic analysis demonstrates that Sid4 is strikingly similar to the fibronectin binding Ig domains of Perlecan/HSPG2. Overall, our data demonstrate a fundamental role for sid4, possibly as a co-factor in extracellular matrix (ECM) interactions, in processes underlying tissue patterning and organogenesis in a vertebrate.

Descriptors: zebrafish embryology, body patterning physiology, extracellular matrix metabolism, gene expression, immunoglobulins genetics, phylogeny, amino acid sequence, apoptosis genetics, base sequence, molecular cloning, cluster analysis, computational biology, DNA primers, immunoglobulins metabolism, in situ hybridization, in situ nick end labeling, microinjections, molecular sequence data, neural crest metabolism, neurons metabolism, rhombencephalon metabolism, DNA sequence analysis.

Dill, K.K. and S.L. Amacher (2005). **Tortuga refines Notch pathway gene expression in the zebrafish presomitic mesoderm at the post-transcriptional level.** *Developmental Biology* 287(2): 225-36. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: We have identified the zebrafish tortuga (tor) gene by an ENU-induced mutation that disrupts the presomitic mesoderm (PSM) expression of Notch pathway genes. In tor mutants, Notch pathway gene expression persists in regions of the PSM where expression is normally off in wild type embryos. The expression of hairy/Enhancer of split-related 1 (her1) is affected first, followed by the delta genes deltaC and deltaD, and finally, by another hairy/Enhancer of split-related gene, her7. In situ hybridization with intron-specific probes for her1 and deltaC indicates that transcriptional bursts of expression are normal in tor mutants, suggesting that tor normally functions to refine her1 and deltaC message levels downstream of transcription. Despite the striking defects in Notch pathway gene expression, somite boundaries form normally in tor mutant embryos, although somitic mesoderm defects are apparent later, when cells mature to form muscle fibers. Thus, while the function of Notch pathway genes is required for proper somite formation, the tor mutant phenotype suggests that precise oscillations of Notch pathway transcripts are not essential for establishing segmental pattern in the presomitic mesoderm.

Descriptors: zebrafish, developmental gene expression regulation, mesoderm metabolism, Notch receptors metabolism, basic helix loop helix transcription factors genetics, basic helix loop helix transcription factors metabolism, body patterning, metabolism, in situ hybridization, membrane membrane proteins metabolism, muscle fibers metabolism, muscle fibers ultrastructure, skeletal muscle cytology, skeletal muscle embryology, skeletal muscle metabolism, mutation, nerve tissue nerve tissue proteins metabolism, messenger RNA metabolism , signal transduction, somites cytology, somites metabolism, transcription factors genetics and metabolism.

Ding, J. and C. Duan (2003). **Igf binding protein-5 is required for the pharyngeal skeleton formation in vivo**. *Integrative and Comparative Biology* 43(6): 1013. ISSN: 1540 7063.

NAL Call Number: QL1.I67

Descriptors: zebrafish, skeletal system, development, alcian blue staining, tissue growth, tissue survival, in vivo pharyngeal skeleton formation.

Dixon, L.J., A.L. McDowell, J.D. Houchins, and J. Bilotta (2004). **Effects of restricted spectral rearing on the development of zebrafish retinal physiology.** *Documenta Ophthalmologica* 109(1): 17-33. ISSN: 0012 4486.

Abstract: Research has shown that rearing in abnormal lighting environments affects both visual behavior and retinal physiology in zebrafish larvae. These studies, however, used only constant dark and constant white light as the experimental rearing conditions. This study assessed the effects of rearing larvae in restricted spectral lighting environments on zebrafish retinal physiology. Larvae were reared in one of seven different lighting environments: cyclic white light (control group), constant blue light, constant green light, constant orange light, cyclic blue light, cyclic green light, and cyclic orange light. Assessment of retinal physiology was done using the electroretinogram (ERG). Results showed that rearing larvae in constant light conditions caused deficits in sensitivity to ultraviolet- and short-wavelength stimuli, but had little effect on sensitivity to middle- and long-wavelength stimuli. Rearing larvae in cyclic light did not cause differences in sensitivity to middle- and long-wavelength stimuli, but did cause extreme deficits in sensitivity to ultraviolet- and short-wavelength stimuli in the cyclic green and orange light-rearing conditions. Sensitivity of the cyclic blue light-rearing group was similar to the control group to stimuli of all wavelengths. The results support the notion that the light-rearing environment impacts the development of the ultraviolet- and short-wavelength cone mechanisms but has little impact on the development of the middle- and long-wavelength cone mechanisms; these effects coincide with the development of the various cone types. This study supports the notion that the zebrafish is a viable model for studying the effects of the lighting environment on visual development.

Descriptors: zebrafish, retina cones physiology, dark adaptation, lighting, retina physiology, behavior physiology, circadian rhythm, electroretinography, larva, ocular physiology, retina radiation effects, sensory thresholds physiology, visual perception physiology.

Dobbs McAuliffe, B., Q. Zhao, and E. Linney (2004). **Feedback mechanisms regulate retinoic acid production and degradation in the zebrafish embryo.** *Mechanisms of Development* 121(4): 339-50. ISSN: 0925-4773.

NAL Call Number: QH607.A1C4

Abstract: Retinoic acid (RA) signaling in vertebrate embryos occurs in a distinct physical and temporal pattern. Regulating this spatial distribution is crucial to the development of the embryo, as RA in excess or in inappropriate tissues is teratogenic. In order to understand how RA availability is determined in zebrafish we have investigated the expression of cyp26a1, an enzyme that inactivates RA, and its relationship to raldh2, one of the enzymes that produce RA from retinal. cyp26a1 expression follows three phases: in presumptive anterior neurectoderm and in a circumblastoporal ring during gastrulation, in the tailbud throughout somitogenesis, and in multiple specific tissue types beginning at mid-somitogenesis and continuing through 48 h postfertilization (hpf). This expression was either adjacent or opposite to those tissues expressing raldh2. We then investigated how RA production might regulate these relationships. Endogenous RA produced by raldhs did not play a role in setting cyp26a1 expression in most tissues. However, exogenous RA regulates expression of both enzymes. cyp26a1 is up regulated in the embryo in a time, concentration, and tissue-dependent manner. Conversely, raldh2 expression is reduced with RA treatment. Tests of the raldh2 promoter in cell transfections proved that RA directly represses its activity. These data demonstrate that the feedback mechanisms regulating production and degradation of RA must be considered in any experiments altering levels of RA in the developing vertebrate embryo.

Descriptors: zebrafish, feedback, biochemical physiology, tretinoin metabolism, aldehyde

oxidoreductases genetics, aldehyde oxidoreductases metabolism, cytochrome P450 enzyme system genetics, cytochrome P450 enzyme system metabolism, gene expression, retinal dehydrogenase, metabolism.

Doitsidou, M. (2005). ***Moleculaire mechanismen die de migratie van primordiale geslachtscellen in zebravis controleren. [Molecular mechanisms governing primordial germ cell migration in zebrafish.]***. Dissertation, Wageningen Universiteit: 80 p.
Descriptors: zebrafish, germ cell, primordial , cell migration.
Notes: Doctoral thesis, English with a summary in Dutch.

Dooley, K.A., A.J. Davidson, and L.I. Zon (2005). **Zebrafish scl functions independently in hematopoietic and endothelial development.** *Developmental Biology* 277(2): 522-36. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The SCL transcription factor is critically important for vertebrate hematopoiesis and angiogenesis, and has been postulated to induce hemangioblasts, bipotential precursors for blood and endothelial cells. To investigate the function of scl during zebrafish hematopoietic and endothelial development, we utilized site-directed, anti-sense morpholinos to inhibit scl mRNA. Knockdown of scl resulted in a loss of primitive and definitive hematopoietic cell lineages. However, the expression of early hematopoietic genes, gata2 and lmo2, was unaffected, suggesting that hematopoietic cells were present but unable to further differentiate. Using gene expression analysis and visualization of vessel formation in live animals harboring an lmo2 promoter-green fluorescent protein reporter transgene (Tg(lmo2:EGFP)), we show that angioblasts were specified normally in the absence of scl, but later defects in angiogenesis were evident. While scl was not required for angioblast specification, forced expression of exogenous scl caused an expansion of both hematopoietic and endothelial gene expression, and a loss of somitic tissue. In cloche and spadetail mutants, forced expression of scl resulted in an expansion of hematopoietic but not endothelial tissue. Surprisingly, in cloche, lmo2 was not induced in response to scl over-expression. Taken together, these findings support distinct roles for scl in hematopoietic and endothelial development, downstream of hemangioblast development.
Descriptors: zebrafish , DNA binding proteins genetics, proteins metabolism, endothelium embryology, developmental gene expression regulation, hematopoiesis physiology, hematopoietic stem cells metabolism, proto oncogene proteins metabolism, transcription factors metabolism, proteins metabolism, antisense elements genetics metabolism, basic helix loop helix transcription factors, cell differentiation physiology, DNA binding proteins genetics, endothelium metabolism, GATA2 transcription factor, green fluorescent proteins metabolism, in situ hybridization, metalloproteins metabolism, microinjections, mutation genetics, proto oncogene reverse transcriptase PCR, T-box domain T-box domain proteins metabolism, transcription factors genetics, trans proteins genetics.

Dorsky, R.I., M. Itoh, R.T. Moon, and A. Chitnis (2003). **Two tcf3 genes cooperate to pattern the zebrafish brain.** *Development* 130(9): 1937-47. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Caudalizing factors operate in the context of Wnt/beta-catenin signaling to induce gene expression in discrete compartments along the rostral-caudal axis of the developing vertebrate nervous system. In zebrafish, basal repression of caudal genes is achieved through the function of Headless (Hdl), a Tcf3 homolog. In this study, we show that a second Tcf3 homolog, Tcf3b, limits caudalization caused by loss of Hdl function and although this Lef/

Tcf family member can rescue hdl mutants, Lef1 cannot. Wnts can antagonize repression mediated by Tcf3 and this derepression is dependent on a Tcf3 beta-catenin binding domain. Systematic changes in gene expression caused by reduced Tcf3 function help predict the shape of a caudalizing activity gradient that defines compartments along the rostral-caudal axis. In addition, Tcf3b has a second and unique role in the morphogenesis of rhombomere boundaries, indicating that it controls multiple aspects of brain development.
Descriptors: zebrafish, brain embryology, hmgb transcription factors genetics, amino acid sequence, cytoskeletal proteins, hmgb proteins metabolism, molecular sequence data, mutation, phenotype, antisense RNA, Tcf transcription factors, transcription factors metabolism, Wnt proteins, proteins.

Dosch, R., D.S. Wagner, K.A. Mintzer, G. Runke, A.P. Wiemelt, and M.C. Mullins (2004). **Maternal control of vertebrate development before the midblastula transition: mutants from the zebrafish I.** *Developmental Cell* 6(6): 771-80. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Maternal factors control development prior to the activation of the embryonic genome. In vertebrates, little is known about the molecular mechanisms by which maternal factors regulate embryonic development. To understand the processes controlled by maternal factors and identify key genes involved, we embarked on a maternal-effect mutant screen in the zebrafish. We identified 68 maternal-effect mutants. Here we describe 15 mutations in genes controlling processes prior to the midblastula transition, including egg development, blastodisc formation, embryonic polarity, initiation of cell cleavage, and cell division. These mutants exhibit phenotypes not previously observed in zygotic mutant screens. This collection of maternal-effect mutants provides the basis for a molecular genetic analysis of the maternal control of embryogenesis in vertebrates.
Descriptors: zebrafish, blastula metabolism, embryology, developmental gene expression regulation genetics, mutation genetics, blastula cytology, cell division genetics, cell polarity genetics, chromosome mapping, cytology, male infertility genetics, oocytes cytology, oocytes metabolism, sex differentiation genetics, sex factors.

Dougan, S.T., R.M. Warga, D.A. Kane, A.F. Schier, and W.S. Talbot (2003). **The role of The zebrafish nodal-related genes squint and cyclops in patterning of mesendoderm.** *Development* 130(9): 1837-51. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Nodal signals, a subclass of the TGFbeta superfamily of secreted factors, induce formation of mesoderm and endoderm in vertebrate embryos. We have examined the possible dorsoventral and animal-vegetal patterning roles for Nodal signals by using mutations in two zebrafish nodal-related genes, squint and cyclops, to manipulate genetically the levels and timing of Nodal activity. squint mutants lack dorsal mesendodermal gene expression at the late blastula stage, and fate mapping and gene expression studies in sqt(-/-); cyc(+/+) and sqt(-/-); cyc(+/-) mutants show that some dorsal marginal cells inappropriately form hindbrain and spinal cord instead of dorsal mesendodermal derivatives. The effects on ventrolateral mesendoderm are less severe, although the endoderm is reduced and muscle precursors are located nearer to the margin than in wild type. Our results support a role for Nodal signals in patterning the mesendoderm along the animal-vegetal axis and indicate that dorsal and ventrolateral mesoderm require different levels of squint and cyclops function. Dorsal marginal cells were not transformed toward more lateral fates in either sqt(-/-); cyc(+/-) or sqt(-/-); cyc(+/+) embryos, arguing against a role for the graded action of Nodal signals in dorsoventral patterning of the mesendoderm. Differential regulation of the cyclops

gene in these cells contributes to the different requirements for nodal-related gene function in these cells. Dorsal expression of cyclops requires Nodal-dependent autoregulation, whereas other factors induce cyclops expression in ventrolateral cells. In addition, the differential timing of dorsal mesendoderm induction in squint and cyclops mutants suggests that dorsal marginal cells can respond to Nodal signals at stages ranging from the mid-blastula through the mid-gastrula.

Descriptors: zebrafish, endoderm metabolism, mesoderm metabolism, transforming growth factor beta metabolism, proteins, cytoskeletal proteins metabolism, gene dosage, developmental gene expression regulation, intracellular signaling peptides and proteins, genetic models, mutation, nervous system embryology, trans activators metabolism, transforming growth factor beta genetics, beta catenin.

Dougan, S.T. and E.A.G. Hagos (2006). **Time-dependent patterning of the germ layers by nodal signals**. *Developmental Biology* 295(1): 440. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish, molecular genetics, development, embryogenesis, Nodal signal, time dependent patterning.

Downes, G.B. and M. Granato (2004). **Acetylcholinesterase function is dispensable for sensory neurite growth but is critical for neuromuscular synapse stability**. *Developmental Biology* 270(1): 232-245. ISSN: 0012-1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish, acetylcholine, acetylcholine receptors, acetylcholinesterase, allelles, biological development, embryonic development, enzymes, freshwater fish, muscles, neurons, neurotransmitters, receptors, sensory neurons, synapses, *Danio rerio*, acetylcholinesterase, neuromuscular synapses.

Draper, B.W., C.M. McCallum, and C.B. Moens (2007). **Nanos1 is required to maintain oocyte production in adult zebrafish.** *Developmental Biology* 305(2): 589-98. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Development of the germline requires the specification and survival of primordial germ cells (PGCs) in the embryo as well as the maintenance of gamete production during the reproductive life of the adult. These processes appear to be fundamental to all Metazoans, and some components of the genetic pathway regulating germ cell development and function are evolutionarily conserved. In both vertebrates and invertebrates, nanos-related genes, which encode RNA-binding zinc finger proteins, have been shown to play essential and conserved roles during germ cell formation. In *Drosophila*, maternally supplied nanos is required for survival of PGCs in the embryo, while in adults, nanos is required for the continued production of oocytes by maintaining germline stem cells self-renewal. In mice and zebrafish, nanos orthologs are required for PGC survival during embryogenesis, but a role in adults has not been explored. We show here that nanos1 in zebrafish is expressed in early stage oocytes in the adult female germline. We have identified a mutation in nanos1 using a reverse genetics method and show that young female nanos mutants contain oocytes, but fail to maintain oocyte production. This progressive loss of fertility in homozygous females is not a phenotype that has been described previously in the zebrafish and underlines the value of a reverse genetics approach in this model system.

Descriptors: zebrafish, oocytes physiology, oogenesis physiology, physiology, oogenesis genetics, stem cells physiology, growth and development, proteins biosynthesis, proteins genetics.

Draper, B.W., D.W. Stock, and C.B. Kimmel (2003). **Zebrafish fgf24 functions with fgf8 to promote posterior mesodermal development.** *Development* 130(19): 4639-54. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Fibroblast growth factor (Fgf) signaling plays an important role during development of posterior mesoderm in vertebrate embryos. Blocking Fgf signaling by expressing a dominant-negative Fgf receptor inhibits posterior mesoderm development. In mice, Fgf8 appears to be the principal ligand required for mesodermal development, as mouse Fgf8 mutants do not form mesoderm. In zebrafish, Fgf8 is encoded by the acerebellar locus, and, similar to its mouse otholog, is expressed in early mesodermal precursors during gastrulation. However, zebrafish fgf8 mutants have only mild defects in posterior mesodermal development, suggesting that it is not the only Fgf ligand involved in the development of this tissue. We report here the identification of an fgf8-related gene in zebrafish, fgf24, that is co-expressed with fgf8 in mesodermal precursors during gastrulation. Using morpholino-based gene inactivation, we have analyzed the function of fgf24 during development. We found that inhibiting fgf24 function alone has no affect on the formation of posterior mesoderm. Conversely, inhibiting fgf24 function in embryos mutant for fgf8 blocks the formation of most posterior mesoderm. Thus, fgf8 and fgf24 are together required to promote posterior mesodermal development. We provide both phenotypic and genetic evidence that these Fgf signaling components interact with no tail and spadetail, two zebrafish T-box transcription factors that are required for the development of all posterior mesoderm. Last, we show that fgf24 is expressed in early fin bud mesenchyme and that inhibiting fgf24 function results in viable fish that lack pectoral fins.

Descriptors: zebrafish embryos, Fgf metabolism, mesoderm physiology, signal transduction physiology, proteins metabolism, amino acid sequence, enhancer elements genetics, fibroblast growth factor 8, Fgf chemistry, Fgf genetics, gastrula cytology, gastrula physiology, developmental gene expression regulation, ligands, mice, molecular sequence data, antisense oligonucleotides metabolism, phylogeny, sequence alignment, T-box domain proteins metabolism, anatomy and histology, proteins chemistry, proteins classification.

Drummond, I.A. (2004). **Zebrafish kidney development.** *Methods in Cell Biology* 76: 501-30. ISSN: 0091-679X.

NAL Call Number: 442P92

Descriptors: zebrafish, kidney embryology, body patterning physiology, cell differentiation physiology, cell lineage, disease models, endothelial cells physiology, epithelial cells physiology, developmental gene expression regulation, homeodomain homeodomain, kidney cytology, kidney physiology, kidney diseases complications, kidney diseases genetics, kidney function tests methods, kidney glomerulus cytology, kidney glomerulus embryology, kidney glomerulus physiology, kidney tubules cytology, kidney tubules embryology, kidney tubules physiology, mesoderm cytology, mesoderm physiology, mutation, physiologic neovascularization physiology, nephrons cytology, nephrons embryology, nephrons physiology, organogenesis, polycystic kidney diseases genetics, polycystic kidney diseases pathology, polycystic kidney diseases physiopathology, regional blood flow physiology.

Du, S.J., J. Rotllant, and X. Tan (2006). **Muscle-specific expression of the smyd1 gene is controlled by its 5.3-kb promoter and 5'-flanking sequence in zebrafish embryos.** *Developmental Dynamics* 235(12): 3306-15. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish, histone lysine n methyltransferase genetics, genetics, 5' flanking

region, genetically modified, base sequence, DNA genetics, developmental gene expression regulation, green fluorescent in situ hybridization, molecular sequence data, fast twitch muscle fibers metabolism, muscle fibers, slow twitch metabolism, skeletal muscle embryology, skeletal muscle metabolism, promoter regions genetics, messenger RNA genetics and metabolism, recombinant fusion proteins genetics.

Du, S.J., H. Li, Y. Bian, and Y. Zhong (2008). **Heat-shock protein 90 alpha 1 is required for organized myofibril assembly in skeletal muscles of zebrafish embryos**. *Proceedings of the National Academy of Sciences, USA* 105(2): 554-559. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, biochemical analysis, chaperones, development, embryos, filaments, heat shock proteins, muscle contraction, myofibrils, myosin, protein folding, skeletal muscle, somites, *Danio rerio*.

Dumstrei, K., R. Mennecke, and E. Raz (2004). **Signaling pathways controlling primordial germ cell migration in zebrafish.** *Journal of Cell Science* 117(Pt 20): 4787-95. ISSN: 0021-9533.
NAL Call Number: QH301.J6
Abstract: During their migration, zebrafish primordial germ cells (PGCs) rely on directional cues provided by the chemokine SDF-1a, whose receptor is CXCR4b. The molecular mechanisms whereby CXCR4b activation is interpreted intracellularly into directional migration are not known. Here we investigate the role of two important biochemical pathways -- G-protein-dependent and phosphoinositide 3-kinase (PI3K)-dependent signaling -- in directing PGC migration in zebrafish. We show that G proteins of the Gi family are essential for directional migration but not for PGC motility. Inhibition of PI3K signaling in PGCs slows down their migration and leads to abnormal cell morphology as well as to reduced stability of filopodia. Invariably, during directed PGC migration, the distribution of the products of PI3K activity - phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P(3)] and/or phosphatidylinositol (3,4)bisphosphate [PtdIns(3,4)P(2)] -- is not polarized, and reducing the level of these 3-phosphoinositides does not affect the ability of PGCs to migrate directionally. We therefore conclude that Gi-dependent signaling is essential for directional migration, whereas the PI3K pathway is important for the actual motility of PGCs.
Descriptors: zebrafish embryos, cell movement physiology, GTP binding protein alpha subunits, gi go metabolism, germ cells physiology, signal transduction physiology, 1 phosphatidylinositol 3 kinase metabolism, cell membrane metabolism, cell shape, germ cells cytology, in situ hybridization, morphogenesis physiology, pertussis toxin metabolism, phosphatidylinositol phosphates metabolism, pseudopodia metabolism, Cxcr4 receptors genetics, Cxcr4 receptors metabolism, recombinant fusion proteins metabolism, anatomy and histology.

Durst, W., C. Henry, and S. Amacher (2004). **Analysis of a novel zebrafish segmentation mutant.** *Developmental Biology* 271(2): 619. ISSN: 0012 1606.
NAL Call Number: 448.8D49
Descriptors: zebrafish, molecular genetics, biochemistry and molecular biophysics, development, segmentation mutant.

Dworkin, S., J.K. Heath, T.A. deJong Curtain, B.M. Hogan, G.J. Lieschke, J. Malaterre, R.G. Ramsay, and T. Mantamadiotis (2007). **CREB activity modulates neural cell proliferation, midbrain-hindbrain organization and patterning in zebrafish.** *Developmental Biology* 307(1): 127-41. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Neural stem/progenitor cells (NPCs) self-renew and differentiate, generating neuronal and non-neuronal (glial) cell lineages. Although a number of factors, including transcription factors, have been shown to be important in the regulation of NPC proliferation and differentiation, the precise molecular networks remain to be identified. The cAMP Response Element-Binding protein (CREB) is a transcription factor important for neuronal survival, differentiation and plasticity. Recent work suggests that CREB activation, via serine phosphorylation in the kinase inducible domain, is important for neurogenesis in the adult rodent brain. We sought to further investigate CREB function in neurogenesis, using the zebrafish (*Danio rerio*). Structural and functional analysis of the zebrafish CREB orthologue showed high conservation with mammalian CREB. Activated (phosphorylated) CREB (pCREB) was localised to all known proliferation zones in the adult zebrafish brain, including actively cycling cells. Furthermore, we found that modulating CREB activity during early zebrafish development caused significant defects in neural proliferation, midbrain-hindbrain organization and body patterning. These findings reveal broader and stage-specific physiological roles of CREB function during vertebrate neural development and proliferation.

Descriptors: zebrafish, body patterning, cell proliferation, cyclic AMP response element binding protein physiology, mesencephalon physiology, neurons cytology, rhombencephalon physiology, central nervous system growth and development, phosphorylation.

Eaton, J.L. and E. Glasgow (2006). **The zebrafish bHLH PAS transcriptional regulator, single-minded 1 (sim1), is required for isotocin cell development.** *Developmental Dynamics* 235(8): 2071-82. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: A wide range of physiological and behavioral processes, such as social, sexual, and maternal behaviors, learning and memory, and osmotic homeostasis are influenced by the neurohypophysial peptides oxytocin and vasopressin. Disruptions of these hormone systems have been linked to several neurobehavioral disorders, including autism, Prader-Willi syndrome, affective disorders, and obsessive-compulsive disorder. Studies in zebrafish promise to reveal the complex network of regulatory genes and signaling pathways that direct the development of oxytocin- and vasopressin-like neurons, and provide insight into factors involved in brain disorders associated with disruption of these systems. Isotocin, which is homologous to oxytocin, is expressed early, in a simple pattern in the developing zebrafish brain. Single-minded 1 (sim1), a member of the bHLH-PAS family of transcriptional regulatory genes, is required for terminal differentiation of mammalian oxytocin cells and is a master regulator of neurogenesis in *Drosophila*. Here we show that sim1 is expressed in the zebrafish forebrain and is required for isotocin cell development. The expression pattern of sim1 mRNA in the embryonic forebrain is dynamic and complex, and overlaps with isotocin expression in the preoptic area. We provide evidence that the role of sim1 in zebrafish neuroendocrine cell development is evolutionarily conserved with that of mammals.

Descriptors: zebrafish, basic helix loop helix transcription factors metabolism, cell differentiation, oxytocin analogs and derivatives, repressor proteins metabolism, genetic transcription genetics, metabolism, basic helix loop helix transcription factors classification, basic helix loop helix transcription factors genetics, body patterning, diencephalon embryology, diencephalon metabolism, developmental gene expression regulation, homeodomain proteins genetics and metabolism, oxytocin metabolism, phylogeny, prosencephalon embryology, prosencephalon metabolism, messenger RNA genetics, repressor proteins classification, repressor spinal nerve roots embryology, spinal nerve roots metabolism, transcription factors genetics and metabolism, genetics, proteins classification.

Eberhart, J.K., M.E. Swartz, T.C. Boling, and C.B. Kimmel (2006). **Hh-dependent pdgf signaling mediates zebrafish palatogenesis**. *Developmental Biology* 295(1): 425. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, biochemistry and molecular biophysics, development, dental and oral system , signaling hierarchy, pdgf, palatogenesis.

Eberhart, J.K., M.E. Swartz, J.G. Crump, and C.B. Kimmel (2006). **Early Hedgehog signaling from neural to oral epithelium organizes anterior craniofacial development**. *Development* 133(6): 1069-1077. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Hedgehog (Hh) signaling plays multiple roles in the development of the anterior craniofacial skeleton. We show that the earliest function of Hh is indirect, regulating development of the stomodeum, or oral ectoderm. A subset of post-migratory neural crest cells, that gives rise to the cartilages of the anterior neurocranium and the pterygoid process of the palatoquadrate in the upper jaw, condenses upon the upper or roof layer of the stomodeal ectoderm in the first pharyngeal arch. We observe that in mutants for the Hh co-receptor smoothened (smo) the condensation of this specific subset of crest cells fails, and expression of several genes is lost in the stomodeal ectoderm. Genetic mosaic analyses with smo mutants show that for the crest cells to condense the crucial target tissue receiving the Hh signal is the stomodeum, not the crest. Blocking signaling with cyclopamine reveals that the crucial stage, for both crest condensation and stomodeal marker expression, is at the end of gastrulation-some eight to ten hours before crest cells migrate to associate with the stomodeum. Two Hh genes, shh and twhh, are expressed in midline tissue at this stage, and we show using mosaics that for condensation and skeletogenesis only the ventral brain primordium, and not the prechordal plate, is an important Hh source. Thus, we propose that Hh signaling from the brain primordium is required for proper specification of the stomodeum and the stomodeum, in turn, promotes condensation of a subset of neural crest cells that will form the anterior neurocranial and upper jaw cartilage. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, nervous system, neural coordination, molecular genetics, biochemistry and molecular biophysics, development, Hedgehog signaling, neural crest cells.

Echeverri, K. and A.C. Oates (2007). **Coordination of symmetric cyclic gene expression during somitogenesis by suppressor of hairless involves regulation of retinoic acid catabolism**. *Developmental Biology* 301(2): 388-403. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, antisense, asymmetry, catabolism, coordination, embryonic development, embryos, enzymes, fibroblast growth factor, freshwater fish, gene expression, genetic abnormalities, hairless, mesoderm, metabolism, Notch protein, oscillations, oscillators, phylogenetics, retinoic acid, segmentation, signal transduction, skeletal muscle, suppressors, transcription factors, vertebrae, somites, somitogenesis, *Danio rerio*.

Eisen, J. (2003). **Zebrafish as a model system. Perspective.** *Developmental Dynamics* 228(3): 299-300. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, research genetic models, genetics.

Eivers, E., K. Mccarthy, C. Glynn, C.M. Nolan, and L. Byrnes (2004). **Insulin-like growth factor (IGF) signalling is required for early dorso-anterior development of the zebrafish embryo**. *International Journal of Developmental Biology* 48(10): 1131-1140. ISSN: 0214

6282.
Abstract: The insulin-like growth factor (IGF) signalling pathway has been highly conserved in animal evolution and, in mammals and *Xenopus*, plays a key role in embryonic growth and development, with the IGF-1 receptor (IGF-1R) being a crucial regulator of the signalling cascade. Here we report the first functional role for the IGF pathway in zebrafish. Expression of mRNA coding for a dominant negative IGF-1R resulted in embryos that were small in size compared to controls and had disrupted head and CNS development. At its most extreme, this phenotype was characterized by a complete loss of head and eye structures, an absence of notochord and the presence of abnormal somites. In contrast, up-regulation of IGF signalling following injection of IGF-1 mRNA, resulted in a greatly expanded development of anterior structures at the expense of trunk and tail. IGF-1R knockdown caused a significant decrease in the expression of Otx2, Rx3, FGF8, Pax6.2 and Ntl, while excess IGF signalling expanded Otx2 expression in presumptive forebrain tissue and widened the Ntl expression domain in the developing notochord. The observation that IGF-1R knockdown reduced expression of two key organizer genes (chordin and goosecoid) suggests that IGF signalling plays a role in regulating zebrafish organizer activity. This is supported by the expression of IGF-1, IGF-2 and IGF-1R in shield-stage zebrafish embryos and the demonstration that IGF signalling influences expression of BMP2b, a gene that plays an important role in zebrafish pattern formation. Our data is consistent with a common pathway for integration of IGF, FGF8 and anti-BMPs in early vertebrate development. Copyright © 2008 CABI
Descriptors: zebrafish, embryonic development, embryos, genes, insulin like growth factor, messenger RNA, phenotypes, regulatory genes, signal transduction, *Danio rerio*, fishes.

Elizondo, M.R., E.L. MacDonald, and D.M. Parichy (2006). **Dwarf zebrafish, altered bone development, and kidney stone formation in trpm7 mutants** . *Developmental Biology* 295(1): 322-323. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, biological development, bones, embryology, freshwater fish, gene expression, kidneys, organogenesis, *Danio rerio*.

Ellertsdottir, E., J. Ganz, K. Durr, N. Loges, F. Biemar, F. Seifert, A.K. Ettl, A.K. Kramer Zucker, R. Nitschke, and W. Driever (2006). **A mutation in the zebrafish Na,K-ATPase subunit atp1a1a.1 provides genetic evidence that the sodium potassium pump contributes to left-right asymmetry downstream or in parallel to nodal flow.** *Developmental Dynamics* 235(7): 1794-808. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryology, Na+ K+ exchanging ATPase physiology, biological transport, body patterning developmental gene expression regulation, membrane membrane proteins metabolism, mutation, Na+ K+ exchanging ATPase biosynthesis, Na+ K+ exchanging ATPase genetics, protein subunits, genetics and metabolism, proteins biosynthesis.

Ellingsen, S., M.A. Laplante, M. Konig, H. Kikuta, T. Furmanek, E.A. Hoivik, and T.S. Becker (2005). **Large-scale enhancer detection in the zebrafish genome.** *Development* 132(17): 3799-811. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Murine retroviral vectors carrying an enhancer detection cassette were used to generate 95 transgenic lines of fish in which reporter expression is observed in distinct patterns during embryonic development. We mapped 65 insertion sites to the as yet unfinished

zebrafish genome sequence. Many integrations map close to previously known developmental genes, including transcription factors of the Pax, Hox, Sox, Pou, Otx, Emx, zinc-finger and bHLH gene families. In most cases, the activated provirus is located in, or within a 15 kb interval around, the corresponding transcriptional unit. The exceptions include four insertions into a gene desert on chromosome 20 upstream of sox11b, and an insertion upstream of otx1. In these cases, the activated insertions are found at a distance of between 32 kb and 132 kb from the coding region. These as well as seven other insertions described here identify genes that have recently been associated with ultra conserved non-coding elements found in all vertebrate genomes.

Descriptors: zebrafish, enhancer elements genetics, genome, genetics, alleles, genetically modified, cultured cells, embryology, metabolism, developmental gene expression regulation genetics , reporter insertional mutagenesis, genetic transcription genetics.

Elsalini, O.A., J. von Gartzen, M. Cramer, and K.B. Rohr (2003). **Zebrafish hhex, nk2.1a, and pax2.1 regulate thyroid growth and differentiation downstream of Nodal-dependent transcription factors.** *Developmental Biology* 263(1): 67-80. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: During zebrafish development, the thyroid primordium initiates expression of molecular markers such as hhex and nk2.1a in the endoderm prior to pharynx formation. As expected for an endodermally derived organ, initiation of thyroid development depends on Nodal signalling. We find that it also depends on three downstream effectors of Nodal activity, casanova (cas), bonnie and clyde (bon), and faust (fau)/gata5. Despite their early Nodal-dependent expression in the endoderm, both hhex and nk2.1a are only required relatively late during thyroid development. In hhex and nk2.1a loss-of-function phenotypes, thyroid development is initiated and arrests only after the primordium has evaginated from the pharyngeal epithelium. Thus, like pax2.1, both hhex and nk2.1a have similarly late roles in differentiation or growth of thyroid follicular cells, and here, we show that all three genes act in parallel rather than in a single pathway. Our functional analysis suggests that these genes have similar roles as in mammalian thyroid development, albeit in a different temporal mode of organogenesis.

Descriptors: zebrafish, DNA binding, homeodomain, thyroid gland embryology, transcription factors physiology, transforming growth factor beta physiology, proteins, cell differentiation, cell movement, endoderm physiology, developmental gene expression regulation, mice, pax2 transcription factor, repressor proteins.

Elworthy, S., J.A. Lister, T.J. Carney, D.W. Raible, and R.N. Kelsh (2003). **Transcriptional regulation of mitfa accounts for the sox10 requirement in zebrafish melanophore development.** *Development* 130(12): 2809-18. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: The transcription factor Sox10 is required for the specification, migration and survival of all nonectomesenchymal neural crest derivatives including melanophores. sox10(-/-) zebrafish lack expression of the transcription factor mitfa, which itself is required for melanophore development. We demonstrate that the zebrafish mitfa promoter has sox10 binding sites necessary for activity in vitro, consistent with studies using mammalian cell cultures that have shown that Sox10 directly regulates Mitf expression. In addition, we demonstrate that these sites are necessary for promoter activity in vivo. We show that reintroduction of mitfa expression in neural crest cells can rescue melanophore development in sox10(-/-) embryos. This rescue of melanophores in sox10(-/-) embryos is quantitatively indistinguishable from rescue in mitfa(-/-) embryos. These findings show that the essential function of sox10 in mel-

anophore development is limited to transcriptional regulation of mitfa. We propose that the dominant melanophore phenotype in Waardenburg syndrome IV individuals with SOX10 mutations is likely to result from failure to activate MITF in the normal number of melanoblasts.

Descriptors: zebrafish, DNA binding proteins genetics, proteins metabolism, high mobility group proteins metabolism, melanophores metabolism, transcription factors , genetic crosses, DNA binding proteins genetics, proteins biosynthesis, DNA binding proteins genetics, developmental gene expression regulation physiology, microphthalmia associated transcription factor, mutation, promoter regions genetics, transcription factors biosynthesis, transcription factors genetics.

Elworthy, S., J.P. Pinto, A. Pettifer, M.L. Cancela, and R.N. Kelsh (2005). **Phox2b function in the enteric nervous system is conserved in zebrafish and is sox10-dependent.** *Mechanisms of Development* 122(5): 659-69. ISSN: 0925-4773.

NAL Call Number: QH607.A1C4

Abstract: Zebrafish lacking functional sox10 have defects in non-ectomesenchymal neural crest derivatives including the enteric nervous system (ENS) and as such provide an animal model for human Waardenburg Syndrome IV. Here, we characterize zebrafish phox2b as a functionally conserved marker of the developing ENS. We show that morpholino-mediated knockdown of Phox2b generates fish modeling Hirschsprung disease. Using markers, including phox2b, we investigate the ontogeny of the sox10 ENS phenotype. As previously shown for melanophore development, ENS progenitor fate specification fails in these mutant fish. However, in addition, we trace back the sox10 mutant ENS defect to an even earlier time point, finding that most neural crest cells fail to migrate ventrally to the gut primordium.

Descriptors: zebrafish, carrier carrier, enteric nervous system embryology, developmental gene expression regulation, high mobility group proteins genetics and physiology, homeodomain, transcription factors physiology, amino acid sequence, carrier proteins metabolism, central nervous system embryology, chromosome mapping, high mobility group proteins metabolism, homeodomain proteins metabolism, in situ hybridization, melanophores metabolism, fluorescence microscopy, genetic models, molecular sequence data, mutation, neural crest metabolism, neurons metabolism, phenotype, phylogeny, amino acid sequence homology, stem cells metabolism, time factors, transcription factors metabolism.

Emoto, Y., H. Wada, H. Okamoto, A. Kudo, and Y. Imai (2005). **Retinoic acid-metabolizing enzyme Cyp26a1 is essential for determining territories of hindbrain and spinal cord in zebrafish.** *Developmental Biology* 278(2): 415-27. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Retinoic acid (RA) plays a critical role in neural patterning and organogenesis in the vertebrate embryo. Here we characterize a mutant of the zebrafish named giraffe (gir) in which the gene for the RA-degrading enzyme Cyp26a1 is mutated. The gir mutant displayed patterning defects in multiple organs including the common cardinal vein, pectoral fin, tail, hindbrain, and spinal cord. Analyses of molecular markers suggested that the lateral plate mesoderm is posteriorized in the gir mutant, which is likely to cause the defects of the common cardinal vein and pectoral fin. The cyp26a1 expression in the rostral spinal cord was strongly upregulated in the gir mutant, suggesting a strong feedback control of its expression by RA signaling. We also found that the rostral spinal cord territory was expanded at the expense of the hindbrain territory in the gir mutant. Such a phenotype is the opposite of that of the mutant for Raldh2, an enzyme that synthesizes RA. We propose a model in which Cyp26a1 attenuates RA signaling in the prospective rostral spinal cord to limit the expression

of hox genes and to determine the hindbrain-spinal cord boundary.
Descriptors: zebrafish embryos, cytochrome P450 enzyme system metabolism, rhombencephalon embryology, spinal cord embryology, tretinoin metabolism, base sequence, body patterning genetics, chromosome mapping, DNA primers, in situ hybridization, morphogenesis, mutation, phenotype, rhombencephalon enzymology, spinal cord enzymology.

England, S.J., G.B. Blanchard, L. Mahadevan, and R.J. Adams (2006). **A dynamic fate map of the forebrain shows how vertebrate eyes form and explains two causes of cyclopia**. *Development* 133(23): 4613-4617. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Mechanisms for shaping and folding sheets of cells during development are poorly understood. An example is the complex reorganisation of the forebrain neural plate during neurulation, which must fold a sheet into a tube while evaginating two eyes from a single contiguous domain within the neural plate. We, for the first time, track these cell rearrangements to show that forebrain morphogenesis differs significantly from prior hypotheses. We postulate a new model for forebrain neurulation and demonstrate how mutations affecting two signalling pathways can generate cyclopic phenotypes by disrupting normal cell movements or introducing new erroneous behaviours. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, nervous system, development, sense organs, cyclopia, congenital disease, etiology, phenotype, morphogenesis, neurulation, dynamic fate map.

Erdmann, B., F.P. Kirsch, F.G. Rathjen, and M.I. More (2003). **N-cadherin is essential for retinal lamination in the zebrafish.** *Developmental Dynamics* 226(3): 570-7. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, cadherins genetics, cadherins metabolism, developmental gene expression regulation, retina embryology, retina physiology, epithelial cells physiology, crystalline lens cytology, crystalline lens embryology, electron microscopy, mutation, photoreceptors, photoreceptors ultrastructure, synapses physiology.

Erickson, T., S. Scholpp, M. Brand, C.B. Moens, and A.J. Waskiewicz (2007). **Pbx proteins cooperate with engrailed to pattern the midbrain-hindbrain and diencephalic-mesencephalic boundaries**. *Developmental Biology* 301(2): 504-517. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, boundaries, developmental stages, embryos, engrailed protein, fibroblast growth factor 8, freshwater fish, hindbrain, mesencephalon, models, neural tube, pattern formation, phenotypes, phylogenetics, proteins, territory, transcription, transcription factors, *Danio rerio*, *Drosophila*.

Eroglu, B., G. Wang, N. Tu, X. Sun, and N.F. Mivechi (2006). **Critical role of Brg1 member of the SWI/SNF chromatin remodeling complex during neurogenesis and neural crest induction in zebrafish.** *Developmental Dynamics* 235(10): 2722-35. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, chromosomal proteins, non-histone physiology, neural crest embryology, transcription factors physiology, cell differentiation genetics, cell differentiation physiology, chromatin metabolism, chromatin immunoprecipitation, chromosomal proteins, non histone genetics, chromosomal proteins, non histone metabolism, DNA helicases genetics, DNA helicases metabolism, DNA helicases physiology, embryology, metabolism, developmental gene expression regulation genetics, immunohistochemistry, in situ hybridization, mutation genetics, neural crest metabolism, organogenesis genetics, organogenesis

physiology, messenger RNA genetics and metabolism, time factors, transcription factors genetics and metabolism and proteins metabolism.

Esguerra, C.V., L. Nelles, L. Vermeire, A. Ibrahimi, A.D. Crawford, R. Derua, E. Janssens, E. Waelkens, P. Carmeliet, D. Collen, and D. Huylebroeck (2007). **Ttrap is an essential modulator of Smad3-dependent Nodal signaling during zebrafish gastrulation and left-right axis determination.** *Development* 134(24): 4381-93 . ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: During vertebrate development, signaling by the TGFbeta ligand Nodal is critical for mesoderm formation, correct positioning of the anterior-posterior axis, normal anterior and midline patterning, and left-right asymmetric development of the heart and viscera. Stimulation of Alk4/EGF-CFC receptor complexes by Nodal activates Smad2/3, leading to left-sided expression of target genes that promote asymmetric placement of certain internal organs. We identified Ttrap as a novel Alk4- and Smad3-interacting protein that controls gastrulation movements and left-right axis determination in zebrafish. Morpholino-mediated Ttrap knockdown increases Smad3 activity, leading to ectopic expression of snail1a and apparent repression of e-cadherin, thereby perturbing cell movements during convergent extension, epiboly and node formation. Thus, although the role of Smad proteins in mediating Nodal signaling is well-documented, the functional characterization of Ttrap provides insight into a novel Smad partner that plays an essential role in the fine-tuning of this signal transduction cascade.
Descriptors: zebrafish, Tgfbeta ligand Nodal, Smad3, Alk4, snail 1-a, e-cadherin, embryo development.

Esni, F., B. Ghosh, A.V. Biankin, J.W. Lin, M.A. Albert, X. Yu, R.J. MacDonald, C.I. Civin , F.X. Real, M.A. Pack, D.W. Ball, and S.D. Leach (2004). **Notch inhibits Ptf1 function and acinar cell differentiation in developing mouse and zebrafish pancreas.** *Development* 131(17): 4213-24. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Notch signaling regulates cell fate decisions in a variety of adult and embryonic tissues, and represents a characteristic feature of exocrine pancreatic cancer. In developing mouse pancreas, targeted inactivation of Notch pathway components has defined a role for Notch in regulating early endocrine differentiation, but has been less informative with respect to a possible role for Notch in regulating subsequent exocrine differentiation events. Here, we show that activated Notch and Notch target genes actively repress completion of an acinar cell differentiation program in developing mouse and zebrafish pancreas. In developing mouse pancreas, the Notch target gene Hes1 is co-expressed with Ptf1-P48 in exocrine precursor cells, but not in differentiated amylase-positive acinar cells. Using lentiviral delivery systems to induce ectopic Notch pathway activation in explant cultures of E10.5 mouse dorsal pancreatic buds, we found that both Hes1 and Notch1-IC repress acinar cell differentiation, but not Ptf1-P48 expression, in a cell-autonomous manner. Ectopic Notch activation also delays acinar cell differentiation in developing zebrafish pancreas. Further evidence of a role for endogenous Notch in regulating exocrine pancreatic differentiation was provided by examination of zebrafish embryos with homozygous mindbomb mutations, in which Notch signaling is disrupted. mindbomb-deficient embryos display accelerated differentiation of exocrine pancreas relative to wild-type clutchmate controls. A similar phenotype was induced by expression of a dominant-negative Suppressor of Hairless [Su(H)] construct, confirming that Notch actively represses acinar cell differentiation during zebrafish pancreatic development. Using transient transfection assays involving a Ptf1-responsive reporter gene,

we further demonstrate that Notch and Notch/Su(H) target genes directly inhibit Ptf1 activity, independent of changes in expression of Ptf1 component proteins. These results define a normal inhibitory role for Notch in the regulation of exocrine pancreatic differentiation.
Descriptors: zebrafish, developmental gene expression regulation, membrane, transcription factors metabolism, adenoviridae genetics, COS cells, cell differentiation, endoderm metabolism, gene transfer techniques, reporter genes, green fluorescent proteins, in situ hybridization, lentivirus genetics, luciferases metabolism, luminescent proteins metabolism, membrane proteins metabolism, mice, confocal microscopy, fluorescence microscopy, pancreas, plasmids metabolism, promoter regions genetics, messenger RNA metabolism, Notch receptors, time factors, transfection, transgenes.

Essner, J.J., J.D. Amack, M.K. Nyholm, E.B. Harris, and H.J. Yost (2005). **Kupffer's vesicle is a ciliated organ of asymmetry in the zebrafish embryo that initiates left-right development of the brain, heart and gut.** *Development* 132(6): 1247-60. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Monocilia have been proposed to establish the left-right (LR) body axis in vertebrate embryos by creating a directional fluid flow that triggers asymmetric gene expression. In zebrafish, dorsal forerunner cells (DFCs) express a conserved ciliary dynein gene (left-right dynein-related1, lrdr1) and form a ciliated epithelium inside a fluid-filled organ called Kupffer's vesicle (KV). Here, videomicroscopy demonstrates that cilia inside KV are motile and create a directional fluid flow just prior to the onset of asymmetric gene expression in lateral cells. Laser ablation of DFCs and surgical disruption of KV provide direct evidence that ciliated KV cells are required during early somitogenesis for subsequent LR patterning in the brain, heart and gut. Antisense morpholinos against lrdr1 disrupt KV fluid flow and perturb LR development. Furthermore, lrdr1 morpholinos targeted to DFC/KV cells demonstrate that Lrdr1 functions in these ciliated cells to control LR patterning. This provides the first direct evidence, in any vertebrate, that impairing cilia function in derivatives of the dorsal organizer, and not in other cells that express ciliogenic genes, alters LR development. Finally, genetic analysis reveals novel roles for the T-box transcription factor no tail and the Nodal signaling pathway as upstream regulators of lrdr1 expression and KV morphogenesis. We propose that KV is a transient embryonic 'organ of asymmetry' that directs LR development by establishing a directional fluid flow. These results suggest that cilia are an essential component of a conserved mechanism that controls the transition from bilateral symmetry to LR asymmetry in vertebrates.
Descriptors: zebrafish, brain embryology, gastrointestinal tract development, heart embryology, body patterning physiology, cilia physiology, transforming growth factor beta genetics, transforming growth factor beta.

Etard, C., M. Behra, R. Ertzer, N. Fischer, S. Jesuthasan, P. Blader, R. Geisler, and U. Straehle (2005). **Mutation in the delta-subunit of the nachr suppresses the muscle defects caused by lack of dystrophin**. *Developmental Dynamics* 234(4): 1016-1025. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Normal motility of the zebrafish embryo requires a large number of gene loci, many of which have human orthologues implicated in myasthenias and other myopathies. We have identified a mutation in the zebrafish that abolishes body motility. Embryos have narrower myofibrils and lack clusters of nicotinic acetylcholine receptors (nAChRs) on the surface of the somitic muscle. We mapped the mutation to the 6-subunit of the nAChR, showing this mutant to be a new allele of the previously named sofa potato (sop). The mutant allele carries a missense mutation in the extracellular domain altering the cysteine at

position 150 to an arginine. The 6-subunit is expressed in all striated muscles in embryonic and early larval stages together with the alpha 1, beta 1, epsilon, and gamma-subunits of nAChR. In contrast to mammals that show switching from the gamma embryonic to the adult E-subunit, the two subunits are coexpressed in zebrafish embryos. We, furthermore, demonstrated that the sop/delta-nAChR mutation is a suppressor of the myopathy caused by lack of Dystrophin. The myofiber detachment phenotype of Dystroglycan-deficient embryos was not suppressed, suggesting that Dystrophin and Dystroglycan play distinct roles in muscle formation and maintenance of muscle integrity. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, molecular genetics, muscular system, myopathy , etiology, myasthenias, nicotinic acetylcholine receptors, genetics.

Etard, C., M. Behra, N. Fischer, D. Hutcheson, R. Geisler, and U. Strahle (2007). **The UCS factor Steif/Unc-45b interacts with the heat shock protein Hsp90a during myofibrillogenesis**. *Developmental Biology* 308(1): 133-143. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, freshwater fish, heat shock, mutations, myosin, phylogenetics, proteins, *Danio rerio*, genetics and evolution.

Evans, T. and A. Holtzinger (2005). **Forming organs from tubes: identification of a common transcriptionally regulated morphogenetic step for many organs.** *Developmental Biology* 283(2): 684. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, biochemistry and molecular biophysics, transcription, embryonic development .

Evans, T.G., Y. Yamamoto, W.R. Jeffery, and P.H. Krone (2004). **The stress-inducible hsp70 is required for normal embryonic lens formation in the zebrafish.** *Developmental Biology* 271(2): 617. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, molecular genetics, biochemistry and molecular biophysics, development, sense organs.

Fadool, J.M. (2003). **Development of a rod photoreceptor mosaic revealed in transgenic zebrafish.** *Developmental Biology* 258(2): 277-90. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The number and distribution of neurons within the vertebrate retina are tightly regulated. This is particularly apparent in the highly ordered, crystalline-like arrangement of the cone photoreceptors in the teleost. In this report, using a transgenic line of zebrafish, a novel and developmentally regulated mosaic pattern of the rod photoreceptors is described. The spatial and temporal expression of EGFP, under the control of the Xenopus rhodopsin gene promoter, was nearly identical to the endogenous rhodopsin. EGFP was first detected in the ventral nasal retinal in an area of precocious neurogenesis referred to as the "ventral patch". Subsequent expression of EGFP was observed in isolated cells sporadically distributed across the dorsal and central retina. However, confocal microscopy and spatial analysis of larval eyes or retinal explants from adults revealed a precise arrangement to the rod photoreceptors. The rod terminals were arranged in regularly spaced rows with clearly identifiable telodendria linking neighboring cells. The rod inner segments projected through the cone mosaic in a predictable pattern. In the adult, the rod mosaic originated near the retinal margin where clusters of rods differentiated around the immature short single cone. In the

embryo, the sporadic differentiation of the rods led to the gradual formation of the mosaic pattern. With the growing interest in neuronal stem cells, revisiting this model of neurogenesis provides an avenue to uncover mechanisms underlying the precise integration of new neuronal elements into a preexisting neural network.
Descriptors: zebrafish, retinal rodsgrowth and development, genetics, growth and development, genetically modified, developmental gene expression regulation, green fluorescent proteins, luminescent proteins genetics and metabolism, neurological models, mosaicism, promoter regions genetics, recombinant proteins genetics and metabolism, rhodopsin genetics, *Xenopus*.

Fadool, J.M., K. Alvarez-Delfin, and A.C. Morris (2006). **Photoreceptor subtype specification and mosaic patterning in zebrafish**. *Developmental Biology* 295(1): 406. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, nervous system, development, sense organs, genetic chimera analysis, cell-cell signaling, neurogenesis, mosaic patterning.

Fadool, J.M., A. Davis, and M. Merritt (2004). **Genetic analysis of photoreceptor cell development in the zebrafish retina.** *Developmental Biology* 271(2): 604. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, development, molecular genetics, sense organs, genetic analysis, genetic techniques, cell-cell interaction.

Fan, X., E.G. Hagos, B. Xu, C. Sias, K. Kawakami, R.D. Burdine, and S.T. Dougan (2007). **Nodal signals mediate interactions between the extra-embryonic and embryonic tissues in zebrafish.** *Developmental Biology* 310(2): 363-78. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: In many vertebrates, extra-embryonic tissues are important signaling centers that induce and pattern the germ layers. In teleosts, the mechanism by which the extra-embryonic yolk syncytial layer (YSL) patterns the embryo is not understood. Although the Nodal-related protein Squint is expressed in the YSL, its role in this tissue is not known. We generated a series of stable transgenic lines with GFP under the control of squint genomic sequences. In all species, nodal-related genes induce their own expression through a positive feedback loop. We show that two tissue specific enhancers in the zebrafish squint gene mediate the response to Nodal signals. Expression in the blastomeres depends upon a conserved Nodal response element (NRE) in the squint first intron, while expression in the extra-embryonic enveloping layer (EVL) is mediated by an element upstream of the transcription start site. Targeted depletion experiments demonstrate that the zebrafish Nodal-related proteins Squint and Cyclops are required in the YSL for endoderm and head mesoderm formation. Thus, Nodal signals mediate interactions between embryonic and extra-embryonic tissues in zebrafish that maintain nodal-related gene expression in the margin. Our results demonstrate a high degree of functional conservation between the extra-embryonic tissues of mouse and zebrafish.
Descriptors: zebrafish embryos, intracellular signaling peptides and proteins metabolism, physiology, genetically modified, base sequence, body patterning, endoderm metabolism, developmental gene expression regulation, intracellular signaling peptides and mesoderm metabolism, molecular sequence data, response elements, signal transduction, proteins genetics.

Fekete, D.M. (2003). **Rocks that roll zebrafish**. *Science* 302(5643): 241-242. ISSN: 0036-8075.
NAL Call Number: 470Sci2
Descriptors: zebrafish, embryo development, calcium carbonate, calcium carbonates, crystals, literature reviews, molecular structure, morphogenesis, otoliths, reviews, *Danio rerio*, zebra fish, biomineralization, starmaker gene.

Feldman, B., J.L. Boorech, H. Noushmehr, M. Kirby, and A.G. Elkahloun (2006). **Facs-assisted microdissection of photolabeled cells to identify germ layer-specific genes**. *Developmental Biology* 295(1): 412-413. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, molecular genetics, development, confocal microscopy, imaging and microscopy techniques, fluorescence activated cell sorting, spectrum analysis techniques, histology and cytology techniques.

Feldman, B. and J. Costa (2006). **A reverse-genetic screen identifying RhoGEF genes that are essential for zebrafish epiboly**. *Developmental Biology* 295(1): 394-395. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, molecular genetics, biochemistry and molecular biophysics, enzymology, biochemistry and molecular biophysics.

Feldman, B., W. Pei, H. Williams, M. Clark, and D.L. Stemple (2006). **Squint protects early embryos from temperature-induced dysmorphology**. *Developmental Biology* 295(1): 442. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, development, molecular genetics, nervous system, holoprosencephaly , nervous system disease, congenital disease, etiology, temperature, phenotype, dysmorphology.

Feldner, J., T. Becker, K. Goishi, J. Schweitzer, P. Lee, M. Schachner, M. Klagsbrun, and C.G. Becker (2005). **Neuropilin-1a is involved in trunk motor axon outgrowth in embryonic zebrafish.** *Developmental Dynamics* 234(3): 535-49. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, axons physiology, motor neurons metabolism, neuropilin 1 metabolism, metabolism , axons drug effects, developmental gene expression regulation drug effects, motor neurons drug effects, neuropilin 1 genetics, phenotype, messenger RNA genetics and metabolism, semaphorin 3a metabolism, vascular endothelialgrowth factor A pharmacology, genetics.

Feldner, J., T. Becker, K. Goishi, J. Schweitzer, P. Lee, M. Schachner, M. Klagsbrun, and C.G. Becker (2005). **Neuropilin-1la is involved in trunk motor axon outgrowth in embryonic zebrafish**. *Developmental Dynamics* 234(3): 535-549. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Neuropilin-1, a receptor for axon-repellent semaphorins and vascular endothelial growth factor (VEGF), functions both in angiogenesis and axon growth. Here, we show strong expression of neuropilin-la in primary motor neurons in the trunk of embryonic zebrafish. Reducing the expression of neuropilin-la using antisense morpholino oligonucleotides induced aberrant branching of motor nerves, additional exit points of motor nerves from the spinal cord, and migration of neurons out of the spinal cord along the motor axon pathway in a dose-dependent manner. These phenotypes could be partially rescued by coin-

jecting neuropilin-la mRNA. Other axons in the spinal cord and head appeared unaffected by the morpholino treatment. In addition, neuropilin-la morpholino treatment disturbed normal formation of blood vessels in the trunk of 24 hours postfertilization embryos, as shown by microangiography. Morpholinos to VEGF also disturbed formation of blood vessels but did not affect motor axons, indicating that correct formation of blood vessels is not needed for the growth of primary motor axons. Morpholinos to the semaphorin 3A homologs semaphorin 3A1 and semaphorin 3A2 also had no effect on motor axon growth. However, combined injections of neuropilin-la morpholino, at a concentration that did not elicit axonal aberrations when injected alone, with VEGF, semaphorin 3A1, or semaphorin 3A2 morpholinos synergistically increased the proportion of embryos showing aberrant motor axon growth. Thus, neuropilin-la in primary motor neurons may integrate signals from several ligands and is needed for proper segmental growth of primary motor nerves in zebrafish. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, vascular endothelial growth factor, development, nervous system, motor axon outgrowth, neuropilin-1, semaphorins.

Feng, J., B. White, O.V. Tyurina, B. Guner, T. Larson, H.Y. Lee, R.O. Karlstrom, and J.D. Kohtz (2004). **Synergistic and antagonistic roles of the sonic Hedgehog N- and C-terminal lipids**. *Development* 131(17): 4357-4370. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: The Shh protein contains both N-terminal and C-terminal lipids. The functional redundancy of these lipid moieties is presently unclear. Here, we compare the relative roles of the N- and C-terminal lipids in early rat striatal neuronal differentiation, membrane association and multimerization, and ventralizing activity in the zebrafish forebrain. We show that these lipid act synergistically in cell tethering and the formation of a large (L) multimer (669 kDa). However, the C-terminal lipid antagonizes the rat striatal neuronal differentiation-inducing activity of the N-terminal lipid. In addition, multimerization is required but not sufficient for the differentiation-inducing activity. Based on the presence of different N- and C-lipid-containing Shh proteins in the rat embryo, and on their different activities, we propose that both N- and C-terminal lipids are required for the formation of multimers involved in long-range signaling, and that the C-terminal lipid may function in long-range signaling by reducing Shh activity until it reaches its long-range target. Comparative analysis of the ventralizing activities of different N- and C-terminal lipid-containing Shh proteins in the zebrafish forebrain shows that the presence of at least one lipid is required for signaling activity, suggesting that lipid modification of Shh is a conserved requirement for signaling in the forebrain of rodents and zebrafish. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, cell biology, development, nervous system, comparative analysis, cell differentiation, Shh protein, forebrain, rats.

Feng, X., E.G. Adiarte, and S.H. Devoto (2006). **Hedgehog acts directly on the zebrafish dermomyotome to promote myogenic differentiation.** *Developmental Biology* 300(2): 736-46. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Vertebrate myogenesis is regulated by signaling proteins secreted from surrounding tissues. One of the most important, Sonic hedgehog, has been proposed to regulate myogenic precursor cell survival, proliferation, and differentiation in a variety of vertebrates. In zebrafish, Hedgehog signaling is both necessary and sufficient for the development of embryonic slow muscle fibers-the earliest differentiating muscle fibers. Here we investigated the function of Hedgehog signaling in another zebrafish myogenic lineage, a dermomyo-

tomal population of cells defined by somitic pax3/7 expression. We found that Hedgehog negatively regulates the number of myogenic precursors expressing pax3/7. Hh also positively regulates the growth of embryonic fast muscle. Unlike Hedgehog's function in regulating the elongation of fast muscle fibers, this regulation is not mediated by embryonic slow muscle fibers. Instead, it is a direct Hedgehog response, cell autonomous to myogenic precursors. The regulation of myogenic precursors and their differentiation into fast fibers have a different critical time period for Hh signaling, and different requirements for specific gli gene family members of Hh activated transcription factors from the earlier promotion of embryonic slow muscle fiber differentiation. We propose that Hedgehog signaling acts at multiple times on different lineages, through different downstream pathways, to promote myogenic differentiation.

Descriptors: zebrafish, cell differentiation physiology, Hedgehog, muscle development physiology, muscle fibers cytology, muscle fibers metabolism, myoblasts cytology, myoblasts metabolism, pax7 transcription factor antagonists and inhibitors, pax7 transcription factor biosynthesis, paired box transcription factors biosynthesis, paired box transcription factors genetics, signal transduction physiology, genetics, Hedgehog signaling.

Fernandez, J., M. Valladares, R. Fuentes, and A. Ubilla (2006). **Reorganization of cytoplasm in the zebrafish oocyte and egg during early steps of ooplasmic segregation.** *Developmental Dynamics* 235(3): 656-71. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: The aim of this work is to determine when and how ooplasmic segregation is initiated in the zebrafish egg. To this end, the organization of the ooplasm and vitelloplasm were examined in oocytes and eggs shortly after activation. Ooplasmic segregation, initiated in the stage V oocyte, led to the formation of ooplasmic domains rich in organelles, and ribonucleoproteins. A linear array of closely arranged peripheral yolk globules separated an outer domain of ectoplasm from an inner domain of interconnected endoplasmic lacunae. The structure of this yolk array and the distribution of microinjected labeled tracers suggests that it may provide a barrier limiting ooplasm transit. Loosely arranged yolk globules at the animal hemisphere allow wide connections between the endoplasm and a preblastodisc domain. Activation caused further segregation of ooplasm, reorganization of endoplasmic lacunae, and blastodisc growth. The presence of an endoplasmic cytoskeleton suggests that these changes may be driven by microtubules and microfilaments.

Descriptors: zebrafish egg, cleavage stage, ovum cytology, cytoplasm ultrastructure, oocytes cytology, ovum cytology, cleavage stage, ovum ultrastructure, cytoplasm physiology, egg yolk cytology, egg yolk physiology, egg yolk ultrastructure, microtubules ultrastructure, oocytes chemistry, oocytes ultrastructure, organelles ultrastructure, ovum chemistry, ovum ultrastructure, ribonucleoproteins analysis.

Field, H.A., P.D. Dong, D. Beis, and D.Y. Stainier (2003). **Formation of the digestive system in zebrafish. II. Pancreas morphogenesis.** *Developmental Biology* 261(1): 197-208. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Recent studies have suggested that the zebrafish pancreas develops from a single pancreatic anlage, located on the dorsal aspect of the developing gut. However, using a transgenic zebrafish line that expresses GFP throughout the endoderm, we report that, in fact, two pancreatic anlagen join to form the pancreas. One anlage is located on the dorsal aspect of the developing gut and is present by 24 h postfertilization (hpf), the second anlage is located on the ventral aspect of the developing gut in a position anterior to the dorsal anlage and is

present by 40 hpf. These two buds merge by 52 hpf to form the pancreas. Using heart and soul mutant embryos, in which the pancreatic anlagen most often do not fuse, we show that the posterior bud generates only endocrine tissue, while the anterior bud gives rise to the pancreatic duct and exocrine cells. Interestingly, at later stages, the anterior bud also gives rise to a small number of endocrine cells usually present near the pancreatic duct. Altogether, these studies show that in zebrafish, as in the other model systems analyzed to date, the pancreas arises from multiple buds. To analyze whether other features of pancreas development are conserved and investigate the influence of surrounding tissues on pancreas development, we examined the role of the vasculature in this process. Contrary to reports in other model systems, we find that, although vascular endothelium is in contact with the posterior bud throughout pancreas development, its absence in cloche mutant embryos does not appear to affect the early morphogenesis or differentiation of the pancreas.

Descriptors: zebrafish embryo, pancreas embryology, genetically modified fish, vascular endothelium embryology, developmental gene expression regulation, green fluorescent proteins, luminescent mutation, pancreas blood supply, pancreas cytology, pancreas metabolism, recombinant genetics.

Field, H.A., E.A. Ober, T. Roeser, and D.Y. Stainier (2003). **Formation of the digestive system in zebrafish. I. Liver morphogenesis.** *Developmental Biology* 253(2): 279-90. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Despite the essential functions of the digestive system, much remains to be learned about the cellular and molecular mechanisms responsible for digestive organ morphogenesis and patterning. We introduce a novel zebrafish transgenic line, the gutGFP line, that expresses GFP throughout the digestive system, and use this tool to analyze the development of the liver. Our studies reveal two phases of liver morphogenesis: budding and growth. The budding period, which can be further subdivided into three stages, starts when hepatocytes first aggregate, shortly after 24 h postfertilization (hpf), and ends with the formation of a hepatic duct at 50 hpf. The growth phase immediately follows and is responsible for a dramatic alteration of liver size and shape. We also analyze gene expression in the developing liver and find a correlation between the expression of certain transcription factor genes and the morphologically defined stages of liver budding. To further expand our understanding of budding morphogenesis, we use loss-of-function analyses to investigate factors potentially involved in this process. It had been reported that no tail mutant embryos appear to lack a liver primordium, as assessed by gata6 expression. However, analysis of gutGFP embryos lacking Ntl show that the liver is in fact present. We also find that, in these embryos, the direction of liver budding does not correlate with the direction of intestinal looping, indicating that the left/right behavior of these tissues can be uncoupled. In addition, we use the cloche mutation to analyze the role of endothelial cells in liver morphogenesis, and find that in zebrafish, unlike what has been reported in mouse, endothelial cells do not appear to be necessary for the budding of this organ.

Descriptors: zebrafish embryo, liver embryology, genetically modified, base sequence, body patterning, digestive system embryology, endothelium embryology, developmental gene expression regulation, green fluorescent proteins, luminescent oligodeoxyribonucleotides genetics, recombinant proteins genetics.

Finkielsztein, A., Q.Z. Sun, C. Hillis, and G.M. Kelly (2006). **Pten in zebrafish gastrulation**. *Developmental Biology* 295(1): 440. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish embryos, molecular genetics, enzymology, sense organs, cell proliferation, cell apoptosis, cell polarization, gastrulation.

Fischer, S., B.W. Draper, and C.J. Neumann (2003). **The zebrafish fgf24 mutant identifies an additional level of Fgf signaling involved in vertebrate forelimb initiation.** *Development* 130(15): 3515-24. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The development of vertebrate limb buds is triggered in the lateral plate mesoderm by a cascade of genes, including members of the Fgf and Wnt families, as well as the transcription factor tbx5. Fgf8, which is expressed in the intermediate mesoderm, is thought to initiate forelimb formation by activating wnt2b, which then induces the expression of tbx5 in the adjacent lateral plate mesoderm. Tbx5, in turn, is required for the activation of fgf10, which relays the limb inducing signal to the overlying ectoderm. We show that the zebrafish fgf24 gene, which belongs to the Fgf8/17/18 subfamily of Fgf ligands, acts downstream of tbx5 to activate fgf10 expression in the lateral plate mesoderm. We also show that fgf24 activity is necessary for the migration of tbx5-expressing cells to the fin bud, and for the activation of shh, but not hand2, expression in the posterior fin bud.
Descriptors: zebrafish, embryonic induction physiology, Fgf genetics, forelimb embryology, cell movement physiology, embryonic induction genetics, Fgf metabolism, growth substances metabolism, limb bud metabolism, mutation, T-box domain proteins metabolism, genetics.

Fisher, S., P. Jagadeeswaran, and M.E. Halpern (2003). **Radiographic analysis of zebrafish skeletal defects.** *Developmental Biology* 264(1): 64-76. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Systematic identification of skeletal dysplasias in model vertebrates provides insight into the pathogenesis of human skeletal disorders and can aid in the identification of orthologous human genes. We are undertaking a mutagenesis screen for skeletal dysplasias in adult zebrafish, using radiography to detect abnormalities in skeletal anatomy and bone morphology. We have isolated chihuahua, a dominant mutation causing a general defect in bone growth. Heterozygous chihuahua fish have phenotypic similarities to human osteogenesis imperfecta, a skeletal dysplasia caused by mutations in the type I collagen genes. Mapping and molecular characterization of the chihuahua mutation indicates that the defect resides in the gene encoding the collagen I(alpha1) chain. Thus, chihuahua accurately models osteogenesis imperfecta at the biologic and molecular levels, and will prove an important resource for studies on the disease pathophysiology. Radiography is a practical screening tool to detect subtle skeletal abnormalities in the adult zebrafish. The identification of chihuahua demonstrates that mutant phenotypes analogous to human skeletal dysplasias will be discovered.
Descriptors: zebrafish, bone and bones abnormalities, bone and bones radiography, collagen Type I genetics, osteogenesis imperfecta genetics, abnormalities, amino acid sequence, disease models, in situ hybridization, molecular sequence data, mutation, osteogenesis imperfecta pathology, phenotype, genetics and metabolism.

Flanagan-Steet, H., M.A. Fox, D. Meyer, and J.R. Sanes (2005). **Neuromuscular synapses can form in vivo by incorporation of initially aneural postsynaptic specializations**. *Development* 132(20): 4471-4481. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Synapse formation requires the coordination of pre- and postsynaptic differentiation. An unresolved question is which steps in the process require interactions between pre- and postsynaptic cells, and which proceed cell-autonomously. One current model is that

factors released from presynaptic axons organize postsynaptic differentiation directly beneath the nerve terminal. Here, we used neuromuscular junctions (NMJs) of the zebrafish primary motor system to test this model. Clusters of neurotransmitter (acetylcholine) receptors (AChRs) formed in the central region of the myotome, destined to be synapse-rich, before axons extended and even when axon extension was prevented. Time-lapse imaging revealed that pre-existing clusters on early-born slow (adaxial) muscle fibers were incorporated into NMJs as axons advanced. Axons were, however, required for the subsequent remodeling and selective stabilization of synaptic clusters that precisely appose post- to presynaptic elements. Thus, motor axons are dispensable for the initial stages of postsynaptic differentiation but are required for later stages. Moreover, many AChR clusters on later-born fast muscle fibers formed at sites that had already been contacted by axons, suggesting heterogeneity in the signaling mechanisms leading to synapse formation by a single axon. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, nervous system, muscular system, development, aneural postsynaptic specialization.

Fleming, A., R. Keynes, and D. Tannahill (2004). **A central role for the notochord in vertebral patterning.** *Development* 131(4): 873-880. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: The vertebrates are defined by their segmented vertebral column, and vertebral periodicity is thought to originate from embryonic segments, the somites. According to the widely accepted 'resegmentation' model, a single vertebra forms from the recombination of the anterior and posterior halves of two adjacent sclerotomes on both sides of the embryo. Although there is supporting evidence for this model in amniotes, it remains uncertain whether it applies to all vertebrates. To explore this, we have investigated vertebral patterning in the zebrafish. Surprisingly, we find that vertebral bodies (centra) arise by secretion of bone matrix from the notochord rather than somites; centra do not form via a cartilage intermediate stage, nor do they contain osteoblasts. Moreover, isolated, cultured notochords secrete bone matrix in vitro, and ablation of notochord cells at segmentally reiterated positions in vivo prevents the formation of centra. Analysis of fss mutant embryos, in which sclerotome segmentation is disrupted, shows that whereas neural arch segmentation is also disrupted, centrum development proceeds normally. These findings suggest that the notochord plays a key, perhaps ancient, role in the segmental patterning of vertebrae. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, cell biology, development, molecular genetics, skeletal system, resegmentation model, segmentation.

Flores, M.V., E.Y. Lam, P. Crosier, and K. Crosier (2006). **A hierarchy of Runx transcription factors modulate the onset of chondrogenesis in craniofacial endochondral bones in zebrafish.** *Developmental Dynamics* 235(11): 3166-76. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryos, cartilage embryology, chondrogenesis genetics, developmental gene expression regulation, transcription factors physiology, growth and development, bone and bones chemistry, bone and bones embryology , branchial region embryology, cartilage chemistry, cartilage metabolism, chondrogenesis drug effects, chemistry, metabolism, endoderm chemistry, endoderm metabolism, face embryology, antisense oligonucleotides pharmacology, skull chemistry, skull embryology, transcription factors analysis, transcription factors genetics, genetics, proteins analysis.

Formstone, C.J. and I. Mason (2005). **Combinatorial activity of Flamingo proteins directs convergence and extension within the early zebrafish embryo via the planar cell polarity pathway.** *Developmental Biology* 282(2): 320-35. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The seven-transmembrane protocadherin, Flamingo, functions in a number of processes during Drosophila development, including planar cell polarity (PCP). To assess the role(s) of Flamingo1/Celsr1 (Fmi1) during vertebrate embryogenesis we have exploited the zebrafish system, identifying two Fmi1 orthologues (zFmi1a and zFmi1b) and employing morpholinos to induce mis-splicing of zebrafish fmi1 mRNAs, to both imitate mutations identified in Drosophila flamingo and generate novel aberrant Flamingo proteins. We demonstrate that in the zebrafish gastrula, Fmi1 proteins function in concert with each other and with the vertebrate PCP proteins, Wnt11 and Strabismus, to mediate convergence and extension during gastrulation, without altering early dorso-ventral patterning. We show that zebrafish Fmi1a promotes extension of the entire antero-posterior axis of the zebrafish gastrula including prechordal plate and ventral diencephalic precursors. However, while we show that control over axial extension is autonomous, we find that Fmi1a is not required within lateral cells undergoing dorsal convergence.
Descriptors: zebrafish embryology, body patterning physiology, cadherins metabolism, cell polarity physiology, signal transduction physiology, proteins metabolism, base sequence, cadherins genetics, molecular cloning, cluster analysis, DNA primers, glycoproteins metabolism, membrane proteins metabolism, molecular sequence data, phylogeny, reverse transcriptase PCR , DNA sequence analysis, Wnt proteins.

Foucher, I., M. Mione, A. Simeone, D. Acampora, L. Bally Cuif, and C. Houart (2006). **Differentiation of cerebellar cell identities in absence of Fgf signalling in zebrafish Otx morphants.** *Development* 133(10): 1891-900. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Although the secreted molecule Fgf8 is a key player of the isthmic organiser function, the mechanisms by which it acts remain unclear. Here, we present evidence indicating that Fgf8 is not instructive in establishing zebrafish cerebellar cell identities, although it is required for proliferation and morphogenesis of this territory. We first show that, as in mouse, lack of Otx function in zebrafish leads to transformation of the presumptive mesencephalon into an extended rhombomere 1 (r1). Expanded Fgf8 expression was proposed to be the cause of this fate transformation. However, this report demonstrates that zebrafish embryos lacking both Otx and fgf8 functions retain an extended r1 and display differentiation of at least two cerebellar cell fates. We show that this is not caused by presence of other Fgfs, which implies that in absence of Otx, Fgf function is not necessary for the differentiation of cerebellar cell types. Otx proteins are therefore potent repressors of cerebellar fates, kept out of r1 progeny by Fgf8. Because Otx transcripts are not present in presumptive r1 territory prior to fgf8 expression, Fgf8 is required to maintain, rather than induce, the posterior boundary of Otx expression. This maintenance is enough to allow cerebellar differentiation.
Descriptors: zebrafish embryology, cell differentiation, cerebellum cytology, Otx transcription factors metabolism, proteins metabolism, cell proliferation, cerebellum embryology fibroblast growth factor 8 metabolism, Fgf metabolism, mesencephalon embryology, microinjections, mutation, antisense oligonucleotides pharmacology, pyrroles pharmacology, rhombencephalon embryology, signal transduction drug effects, metabolism.

Francisco Simao, M., M. Perez Camps, and F. Garcia Ximenez (2007). **Zebrafish embryo development can be reversibly arrested at the MBT stage by exposure to a water temperature of 16 degrees C**. *Spanish Journal of Agricultural Research* 5(2): 181-185. ISSN: 1695 971X.
NAL Call Number: S3.S636
Abstract: Germline chimaerism (intra or interspecific) is a technique of great potential in aquaculture. It allows specimens to be obtained that produce gametes whose origins lie in the cells of the donor organism. Chimaerism is usually performed at the mid blastula transition (MBT) stage since this is the last in which embryonic cells remain completely totipotent. Zebrafish are photoperiodic in their egg-laying behaviour and show rapid embryonic development. For chimaerism to be successful, it is of interest to establish the maximum time over which embryonic development can be reversibly arrested. This paper reports the effect on survival of subjecting zebrafish embryos at different stages of development to a water temperature of 16 degrees C for different lengths of time. The maximum exposure time after which these embryos were able to resume development following low-temperature-induced developmental arrest became shorter as the embryonic stage exposed became earlier. At the MBT stage, the maximum safe exposure time was 2 h; longer exposure times led to problems in development and survival. Copyright © 2008 CABI
Descriptors: zebrafish, chimaeras, embryonic development, embryos, fish eggs, survival, water temperature, *Danio rerio*.
Language of Text: Spanish.

Freisinger, C.M. and D.C. Slusarski (2005). **Calcium signaling and regulation of G-proteins in zebrafish development**. *Federation of American Societies for Experimental Biology Journal* 19(5, Suppl. S, Part 2): A1338. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: Progression from egg to embryo requires a dynamic interaction of signal transduction networks. We combine cellular, molecular and genetic analysis with high-resolution calcium imaging to investigate how signaling networks influence embryonic patterning. Inhibition of maternal endogenous Ca2+ release induces hyperdorsalized phenotypes in the zebrafish (*Danio rerio*). We have linked calciummodulation with Non-canonical Writ signaling and defined a requirement for G-protein signaling.We investigate the regulation of downstream signaling components such as regulators of G-protein Signaling (RGS) and Ca2+-sensing proteins in early zebrafish development. Cloning and characterization of RGS family members will be discussed as well as loss-of-function analyses. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryo, development, calcium, g-proteins, signal transduction networks.

French, C.R., T. Erickson, D. Callander, K.M. Berry, R. Koss, D.W. Hagey, J. Stout, K. Wuennenberg Stapleton, J. Ngai, C.B. Moens, and A.J. Waskiewicz (2007). **Pbx homeodomain proteins pattern both the zebrafish retina and tectum.** *BioMed Central Developmental Biology* 7: 85. ISSN: 1471 213X.
NAL Call Number: QH491.B63
Abstract: BACKGROUND: Pbx genes encode TALE class homeodomain transcription factors that pattern the developing neural tube, pancreas, and blood. Within the hindbrain, Pbx cooperates with Hox proteins to regulate rhombomere segment identity. Pbx cooperates with Eng to regulate midbrain-hindbrain boundary maintenance, and with MyoD to control fast muscle cell differentiation. Although previous results have demonstrated that Pbx is required for proper eye size, functions in regulating retinal cell identity and patterning

have not yet been examined. RESULTS: Analysis of retinal ganglion cell axon pathfinding and outgrowth in pbx2/4 null embryos demonstrated a key role for pbx genes in regulating neural cell behavior. To identify Pbx-dependent genes involved in regulating retino-tectal pathfinding, we conducted a microarray screen for Pbx-dependent transcripts in zebrafish, and detected genes that are specifically expressed in the eye and tectum. A subset of Pbx-dependent retinal transcripts delineate specific domains in the dorso-temporal lobe of the developing retina. Furthermore, we determined that some Pbx-dependent transcripts also require Meis1 and Gdf6a function. Since gdf6a expression is also dependent on Pbx, we propose a model in which Pbx proteins regulate expression of the growth factor gdf6a, which in turn regulates patterning of the dorso-temporal lobe of the retina. This, in concert with aberrant tectal patterning in pbx2/4 null embryos, may lead to the observed defects in RGC outgrowth. CONCLUSION: These data define a novel role for Pbx in patterning the vertebrate retina and tectum in a manner required for proper retinal ganglion cell axon outgrowth.
Descriptors: zebrafish, body patterning genetics, DNA binding proteins genetics, homeodomain retina embryology, superior colliculus embryology, proteins genetics developmental gene expression regulation, in situ hybridization, molecular probes, oligonucleotide array sequence analysis, retinal ganglion cells physiology, genetics.

Fricke, C. and C.B. Chien (2005). **Cloning of full-length zebrafish dcc and expression analysis during embryonic and early larval development.** *Developmental Dynamics* 234(3): 732-9. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, developmental gene expression regulation, larva genetics, larva growth and development, cell surface receptors genetics, cell surface receptors metabolism, genetics, amino acid sequence, molecular cloning, embryology, metabolism, molecular sequence data, organ specificity, phylogeny, cell surface receptors chemistry, sequence alignment, amino acid sequence homology, time factors, growth and development, proteins chemistry.

Fujimoto, T., T. Kataoka, S. Otani, T. Saito, T. Aita, E. Yamaha, and K. Arai (2004). **Embryonic stages from cleavage to gastrula in the loach *Misgurnus anguillicaudatus*.** *Zoological Science* 21(7): 747-755. ISSN: 0289 0003.
NAL Call Number: 442P92
Abstract: Early developmental staging from the zygote stage to the gastrula is a basic step for studying embryonic development and biotechnology. We described the early embryonic development of the loach, Misgurnus anguillicaudatus, based on morphological features and gene expression. Synchronous cleavage was repeated for 9 cycles about every 27 min at 20 degrees C after the first cleavage. After the 10th synchronous cleavage, asynchronous cleavage was observed 5.5 h post-fertilization (hpf), indicating the mid-blastula transition. The yolk syncytial layer (YSL) was formed at this time. Expressions of goosecoid and no tail were detected by whole-mount in situ hybridization from 6 hpf. This time corresponded to the late-blastula period. Thereafter, epiboly started and a blastoderm covered over the yolk cell at 8 hpf. At 10 hpf, the germ ring and the embryonic shield were formed, indicating the stage of early gastrula. Afterward, the epiboly advanced at the rate of 10% of the yolk cell each hour. The blastoderm covered the yolk cell completely at 15 hpf. The embryonic development of the loach resembled that of the zebrafish in terms of morphological change and gene expression. Therefore, it is possible that knowledge of the developmental stages of the zebrafish might be applicable to the loach. Copyright © 2008 CABI
Descriptors: zebrafish, cleavage, cytology, developmental stages, embryonic development,

embryos, fertilization, gene expression, genes, histology, morphology, zygotes, *Misgurnus anguillicaudatus*, loach.

Fukuda, K. and Y. Kikuchi (2005). **Review: Endoderm development in vertebrates: fate mapping, induction and regional specification**. *Development Growth & Differentiation* 47(6): 343-355. ISSN: 0012-1592.
NAL Call Number: QL951.E4
Descriptors: zebrafish, amphibiotic species, biological development, digestive system, freshwater fish, glands, growth, respiratory system, skin, specifications, *Danio rerio*, *Xenopus*.
Notes: Review article.

Funfak, A., A. Brosing, M. Brand, and J.M. Kohler (2007). **Micro fluid segment technique for screening and development studies on *Danio rerio* embryos.** *Lab on a Chip* 7(9): 1132-8. ISSN: 1473-0197.
Abstract: The applicability of micro fluid segments for studying the behaviour of multicellular systems, in particular embryonic development, has been investigated. It was found that eggs from the zebrafish *Danio rerio* can be introduced into micro fluid segments without serious damage by using perfluoromethyldecalin (PP9) as the carrier liquid and Teflon (PTFE) as the tube material. The development processes of fish embryos were observed over a time period of 80 hours, until hatching time. After five days, the fish larvae were brought out of the micro fluid segments and transferred into breeding reservoirs. Effects of the membrane-damaging anionic surfactant sodium dodecyl sulfate (SDS) alone and SDS with the addition of CuCl(2) (copper(II) chloride) were investigated. By analyzing different end points, we found inhibiting and also supporting effects on the development of the embryos. Low SDS concentrations with and without copper(II) ions were supportive, while higher SDS concentrations led to negative impacts on the development of the embryos. The results showed that automated micro screening processes with complex biological systems can be performed using microfluidic systems and are applicable for future toxicological and drug screening studies.
Descriptors: zebrafish, biological assay instrumentation, embryo culture techniques instrumentation, embryonic development physiology, microfluidic analytical techniques instrumentation, growth and development, biological assay methods, copper administration and dosage, embryo culture techniques methods, embryonic development drug effects, equipment design, equipment failure analysis, microfluidic analytical techniques methods, sodium dodecyl sulfate administration and dosage.

Furthauer, M., J. Van Celst, C. Thisse, and B. Thisse (2004). **Fgf signalling controls the dorsoventral patterning of the zebrafish embryo.** *Development* 131(12): 2853-64. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The establishment of dorsoventral (DV) patterning in vertebrate embryos depends on the morphogenic activity of a group of Tgfbeta superfamily members, the bone morphogenetic proteins (Bmps) (which specify ventral cell fates), and on their interaction with their dorsally secreted cognate inhibitors chordin and noggin. In the zebrafish, genetic analysis has revealed that Bmp2b and Bmp7, as well as their antagonist chordin, are required for proper DV patterning. The expression of Bmp genes is initially activated in the whole blastula. Well before the beginning of gastrulation, Bmp gene expression progressively disappears from the dorsal side to become restricted to the ventral part of the embryo. We show that this early restriction of Bmp gene expression, which occurs independently of noggin and chordin, is an essential step in the establishment of DV patterning. The progressive ventral restriction

of Bmp gene transcripts is coincident with the spreading of Fgf activity from the dorsal side of the embryo, suggesting that Fgf signalling is implicated in dorsal downregulation of Bmp gene expression. In accordance with this, activation of the Fgf/Ras/Mapk-signalling pathway inhibits ventral Bmp gene expression, thereby causing a dorsalisation of the embryo. Conversely, inhibition of Fgf signalling causes Bmp gene expression to expand dorsally, leading to an expansion of ventral cell fates. In accordance with an important role of Fgf signalling in the DV patterning of the zebrafish, we show that loss of Fgf8 function enhances the ventralisation of chordin-deficient embryos. Our results thereby demonstrate that pre-gastrula stage Fgf-signalling is essential to delimit the expression domain of the genes encoding the functional morphogen of the dorsoventral axis of the early zebrafish embryo.
Descriptors: zebrafish, body patterning physiology, transforming growth factor beta physiology, blastomeres cytology, blastomeres physiology, bone morphogenetic bone morphogenetic, cytology, physiology, fibroblast growth factor 8, Fgf genetics, developmental gene expression regulation genetics, morphogenesis, organ culture techniques, signal transduction, genetic transcription genetics.

Furutani Seiki, M., T. Sasado, C. Morinaga, H. Suwa, K. Niwa, H. Yoda, T. Deguchi, Y. Hirose, A. Yasuoka, T. Henrich, T. Watanabe, N. Iwanami, D. Kitagawa, K. Saito, S. Asaka, M. Osakada, S. Kunimatsu, A. Momoi, H. Elmasri, C. Winkler, M. Ramialison, F. Loosli, R. Quiring, M. Carl, C. Grabher, and S. Winkler (2004). **A systematic genome-wide screen for mutations affecting organogenesis in medaka, *Oryzias latipes***. *Mechanisms of Development* 121(7/8): 647-658. ISSN: 0925 4773.
NAL Call Number: QH607.A1C4
Abstract: A large-scale mutagenesis screen was performed in Medaka to identify genes acting in diverse developmental processes. Mutations were identified in homozygous F3 progeny derived from ENU-treated founder males. In addition to the morphological inspection of live embryos, other approaches were used to detect abnormalities in organogenesis and in specific cellular processes, including germ cell migration, nerve tract formation, sensory organ differentiation and DNA repair. Among 2031 embryonic lethal mutations identified, 312 causing defects in organogenesis were selected for further analyses. From these, 126 mutations were characterized genetically and assigned to 105 genes. The similarity of the development of Medaka and zebrafish facilitated the comparison of mutant phenotypes, which indicated that many mutations in Medaka cause unique phenotypes so far unrecorded in zebrafish. Even when mutations of the two fish species cause a similar phenotype such as one-eyed-pinhead or parachute, more genes were found in Medaka than in zebrafish that produced the same phenotype when mutated. These observations suggest that many Medaka mutants represent new genes and, therefore, are important complements to the collection of zebrafish mutants that have proven so valuable for exploring genomic function in development. Copyright © 2008 CABI
Descriptors: zebrafish, abnormalities, DNA, embryos, genes, genomes, morphology, mutagenesis, mutations, organogenesis, phenotypes, species differences, *Danio rerio*, *Oryzias latipes*.
Notes: Special issue.Medaka.

Furutani Seiki, M. and J. Wittbrodt (2004). **Medaka and zebrafish, an evolutionary twin study.** *Mechanisms of Development* 121(7-8): 629-637. ISSN: 0925 4773.
NAL Call Number: QH607.A1C4
Descriptors: zebrafish, *Danio rerio,* medaka, *Oryzias latipess*, animals as tools, developmental

genetics model species comparison, review, literature review, development, developmental genetics, genetics.

Gajewski, M., D. Sieger, B. Alt, C. Leve, S. Hans, C. Wolff, K.B. Rohr, and D. Tautz (2003). **Anterior and posterior waves of cyclic her1 gene expression are differentially regulated in the presomitic mesoderm of zebrafish.** *Development* 130(18): 4269-78. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Somite formation in vertebrates depends on a molecular oscillator in the presomitic mesoderm (PSM). In order to get a better insight into how oscillatory expression is achieved in the zebrafish *Danio rerio*, we have analysed the regulation of her1 and her7, two bHLH genes that are co-expressed in the PSM. Using specific morpholino oligonucleotide mediated inhibition and intron probe in situ hybridisation, we find that her7 is required for initiating the expression in the posterior PSM, while her1 is required to propagate the cyclic expression in the intermediate and anterior PSM. Reporter gene constructs with the her1 upstream sequence driving green fluorescent protein (GFP) expression show that separable regulatory regions can be identified that mediate expression in the posterior versus intermediate and anterior PSM. Our results indicate that the cyclic expression is generated at the transcriptional level and that the resulting mRNAs have a very short half-life. A specific degradation signal for her1 mRNA must be located in the 5'-UTR, as this region also destabilises the GFP mRNA such that it mimics the dynamic pattern of the endogenous her1 mRNA. In contrast to the mRNA, GFP protein is stable and we find that all somitic cells express the protein, proving that her1 mRNA is transiently expressed in all cells of the PSM.
Descriptors: zebrafish, developmental gene expression regulation, mesoderm physiology, somites physiology, transcription factors metabolism, proteins metabolism, genetically modified, reporter genes, in situ hybridization, antisense oligonucleotides metabolism, phylogeny, promoter regions genetics, transcription factors classification, transcription factors genetics, growth and development, proteins classification.

Galloway, J.L., R.A. Wingert, C. Thisse, B. Thisse, and L.I. Zon (2005). **Loss of gata1 but not gata2 converts erythropoiesis to myelopoiesis in zebrafish embryos.** *Developmental Cell* 8(1): 109-16. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: The differentiation of hematopoietic progenitors into erythroid or myeloid cell lineages is thought to depend upon relative levels of the transcription factors gata1 and pu.1. While loss-of-function analysis shows that gata1 is necessary for terminal erythroid differentiation, no study has demonstrated that loss of gata1 alters myeloid differentiation during ontogeny. Here we provide in vivo evidence that loss of Gata1, but not Gata2, transforms primitive blood precursors into myeloid cells, resulting in a massive expansion of granulocytic neutrophils and macrophages at the expense of red blood cells. In addition to this fate change, expression of many erythroid genes was found to be differentially dependent on Gata1 alone, on both Gata1 and Gata2, or independent of both Gata factors, suggesting that multiple pathways regulate erythroid gene expression. Our studies establish a transcriptional hierarchy of Gata factor dependence during hematopoiesis and demonstrate that gata1 plays an integral role in directing myelo-erythroid lineage fate decisions during embryogenesis.
Descriptors: zebrafish embryos, DNA binding , erythropoiesis physiology, myelopoiesis physiology, transcription factors physiology, metabolism, genetically modified, cell death physiology, cell differentiation physiology, erythroid progenitor cells metabolism, erythroid specific DNA binding proteins genetics, factors, flow cytometry methods, GATA1 transcription factor, developmental gene expression regulation physiology, immunohistochemistry

methods, in situ hybridization methods, in situ nick end labeling methods, microinjections methods, transcription factors metabolism.

Gamse, J.T., Y.S. Kuan, M. Macurak, C. Brosamle, B. Thisse, C. Thisse, and M.E. Halpern (2005). **Directional asymmetry of the zebrafish epithalamus guides dorsoventral innervation of the midbrain target.** *Development* 132(21): 4869-81. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The zebrafish epithalamus, consisting of the pineal complex and flanking dorsal habenular nuclei, provides a valuable model for exploring how left-right differences could arise in the vertebrate brain. The parapineal lies to the left of the pineal and the left habenula is larger, has expanded dense neuropil, and distinct patterns of gene expression from the right habenula. Under the influence of Nodal signaling, positioning of the parapineal sets the direction of habenular asymmetry and thereby determines the left-right origin of habenular projections onto the midbrain target, the interpeduncular nucleus (IPN). In zebrafish with parapineal reversal, neurons from the left habenula project to a more limited ventral IPN region where right habenular axons would normally project. Conversely, efferents from the right habenula adopt a more extensive dorsoventral IPN projection pattern typical of left habenular neurons. Three members of the leftover-related KCTD (potassium channel tetramerization domain containing) gene family are expressed differently by the left and right habenula, in patterns that define asymmetric subnuclei. Molecular asymmetry extends to protein levels in habenular efferents, providing additional evidence that left and right axons terminate within different dorsoventral regions of the midbrain target. Laser-mediated ablation of the parapineal disrupts habenular asymmetry and consequently alters the dorsoventral distribution of innervating axons. The results demonstrate that laterality of the dorsal forebrain influences the formation of midbrain connections and their molecular properties.
Descriptors: zebrafish embryology, body patterning, epithalamus embryology, mesencephalon embryology, nervous system embryology epithalamus physiology, habenula embryology, neurons, pineal gland.

Gamse, J.T., C. Thisse, B. Thisse, and M.E. Halpern (2003). **The parapineal mediates left-right asymmetry in the zebrafish diencephalon.** *Development* 130(6): 1059-68. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The dorsal diencephalon (or epithalamus) of larval zebrafish displays distinct left-right asymmetries. The pineal complex consists of the pineal organ anlage and an unpaired, left-sided accessory organ - the parapineal. The neighboring brain nuclei, the left and right dorsal habenulae, show consistent differences in their size, density of neuropil and gene expression. Mutational analyses demonstrate a correlation between the left-right position of the parapineal and the laterality of the habenular nuclei. We show that selective ablation of the parapineal organ results in the loss of habenular asymmetry. The left-sided parapineal therefore influences the left-right identity of adjacent brain nuclei, indicating that laterality of the dorsal diencephalon arises in a step-wise fashion.
Descriptors: zebrafish embryology, diencephalon embryology, diencephalon growth and development, embryonic induction physiology, nerve tissue nerve tissue proteins metabolism.

Gamse, J.T., K. Santhakumar, C. Thisse, B. Thisse, and M.E. Halpern (2005). **Zebrafish from beyond establishes left-right asymmetry of the zebrafish brain.** *Developmental Biology* 283(2): 589. ISSN: 0012 1606.
NAL Call Number: 442.8D49

Descriptors: zebrafish, molecular genetics, biochemistry and molecular biophysics, brain asymmetry, nervous system, neural coordination, two hybrid screening, genetic techniques, phenotype, left right asymmetry.

Gansner, J.M., B.A. Mendelsohn, K.A. Hultman, S.L. Johnson, and J.D. Gitlin (2007). **Essential role of lysyl oxidases in notochord development**. *Developmental Biology* 307(2): 202-213. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, developmental stages, embryonic development, freshwater fish, genes, genetic abnormalities, inhibitors, parturition, *Danio rerio*.

Gao, Y., P. Li, and L. Li (2005). **Transgenic zebrafish that express tyrosine hydroxylase promoter in inner retinal cells.** *Developmental Dynamics* 233(3): 921-9. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: We have generated a transgenic zebrafish line [Tg(Th:GFP)] that expresses green fluorescence proteins (GFP) driven by rat tyrosine hydroxylase (TH) promoter. In zebrafish, the transgene was expressed as early as 16 hr postfertilization (hpf). The first transgene expression was detected in the midbrain. Within a few hours of development, the expression spread to the forebrain and hindbrain. In the retina, the first transgene expression was detected at approximately 40 hpf, at which time a single GFP-positive cell was seen in the ventral-nasal patch of the retina. In late development, GFP spread across the inner retina. GFP was found in retinal cells that expressed TH or phenylethanolamine N-methyl-transferase (PNMT), the first and last enzymes for synthesis of catecholamine, respectively. This suggests that the transgene is expressed in catecholaminergic neurons. Of interest, GFP was also detected in some retinal cells that release gamma-aminobutyric acid. These latter data suggest that the transgene may also be expressed in noncatecholaminergic cells.
Descriptors: zebrafish embryos, developmental gene expression regulation, promoter regions genetics, retina cytology, retina metabolism, tyrosine 3 monooxygenase genetics, genetics, genetically modified, brain embryology, brain enzymology, reporter phenylethanolamine n methyltransferase genetics, phenylethanolamine N methyltransferase metabolism, rats, retina embryology, retina enzymology, trans tyrosine 3 monooxygenase metabolism, gamma aminobutyric acid metabolism.

Garcia Crespo, D. and E. Vecino (2004). **Differential expression of calretinin in the developing and regenerating zebrafish visual system.** *Histology and Histopathology* 19(4): 1193-9. ISSN: 0213-3911.
Abstract: Calretinin is a calcium-binding protein which participates in a variety of functions including calcium buffering and neuronal protection. It also serves as a developmental marker of retinal ganglion cells (RGCs). In order to study the role of calretinin in the development and regeneration of RGCs, we have studied its pattern of expression in the retina at different developmental stages, as well as during optic nerve regeneration by means of immunohistochemistry. During development, calretinin is found for the first time in RGCs when they connect with the optic tectum. Optic nerves from adult zebrafish were crushed and after different survival times, calretinin expression in the retina, optic nerve tract and optic tectum was studied. From the day of crushing to 10 days later, calretinin expression was found to be downregulated within RGCs and their axons, as was also observed during the early developmental stages of RGCs, when they are not committed to a definite cell phenotype. Moreover, 13 days after lesion, when the regenerating axons arrived at the optic tectum, a recovery of calretinin immunoreactivity within the RGCs was observed. These results indicate that calre-

tinin may play an important role during optic nerve regeneration, Thus, the down-regulation of Calretinin during the growth of the RGC axons towards the target during development as well as during their regeneration after injury, indicates that an increase the availability of cytosolic calcium is integral to axon outgrowth thus recapitulating the pattern observed during development.
Descriptors: zebrafish, calcium binding protein, vitamin D dependent metabolism, eye proteins metabolism, optic nerve growth and development, optic nerve metabolism, retina growth and development, retina metabolism, calcium metabolism, cell differentiation, immunohistochemistry, nerve regeneration, optic nerve physiology, retina physiology, retinal ganglion cells cytology, retinal ganglion cells metabolism, superior colliculus growth and development, superior colliculus metabolism, time factors, anatomy and histology, growth and development.

Gardiner, M.R., D.F. Daggett, L.I. Zon, and A.C. Perkins (2005). **Zebrafish KLF4 is essential for anterior mesendoderm/pre-polster differentiation and hatching.** *Developmental Dynamics* 234(4): 992-6. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, cell differentiation physiology, endoderm physiology, developmental gene expression regulation, kruppel like transcription factors metabolism, proteins metabolism, endoderm metabolism, in situ hybridization, antisense oligonucleotides.

Gardiner, M.R., M.M. Gongora, S.M. Grimmond, and A.C. Perkins (2007). **A global role for zebrafish klf4 in embryonic erythropoiesis.** *Mechanisms of Development* 124(9-10): 762-74. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: There are two waves of erythropoiesis, known as primitive and definitive waves in mammals and lower vertebrates including zebrafish. The founding member of the Kruppel-like factor (KLF) family of CACCC-box binding proteins, EKLF/Klf1, is essential for definitive erythropoiesis in mammals but only plays a minor role in primitive erythropoiesis. Morpholino knockdown experiments have shown a role for zebrafish klf4 in primitive erythropoiesis and hatching gland formation. In order to generate a global understanding of how klf4 might influence gene expression and differentiation, we have performed expression profiling of klf4 morphants, and then performed validation of many putative target genes by qRT-PCR and whole mount in situ hybridization. We found a critical role for klf4 in embryonic globin, heme synthesis and hatching gland gene expression. In contrast, there was an increase in expression of definitive hematopoietic specific genes such as larval globin genes, runx1 and c-myb from 24 hpf, suggesting a selective role for klf4 in primitive rather than definitive erythropoiesis. In addition, we show klf4 preferentially binds CACCC box elements in the primitive zebrafish beta-like globin gene promoters. These results have global implications for primitive erythroid gene regulation by KLF-CACCC box interactions.
Descriptors: zebrafish, erythropoiesis physiology, Kruppel-like transcription factors physiology, gene expression profiling, developmental gene expression regulation physiology, globins biosynthesis and genetics, heme biosynthesis, kruppel like transcription factors blood, physiology, blood proteins.

Geldmacher Voss, B., A.M. Reugels, S. Pauls, and J.A. Campos Ortega (2003). **A 90-degree rotation of the mitotic spindle changes the orientation of mitoses of zebrafish neuroepithelial cells.** *Development* 130(16): 3767-80. ISSN: 0950 1991.
NAL Call Number: QL951.D38

Abstract: In the neural plate and neural tube in the trunk region of the zebrafish embryo, dividing cells are oriented parallel to the plane of the neuroepithelium, while in neural keel/rod, cells divide perpendicular to it. This change in the orientation of mitosis is brought about by a 90 degrees rotation of the mitotic spindle. As the two halves of the neural primordium in keel/rod stage are in apposition, the perpendicular orientation of mitoses in this stage determines that daughter cells become allocated to both sides of the neural tube. To assess the role played by cell junctions in controlling the orientation of dividing cells, we studied the expression of components of adherens and tight junctions in the neuroepithelial cells. We find that these proteins are distributed irregularly at the neural plate stage and become polarised apically in the cell membrane only during the keel/rod stage. The stereotypic orientation of mitoses is perturbed only weakly upon loss of function of the cell junction components ASIP and aPKClambda, suggesting that mitotic orientation depends in part on the integrity of cell junctions and the polarity of the epithelium as a whole. However, the 90-degree rotation of the spindle does not require perfectly polarised cell junctions between the neuroepithelial cells.
Descriptors: zebrafish embryology, cell polarity, central nervous system embryology, epithelial cells physiology, mitosis physiology, mitotic spindle apparatus physiology, biological markers, carrier proteins genetics and metabolism, cell movement physiology, central nervous system cytology and physiology, epithelial cells cytology, isoenzymes, microinjections, antisense oligonucleotides genetics and metabolism, protein kinase C metabolism, messenger RNA genetics metabolism, recombinant fusion proteins metabolism, transgenes and metabolism.

Gemballa, S., K. Hagen, K. Roeder, M. Rolf, and K. Treiber (2003). **Structure and evolution of the horizontal septum in vertebrates**. *Journal of Evolutionary Biology* 16(5): 966-975. ISSN: 1010-061X.
NAL Call Number: QH359.J68
Descriptors: zebrafish, animal morphology, bioenergetics, biological development, collagen, evolution, freshwater fish, phylogeny, *Danio rerio*.

Georgijevic, S., Y. Subramanian, E.L. Rollins, O. Starovic Subota, A.C. Tang, and S.J. Childs (2007). **Spatiotemporal expression of smooth muscle markers in developing zebrafish gut.** *Developmental Dynamics* 236(6): 1623-32. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, digestive system embryology and metabolism, developmental gene expression regulation, smooth muscle metabolism, aging physiology, air sacs embryology and metabolism, amino acid sequence, biological markers, conserved sequence, embryology, metabolism, molecular sequence data, phylogeny, sequence alignment, time factors, proteins chemistry and genetics.

Gering, M. and R. Patient (2005). **Hedgehog signaling is required for adult blood stem cell formation in zebrafish embryos.** *Developmental Cell* 8(3): 389-400. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Studies with embryonic explants and embryonic stem cells have suggested a role for Hedgehog (Hh) signaling in hematopoiesis. However, targeted deletion of Hh pathway components in the mouse has so far failed to provide in vivo evidence. Here we show that zebrafish embryos mutant in the Hh pathway or treated with the Hh signaling inhibitor cyclopamine display defects in adult hematopoietic stem cell (HSC) formation but not in primitive hematopoiesis. Hh is required in the trunk at three consecutive stages during vas-

cular development: for the medial migration of endothelial progenitors of the dorsal aorta (DA), for arterial gene expression, and for the formation of intersomitic vessel sprouts. Interference with Hh signaling during the first two stages also interferes with HSC formation. Furthermore, HSC and DA formation also share Vegf and Notch requirements, which further distinguishes them from primitive hematopoiesis and underlines their close relationship during vertebrate development.

Descriptors: zebrafish embryo, hematopoiesis physiology, hematopoietic stem cells cytology, signal transduction physiology, T lymphocytes cytology, trans activators metabolism, body patterning drug effects, body patterning physiology, cell movement drug effects, cell movement physiology, core bindingfactor alpha 2 subunit, DNA binding proteins genetics, proteins metabolism, developmental gene expression regulation drug effects, developmental gene expression regulation physiology, Hedgehog proteins, hematopoiesis drug effects, membrane proteins metabolism, mutation genetics, physiologic neovascularization drug effects, physiologic neovascularization physiology, proto oncogene proteins metabolism, Notch receptors, signal transduction drug effects, trans activators genetics, transcription factors metabolism, vascular endothelialgrowth factor A metabolism, veratrum alkaloids toxicity.

Gering, M., Y. Yamada, T.H. Rabbitts, and R.K. Patient (2003). **Lmo2 and Scl/tal1 convert non-axial mesoderm into haemangioblasts which differentiate into endothelial cells in the absence of Gata1.** *Development* 130(25): 6187-6199. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Descriptors: zebrafish, biochemistry and molecular biophysics, blood and lymphatics: transport and circulation, cardiovascular system, development, angiogenic remodeling, erythropoiesis, hematopoiesis, myelopoiesis, vasculogenesis.

Germanguz, I., D. Lev, T. Waisman, C.H. Kim, and I. Gitelman (2007). **Four twist genes in zebrafish, four expression patterns.** *Developmental Dynamics* 236(9): 2615-26. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryos, developmental biology methods, developmental gene expression regulation, twist transcription factor biosynthesis, amino acid sequence, body patterning, limb buds embryology, mesoderm metabolism, molecular sequence data, neural crest embryology, phylogeny, messenger RNA metabolism, amino acid sequence homology, twist transcription factor physiology.

Gestri, G., M. Carl, I. Appolloni, S.W. Wilson, G. Barsacchi, and M. Andreazzoli (2005). **Six3 functions in anterior neural plate specification by promoting cell proliferation and inhibiting BMP4 expression**. *Development* 132(10): 2401-2413. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Although it is well established that Six3 is a crucial regulator of vertebrate eye and forebrain development, it is unknown whether this homeodomain protein has a role in the initial specification of the anterior neural plate. In this study, we show that exogenous Six3 can expand the anterior neural plate in both *Xenopus* and zebrafish, and that this occurs in part through Six3-dependent transcriptional regulation of the cell cycle regulators cyclinD1 and p27Xic1, as well as the anti-neurogenic genes Zic2 and Xhairy2. However, Six3 can still expand the neural plate in the presence of cell cycle inhibitors and we show that this is likely to be due to its ability to repress the expression of Bmp4 in ectoderm adjacent to the anterior neural plate. Furthermore, exogenous Six3 is able to restore the size of the anterior neural plate in chordino mutant zebrafish, indicating that it has the ability to promote

anterior neural development by antagonising the activity of the BMP pathway. On its own, Six3 is unable to induce neural tissue in animal caps, but it can do so in combination with Otx2. These results suggest a very early role for Six3 in specification of the anterior neural plate, through the regulation of cell proliferation and the inhibition of BMP signalling. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, nervous system, neural coordination, molecular genetics, cell proliferation, cell fate, *Xenopus*.

Ghiselli, G. and S.A. Farber (2005). **D-glucuronyl C5-epimerase acts in dorso-ventral axis formation in zebrafish.** *BioMed Central Developmental Biology* 5: 19. ISSN: 1471 213X.
NAL Call Number: QH491.B63
Abstract: BACKGROUND: Heparan sulfate (HS) is an ubiquitous component of the extracellular matrix that binds and modulates the activity of growth factors, cytokines and proteases. Animals with defective HS biosynthesis display major developmental abnormalities however the processes that are affected remain to be defined. D-glucuronyl-C5-epimerase (Glce) is a key HS chain modifying enzyme that catalyses the conversion of glucuronic acid into iduronic acid, a biosynthetic step that enhances HS biological activity. In this study the role of Glce during early zebrafish development has been investigated. RESULTS: Two Glce-like proteins (Glce-A and -B) are expressed in zebrafish at all times. They are the products of two distinct genes that, based on chromosomal mapping, are both orthologues of the same single human gene. Transcripts for both proteins were detected in fertilized zebrafish embryos prior to the onset of zygotic transcription indicating their maternal origin. At later developmental stages the epimerases are expressed widely throughout gastrulation and then become restricted to the hindbrain at 24 h post-fertilization. By monitoring the expression of well characterized marker genes during gastrulation, we have found that misexpression of Glce causes a dose-dependent expansion of the ventral structures, whereas protein knockdown using targeted antisense morpholino oligonucleotides promotes axis dorsalization. The ventralizing activity of Bmp2b is enhanced by Glce overexpression whereas Glce knockdown impairs Bmp2b activity. CONCLUSION: Glce activity is an important determinant of of dorso-ventral axis formation and patterning in zebrafish. In particular Glce acts during gastrulation by affecting Bmp-mediated cell specification. The results obtained further corroborate the concept that HS encodes information that affect morphogenesis during early vertebrate development.
Descriptors: zebrafish, body patterning, carbohydrate epimerases physiology, bone morphogenetic embryonic development, heparitin sulfate.

Gibert, Y., A. Gajewski, A. Meyer, and G. Begemann (2006). **Induction and prepatterning of the zebrafish pectoral fin bud requires axial retinoic acid signaling.** *Development* 133(14): 2649-59. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Vertebrate forelimbs arise as bilateral appendages from the lateral plate mesoderm (LPM). Mutants in aldh1a2 (raldh2), an embryonically expressed gene encoding a retinoic acid (RA)-synthesizing enzyme, have been used to show that limb development and patterning of the limb bud are crucially dependent on RA signaling. However, the timing and cellular origin of RA signaling in these processes have remained poorly resolved. We have used genetics and chemical modulators of RA signaling to resolve these issues in the zebrafish. By rescuing pectoral fin induction in the aldh1a2/neckless mutant with exogenous RA and by blocking RA signaling in wild-type embryos, we find that RA acts as a permissive signal that is required during the six- to eight-somite stages for pectoral fin induction. Cell-

transplantation experiments show that RA production is not only crucially required from flanking somites, but is sufficient to permit fin bud initiation when the trunk mesoderm is genetically ablated. Under the latter condition, intermediate mesoderm alone cannot induce the pectoral fin field in the LPM. We further show that induction of the fin field is directly followed by a continued requirement for somite-derived RA signaling to establish a prepattern of anteroposterior fates in the condensing fin mesenchyme. This process is mediated by the maintained expression of the transcription factor hand2, through which the fin field is continuously posteriorized, and lasts up to several hours prior to limb-budding. Thus, RA signaling from flanking somites plays a dual early role in the condensing limb bud mesenchyme.

Descriptors: zebrafish embryos, body patterning, embryonic induction, anatomy and histology, embryonic structures physiology, signal transduction physiology, tretinoin metabolism, in situ hybridization, mesoderm cytology, mesoderm physiology, morphogenesis, somites physiology, time factors, genetics.

Gilardelli, C.N., O. Pozzoli, P. Sordino, G. Matassi, and F. Cotelli (2004). **Functional and hierarchical interactions among zebrafish vox/vent homeobox genes.** *Developmental Dynamics* 230(3): 494-508. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish, homeobox genes, transcription factors genetics and, amino acid and base sequence, body patterning genetics, bone morphogenetic proteins metabolism, drug effects, developmental gene expression regulation, , microinjections, molecular sequence data, antisense oligonucleotides pharmacology, phylogeny, tertiary protein structure, amino acid sequence homology, genetics, proteins chemistry.

Gillhouse, M., M. Wagner Nyholm, H. Hikasa, S.Y. Sokol, and Y. Grinblat (2004). **Two Frodo/Dapper homologs are expressed in the developing brain and mesoderm of zebrafish.** *Developmental Dynamics* 230(3): 403-9. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryos, brain metabolism, mesoderm metabolism, proteins genetics and metabolism, signal transducing adaptor proteins, amino acid sequence, carrier proteins molecular evolution, molecular sequence data, open reading frames, tertiary protein structure, amino acid sequence homology, *Xenopus* proteins, proteins chemistry.

Gingerich, J.L., R. Lindeman, E. Putiri, K. Stolzmann, and F. Pelegri (2006). **Analysis of axis induction mutant embryos reveals morphogenetic events associated with zebrafish yolk extension formation**. *Developmental Dynamics* 235(10): 2749-2760. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: We analyze patterning and morphogenetic events during somitogenesis in hecate mutant embryos, which exhibit early axis induction defects. The posterior region, in the absence of a dorsal axis, is capable of forming organized gene expression patterns. The aberrant morphogenesis of mutant embryos is associated with anteriorly directed cell movements, underlying the enveloping layer, from the posterior region. In both wild-type and mutant embryos, these changes result in an accumulation of cells, whose location correlates with a constriction in the posterior yolk cell, which in the wild-type corresponds to the yolk extension. The region encompassing the constriction corresponds to a region of expression of zangptl2 in the yolk syncytial layer, which expands anteriorly together with the anteriorly migrating tail bud-derived cell population. Our data indicate that yolk extension formation is associated with coordinated changes involving the anterior migration of cells from the pos-

terior region, changes in surface cellular layers, and inductive gene expression events in the YSL. Copyright © 2008 CABI

Descriptors: zebrafish, embryonic development, embryos, fish eggs, gene expression, genes, morphogenesis, mutants, *Danio rerio*.

Glavic, A., S.M. Honore, C.G. Feijoo, F. Bastidas, M.L. Allende, and R. Mayor (2004). **Role of BMP signaling and the homeoprotein Iroquois in the specification of the cranial placodal field** . *Developmental Biology* 272(1): 89-103. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Different types of placodes originate at the anterior border of the neural plate but it is still an unresolved question whether individual placodes arise as distinct ectodermal specializations in situ or whether all or a subset of the placodes originate from a common preplacodal field. We have analyzed the expression and function of the homeoprotein Iro I in *Xenopus* and zebrafish embryos, and we have compared its expression with several preplacodal and placodal markers. Our results indicate that the iro1 genes are expressed in the preplacodal region, being one of the earliest markers for this area. We show that an interaction between the neural plate and the epidermis is able to induce the expression of several preplacodal markers, including Xiro I, by a similar mechanism to that previously shown for neural crest induction. In addition, we analyzed the role of BMP in the specification of the preplacodal field by studying the expression of the preplacodal markers Six), Xiro1, and several specific placodal markers. We experimentally modified the level of BMP activity by three different methods. First, we implanted beads soaked with noggin in early neurula stage *Xenopus* embryos; second, we injected the mRNA that encodes a dominant negative of the BMP receptor into *Xenopus* and zebrafish embryos;, and third, we grafted cells expressing chordin into zebrafish embryos. The results obtained using all three methods show that a reduction in the level of BMP activity leads to an expansion of the preplacodal and placodal region similar to what has been described for neural crest regions. By using conditional constructs of Xiro1, we performed gain and loss of function experiments. We show that Xiro1 play an important role in the specification of both the preplacodal field as well as individual placodes. We have also used inducible dominant negative and activator constructs of Notch signaling components to analyze the role of these factors on placodal development. Our results indicate that the a precise level of BMP activity is required to induce the neural plate border, including placodes and neural crest cells, that in this border the iro1 I gene is activated, and that this activation is required for the specification of the placodes. Copyright 2004 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, biochemistry and molecular biophysics, development, integumentary system, chemical coordination and homeostasis, nervous system, developmental biology, neurogenesis, *Xenopus*.

Gleason, M.R., R. Armisen, M.A. Verdecia, H. Sirotkin, P. Brehm, and G. Mandel (2004). **A mutation in SERCA underlies motility dysfunction in accordion zebrafish.** *Developmental Biology* 276(2): 441-51. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Zebrafish acquire the ability for fast swimming early in development. The motility mutant accordion (acc) undergoes exaggerated and prolonged contractions on both sides of the body, interfering with the acquisition of patterned swimming responses. Our whole cell recordings from muscle indicate that the defect is not manifested in neuromuscular transmission. However, imaging of skeletal muscle of larval acc reveals greatly prolonged calcium transients and associated contractions in response to depolarization. Positional cloning of acc

identified a serca mutation as the cause of the acc phenotype. SERCA is a sarcoplasmic reticulum transmembrane protein in skeletal muscle that mediates calcium re-uptake from the myoplasm. The mutation in SERCA, a serine to phenylalanine substitution, is likely to result in compromised protein function that accounts for the observed phenotype. Indeed, direct evidence that mutant SERCA causes the motility dysfunction was provided by the finding that wild type fish injected with an antisense morpholino directed against serca, exhibited accordion-like contractions and impaired swimming. We conclude that the motility dysfunction in embryonic and larval accordion zebrafish stems directly from defective calcium transport in skeletal muscle rather than defective CNS drive.

Descriptors: zebrafish, calcium transporting ATPases genetics, calcium transporting ATPases metabolism, swimming physiology, amino acid sequence, calcium metabolism, calcium transporting ATPases chemistry, in situ hybridization, molecular models, molecular sequence data, muscle contraction physiology and cytology, skeletal muscle physiology, mutation, antisense oligonucleotides genetics, antisense oligonucleotides metabolism, patch clamp techniques, phenotype, tertiary protein structure, anatomy, histology and genetics.

Glickman, N.S., C.B. Kimmel, M.A. Jones, and R.J. Adams (2003). **Shaping the zebrafish notochord.** *Development* 130(5): 873-87. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Promptly after the notochord domain is specified in the vertebrate dorsal mesoderm, it undergoes dramatic morphogenesis. Beginning during gastrulation, convergence and extension movements change a squat cellular array into a narrow, elongated one that defines the primary axis of the embryo. Convergence and extension might be coupled by a highly organized cellular intermixing known as mediolateral intercalation behavior (MIB). To learn whether MIB drives early morphogenesis of the zebrafish notochord, we made 4D recordings and quantitatively analyzed both local cellular interactions and global changes in the shape of the dorsal mesodermal field. We show that MIB appears to mediate convergence and can account for extension throughout the dorsal mesoderm. Comparing the notochord and adjacent somitic mesoderm reveals that extension can be regulated separately from convergence. Moreover, mutational analysis shows that extension does not require convergence. Hence, a cellular machine separate from MIB that can drive dorsal mesodermal extension exists in the zebrafish gastrula. The likely redundant control of morphogenesis may provide for plasticity at this critical stage of early development.

Descriptors: zebrafish embryology, cell movement physiology, morphogenesis, notochord embryology, cell differentiation physiology, cell size, fluorescent dyes metabolism, kinetics, confocal microscopy, notochord growth and development, T-box domain proteins genetics and metabolism, time factors, growth and development, metabolism.

Gnugge, L., D. Meyer, and W. Driever (2004). **Pancreas development in zebrafish.** *Methods in Cell Biology* 76: 531-51. ISSN: 0091-679X.

NAL Call Number: 442P92

Descriptors: zebrafish, pancreas embryology, body patterning genetics and physiology, cell differentiation physiology, endoderm physiology, endothelial cells physiology, gene expression regulation, Islets of Langerhans cytology and embryology, Islets of Langerhans growth and development, mutation, notochord physiology, organogenesis, pancreas growth and development, pancreas physiology and exocrine cytology, exocrine pancreas embryology, exocrine pancreas growth and development, phenotype, transcription factors physiology, zebrafish growth and development.

Godinho, L., J.S. Mumm, P.R. Williams, E.H. Schroeter, A. Koerber, S.W. Park, S.D. Leach, and R.O. Wong (2005). **Targeting of amacrine cell neurites to appropriate synaptic laminae in the developing zebrafish retina.** *Development* 132(22): 5069-79. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Cellular mechanisms underlying the precision by which neurons target their synaptic partners have largely been determined based on the study of projection neurons. By contrast, little is known about how interneurons establish their local connections in vivo. Here, we investigated how developing amacrine interneurons selectively innervate the appropriate region of the synaptic neuropil in the inner retina, the inner plexiform layer (IPL). Increases (ON) and decreases (OFF) in light intensity are processed by circuits that are structurally confined to separate ON and OFF synaptic sublaminae within the IPL. Using transgenic zebrafish in which the majority of amacrine cells express fluorescent protein, we determined that the earliest amacrine-derived neuritic plexus formed between two cell populations whose somata, at maturity, resided on opposite sides of this plexus. When we followed the behavior of individual amacrine cells over time, we discovered that they exhibited distinct patterns of structural dynamics at different stages of development. During cellular migration, amacrine cells exhibited an exuberant outgrowth of neurites that was undirected. Upon reaching the forming IPL, neurites extending towards the ganglion cell layer were relatively more stable. Importantly, when an arbor first formed, it preferentially ramified in either the inner or outer IPL corresponding to the future ON and OFF sublaminae, and maintained this stratification pattern. The specificity by which ON and OFF amacrine interneurons innervate their respective sublaminae in the IPL contrasts with that observed for projection neurons in the retina and elsewhere in the central nervous system.

Descriptors: zebrafish embryology, amacrine cells embryology, neurites physiology, retina embryology, synapses physiology, amacrine cells cytology, genetically modified, reporter genes, synapses genetics.

Goishi, K. and M. Klagsbrun (2004). **Vascular endothelial growth factor and its receptors in embryonic zebrafish blood vessel development.** *Current Topics in Developmental Biology* 62: 127-52. ISSN: 0070 2153.

NAL Call Number: QL951.C8

Abstract: There is intense interest in how blood vessel development is regulated. A number of vascular growth factors and their receptors have been described. The vascular endothelial growth factor (VEGF) and its receptors are major contributors to normal mammalian vascular development. These receptors include VEGFR-1, VEGFR-2, VEGFR-3, neuropilin-1 (NRP1), and NRP2. The function of these genes have been determined to some degree in mouse gene targeting studies. These knockouts are embryonically lethal, and early death can be attributed in part to lack of normal blood and lymphatic vessel development. More recently, it has been demonstrated that zebrafish are an excellent model for studying the genes and proteins that regulate embryonic vascular development. Zebrafish have a number of advantages compared to mice, including rapid embryonic development and the ability to examine and manipulate embryos outside of the animal. In this review, we describe some of the earlier mouse VEGF/receptor functional studies and emphasize the development of the zebrafish vasculature. We describe the zebrafish vasculature, zebrafish VEGF and VEGF receptors, advantages of the zebrafish model, resources, and methods of determining growth factor and receptor function.

Descriptors: zebrafish embryology, blood vessels embryology, blood vessels metabolism, vascular endothelial growth factor receptor A metabolism, vascular endothelialgrowth factor A metabolism, immunophilins and semaphorins metabolism, genetics.

Goishi, K., P. Lee, A.J. Davidson, E. Nishi, L.I. Zon, and M. Klagsbrun (2003). **Inhibition of zebrafish epidermal growth factor receptor activity results in cardiovascular defects.** *Mechanisms of Development* 120(7): 811-22. ISSN: 0925-4773.

NAL Call Number: QH607.A1C4

Abstract: The physiological role of any of the epidermal growth factor (EGF) receptor tyrosine kinases has yet to be determined in zebrafish. We isolated a zebrafish homologue of EGFR (egfr) that shows a 63% amino acid overall identity to human EGFR but with 90% amino acid identity in the kinase domain. Whole mount in situ hybridization showed ubiquitous distribution of egfr transcripts during gastrulation, somitogenesis and later stages. When expressed in Chinese hamster ovary cells, zebrafish Egfr was a functional receptor that responded to EGF by receptor tyrosine phosphorylation and activation of MAP kinase. The function of zebrafish Egfr in vivo was determined by inhibiting its activity using EGFR kinase inhibitors and antisense morpholinos (MO), which inhibited Egfr kinase activity and translation of egfr messenger RNA into protein, respectively. The zebrafish is a particularly excellent model for studying cardiovascular development because zebrafish are transparent allowing direct visualization of the heart and circulation in the blood vessels. Inhibition of zebrafish Egfr activity in vivo impeded blood flow via the outflow tract into the aorta and impeded circulation in the axial and intersegmental vessels by 80 h post-fertilization. Analysis of the heart showed that the heart chambers and pericardial sacs were dilated and the outflow tracts were narrowed. Together these results suggested that zebrafish Egfr has a cardiovascular function in the developing zebrafish that is required for normal circulation.

Descriptors: zebrafish embryology, rats, cardiovascular abnormalities metabolism, epidermal growth factor receptor metabolism, amino acid sequence, cardiovascular abnormalities etiology, molecular sequence data, pyrimidines pharmacology, pyrroles pharmacology, receptor, epidermalgrowth factor antagonists and inhibitors, tyrphostins pharmacology, metabolism.

Goishi, K., A. Shimizu, G. Najarro, S. Watanabe, R. Rogers, L.I. Zon, and M. Klagsbrun (2006). **Alpha A-crystallin expression prevents gamma-crystallin insolubility and cataract formation in the zebrafish cloche mutant lens.** *Development* 133(13): 2585-93. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Cataracts, the loss of lens transparency, are the leading cause of human blindness. The zebrafish embryo, with its transparency and relatively large eyes, is an excellent model for studying ocular disease in vivo. We found that the zebrafish cloche mutant, both the cloche(m39) and cloche(S5) alleles, which have defects in hematopoiesis and blood vessel development, also have lens cataracts. Quantitative examination of the living zebrafish lens by confocal microscopy showed significant increases in lens reflectance. Histological analysis revealed retention of lens fiber cell nuclei owing to impeded terminal differentiation. Proteomics identified gamma-crystallin as a protein that was substantially diminished in cloche mutants. Crystallins are the major structural proteins in mouse, human and zebrafish lens. Defects in crystallins have previously been shown in mice and humans to contribute to cataracts. The loss of gamma-crystallin protein in cloche was not due to lowered mRNA levels but rather to gamma-crystallin protein insolubility. AlphaA-crystallin is a chaperone that protects proteins from misfolding and becoming insoluble. The cloche lens is deficient in both alphaA-crystallin mRNA and protein during development from 2-5 dpf. Overexpression of exogenous alphaA-crystallin rescued the cloche lens phenotype, including solubilization of gamma-crystallin, increased lens transparency and induction of lens fiber cell differentiation. Taken together, these results indicate that alphaA-crystallin expression is required for normal lens development and demonstrate that cataract formation can be prevented in vivo. In addi-

tion, these results show that proteomics is a valuable tool for detecting protein alterations in zebrafish.

Descriptors: zebrafish embryos, cataract veterinary, fish diseases genetics, crystalline lens pathology, alpha crystallins genetics, gamma crystallins genetics, amino acid and base sequence, cataract genetics, molecular cloning, DNA primers molecular sequence data, mutation, reverse transcriptase PCR and metabolism, gamma crystallins metabolism.

Goldsmith, M.I., S. Fisher, R. Waterman, and S.L. Johnson (2003). **Saltatory control of isometric growth in the zebrafish caudal fin is disrupted in long fin and rapunzel mutants.** *Developmental Biology* 259(2): 303-17. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Zebrafish fins grow by sequentially adding new segments of bone to the distal end of each fin ray. In wild type zebrafish, segment addition is regulated such that an isometric relationship is maintained between fin length and body length over the lifespan of the growing fish. Using a novel, surrogate marker for fin growth in conjunction with cell proliferation assays, we demonstrate here that segment addition is not continuous, but rather proceeds by saltation. Saltation is a fundamental growth mechanism shared by disparate vertebrates, including humans. We further demonstrate that segment addition proceeds in conjunction with cyclic bursts of cell proliferation in the distal fin ray mesenchyme. In contrast, cells in the distal fin epidermis proliferate at a constant rate throughout the fin ray growth cycle. Finally, we show that two separate fin overgrowth mutants, long fin and rapunzel, bypass the stasis phase of the fin ray growth cycle to develop asymmetrical and symmetrical fin overgrowth, respectively.

Descriptors: zebrafish, fin rays, body patterning genetics, genetics, growth and development, aging genetics, biological markers, body constitution genetics, body height genetics, cell division, extremities growth and development, mutation, phenotype.

Goldsmith, M.I., M.K. Iovine, T. O'Reilly Pol, and S.L. Johnson (2006). **A developmental transition in growth control during zebrafish caudal fin development.** *Developmental Biology* 296(2): 450-7. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: A long-standing question in developmental biology is how do growing and developing animals achieve form and then maintain it. We have revealed a critical transition in growth control during zebrafish caudal fin development, wherein a switch from allometric to isometric growth occurs. This morphological transition led us to hypothesize additional physiological changes in growth control pathways. To test this, we fasted juvenile and adult zebrafish. Juvenile fins continued allometric growth until development of the mature bilobed shape was completed. In contrast, the isometric growth of mature adult fins arrested within days of initiating a fast. We explored the biochemical basis of this difference in physiology between the two phases by assessing the sensitivity to rapamycin, a drug that blocks a nutrient-sensing pathway. We show that the nutrition-independent, allometric growth phase is resistant to rapamycin at 10-fold higher concentrations than are effective at arresting growth in the nutrition-dependent, isometric growth phase. We thus link a morphological transition in growth control between allometric and isometric growth mechanisms to different physiological responses to nutritional state of the animal and finally to different pharmacological responses to a drug (rapamycin) that affects the nutrition-sensing mechanism described from yeast to human.

Descriptors: zebrafish, caudal fin development, tail growth and development, cell proliferation drug effects, functional laterality physiology, mesoderm drug effects, mesoderm

physiology, drug effects, nutritional status physiology, sirolimus administration and dosage, tail cytology, physiology.

Goldsmith, P. and W.A. Harris (2003). **The zebrafish as a tool for understanding the biology of visual disorders.** *Seminars in Cell and Developmental Biology* 14(1): 11-8. ISSN: 1084-9521.
NAL Call Number: QH573
Abstract: Retinal degenerations are the commonest cause of blindness in the Western world, affecting 5% of the population, yet remain largely untreatable. A better understanding of the mechanisms of disease is needed. Zebrafish fill a gap in the current repertoire of models, offering genetic tractability in a vertebrate. Their retina has many similarities with a human retina. Importantly, unlike rodents, they have rich colour vision, offering the potential to model the macular degenerations. A variety of physiological assays, genetic manipulations and histological tools have been developed and useful models of human disease created.
Descriptors: zebrafish, vision disorders etiology, blindness etiology, laboratory techniques and procedures, animal models, retina anatomy and histology, retina pathology and degeneration etiology, vision disorders genetics and pathology, genetics.

Gomez-Skarmeta, J.L., B. Lenhard, and T.S. Becker (2006). **New technologies, new findings, and new concepts in the study of vertebate cis-regulatory sequences**. *Developmental Dynamics* 235(4): 870-885. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: All vertebrates share a similar early embryonic body plan and use the same regulatory genes for their development. The availability of numerous sequenced vertebrate genomes and significant advances in bioinformatics have resulted in the finding that the genomic regions of many of these developmental regulatory genes also contain highly conserved noncoding sequence. In silico discovery of conserved noncoding regions and of transcription factor binding sites as well as the development of methods for high throughput transgenesis in *Xenopus* and zebrafish are dramatically increasing the speed with which regulatory elements can be discovered, characterized, and tested in the context of whole live embryos. We review here some of the recent technological developments that will likely lead to a surge in research on how vertebrate genomes encode regulation of transcriptional activity, how regulatory sequences constrain genomic architecture, and ultimately how vertebrate form has evolved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryo, molecular genetics, development, evolution and adaptation, gene expression, embryonic development, cis regulatory sequence, technological developments, *Xenopus*.

Gore, A.V., S. Tao, J. Tian, and K. Sampath (2004). **Vg1 restricts endoderm cell fate and movement during zebrafish gastrulation.** *Developmental Biology* 271(2): 616. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, molecular biophysics, development, gastrulation, endoderm cells.

Granato, M. (2003). **On best behavior: cadherins drive slow muscle to their limits.** *Developmental Cell* 5(6): 819-820. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Descriptors: zebrafish, biochemistry and molecular biophysics, cell biology, development, muscular system.

Grande, T. and B. Young (2004). **The ontogeny and homology of the Weberian apparatus in the zebrafish *Danio rerio* (*Ostariophysi: Cypriniformes*).** *Zoological Journal of the Linnean Society* 140(2): 241-254. ISSN: 0024 4082.
NAL Call Number: 410.9 L64
Abstract: The ontogeny of the Weberian apparatus was examined in the zebrafish, *Danio rerio*, using both cleared and stained specimens and histology. Over 300 individuals from four independent zebrafish lineages, ranging in size from 3 to 28 mm TL, were examined for this study. Results provide a basic understanding of the development of the Weberian apparatus in the wild-type zebrafish. Our results, in conjunction with those already published, point to substantial variation in the development of the Weberian apparatus among otophysans and new interpretations of the homology of certain ossicles (e.g. tripus and claustrum). Hypotheses of homology among various Weberian ossicles are considered and represent an important step in understanding the evolution of sound transmission in ostariophysan fishes.
Descriptors: zebrafish, *Danio rerio*, ear, Weberian apparatus, ontogeny, homology, development, homology.

Grandel, H., J. Kaslin, J. Ganz, I. Wenzel, and M. Brand (2006). **Neural stem cells and neurogenesis in the adult zebrafish brain: origin, proliferation dynamics, migration and cell fate.** *Developmental Biology* 295(1): 263-77. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Lifelong neurogenesis in vertebrates relies on stem cells producing proliferation zones that contain neuronal precursors with distinct fates. Proliferation zones in the adult zebrafish brain are located in distinct regions along its entire anterior-posterior axis. We show a previously unappreciated degree of conservation of brain proliferation patterns among teleosts, suggestive of a teleost ground plan. Pulse chase labeling of proliferating populations reveals a centrifugal movement of cells away from their places of birth into the surrounding mantle zone. We observe tangential migration of cells born in the ventral telencephalon, but only a minor rostral migratory stream to the olfactory bulb. In contrast, the lateral telencephalic area, a domain considered homologous to the mammalian dentate gyrus, shows production of interneurons and migration as in mammals. After a 46-day chase, newborn highly mobile cells have moved into nuclear areas surrounding the proliferation zones. They often show HuC/D immunoreactivity but importantly also more specific neuronal identities as indicated by immunoreactivity for tyrosine hydroxylase, serotonin and parvalbumin. Application of a second proliferation marker allows us to recognize label-retaining, actively cycling cells that remain in the proliferation zones. The latter population meets two key criteria of neural stem cells: label retention and self renewal.
Descriptors: zebrafish, brain cytology, neurons cytology, stem cells cytology, age factors, bromodeoxyuridine metabolism, cell differentiation and movement, cell proliferation, immunohistochemistry methods, neurons physiology, proliferating cell nuclear antigen metabolism, stem cells physiology.

Greiling, T.M.S. and J.I. Clark (2008). **The transparent lens and cornea in the mouse and zebra fish eye**. *Seminars in Cell & Developmental Biology* 19(2): 94-99. ISSN: 1084-9521.
NAL Call Number: QH573
Descriptors: zebrafish , mouse, transparent lens, cornea, eye, quasielastic laser light scattering spectroscopy, QLS.

Gribble, S.L., O.B. Nikolaus, and R.I. Dorsky (2007). **Regulation and function of Dbx genes in the zebrafish spinal cord.** *Developmental Dynamics* 236(12): 3472-83. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Dbx homeodomain proteins are important for spinal cord dorsal/ventral patterning and the production of multiple spinal cord cell types. We have examined the regulation and function of Dbx genes in the zebrafish. We report that Hedgehog signaling is not required for the induction or maintenance of these genes; in the absence of Hedgehog signaling, dbx1a/1b/2 are expanded ventrally with concomitant increases in postmitotic neurons that differentiate from this domain. Also, we find that retinoic acid signaling is not required for the induction of Dbx expression. Furthermore, we are the first to report that knockdown of Dbx1 function causes a dorsal expansion of nkx6.2, which is thought to be the cross-repressive partner of Dbx1 in mouse. Our data confirm that the dbx1a/1b/2 domain in zebrafish spinal cord development behaves similarly to amniotes, while extending knowledge of Dbx1 function in spinal cord patterning. Developmental Dynamics 236:3472-3483, 2007.
Descriptors: zebrafish, Dbx genes, spinal cord cell types, retinoic acid, amniotes.

Griffin, K.J. and D. Kimelman (2003). **Interplay between FGF, one-eyed pinhead, and T-box transcription factors during zebrafish posterior development.** *Developmental Biology* 264(2): 456-66. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The zebrafish T-box transcription factors spadetail (spt) and the brachyury ortholog no tail (ntl) are together essential for posterior mesoderm formation. In addition to being functionally redundant, spt and ntl also genetically interact with zygotic mutant alleles of one-eyed pinhead (Zoep), leading to synergistic mesodermal defects. Here we have used genetic and pharmacological assays to address the mechanism of these interactions. We show that Zoep and ntl are together required upstream of spt expression, accounting for the severity of the mesodermal defects in Zoep;ntl embryos. Since Xenopus brachyury is proposed to regulate fgf expression, and FGF signaling is required for spt expression, we analyzed the involvement of the FGF signaling pathway in these genetic interactions. Using a specific inhibitor of FGFR activity to indirectly assay the strength of FGF signaling in individual embryos, we found that spt and ntl mutant embryos were both hypersensitive to the FGFR inhibitor. This hypersensitivity is consistent with the possibility that Spt and Ntl function upstream of FGF signaling. Furthermore, we show that minor pharmacological or genetic perturbations in FGF signaling are sufficient to dramatically enhance the Zoep mutant phenotype, providing a plausible explanation for why Zoep genetically interacts with spt and ntl. Finally, we show that Zoep and ace/fgf8 function are essential for the formation of all posterior tissues, including spinal cord. Taken together, our data provide strong in vivo support for the regulation of FGF signaling by T-box transcription factors, and the cooperative activity of Oep and FGF signaling during the formation of posterior structures.
Descriptors: zebrafish embryos, tail development, homeodomain, fibroblast receptors growth factor physiology, T-box domain, transcription factors physiology, Xenopus proteins, fibroblast growth factor 8, Fgf physiology, mesoderm physiology, pyrroles pharmacology, signal transduction.

Grima, R. and S. Schnell (2007). **Can tissue surface tension drive somite formation?** *Developmental Biology* 307(2): 248-257. ISSN: 0012-1606.
NAL Call Number: 442.8D49

Descriptors: zebrafish, tissue surface tension, adhesion, developmental stages, freshwater fish, tissues, *Danio rerio*.

Grimes, A.C., H.A. Stadt, I.T. Shepherd, and M.L. Kirby (2006). **Solving an enigma: arterial pole development in the zebrafish heart.** *Developmental Biology* 290(2): 265-76. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: It is a widely held belief that the arterial pole of the zebrafish heart is unusual among models of comparative cardiogenesis. This is based, in part, on the report that the bulbus arteriosus undergoes a striated-to-smooth muscle phenotypic transition during development. An implication of this is that the zebrafish, a model almost ubiquitously accepted in other fields of comparative biology, may be poorly suited to the study of conotruncal abnormalities in human disease. However, while the use of atrioventricular-specific molecular markers has allowed extensive characterization of the development of the atrium and ventricle, the lack of any bulbus-specific markers has meant that this region of the zebrafish heart is poorly characterized and quite possibly misunderstood. We have discovered that the fluorescent nitric oxide indicator 4,5-diaminofluorescein diacetate (DAF-2DA) specifically labels the bulbus arteriosus throughout development from approximately 48 h post-fertilization. Therefore, using DAF-2DA and an immunohistochemical approach, we attempted to further characterize the development of the bulbus. We have concluded that no such phenotypic transition occurs, that contrary to current thinking, aspects of zebrafish arterial pole development are evolutionarily conserved, and that the bulbus should not be considered a chamber, being more akin to the arterial trunk(s) of higher vertebrates.

Descriptors: zebrafish hearts, arteries embryology, bulbus arteriosus, developmental gene expression regulation, heart embryology, body patterning, chick embryo, fluorescein pharmacology, genetic markers, immunohistochemistry, fluorescence microscopy, smooth muscle metabolism, myocardium metabolism, nitric oxide metabolism, paraffin chemistry, phenotype, time factors.

Groves, J.A., C.L. Hammond, and S.M. Hughes (2005). **Fgf8 drives myogenic progression of a novel lateral fast muscle fibre population in zebrafish.** *Development* 132(19): 4211-22. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Fibroblast growth factors (Fgfs) have long been implicated in regulating vertebrate skeletal muscle differentiation, but their precise role(s) in vivo remain unclear. Here, we show that Fgf8 signalling in the somite is required for myod expression and terminal differentiation of a subset of fast muscle cells in the zebrafish lateral somite. In the absence of Fgf8, lateral somite cells transiently express myf5 but fail to make muscle and remain in a dermomyotome-like state characterised by pax3 and meox expression. Slow muscle fibres form and commence normal migration in the absence of Fgf8, but fail to traverse the expanded undifferentiated lateral somite. The Fgf8-independent residual population of medial fast muscle fibres is not Hedgehog dependent. However, Fgf8-independent medial fast muscle precursors are lacking in floatinghead mutants, suggesting that they require another ventral midline-derived signal. We conclude that Fgf8 drives terminal differentiation of a specific population of lateral muscle precursor cells within the early somite.

Descriptors: zebrafish, fibroblast growth factor 8 physiology, fast twitch muscle fibers physiology, myoblasts physiology, somites physiology, body patterning physiology, cell differentiation, metabolism, Hedgehog proteins, fast twitch muscle fibers cytology, Myod protein biosynthesis, myoblasts metabolism, myogenic regulatory factor 5 biosynthesis, myogenin

metabolism, paired box transcription factors biosynthesis, signal transduction, trans activators physiology, proteins biosynthesis.

Grozea, A., I. Vintila, M. Bura, A. Bencsik, D. Ilie , V. Caraba, I. Banatean Dunea, and R. Muscalu Nagy (2003). **Research regarding the production of fish chimeras by means of blastomere transplant in *Cyprinidae***. *Buletinul Universitatii De Stiinte Agricole Si Medicina Veterinara Cluj Napoca, Seria Zootehnie Si Biotehnologii* 59: 265-269. ISSN: 1454 2382.
NAL Call Number: S469.R6 B85
Abstract: This paper describes the creation of chimaeras by transferring the blastomeres of zebrafish (*Danio rerio*) and carp (*Cyprinus carpio*) donor embryos to recipient embryos. Dechorionated embryos from zebrafish standard (pigmented, S) and carp Lausitz (with scales, L) donor lines were cultured for 72 h, after which their blastomeres were removed and transplanted into the recipient embryos of b4 (albino, A) and Eischgrund and zebrafish and carp lines, respectively, by microinjection. These embryos were cultured and monitored at 3-h intervals until hatching. It was shown that out of 97 manipulated zebrafish embryos, 17, (17.5%), 3 (3.1%) and 1 (1.03%) reached the segmentation, pharingula and hatching periods, respectively, while only 3 (21.43%) out of 14 carp embryos reached the segmentation period and none survived to the pharingula and hatching periods. The low viability and survival of embryos to the hatching stage obtained in this study suggests that some problems still occur in the transplantation procedure. Copyright © 2008 CABI
Descriptors: zebrafish, carp, chimaeras, embryo culture, embryonic development, embryos, in vitro culture, lines, micromanipulation, transplantation, *Danio rerio*.

Gulati Leekha, A. and D. Goldman (2006). **A reporter-assisted mutagenesis screen using alpha 1-tubulin-GFP transgenic zebrafish uncovers missteps during neuronal development and axonogenesis.** *Developmental Biology* 296(1): 29-47. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Alpha1-tubulin expression occurs in a neural-specific, temporally regulated, and regeneration-inducible fashion in zebrafish. A GFP reporter driven by the alpha1-tubulin promoter in transgenic zebrafish acts as a stable, in vivo molecular tag that follows neuronal development from birth/specification through postmitotic differentiation to axonal outgrowth and synaptogenesis. We exploited this transgenic system in a reporter expression-dependent (morphology-independent) mutagenesis screen to identify disruptions in genetic loci essential for neuronogenesis and axon elaboration, which would manifest as visually appreciable perturbations in GFP fluorescence. Thirty-two such recessive mutations were obtained, a subset of which was screened through a secondary RNA quantification-based assay to eliminate housekeeping gene defects. Three representative loci, when characterized in detail, were found to exhibit missteps in discrete, sequential stages of embryonic neuronal development. Mutation in sookshma panneurally diminishes the neural precursor pool by affecting cell proliferation in the developing embryo while patterning along the neuraxis remains unperturbed. Disruption of drishti on the other hand ameliorates the mitotic neural population by affecting cell cycle exit of progenitors and stalling their progression to the postmitotic neuronal stage, without impairing subsequent cell fate determination or differentiation. Finally, dhruva is required during neuronal differentiation for axonal branching and terminal innervation in spinal motoaxons and the retinotectal projection. Molecular identification of these loci and analysis of the remaining mutational repertoire will offer unique insights into the genetic inputs that go on to make a mature, differentiated neuron.
Descriptors: zebrafish, goldfish, axons physiology, cell differentiation genetics, reporter genes, green fluorescent mutagenesis, neurons cytology, tubulin genetics, genetics, genetically

modified, goldfish genetics, mutation , neurons physiology, stem cells cytology, stem cells physiology, transgenes, tubulin biosynthesis.

Gulati-Leekha, A. and D. Goldman (2006). **Uncovering genes essential for neuronal development in zebrafish using a GFP-based forward genetic screen**. *Developmental Biology* 295(1): 440-441. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, molecular genetics, nervous system, neural coordination, genetic screen, genetic techniques, cell proliferation, cell cycle, synaptogenesis, neurogenesis, neuronal development, GFP.

Gupta, S., H. Zhu, L.I. Zon, and T. Evans (2006). **BMP signaling restricts hemato-vascular development from lateral mesoderm during somitogenesis**. *Development* 133(11): 2177-2187. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The bone morphogenetic protein (BMP) signaling pathway is essential during gastrulation for the generation of ventral mesoderm, which makes it a challenge to de. ne functions for this pathway at later stages of development. We have established an approach to disrupt BMP signaling specifically in lateral mesoderm during somitogenesis, by targeting a dominant-negative BMP receptor to Lmo2+ cells in developing zebrafish embryos. This results in expansion of hematopoietic and endothelial cells, while restricting the expression domain of the pronephric marker pax2.1. Expression of a constitutively active receptor and transplantation experiments were used to confirm that BMP signaling in lateral mesoderm restricts subsequent hemato-vascular development. The results show that the BMP signaling pathway continues to function after cells are committed to a lateral mesoderm fate, and influences subsequent lineage decisions by restricting hemato-vascular fate in favor of pronephric development. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, blood and lymphatics, transport and circulation, development, molecular genetics, cardiovascular system, transport and circulation, somitogenesis, hemato vascular development, zebrafish embryos.

Haas, P. and D. Gilmour (2006). **Chemokine signaling mediates self-organizing tissue migration in the zebrafish lateral line.** *Developmental Cell* 10(5): 673-80. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: The shape of most complex organ systems arises from the directed migration of cohesive groups of cells. Here, we dissect the role of the chemokine guidance receptor Cxcr4b in regulating the collective migration of one such cohesive tissue, the zebrafish lateral line primordium. Using in vivo imaging, we show that the shape and organization of the primordium is surprisingly labile, and that internal cell movements are uncoordinated in embryos with reduced Cxcr4b signaling. Genetic mosaic experiments reveal that single cxcr4b mutant cells can migrate in a directional manner when placed in wild-type primordia, but that they are specifically excluded from the leading edge. Moreover, a remarkably small number of SDF1a-responsive cells are able to organize an entire cxcr4b mutant primordium to restore migration and organogenesis in the lateral line. These results reveal a role for chemokine signaling in mediating the self-organizing migration of tissues during morphogenesis.
Descriptors: zebrafish, body patterning, Cxc chemokines metabolism, embryology, metabolism, signal transduction, metabolism, cell communication, cell movement, cytology, Cxcr4 receptors metabolism.

Habeck, H., A.M. Vogel, and S. Schulte-Merker (2003). **Getting connected.** *Developmental Cell* 5(5): 669-670. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Descriptors: zebrafish, cardiovascular system, development, multiphoton time lapse imaging, imaging and microscopy techniques, angiogenesis.

Hadrys, T., B. Punnamoottil, M. Pieper, H. Kikuta, G. Pezeron, T.S. Becker, V. Prince, R. Baker , and S. Rinkwitz (2006). **Conserved co-regulation and promoter sharing of hoxb3a and hoxb4a in zebrafish.** *Developmental Biology* 297(1): 26-43. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The expression of zebrafish hoxb3a and hoxb4a has been found to be mediated through five transcripts, hoxb3a transcripts I-III and hoxb4a transcripts I-II, driven by four promoters. A "master" promoter, located about 2 kb downstream of hoxb5a, controls transcription of a pre-mRNA comprising exon sequences of both genes. This unique gene structure is proposed to provide a novel mechanism to ensure overlapping, tissue-specific expression of both genes in the posterior hindbrain and spinal cord. Transgenic approaches were used to analyze the functions of zebrafish hoxb3a/hoxb4a promoters and enhancer sequences containing regions of homology that were previously identified by comparative genomics. Two neural enhancers were shown to establish specific anterior expression borders within the hindbrain and mediate expression in defined neuronal populations derived from hindbrain rhombomeres (r) 5 to 8, suggesting a late role of the genes in neuronal cell lineage specification. Species comparison showed that the zebrafish hoxb3a r5 and r6 enhancer corresponded to a sequence within the mouse HoxA cluster controlling activity of Hoxa3 in r5 and r6, whereas a homologous region within the HoxB cluster activated Hoxb3 expression but limited to r5. We conclude that the similarity of hoxb3a/Hoxa3 regulatory mechanisms reflect the shared descent of both genes from a single ancestral paralog group 3 gene.
Descriptors: zebrafish embryos, developmental gene expression regulation, homeodomain promoter regions genetics, genetically modified, cell differentiation genetics enhancer elements, exons, homeodomain proteins metabolism, mice, molecular sequence data, reverse transcriptase PCR, rhombencephalon cytology, rhombencephalon embryology, nucleic acid sequence homology, *Xenopus* proteins metabolism.

Hadzhiev, Y., Z. Lele, S. Schindler, S.W. Wilson, P. Ahlberg, U. Strahle, and F. Muller (2007). **Hedgehog signaling patterns the outgrowth of unpaired skeletal appendages in zebrafish.** *BioMed Central Developmental Biology* 7: 75. ISSN: 1471 213X.
NAL Call Number: QH491.B63
Abstract: BACKGROUND: Little is known about the control of the development of vertebrate unpaired appendages such as the caudal fin, one of the key morphological specializations of fishes. Recent analysis of lamprey and dogshark median fins suggests the co-option of some molecular mechanisms between paired and median in *Chondrichthyes*. However, the extent to which the molecular mechanisms patterning paired and median fins are shared remains unknown. RESULTS: Here we provide molecular description of the initial ontogeny of the median fins in zebrafish and present several independent lines of evidence that Sonic hedgehog signaling emanating from the embryonic midline is essential for establishment and outgrowth of the caudal fin primordium. However, gene expression analysis shows that the primordium of the adult caudal fin does not harbor a Sonic hedgehog-expressing domain equivalent to the Shh secreting zone of polarizing activity (ZPA) of paired appendages. CONCLUSION: Our results suggest that Hedgehog proteins can regulate skeletal appendage outgrowth independent of a ZPA and demonstrates an unexpected mechanism for

mediating Shh signals in a median fin primordium. The median fins evolved before paired fins in early craniates, thus the patterning of the median fins may be an ancestral mechanism that controls the outgrowth of skeletogenic appendages in vertebrates.

Descriptors: zebrafish embryos, body patterning physiology, Hedgehog, signal transduction physiology, body patterning genetics, drug effects, embryology and metabolism, extremities embryology, developmental gene expression regulation drug effects, green fluorescent proteins genetics and metabolism, Hedgehog in situ hybridization, limb bud embryology, limb bud metabolism, mutation, reverse transcriptase PCR, signal transduction genetics, veratrum alkaloids pharmacology.

Hagos, E.G. and S.T. Dougan (2007). **Time-dependent patterning of the mesoderm and endoderm by Nodal signals in zebrafish.** *BioMed Central Developmental Biology* 7: 22. ISSN: 1471 213X.

NAL Call Number: QH491.B63

Abstract: BACKGROUND: The vertebrate body plan is generated during gastrulation with the formation of the three germ layers. Members of the Nodal-related subclass of the TGF-beta superfamily induce and pattern the mesoderm and endoderm in all vertebrates. In zebrafish, two nodal-related genes, called squint and cyclops, are required in a dosage-dependent manner for the formation of all derivatives of the mesoderm and endoderm. These genes are expressed dynamically during the blastula stages and may have different roles at different times. This question has been difficult to address because conditions that alter the timing of nodal-related gene expression also change Nodal levels. We utilized a pharmacological approach to conditionally inactivate the ALK 4, 5 and 7 receptors during the blastula stages without disturbing earlier signaling activity. This permitted us to directly examine when Nodal signals specify cell types independently of dosage effects. RESULTS: We show that two drugs, SB-431542 and SB-505124, completely block the response to Nodal signals when added to embryos after the mid-blastula transition. By blocking Nodal receptor activity at later stages, we demonstrate that Nodal signaling is required from the mid-to-late blastula period to specify sequentially, the somites, notochord, blood, Kupffer's vesicle, hatching gland, heart, and endoderm. Blocking Nodal signaling at late times prevents specification of cell types derived from the embryo margin, but not those from more animal regions. This suggests a linkage between cell fate and length of exposure to Nodal signals. Confirming this, cells exposed to a uniform Nodal dose adopt progressively more marginal fates with increasing lengths of exposure. Finally, cell fate specification is delayed in squint mutants and accelerated when Nodal levels are elevated. CONCLUSION: We conclude that (1) Nodal signals are most active during the mid-to-late blastula stages, when nodal-related gene expression and the movement of responding cells are at their most dynamic; (2) Nodal signals specify cell fates along the animal-vegetal axis in a time-dependent manner; (3) cells respond to the total cumulative dose of Nodal signals to which they are exposed, as a function of distance from the source and duration of exposure.

Descriptors: zebrafish embryos, body patterning genetics, endoderm physiology, intracellular signaling peptides and mesoderm physiology, benzamides pharmacology, benzodioxoles pharmacology, blastula drug effects, dioxoles pharmacology, imidazoles pharmacology, intracellular signaling peptides and proteins antagonists and inhibitors, pyridines pharmacology, time factors.

Hagos, E.G., X. Fan, and S.T. Dougan (2007). **The role of maternal Activin-like signals in zebrafish embryos.** *Developmental Biology* 309(2): 245-58. ISSN: 0012-1606.

NAL Call Number: 442.8D49

Abstract: Maternal Activin-like proteins, a subgroup of the TGF-beta superfamily, play a key role in establishing the body axes in many vertebrates, but their role in teleosts is unclear. At least two maternal Activin-like proteins are expressed in zebrafish, including the Vg1 orthologue, zDVR-1, and the nodal-related gene, Squint. Our analysis of embryos lacking both maternal and zygotic squint function revealed that maternal squint is required in some genetic backgrounds for the formation of dorsal and anterior tissues. Conditional inactivation of the ALK4, 5 and 7 receptors by SB-505124 treatment during the cleavage stages ruled out a role for maternal Squint, zDVR-1, or other Activin-like ligands before the mid-blastula transition, when the dorsal axis is established. Furthermore, we show that maternal Squint and zDVR-1 are not required during the cleavage stages to induce zygotic nodal-related gene expression. nodal-related gene expression decreases when receptor inhibition continues past the mid-blastula transition, resulting in a progressive loss of mesoderm and endoderm. We conclude that maternally expressed Activin-like signals do not act before the mid-blastula transition in zebrafish, but do have a variably penetrant role in the later stages of axis formation. This contrasts with the early role for these signals during Xenopus development.
Descriptors: zebrafish embryos, Vg1, orthologue, zDVr-1, Nodal-related gene, ALK4, 5 and 7 receptors, SB-505124 treatment.

Haines, L., C. Neyt, P. Gautier, D.G. Keenan, R.J. Bryson Richardson, G.E. Hollway, N.J. Cole, and P.D. Currie (2004). **Met and Hgf signaling controls hypaxial muscle and lateral line development in the zebrafish.** *Development* 131(19): 4857-69. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Somites give rise to a number of different embryonic cell types, including the precursors of skeletal muscle populations. The lateral aspect of amniote and fish somites have been shown to give rise specifically to hypaxial muscle, including the appendicular muscle that populates fins and limbs. We have investigated the morphogenetic basis for formation of specific hypaxial muscles within the zebrafish embryo and larvae. Transplantation experiments have revealed a developmentally precocious commitment of cells derived from pectoral fin level somites to forming hypaxial and specifically appendicular muscle. The fate of transplanted somites cannot be over-ridden by local inductive signals, suggesting that somitic tissue may be fixed at an early point in their developmental history to produce appendicular muscle. We further show that this restriction in competence is mirrored at the molecular level, with the exclusive expression of the receptor tyrosine kinase met within somitic regions fated to give rise to appendicular muscle. Loss-of-function experiments reveal that Met and its ligand, hepatocyte growth factor, are required for the correct morphogenesis of the hypaxial muscles in which met is expressed. Furthermore, we demonstrate a requirement for Met signaling in the process of proneuromast deposition from the posterior lateral line primordia.
Descriptors: zebrafish, hepatocyte growth factor metabolism, skeletal muscle growth and development, skeletal muscle metabolism, proto oncogene proteins C met metabolism, zebrafish growth and development, metabolism, genetically modified, base sequence, DNA genetics, developmental gene expression regulation, hepatocyte growth factor genetics, morphogenesis, skeletal muscle embryology, phylogeny, proto oncogene proteins C met genetics, signal transduction, somites metabolism, somites transplantation.

Hall, C., M.V. Flores, G. Murison, K. Crosier, and P. Crosier (2006). **An essential role for zebrafish Fgfrl1 during gill cartilage development.** *Mechanisms of Development* 123(12): 925-40. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4

Abstract: The vertebrate craniofacial skeleton develops via a complex process involving signaling cascades in all three germ layers. Fibroblast growth factor (FGF) signaling is essential for several steps in pharyngeal arch development. In zebrafish, Fgf3 and Fgf8 in the mesoderm and hindbrain have an early role to pattern the pouch endoderm, influencing craniofacial integrity. Endodermal FGF signaling is required for the differentiation and survival of postmigratory neural crest cells that form the pharyngeal skeleton. We identify a novel role for zebrafish Fgf receptor-like 1a (Fgfrl1a) that is indispensable during gill cartilage development. We show that depletion of Fgfrl1a is sufficient to abolish cartilage derivatives of the ceratobranchials. Using an Fgfrl1a-deficient model, we analyzed expression of genes critical for chondrogenesis in the different compartments of the developing pharyngeal arch. Fgfrl1a-depleted animals demonstrate typical neural crest specification and migration to populate the arch primordia as well as normal pouch segmentation. However, in the absence of Fgfrl1a, larvae fail to express the transcription factor glial cells missing 2 (gcm2), a gene necessary for cartilage and gill filament formation, in the ectodermal lining of the branchial arches. In addition, two transcription factors essential for chondrogenesis, sox9a and runx2b, fail to express within the mesenchymal condensations of the branchial arches. A duplicate zebrafish gene, fgfrl1b, has now been identified. We show that Fgfrl1b is also required for proper formation of all ventral cartilage elements and acts cooperatively with Fgfrl1a during gill cartilage formation.
Descriptors: zebrafish, cartilage embryology, gills , fibroblast receptors growth factor physiology, amino acid sequence, branchial region chemistry and embryology, cartilage chemistry, cell movement genetics, DNA binding proteins genetics, proteins analysis and genetics, DNA binding proteins genetics, proteins metabolism, ectoderm chemistry and metabolism, embryonic development genetics, gills chemistry, hmgb proteins analysis, hmgb hmgb proteins metabolism, molecular sequence data, neural crest cytology, phylogeny, fibroblast receptorsgrowth factor analysis, fibroblast receptors growth factor genetics, transcription factors analysis and genetics and metabolism.

Hall, C., M.V. Flores, T. Storm, K. Crosier, and P. Crosier (2007). **The zebrafish lysozyme C promoter drives myeloid-specific expression in transgenic fish.** *BioMed Central Developmental Biology* 7: 42. ISSN: 1471 213X.
NAL Call Number: QH491.B63
Abstract: BACKGROUND: How different immune cell compartments contribute to a successful immune response is central to fully understanding the mechanisms behind normal processes such as tissue repair and the pathology of inflammatory diseases. However, the ability to observe and characterize such interactions, in real-time, within a living vertebrate has proved elusive. Recently, the zebrafish has been exploited to model aspects of human disease and to study specific immune cell compartments using fluorescent reporter transgenic lines. A number of blood-specific lines have provided a means to exploit the exquisite optical clarity that this vertebrate system offers and provide a level of insight into dynamic inflammatory processes previously unavailable. RESULTS: We used regulatory regions of the zebrafish lysozyme C (lysC) gene to drive enhanced green fluorescent protein (EGFP) and DsRED2 expression in a manner that completely recapitulated the endogenous expression profile of lysC. Labeled cells were shown by co-expression studies and FACS analysis to represent a subset of macrophages and likely also granulocytes. Functional assays within transgenic larvae proved that these marked cells possess hallmark traits of myelomonocytic cells, including the ability to migrate to inflammatory sources and phagocytose bacteria. CONCLUSION: These reporter lines will have utility in dissecting the genetic determinants of commitment to the myeloid lineage and in further defining how lysozyme-expressing cells

participate during inflammation.
Descriptors: zebrafish, genetically modified genetics , developmental gene expression, regulation, muramidase genetics, myeloid cells cytology, promoter regions genetics metabolism, cell lineage, molecular cloning reporter genes, green fluorescent proteins genetics and metabolism, immunohistochemistry, in situ hybridization, muramidase metabolism.

Halloran, M.C. and J.D. Berndt (2003). **Current progress in neural crest cell motility and migration and future prospects for the zebrafish model system.** *Developmental Dynamics* 228(3): 497-513. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, neural crest physiology, cell adhesion, cell movement physiology, chemotaxis physiology, cytoskeleton physiology, mutagenesis, genetics.

Hamade, A., M. Deries, G. Begemann, L. Bally-Cuif, C. Genet, F. Sabatier, A. Bonnieu, and X. Cousin (2006). **Retinoic acid activates myogenesis in vivo through Fgf8 signalling**. *Developmental Biology* 289(1): 127-140. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Retinoic acid (RA) has been shown to regulate muscle differentiation in vitro. Here, we have investigated the role of RA signalling during embryonic myogenesis in zebrafish. We have altered RA signalling from gastrulation stages onwards by either inhibiting endogenous RA synthesis using an inhibitor of retinaldehyde dehydrogenases (DEAB) or by addition of exogenous RA. DEAB reduces expression of the myogenic markers myoD and myogenin in somites, whereas RA induces increased expression of these genes and strongly induces premature myoD expression in the presomitic mesoderm (psm). The expression dynamics of myf5 in presomitic and somitic mesoderm suggest that RA promotes muscle differentiation, a role supported by the fact that RA activates expression of fast myosin, while DEAB represses it. We identify Fgf8 as a major relay factor in RA-mediated activation of myogenesis. We show that fgf8 expression in somites and anterior psm is regulated by RA, and find that in the absence of Fgf8 signalling in the acerebellar mutant RA fails to promote myoD expression. We propose that, in the developing embryo, localised synthesis of RA by Raldh2 in the anterior psm and in somites activates fgf8 expression which in turn induces the expression of myogenic genes and fast muscle differentiation. (c) 2005 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, molecular genetics, muscular system: movement and support, myogenesis , muscle differentiation, embryonic myogenesis.

Hammerschmidt, M., C. Kramer, M. Nowak, W. Herzog, and J. Wittbrodt (2003). **Loss of maternal Smad5 in zebrafish embryos affects patterning and morphogenesis of optic primordia.** *Developmental Dynamics* 227(1): 128-33. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, body patterning, DNA binding proteins genetics, proteins metabolism, eye embryology, phosphoproteins metabolism, trans activators metabolism, genetically modified, bone morphogenetic proteins genetics and metabolism, DNA binding proteins genetics, eye anatomy and histology, in situ hybridization, morphogenesis, phenotype, phosphosignal transduction, Smad5 protein, trans activators genetics.

Hammond, C.L., Y. Hinits, D.P. Osborn, J.E. Minchin, G. Tettamanti, and S.M. Hughes (2007). **Signals and myogenic regulatory factors restrict pax3 and pax7 expression to dermomyotome-like tissue in zebrafish.** *Developmental Biology* 302(2): 504-21. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Pax3/7 paired homeodomain transcription factors are important markers of muscle stem cells. Pax3 is required upstream of myod for lateral dermomyotomal cells in the amniote somite to form particular muscle cells. Later Pax3/7-dependent cells generate satellite cells and most body muscle. Here we analyse early myogenesis from, and regulation of, a population of Pax3-expressing dermomyotome-like cells in the zebrafish. Zebrafish pax3 is widely expressed in the lateral somite and, along with pax7, becomes restricted anteriorly and then to the external cells on the lateral somite surface. Midline-derived Hedgehog signals appear to act directly on lateral somite cells to repress Pax3/7. Both Hedgehog and Fgf8, signals that induce muscle formation within the somite, suppress Pax3/7 and promote expression of myogenic regulatory factors (MRFs) myf5 and myod in specific muscle precursor cell populations. Loss of MRF function leads to loss of myogenesis by specific populations of muscle fibres, with parallel up-regulation of Pax3/7. Myod is required for lateral fast muscle differentiation from pax3-expressing cells. In contrast, either Myf5 or Myod is sufficient to promote slow muscle formation from adaxial cells. Thus, myogenic signals act to drive somite cells to a myogenic fate through up-regulation of distinct combinations of MRFs. Our data show that the relationship between Pax3/7 genes and myogenesis is evolutionarily ancient, but that changes in the MRF targets for particular signals contribute to myogenic differences between species.

Descriptors: zebrafish embryos, Fgf physiology , Hedgehog, Myod protein physiology, myogenic regulatory factor 5 physiology, pax7 transcription factor biosynthesis, paired box transcription factors biosynthesis, physiology, proteins biosynthesis and physiology, cell proliferation developmental gene expression regulation, muscle development, muscle fibers physiology, mutation, neural crest embryology and metabolism, pax7 transcription factor genetics, paired box transcription factors genetics, signal transduction, somites physiology.

Hammond, K.L., H.E. Loynes, A.A. Folarin, J. Smith, and T.T. Whitfield (2003). **Hedgehog signalling is required for correct anteroposterior patterning of the zebrafish otic vesicle.** *Development* 130(7): 1403-17. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Currently, few factors have been identified that provide the inductive signals necessary to transform the simple otic placode into the complex asymmetric structure of the adult vertebrate inner ear. We provide evidence that Hedgehog signalling from ventral midline structures acts directly on the zebrafish otic vesicle to induce posterior otic identity. We demonstrate that two strong Hedgehog pathway mutants, chameleon (con(tf18b)) and slow muscle omitted (smu(b641)) exhibit a striking partial mirror image duplication of anterior otic structures, concomitant with a loss of posterior otic domains. These effects can be phenocopied by overexpression of patched1 mRNA to reduce Hedgehog signalling. Ectopic activation of the Hedgehog pathway, by injection of sonic hedgehog or dominant-negative protein kinase A RNA, has the reverse effect: ears lose anterior otic structures and show a mirror image duplication of posterior regions. By using double mutants and antisense morpholino analysis, we also show that both Sonic hedgehog and Tiggy-winkle hedgehog are involved in anteroposterior patterning of the zebrafish otic vesicle.

Descriptors: zebrafish, ear embryology, embryonic induction physiology, trans activators metabolism, ear abnormalities, ganglia embryology, Hedgehog proteins, cell surface receptors metabolism, proteins.

Han, T.M., X. Xu, J. Leigh, and S.L. Amacher (2004). **Zebrafish T-box genes and floor plate formation.** *Developmental Biology* 271(2): 588. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, nervous system, neural coordination, development, molecular genetics.

Hanaoka, R., Y. Ohmori, K. Uyemura, T. Hosoya, Y. Hotta, T. Shirao, and H. Okamoto (2004). **Zebrafish gcmb is required for pharyngeal cartilage formation.** *Mechanisms of Development* 121(10): 1235-47. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: The glial cells missing (gcm) gene in Drosophila encodes a GCM-motif transcription factor that functions as a binary switch to select between glial and neuronal cell fates. To understand the function of gcm in vertebrates, we isolated the zebrafish gcmb and analyzed the function of this gene using antisense morpholino oligonucleotides against gcmb mRNA (gcmb-MO) and transgenic overexpression. Zebrafish gcmb is expressed in the pharyngeal arch epithelium and in cells of the macrophage lineage. gcmb-MO-injected larvae show significantly reduced branchial arch cartilages. fgf3-MO-injected larvae display a similar phenotype to that of gcmb-MO-injected larvae with respect to the lack of pharyngeal cartilage formation. In addition, gcmb expression in the pharyngeal arches is down-regulated in fgf3-MO-injected larvae. The gcmb transgenic larvae show a protrusion of the lower jaw and abnormal spatial arrangement of the pharyngeal cartilage elements. These results suggest that gcmb is required for normal pharyngeal cartilage formation in zebrafish and that its expression is dependent on fgf3 activity.
Descriptors: zebrafish embryos, branchial region growth and development, cartilage growth and development, neuropeptides genetics, trans activators genetics, growth and development, amino acid sequence, genetically modified, base sequence, molecular cloning, DNA binding proteins genetics, proteins, fibroblast growth factor 3, Fgf genetics, developmental gene expression regulation, in situ hybridization, larva drug effects, larva growth and development, molecular sequence data, oligodeoxyribonucleotides, antisense genetics, oligodeoxyribonucleotides, antisense pharmacology, phenotype, amino acid sequence homology, transcription factors.

Hans, S., N. Scheer, I. Riedl, E. v Weizsacker, P. Blader, and J.A. Campos Ortega (2004). **Her3, a zebrafish member of the hairy-E(spl) family, is repressed by Notch signalling.** *Development* 131(12): 2957-69. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: her3 encodes a zebrafish bHLH protein of the Hairy-E(Spl) family. During embryogenesis, the gene is transcribed exclusively in the developing central nervous system, according to a fairly simple pattern that includes territories in the mesencephalon/rhombencephalon and the spinal cord. In all territories, the her3 transcription domain encompasses regions in which neurogenin 1 (neurog1) is not transcribed, suggesting regulatory interactions between the two genes. Indeed, injection of her3 mRNA leads to repression of neurog1 and to a reduction in the number of primary neurones, whereas her3 morpholino oligonucleotides cause ectopic expression of neurog1 in the rhombencephalon. Fusions of Her3 to the transactivation domain of VP16 and to the repression domain of Engrailed show that Her3 is indeed a transcriptional repressor. Dissection of the Her3 protein reveals two possible mechanisms for transcriptional repression: one mediated by the bHLH domain and the C-terminal WRPW tetrapeptide; and the other involving the N-terminal domain and the orange domain. Gel retardation assays suggest that the repression of neurog1 transcription

occurs by binding of Her3 to specific DNA sequences in the neurog1 promoter. We have examined interrelationships of her3 with members of the Notch signalling pathway by the Gal4-UAS technique and mRNA injections. The results indicate that Her3 represses neurog1 and, probably as a consequence of the neurog1 repression, deltaA, deltaD and her4. Moreover, Her3 represses its own transcription as well. Surprisingly, and in sharp contrast to other members of the E(spl) gene family, transcription of her3 is repressed rather than activated by Notch signalling.
Descriptors: zebrafish embryos, membrane, morphogenesis physiology, repressor base sequence, basic helix loop helix transcription factors and genetics, molecular cloning, DNA primers, developmental gene expression regulation, helix loop helix motifs genetics, nerve tissue nervous system embryology, plasmids genetics, PCR methods, promoter regions genetics, messenger RNA genetics, Notch receptors.

Hans, S., J. Christison, D. Liu, and M. Westerfield (2007). **Fgf-dependent otic induction requires competence provided by Foxi1 and Dlx3b**. *Bio Med Central Developmental Biology* 7: 5. ISSN: 1471-213X.
NAL Call Number: QH491.B63
Descriptors: zebrafish, ablation, bone morphogenetic proteins, cytology, developmental stages, fibroblast growth factor, fibroblast growth factor 3, fibroblast growth factor 8, freshwater fish, gastrulation, growth, hindbrain, HSP70 protein, inner ear, neural plate, promoters, retinoic acid, signal transduction, vesicles, otic placode, *Danio rerio*.

Hans, S., D. Liu, and M. Westerfield (2004). **Pax8 and Pax2a function synergistically in otic specification, downstream of the Foxi1 and Dlx3b transcription factors**. *Development* 131(20): 5091-5102. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The vertebrate inner ear arises from an ectodermal thickening, the otic placode, that forms adjacent to the presumptive hindbrain. Previous studies have suggested that competent ectodermal cells respond to Fgf signals from adjacent tissues and express two highly related paired box transcription factors Pax2a and Pax8 in the developing placode. We show that compromising the functions of both Pax2a and Pax8 together blocks zebrafish ear development, leaving only a few residual otic cells. This suggests that Pax2a and Pax8 are the main effectors downstream of Fgf signals. Our results further provide evidence that pax8 expression and pax2a expression are regulated by two independent factors, Foxi1 and Dlx3b, respectively. Combined loss of both factors eliminates all indications of otic specification. We suggest that the Foxi1-Pax8 pathway provides an early 'jumpstart' of otic specification that is maintained by the Dlx3b-Pax2a pathway. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, cell biology, ear development, molecular genetics, sense organs, otic specification.

Hardison, A.L., L. Lichten, S. Banerjee Basu, T.S. Becker, and S.M. Burgess (2005). **The zebrafish gene claudinj is essential for normal ear function and important for the formation of the otoliths.** *Mechanisms of Development* 122(7-8): 949-58. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: We have identified a mutation in the zebrafish gene claudinj generated by retroviral integration. Mutant embryos display otoliths severely reduced in size, no response to tapping stimulus, and an inability to balance properly suggesting vestibular and hearing dysfunction. Antisense in situ hybridization to the cldnj gene showed expression first in the otic placode and later asymmetric expression in the otic vesicle. Morpholino inhibition of

claudinj expression showed similar defects in otolith formation. Phylogenetic analysis of claudin sequences from multiple species demonstrates that claudinj was part of a gene expansion that began in the common ancestor of fish and humans, but additional fish specific gene duplications must have also occurred.
Descriptors: zebrafish, mutant embryos, ear embryology, ear physiology, genetically modified, ear anatomy and histology, embryology, developmental gene expression regulation, hearing genetics and physiology, mutation genetics, phenotype, phylogeny, rhombencephalon embryology and metabolism, proteins classification.

Hardy, M.E., L.V. Ross, and C.B. Chien (2007). **Focal gene misexpression in zebrafish embryos induced by local heat shock using a modified soldering iron.** *Developmental Dynamics* 236(11): 3071-6. ISSN: 1058-8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, gene, misexpression of genes, heat shock, soldering iron, development, repressor systems, isolated areas of embryo.

Harrington, M.J., M. Tsang, I. Dawid, M. Halpern, and R. Brewster (2005). **Role of desmosomal cadherins in zebrafish vascular morphogenesis.** *Developmental Biology* 283(2): 689. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, cardiovascular system, transport and circulation, molecular genetics, decreased blood circulation, pericardial edema, abnormal vessel morphology.

Harvey, S.A. and M.P.O. Logan (2006). **SALL4 acts downstream of TBX5 and is required for pectoral fin outgrowth**. *Development* 133(6): 1165-1173. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Okihiro syndrome (OS) is defined by forelimb defects associated with the eye disorder Duane anomaly and results from mutations in the gene SALL4. Forelimb defects in individuals with OS range from subtle thumb abnormalities to truncated limbs. Mutations in the T-box transcription factor TBX5 cause Holt-Oram syndrome (HOS), which results in forelimb and heart defects. Although mutations in TBX5 result in HOS, it has been predicted that these mutations account for only similar to 30% of all individuals with HOS. Individuals with OS and HOS limb defects are very similar, in fact, individuals with mutations in SALL4 have in some cases previously been diagnosed with HOS. Using zebrafish as a model, we have investigated the function of sall4 and the relationship between sall4 and tbx5, during forelimb development. We demonstrate that sall4 and a related gene sall1 act downstream of tbx5 and are required for pectoral fin development. Our studies of Sall gene family redundancy and tbx5 offer explanations for the similarity of individuals with OS and HOS limb defects. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, forelimb defects, molecular genetics, Holt-Oram syndrome, congenital disease, Okihiro syndrome, eye disease, genetic disease, forelimb development.

Hashiguchi, Y. and M. Nishida (2006). **Evolution and origin of vomeronasal-type odorant receptor gene repertoire in fishes**. *Bio Med Central Evolutionary Biology* 6(1) ISSN: 1471-2148.
NAL Call Number: QH359.B63
Descriptors: zebrafish, chromosomes, evolution, evolutionary genetics, freshwater fish, gene clusters , gene families, genomes, nucleotide sequence, olfaction, olfactory epithelium, per-

ception, phylogenetics, phylogeny, receptors, vomeronasal organ, odorant receptors, *Danio rerio*, Fugu, *Oryzias latipes*, Teleostei, *medaka*.

Hashimoto, H., M. Rebagliati, N. Ahmad, O. Muraoka, T. Kurokawa, M. Hibi, and T. Suzuki (2004). **The Cerberus/Dan-family protein Charon is a negative regulator of Nodal signaling during left-right patterning in zebrafish.** *Development* 131(8): 1741-53. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: We have isolated a novel gene, charon, that encodes a member of the Cerberus/Dan family of secreted factors. In zebrafish, Fugu and flounder, charon is expressed in regions embracing Kupffer's vesicle, which is considered to be the teleost fish equivalent to the region of the mouse definitive node that is required for left-right (L/R) patterning. Misexpression of Charon elicited phenotypes similar to those of mutant embryos defective in Nodal signaling or embryos overexpressing Antivin(Atv)/Lefty1, an inhibitor for Nodal and Activin. Charon also suppressed the dorsalizing activity of all three of the known zebrafish Nodal-related proteins (Cyclops, Squint and Southpaw), indicating that Charon can antagonize Nodal signaling. Because Southpaw functions in the L/R patterning of lateral plate mesoderm and the diencephalon, we asked whether Charon is involved in regulating L/R asymmetry. Inhibition of Charon's function by antisense morpholino oligonucleotides (MOs) led to a loss of L/R polarity, as evidenced by bilateral expression of the left side-specific genes in the lateral plate mesoderm (southpaw, cyclops, atv/lefty1, lefty2 and pitx2) and diencephalon (cyclops, atv/lefty1 and pitx2), and defects in early (heart jogging) and late (heart looping) asymmetric heart development, but did not disturb the notochord development or the atv/lefty1-mediated midline barrier function. MO-mediated inhibition of both Charon and Southpaw led to a reduction in or loss of the expression of the left side-specific genes, suggesting that Southpaw is epistatic to Charon in left-side formation. These data indicate that antagonistic interactions between Charon and Nodal (Southpaw), which take place in regions adjacent to Kupffer's vesicle, play an important role in L/R patterning in zebrafish.

Descriptors: zebrafish embryos, body patterning physiology, signal transduction physiology, transforming growth factor beta metabolism, amino acid sequence, heart embryology, congenital heart defects genetics, intercellular signaling peptides and proteins, Kupffer cells metabolism, molecular sequence data, *Xenopus* proteins.

Hashimoto, Y., S. Maegawa, T. Nagai, E. Yamaha, H. Suzuki, K. Yasuda, and K. Inoue (2004). **Localized maternal factors are required for zebrafish germ cell formation.** *Developmental Biology* 268(1): 152-61. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Maternally supplied factors in fertilized eggs play essential roles in the establishment of primordial germ cells. In zebrafish, cytoplasm at the distal ends of the first and second cleavage furrows has been assumed to contain germ lineage determinants, since maternal transcripts of germ lineage-specific genes are localized to ends of the cleavage furrows. To investigate whether these parts of cytoplasm are required for germ cell formation, we removed all four regions of the cytoplasm by glass capillary at the 4-cell stage. Histological analysis revealed that the ablation of cytoplasm at the ends of the cleavage planes resulted in a severe reduction in the number of germ cells. In addition, the expression of germ lineage markers was eliminated by cytoplasmic ablation. These results demonstrated that cytoplasm at the distal ends of cleavage furrows is essential for germ cell formation. We also found novel localization patterns for zDazl and brul mRNAs along the cleavage planes. Our findings provide the first direct evidence that localized cytoplasmic factors are indispensable for germ

cell establishment in zebrafish.
Descriptors: zebrafish , genomic imprinting, germ cells cytology, in situ hybridization, messenger RNA genetics , RNA binding proteins genetics.

Heicklen Klein, A., L.J. McReynolds, and T. Evans (2005). **Using the zebrafish model to study GATA transcription factors.** *Seminars in Cell and Developmental Biology* 16(1): 95-106. ISSN: 1084-9521.
NAL Call Number: QH573
Abstract: The zebrafish is an established animal model system that profits from the availability of strong experimental approaches in both genetics and embryology. As a vertebrate, zebrafish can be used to model many aspects of human development and disease. GATA transcription factors play important roles in the development of many organ systems, including those for hematopoietic, cardiovascular, reproductive, and gut-endoderm derived tissues. The six vertebrate GATA factors are highly conserved in zebrafish at the level of sequence, expression pattern, and function. The identification of mutants, establishment of transgenic GFP reporter fish, and the ease of performing loss- and gain-of-function experiments have all contributed new insight into our understanding of the regulation and function of GATA factors. We review recent advances toward this goal using the zebrafish system with a focus on hematopoiesis and cardiogenesis, and suggest how comparative genetics using the zebrafish genes might reveal core conserved properties, as well as changes in gene function that reflect different morphogenetic programs utilized by various vertebrate embryos.
Descriptors: zebrafish, DNA binding proteins genetics, models, transcription factors genetics, genetics, research animal, amino acid sequence, erythroid specific DNA binding proteins genetics, factors, GATA2 transcription factor, GATA3 transcription factor, GATA4 transcription factor, GATA5 transcription factor, GATA6 transcription factor, heart growth and development, hematopoiesis, molecular sequence data, sequence alignment, trans activators genetics, vertebrates growth and development.

Heinze, K.G., S. Scholpp, M. Brand, and P. Schwille (2004). **Probing intercellular pathways and propagation of Fgf8 signalling protein during embryogenesis by FCS.** *Biophysical Journal* 86(1): 473a. ISSN: 0006 3495.
NAL Call Number: 442.8 B5238
Abstract: To exert their important functions, expression and propagation of signalling proteins have to be tightly regulated in space and time. A variety of their key physical parameters like concentration and mobility are conveniently accessible by Fluorescence Correlation Spectroscopy (FCS) up to the single molecule level. Being non-invasive and to be used under equilibrium conditions over a wide concentration range, FCS can even be applied in vivo. To illustrate its potential for a quantitative approach towards developmental biology, we present an analysis of Fgf8 protein propagation through developing tissue of a living zebrafish embryo. In vertebrate embryogenesis, Fgf8 acts as a key signalling molecule in various developmental processes, such as maintenance of mesoderm, and induction, patterning and differentiation of different subdivison of the brain, inner ear and heart. In spite of its importance as a secreted signalling molecule, only little is known about the cell biological mechanisms controlling extracellular spreading and efficiency of Fgf8 in tissue. Recently it has been shown that Fgf8 has the ability to travel over a long distance and is internalised via receptor-mediated endocytosis. We address the question what the mechanism behind the propagation is. In the extracellular matrix, we investigated the concentration and mobility of Fgf8 tagged with the chromophore Cy3, and present further evidence that Fgf8 may operate by diffusion. Furthermore, intracellular measurements verify the internalisation into vesicular

structures, most likely early endosomes. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, molecular biophysics, development, fluorescence correlation spectroscopy, FCS, spectrum analysis techniques, embryogenesis .

Henry, C.A. and S.L. Amacher (2004). **Zebrafish slow muscle cell migration induces a wave of fast muscle morphogenesis.** *Developmental Cell* 7(6): 917-23. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: The specification and morphogenesis of slow and fast twitch muscle fibers are crucial for muscle development. In zebrafish, Hedgehog is required for slow muscle fiber specification. However, less is known about signals that promote development of fast muscle fibers, which constitute the majority of somitic cells. We show that when Hedgehog signaling is blocked, fast muscle cell elongation is disrupted. Using genetic mosaics, we show that Hedgehog signal perception is required by slow muscle cells but not by fast muscle cells for fast muscle cell elongation. Furthermore, we show that slow muscle cells are sufficient to pattern the medial to lateral wave of fast muscle fiber morphogenesis even when fast muscle cells cannot perceive the Hedgehog signal. Thus, the medial to lateral migration of slow muscle fibers through the somite creates a morphogenetic signal that patterns fast muscle fiber elongation in its wake.
Descriptors: zebrafish embryos, fast twitch muscle fibers cytology, muscle fibers, slow twitch cytology, cell movement, genotype, Hedgehog proteins, fluorescence microscopy, morphogenesis, muscle development, signal transduction, time factors, trans activators physiology.

Henry, C.A., I.M. McNulty, W.A. Durst, S.E. Munchel, and S.L. Amacher (2005). **Interactions between muscle fibers and segment boundaries in zebrafish.** *Developmental Biology* 287(2): 346-60. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The most obvious segmental structures in the vertebrate embryo are somites: transient structures that give rise to vertebrae and much of the musculature. In zebrafish, most somitic cells give rise to long muscle fibers that are anchored to intersegmental boundaries. Therefore, this boundary is analogous to the mammalian tendon in that it transduces muscle-generated force to the skeletal system. We have investigated interactions between somite boundaries and muscle fibers. We define three stages of segment boundary formation. The first stage is the formation of the initial epithelial somite boundary. The second "transition" stage involves both the elongation of initially round muscle precursor cells and somite boundary maturation. The third stage is myotome boundary formation, where the boundary becomes rich in extracellular matrix and all muscle precursor cells have elongated to form long muscle fibers. It is known that formation of the initial epithelial somite boundary requires Notch signaling; vertebrate Notch pathway mutants show severe defects in somitogenesis. However, many zebrafish Notch pathway mutants are homozygous viable suggesting that segmentation of their larval and adult body plans at least partially recovers. We show that epithelial somite boundary formation and slow-twitch muscle morphogenesis are initially disrupted in after eight (aei) mutant embryos (which lack function of the Notch ligand, DeltaD); however, myotome boundaries form later ("recover") in a Hedgehog-dependent fashion. Inhibition of Hedgehog-induced slow muscle induction in aei/deltaD and deadly seven (des)/notch1a mutant embryos suggests that slow muscle is necessary for myotome boundary recovery in the absence of initial epithelial somite boundary formation. Because we have previously demonstrated that slow muscle migration triggers fast muscle cell elongation in zebrafish, we hypothesize that migrating slow muscle facilitates myotome boundary formation in aei/deltaD mutant embryos by patterning coordinated fast muscle cell elongation. In

addition, we utilized genetic mosaic analysis to show that somite boundaries also function to limit the extent to which fast muscle cells can elongate. Combined, our results indicate that multiple interactions between somite boundaries and muscle fibers mediate zebrafish segmentation.

Descriptors: zebrafish embryos, muscle fibers physiology, somites physiology, body patterning physiology, cell shape, Hedgehog proteins, morphogenesis physiology, mutation, Notch receptors genetics, Notch receptors metabolism, signal transduction, trans activators genetics, trans activators metabolism.

Her, G.M., Y.H. Yeh, and J.L. Wu (2003). **435-bp liver regulatory sequence in the liver fatty acid binding protein (L-FABP) gene is sufficient to modulate liver regional expression in transgenic zebrafish.** *Developmental Dynamics* 227(3): 347-56. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: transgenic zebrafish, carrier carrier, gene expression regulation, liver metabolism, neoplasm proteins, nerve tissue proteins, proteins, base sequence, binding sites, DNA binding proteins genetics, proteins metabolism, fatty acid binding proteins, gene deletion, green fluorescent proteins, hepatocyte nuclear factor 1, hepatocyte nuclear factor 1 alpha, hepatocyte nuclear factor 1 beta, hepatocyte nuclear factor 3 beta, hepatocytes metabolism, luminescent proteins metabolism, mice, genetic models, molecular sequence data, nuclear proteins metabolism, promoter regions genetics, protein binding, rats, recombinant fusion proteins metabolism, nucleic acid sequence homology, transcription factors metabolism, genetic transcription, transgenes.

Her, G.M., Y.H. Yeh, and J.L. Wu (2004). **Functional conserved elements mediate intestinal-type fatty acid binding protein (I-FABP) expression in the gut epithelia of zebrafish larvae.** *Developmental Dynamics* 230(4): 734-42. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish, carrier, developmental gene expression regulation, larva metabolism, base sequence, binding sites, CCAAT enhancer binding proteins metabolism, carrier proteins chemistry, conserved sequence, DNA metabolism, enhancer elements genetics, fatty acid binding proteins, gene deletion, reporter genes, green fluorescent proteins metabolism, intestines embryology , molecular sequence data, promoter regions genetics, tertiary protein structure, nucleic acid sequence homology, transgenes.

Hernandez, L.P., S.E. Patterson, and S.H. Devoto (2005). **The development of muscle fiber type identity in zebrafish cranial muscles.** *Anatomy and Embryology* 209(4): 323-34. ISSN: 0340 2061.

NAL Call Number: QL951

Abstract: Cranial skeletal muscles underlie breathing, eating, and eye movements. In most animals, at least two types of muscle fibers underlie these critical functions: fast and slow muscle fibers. We describe here the anatomical distribution of slow and fast twitch muscle in the zebrafish (Danio rerio) head in the adult and at an early larval stage just after feeding has commenced. We found that all but one of the cranial muscles examined contain both slow and fast muscle fibers, but the relative proportion of slow muscle in each varies considerably. As in the trunk, slow muscle fibers are found only in an anatomically restricted zone of each muscle, usually on the periphery. The relative proportion of slow and fast muscle in each cranial muscle changes markedly with development, with a pronounced decrease in the proportion of slow muscle with ontogeny. We discuss our results in relation to the functional roles of each muscle in larval and adult life and compare findings among a variety of verte-

brates.
Descriptors: zebrafish embryos, skeletal muscle embryology, cell differentiation, fish larva, adults, fast twitch muscle fibers, muscle fibers, slow twitch.

Hernandez Lagunas, L., I.F. Choi, T. Kaji, P. Simpson, C. Hershey, Y. Zhou, L. Zon, M. Mercola, and K.B. Artinger (2005). **Zebrafish narrowminded disrupts the transcription factor prdm1 and is required for neural crest and sensory neuron specification.** *Developmental Biology* 278(2): 347-57. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Specification of both neural crest cells and Rohon-Beard (RB) sensory neurons involves a complex series of interactions between the neural and non-neural ectoderm. The molecular mechanisms directing this process are not well understood. The zebrafish narrowminded (nrd) mutation is unique, since it is one of two mutations in which defects are observed in both cell populations: it leads to a complete absence of RB neurons and a reduction in neural crest cells and their derivatives. Here, we show that nrd is a mutation in prdm1, a SET/zinc-finger domain transcription factor. A Morpholino-mediated depletion of prdm1 phenocopies the nrd mutation, and conversely overexpression of prdm1 mRNA rescues the nrd RB sensory neuron and neural crest phenotype. prdm1 is expressed at the border of the neural plate within the domain where neural crest cells and RB sensory neurons form. Analysis of prdm1 function by overexpression indicates that prdm1 functions to promote the cell fate specification of both neural crest cells and RB sensory neurons, most likely as a downstream effector of the BMP signaling pathway.
Descriptors: zebrafish, nerve tissue neural crest physiology, afferent neurons cytology, repressor transcription factors genetics, base sequence, basic helix loop helix transcription factors, chromosome mapping, DNA primers, DNA binding proteins genetics, proteins, physiology, in situ hybridization, nuclear proteins, restriction mapping, zinc fingers.

Hernandez, R.E., H.A. Rikhof, R. Bachmann, and C.B. Moens (2004). **Vhnf1 integrates global retinoic acid patterning and local FGF signals to direct posterior hindbrain development in zebrafish.** *Development* 131(18): 4511-20. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The vertebrate hindbrain is transiently divided along the anterior-posterior axis into seven morphologically and molecularly distinct segments, or rhombomeres, that correspond to Hox expression domains. The establishment of a proper 'hox code' is required for the development of unique rhombomere identities, including specification of neuronal fates. valentino (val), the zebrafish ortholog of mafB/Kreisler (Kr), encodes a bZip transcription factor that is required cell autonomously for the development of rhombomere (r) 5 and r6 and for activation of Hox group 3 gene expression. Recent work has demonstrated that the expression of val itself depends on three factors: retinoic acid (RA) signals from the paraxial mesoderm; fibroblast growth factor (Fgf) signals from r4; and variant hepatocyte nuclear factor 1 (vhnf1, also known as tcf2), a homeodomain transcription factor expressed posterior to the r4-5 boundary. We have investigated the interactions between these inputs onto val expression in the developing zebrafish hindbrain. We show that RA induces val expression via activation of vhnf1 expression in the hindbrain. Fgf signals from r4, acting through the MapK pathway, then cooperate with Vhnf1 to activate val expression and subsequent r5 and r6 development. Additionally, vhnf1 and val function as part of a multistep process required for the repression of r4 identity in the posterior hindbrain. vhnf1 acts largely independently of val to repress the r4 'hox code' posterior to the r4-5 boundary and therefore to block acquisition of r4-specific neuronal fates in the posterior hindbrain. However, vhnf1 is not

able to repress all aspects of r4 identity equivalently. val is required downstream of vhnf1 to repress r4-like cell-surface properties, as determined by an 'Eph-ephrin code', by repressing ephrin-B2a expression in r5 and r6. The different requirements for vhnf1 and val to repress hoxb1a and ephrin-B2a, respectively, demonstrate that not all aspects of an individual rhombomere's identity are regulated coordinately.

Descriptors: zebrafish embryos, DNA binding proteins genetics, proteins metabolism, Fgf metabolism, rhombencephalon embryology, rhombencephalon metabolism, transcription factors metabolism, tretinoin metabolism, body patterning, DNA binding proteins genetics, cytology, embryology, metabolism, ephrin B2 metabolism, fibroblast growth factor 3, fibroblast growth factor 8, Fgf deficiency, Fgf genetics, developmental gene expression regulation, hepatocyte nuclear factor 1 beta, homeodomain proteins metabolism, mafb transcription factor, mosaicism genetics, mutation genetics, nerve tissue nerve tissue proteins metabolism, rhombencephalon cytology , signal transduction, transcription factors genetics.

Herzog, W., C. Sonntag, S. Von der Hardt, H.H. Roehl, Z.M. Varga, and M. Hammerschmidt (2004). **Fgf3 signaling from the ventral diencephalon is required for early specification and subsequent survival of the zebrafish adenohypophysis.** *Development* 131(15): 3681-92. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: The pituitary gland consists of two major parts: the neurohypophysis, which is of neural origin; and the adenohypophysis, which is of non-neural ectodermal origin. Development of the adenohypophysis is governed by signaling proteins from the infundibulum, a ventral structure of the diencephalon that gives rise to the neurohypophysis. In mouse, the fibroblast growth factors Fgf8, Fgf10 and Fgf18 are thought to affect multiple processes of pituitary development: morphogenesis and patterning of the adenohypophyseal anlage; and survival, proliferation and differential specification of adenohypophyseal progenitor cells. Here, we investigate the role of Fgf3 during pituitary development in the zebrafish, analyzing lia/fgf3 null mutants. We show that Fgf3 signaling from the ventral diencephalon is required in a non-cell autonomous fashion to induce the expression of lim3, pit1 and other pituitary-specific genes in the underlying adenohypophyseal progenitor cells. Despite the absence of such early specification steps, fgf3 mutants continue to form a distinct pituitary anlage of normal size and shape, until adenohypophyseal cells die by apoptosis. We further show that Sonic Hedgehog (Shh) cannot rescue pituitary development, although it is able to induce adenohypophyseal cells in ectopic placodal regions of fgf3 mutants, indicating that Fgf3 does not act via Shh, and that Shh can act independently of Fgf3. In sum, our data suggest that Fgf3 signaling primarily promotes the transcriptional activation of genes regulating early specification steps of adenohypophyseal progenitor cells. This early specification seems to be essential for the subsequent survival of pituitary cells, but not for pituitary morphogenesis or pituitary cell proliferation.

Descriptors: zebrafish embryos, cell survival physiology, diencephalon embryology, Fgf metabolism, morphogenesis physiology, anterior pituitary gland embryology, signal transduction physiology, amino acid sequence, apoptosis, body patterning, cell lineage, diencephalon metabolism, embryonic structures abnormalities, anatomy and histology, fibroblast growth factor 10, fibroblast growth factor 3, Fgf genetics, developmental gene expression regulation, Hedgehog proteins, in situ hybridization, mice, molecular sequence data, anterior pituitary gland cytology, sequence alignment, trans activators genetics, trans activators metabolism.

Herzog, W., X. Zeng, Z. Lele, C. Sonntag, J.W. Ting, C.Y. Chang, and M. Hammerschmidt (2003). **Adenohypophysis formation in the zebrafish and its dependence on sonic Hedgehog.** *Developmental Biology* 254(1): 36-49. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Formation of the adenohypophysis in mammalian embryos occurs via an invagination of the oral ectoderm to form Rathke's pouch, which becomes exposed to opposing dorsoventral gradients of signaling proteins governing specification of the different hormone-producing pituitary cell types. One signal promoting pituitary cell proliferation and differentiation to ventral cell types is Sonic hedgehog (Shh) from the oral ectoderm. To study pituitary formation and patterning in zebrafish, we cloned four cDNAs encoding different pituitary hormones, prolactin (prl), proopiomelancortin (pomc), thyroid stimulating hormone (tsh), and growth hormone (gh), and analyzed their expression patterns relative to that of the pituitary marker lim3. prl and pomc start to be expressed at the lateral edges of the lim3 expression domain, before pituitary cells move into the head. This indicates that patterning of the pituitary anlage and terminal differentiation of pituitary cells starts while cells are still organized in a placodal fashion at the anterior edge of the developing brain. Following the expression pattern of prl and pomc during development, we show that no pituitary-specific invagination equivalent to Rathke's pouch formation takes place. Rather, pituitary cells move inwards together with stomodeal cells during oral cavity formation, with medial cells of the placode ending up posterior and lateral cells ending up anterior, resulting in an anterior-posterior, rather than a dorsoventral, patterning of the adenohypophysis. Carrying out loss- and gain-of-function experiments, we show that Shh from the ventral diencephalon plays a crucial role during induction, patterning, and growth of the zebrafish adenohypophysis. The phenotypes are very similar to those obtained upon pituitary-specific inactivation or overexpression of Shh in mouse embryo, suggesting that the role of Shh during pituitary development has been largely conserved between fish and mice, despite the different modes of pituitary formation in the two vertebrate classes.

Descriptors: zebrafish embryology, anterior pituitary gland embryology, trans activators physiology, amino acid and base sequence, molecular cloning , DNA primers, complementary DNA, Hedgehog proteins, molecular sequence data, morphogenesis, oligopeptides chemistry, oligopeptides genetics, pro opiomelanocortin chemistry, pro opiomelanocortin genetics, amino acid sequence homology, thyrotropin chemistry, thyrotropin genetics.

Hirata, H., L. Saint Amant, J. Waterbury, W. Cui, W. Zhou, Q. Li, D. Goldman, M. Granato, and J.Y. Kuwada (2004). **Accordion, a zebrafish behavioral mutant, has a muscle relaxation defect due to a mutation in the ATPase Ca2+ pump SERCA1.** *Development* 131(21): 5457-68. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: When wild-type zebrafish embryos are touched at 24 hours post-fertilization (hpf), they typically perform two rapid alternating coils of the tail. By contrast, accordion (acc) mutants fail to coil their tails normally but contract the bilateral trunk muscles simultaneously to shorten the trunk, resulting in a pronounced dorsal bend. Electrophysiological recordings from muscles showed that the output from the central nervous system is normal in mutants, suggesting a defect in muscles is responsible. In fact, relaxation in acc muscle is significantly slower than normal. In vivo imaging of muscle Ca2+ transients revealed that cytosolic Ca2+ decay was significantly slower in acc muscle. Thus, it appears that the mutant behavior is caused by a muscle relaxation defect due to the impairment of Ca2+ re-uptake. Indeed, acc mutants carry a mutation in atp2a1 gene that encodes the sarco(endo)plasmic reticulum Ca2+-ATPase 1 (SERCA1), a Ca2+ pump found in the muscle sarcoplasmic

reticulum (SR) that is responsible for pumping Ca2+ from the cytosol back to the SR. As SERCA1 mutations in humans lead to Brody disease, an exercise-induced muscle relaxation disorder, zebrafish accordion mutants could be a useful animal model for this condition.
Descriptors: zebrafish embryos, behavior physiology, calcium transporting ATPases genetics and metabolism, muscles physiology, mutation genetics, amino acid sequence, calcium metabolism, calcium transporting ATPases chemistry, central nervous system metabolism, electrophysiology, physiology, developmental gene expression regulation, molecular sequence data, morphogenesis, muscle relaxation physiology, neuromuscular junction metabolism, messenger RNA genetics and metabolism, sarcoplasmic reticulum calcium transporting ATPases, sequence alignment, time factors.

Hirata, H., T. Watanabe, J. Hatakeyama, S.M. Sprague, L. Saint Amant, A. Nagashima, W.W. Cui, W. Zhou, and J.Y. Kuwada (2007). **Zebrafish relatively relaxed mutants have a ryanodine receptor defect, show slow swimming and provide a model of multi-minicore disease.** *Development* 134(15): 2771-81. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Wild-type zebrafish embryos swim away in response to tactile stimulation. By contrast, relatively relaxed mutants swim slowly due to weak contractions of trunk muscles. Electrophysiological recordings from muscle showed that output from the CNS was normal in mutants, suggesting a defect in the muscle. Calcium imaging revealed that Ca(2+) transients were reduced in mutant fast muscle. Immunostaining demonstrated that ryanodine and dihydropyridine receptors, which are responsible for Ca(2+) release following membrane depolarization, were severely reduced at transverse-tubule/sarcoplasmic reticulum junctions in mutant fast muscle. Thus, slow swimming is caused by weak muscle contractions due to impaired excitation-contraction coupling. Indeed, most of the ryanodine receptor 1b (ryr1b) mRNA in mutants carried a nonsense mutation that was generated by aberrant splicing due to a DNA insertion in an intron of the ryr1b gene, leading to a hypomorphic condition in relatively relaxed mutants. RYR1 mutations in humans lead to a congenital myopathy, multi-minicore disease (MmD), which is defined by amorphous cores in muscle. Electron micrographs showed minicore structures in mutant fast muscles. Furthermore, following the introduction of antisense morpholino oligonucleotides that restored the normal splicing of ryr1b, swimming was recovered in mutants. These findings suggest that zebrafish relatively relaxed mutants may be useful for understanding the development and physiology of MmD.
Descriptors: zebrafish embryos, disease models, muscular diseases genetics and pathology, ryanodine receptor calcium release channel genetics, swimming, genetically modified, base sequence, calcium signaling physiology, central nervous system physiology, molecular sequence data, muscle contraction genetics, fast twitch muscle fibers metabolism, fast twitch muscle fibers physiology, congenital muscular diseases , muscular diseases physiopathology, protein isoforms genetics, RNA splice sites genetics, ryanodine receptor calcium release channel metabolism.

Hirata, M., K. Nakamura, T. Kanemaru, Y. Shibata, and S. Kondo (2003). **Pigment cell organization in the hypodermis of zebrafish.** *Developmental Dynamics* 227(4): 497-503. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, chromatophores cytology, subcutaneous tissue anatomy and histology, chromatophores ultrastructure, histological techniques, electron microscopy, morphogenesis, subcutaneous tissue embryology.

Hirata, M., K.I. Nakamura, and S. Kondo (2005). **Pigment cell distributions in different tissues of the zebrafish, with special reference to the striped pigment pattern**. *Developmental Dynamics* 234(2): 293-300. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: The orderly pigment pattern of zebrafish (*Danio rerio*) is a good model system for studying how spatial patterns form in animals. Recent molecular genetic studies have shown that interactions between the pigment cells play major roles in pattern formation. In the present study, we performed comparative transmission electron microscopy of pigment cells, in order to clarify the structural interactions of pigment cells in tissues with and without a striped pattern. In patterned tissues, pigment cells were distributed as a one-cell-thick sheet. The layer order of the sheets is always kept strictly. In tissues without a striped pattern, the layer order was often disturbed or the cells were distributed in a scattered, double-sheeted, or an accumulated pile. Our observations suggest that the underlying mechanism that controls the vertical order of the pigment cells is related to that controlling the stripe pattern. Copyright © 2008 CABI

Descriptors: zebrafish, animal tissues, color, electron microscopy, genes, genetic effects, molecular genetics, pigments, *Danio rerio*, fishes.

Hirata, T., Y. Suda, K. Nakao, M. Narimatsu, T. Hirano, and M. Hibi (2004). **Zinc finger gene fez-like functions in the formation of subplate neurons and thalamocortical axons**. *Developmental Dynamics* 230(3): 546-556. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: fez-like (fezl) is a forebrain-expressed zinc finger gene required for the formation of the hypothalamic dopaminergic and serotonergic (monoaminergic) neurons in zebrafish. To reveal its function in mammals, we analyzed the expression of the mouse orthologue of fezl and generated fezl-deficient mice by homologous recombination. Mouse fezl was expressed specifically in the forebrain from embryonic day 8.5. At mid-gestation, fezi expression was detected in subdomains of the forebrain, including the dorsal telencephalon and ventral diencephalon. Unlike the zebrafish fezl mutant too few, the fezl-deficient mice displayed normal development of hypothalamic monoaminergic neurons, but showed abnormal "hyperactive" behavior. In fezl-/- mice, the thalamocortical axons (TCA) were reduced in number and aberrantly projected to the cortex. These mutants had a reduced number of subplate neurons, which are involved in guiding the TCA from the dorsal thalamus, although the subplate neurons were born normally. These results suggest that fezl is required for differentiation or survival of the subiplate neurons, and reduction of the subplate neurons in fezl-deficient mice leads to abnormal development of the TCA, providing a possible link between the transcriptional regulation of forebrain development and hyperactive behavior. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, development, molecular genetics, nervous system, hyperactive behavior, subplate neurons.

Ho, D.M., J. Chan, P. Bayliss, and M. Whitman (2006). **Inhibitor-resistant type I receptors reveal specific requirements for TGF-beta signaling in vivo**. *Developmental Biology* 295(2): 730-742. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Activin/nodal-like TGF-beta superfamily ligands signal through the type I receptors Alk4, Alk5, and Alk7, and are responsible for mediating a number of essential processes in development. SB-431542, a chemical inhibitor of activin/nodal signaling, acts by specifically interfering with type I receptors. Here, we use inhibitor-resistant mutant receptors

to examine the efficacy and specificity of SB-431542 in *Xenopus* and zebrafish embryos. Treatment with SB-431542 eliminates Smad2 phosphorylation in vivo and generates a phenotype very similar to those observed in genetic mutants in the nodal signaling pathway. Inhibitor-resistant Alk4 efficiently rescues Smad2 signaling, developmental phenotype, and marker gene expression after inhibitor treatment. This system was used to examine type I receptor specificity for several activin/nodal ligands. We find that Alk4 can efficiently rescue signaling by a wide range of ligands, while Alk7 can only weakly rescue signaling by the same ligands. In whole embryos, nodal signaling during gastrulation can be rescued with Alk4, but not Alk7, while Alk5 can only mediate signaling by ligands expressed later in development. The combination of the ALK inhibitor SB-431542 with inhibitor-resistant ALKs provides a powerful set of tools for examining nodal/activin signaling during embryogenesis. (c) 2006 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, phenotype development, embryogenesis, gastrulation, Nodal signaling, Tgf-beta signaling, *Xenopus*, zebrafish.

Ho, R.K. and I. Skromne (2006). **The role of caudal transcription factors during segmentation of the nervous system and paraxial mesoderm**. *Developmental Biology* 295(1): 332. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, development, molecular genetics, nervous system, segmentation, signaling cue.

Hogan, B.M., M.P. Hunter, A.C. Oates, M.O. Crowhurst, N.E. Hall, J.K. Heath, V.E. Prince, and G.J. Lieschke (2004). **Zebrafish gcm2 is required for gill filament budding from pharyngeal ectoderm.** *Developmental Biology* 276(2): 508-22. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The pharyngeal arches give rise to multiple organs critical for diverse processes, including the thymus, thyroid and parathyroids. Several molecular regulators of thymus and thyroid organogenesis are strikingly conserved between mammals and zebrafish. However, land animals have parathyroids whereas fish have gills. The murine transcription factor Glial cells missing 2 (Gcm2) is expressed specifically in the parathyroid primordium in the endodermal epithelium of the third pharyngeal pouch, and in both mice and humans is required for normal development of parathyroid glands. The molecular regulation of fish gill organogenesis remains to be described. We report the expression of gcm2 in the zebrafish pharyngeal epithelium and a requirement for Hox group 3 paralogs for gcm2 expression. Strikingly, zebrafish gcm2 is expressed in the ectodermal portion of the pharyngeal epithelium and is required for the development of the gill filament buds, precursors of fish-specific gill filaments. This study identifies yet another role for a GCM gene in embryonic development and indicates a role for gcm2 during the evolution of divergent pharyngeal morphologies.
Descriptors: zebrafish embryology, ectoderm physiology, gills embryology, neuropeptides metabolism, pharynx embryology, trans activators metabolism, proteins metabolism, amino acid and base sequence, DNA binding proteins genetics, proteins, epithelium metabolism, developmental gene expression regulation, homeodomain proteins genetics and metabolism, in situ hybridization , mice, molecular sequence data, morphogenesis , neuropeptides classification, neuropeptides genetics, antisense oligonucleotides genetics, antisense oligonucleotides metabolism, parathyroid glands embryology, phylogeny, sequence alignment, trans activators classification, trans activators genetics, transcription factors, proteins classification.

Holley, S.A. (2007). **The genetics and embryology of zebrafish metamerism.** *Developmental Dynamics* 236(6): 1422-49. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryo, embryology, metabolism, developmental gene expression regulation genetics, mesoderm metabolism, genetics, cell movement, cell polarity, mesoderm cytology, metabolism.

Hollway, G.E., R.J. Bryson Richardson, S. Berger , N.J. Cole, T.E. Hall, and P.D. Currie (2007). **Whole-somite rotation generates muscle progenitor cell compartments in the developing zebrafish embryo.** *Developmental Cell* 12(2): 207-19. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Somites are transient, mesodermally derived structures that give rise to a number of different cell types within the vertebrate embryo. To achieve this, somitic cells are partitioned into lineage-restricted domains, whose fates are determined by signals secreted from adjacent tissues. While the molecular nature of many of the inductive signals that trigger formation of different cell fates within the nascent somite has been identified, less is known about the processes that coordinate the formation of the subsomitic compartments from which these cells arise. Utilizing a combination of vital dye-staining and lineage-tracking techniques, we describe a previously uncharacterized, lineage-restricted compartment of the zebrafish somite that generates muscle progenitor cells for the growth of appendicular, hypaxial, and axial muscles during development. We also show that formation of this compartment occurs via whole-somite rotation, a process that requires the action of the Sdf family of secreted cytokines.
Descriptors: zebrafish, body patterning physiology, cell compartmentation, cytology, muscle cells cytology, somites physiology, stem cells cytology, cell lineage, embryology, developmental gene expression regulation, muscle cells metabolism , pax7 transcription factor metabolism, rotation, signal transduction, somites cytology, stem cells metabolism.

Hollway, G.E., J. Maule, P. Gautier, T.M. Evans, D.G. Keenan, C. Lohs, D. Fischer, C. Wicking, and P.D. Currie (2006). **Scube2 mediates Hedgehog signalling in the zebrafish embryo.** *Developmental Biology* 294(1): 104-18. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The Hedgehog family of secreted morphogens specifies the fate of a large number of different cell types within invertebrate and vertebrate embryos, including the muscle cell precursors of the embryonic myotome of zebrafish. Formation of Hedgehog-sensitive muscle fates is disrupted within homozygous zebrafish mutants of the "you"-type class, the majority of which disrupt components of the Hedgehog (HH) signal transduction pathway. We have undertaken a phenotypic and molecular characterisation of one of these mutants, you, which we show results from mutations within the zebrafish orthologue of the mammalian gene scube2. This gene encodes a member of the Scube family of proteins, which is characterised by several protein motifs including EGF and CUB domains. Epistatic and molecular analyses position Scube2 function upstream of Smoothened (Smoh), the signalling component of the HH receptor complex, suggesting that Scube2 may act during HH signal transduction prior to, or during, receipt of the HH signal at the plasma membrane. In support of this model we show that scube2 has homology to cubilin, which encodes an endocytic receptor involved in protein trafficking suggesting a possible mode of function for Scube2 during HH signal transduction.
Descriptors: zebrafish embryos, extracellular matrix, trans activators physiology, Hedgehog

proteins, mutation, protein transport, cell surface receptors, G protein Coupled receptors physiology, signal transduction.

Holmberg, A., T. Schwerte, R. Fritsche, B. Pelster, and S. Holmgren (2003). **Ontogeny of intestinal motility in correlation to neuronal development in zebrafish embryos and larvae.** *Journal of Fish Biology* 63(2): 318-331. ISSN: 0022 1112.
NAL Call Number: QL614.J68
Abstract: For the first time, spontaneous intestinal activity was demonstrated and quantified before the onset of exogenous feeding in zebrafish *Danio rerio* embryos and larvae in vivo, using digital motion analysis. At 3 days post fertilization (dpf), erratic and spontaneous contraction waves were observed in the gut. Later (4-7 dpf), more distinct contraction patterns were distinguished, and anterograde and retrograde contraction waves projecting anally and orally along the intestine, respectively, as well as local rectal contraction waves could be identified and quantified. The frequency of both anterograde intestinal and local rectal contractions increased significantly during the first days of development. There was a tendency towards shorter anterograde contraction waves in the first dpf stage investigated, but the velocity of the waves did not differ significantly between the different dpf stages. The presence of developing neurones in the gut of zebrafish was established using immunohistochemistry, staining for a suite of marker proteins (Hu C/D, HNK-1 and acetylated tubulin). Structural neurones were present in the developing gut from the first dpf stage investigated (2 dpf). In conclusion, during the period (3-7 dpf) when erratic contraction waves turn into a more organized pattern of motility there is also a pronounced development of the innervation, suggesting a correlation in time of the development of gut motility and its neuronal control.
Descriptors: zebrafish, *Danio rerio*, neurons , neuronal development, digestive system, intestinal motility, ontogeny in relation to neuronal development in embryos and larvae, embryo development, larval development.

Holmberg, A., T. Schwerte, B. Pelster, and S. Holmgren (2004). **Ontogeny of the gut motility control system in zebrafish *Danio rerio* embryos and larvae.** *Journal of Experimental Biology* 207(Pt 23): 4085-94. ISSN: 0022-0949.
NAL Call Number: 442.8B77
Abstract: Using digital motion analysis, the ontogeny of the cholinergic, tachykinin and pituitary adenylate cyclase-activating polypeptide (PACAP) control systems was studied in zebrafish *Danio rerio* larvae, in vivo. For the first time we show that the regular propagating anterograde waves that occur in the zebrafish larval gut before and around the onset [at 5-6 days post fertilization (d.p.f.)] of feeding are modulated by acetylcholine or atropine, PACAP and NKA (neurokinin A). At 3 d.p.f., when no spontaneous motility has developed, application of acetylcholine did not affect the gut. However, at 4 d.p.f., acetylcholine increased and atropine reduced the frequency of propagating anterograde waves. At 5 d.p.f., NKA increased and PACAP reduced the wave frequency. This suggests that both excitatory and inhibitory pathways develop at an early stage in the gut, independent of exogenous feeding. Immunohistochemistry established the presence of gut neurons expressing PACAP and NKA in the proximal part of the developing gut from the first stage investigated (2 d.p.f.) and before regular motility was observed. 1 d.p.f. (PACAP) or 2 d.p.f. (NKA) stages later the whole gut was innervated. This supports physiological results that gut motility is under neuronal control during the period when regular motility patterns develop.
Descriptors: zebrafish, gastrointestinal motility drug effects, smooth muscle drug effects, acetylcholine pharmacology, atropine pharmacology, gastrointestinal motility physiology,

immunohistochemistry, larva physiology, smooth muscle physiology, nerve growth factors pharmacology, neurokinin a pharmacology, neuropeptides pharmacology, neurotransmitter agents pharmacology, pituitary adenylate cyclase activating polypeptide, vasoactive intestinal peptide receptors, proteins.

Holmqvist, B., B. Ellingsen, J. Forsell, I. Zhdanova, and P. Alm (2004). **The early ontogeny of neuronal nitric oxide synthase systems in the zebrafish.** *Journal of Experimental Biology* 207(6): 923-935. ISSN: 0022 0949.
NAL Call Number: 442.8B77
Abstract: To examine a putative role for neuronal nitric oxide synthase (nNOS) in early vertebrate development we investigated nNOS mRNA expression and cGMP production during development of the zebrafish *Danio rerio*. The nNOS mRNA expression in the central nervous system (CNS) and periphery showed a distinct spatio-temporal pattern in developing zebrafish embryo and young larvae. nNOS mRNA expression was first detected at 19 h postfertilisation (h.p.f.), in a bilateral subpopulation of the embryonic ventrorostral cell cluster in the forebrain. The number of nNOS mRNA-expressing cells in the brain slowly increased, also appearing in the ventrocaudal cell cluster from about 26 h.p.f., and in the dorsorostral and hindbrain cell cluster and in the medulla at 30 h.p.f. A major increase in nNOS mRNA expression started at about 40 h.p.f., and by 55 h.p.f. the expression constituted cell populations in differentiated central nuclei and in association with the proliferation zones of the brain, and in the medulla and retina. In parts of the skin, nNOS mRNA expression started at 20 h.p.f. and ended at 55 h.p.f. Between 40 and 55 h.p.f., nNOS mRNA expression started in peripheral organs, forming distinct populations after hatching within or in the vicinity of the presumptive swim bladder, enteric ganglia, and along the alimentary tract and nephritic ducts. Expression of nNOS mRNA correlated with the neuronal differentiation pattern and with the timing and degree of cGMP production. These studies indicate spatio-temporal actions by NO during embryogenesis in the formation of the central and peripheral nervous system, with possible involvement in processes such as neurogenesis, organogenesis and early physiology.
Descriptors: zebrafish embryos, *Danio rerio*, enzymes, nitric oxide synthase systems, nervous system, development, early ontogeny of nitric oxide synthase systems, young larvae.

Holtzinger, A. and T. Evans (2005). **Novel functions for GATA4 revealed by the zebrafish model.** *Developmental Biology* 283(2): 684. ISSN: 0012 1606.
NAL Call Number: 442.8 D49
Descriptors: zebrafish, cardiovascular system, transport and circulation, development, hematopoiesis, zebrafish model , GATA4.

Holzschuh, J., A. Barrallo Gimeno, A.K. Ettl, K. Durr, E.W. Knapik, and W. Driever (2003). **Noradrenergic neurons in the zebrafish hindbrain are induced by retinoic acid and require tfap2a for expression of the neurotransmitter phenotype.** *Development* 130(23): 5741-54. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Tfap2a is a transcriptional activator expressed in many different cell types, including neurons, neural crest derivatives and epidermis. We show that mutations at the zebrafish locus previously called mont blanc (mob) or lockjaw (low) encode tfap2a. The mutant phenotype reveals that tfap2a is essential for the development of hindbrain noradrenergic (NA) neurons of the locus coeruleus, medulla and area postrema, as well as for sympathetic NA neurons, epibranchial placode derived visceral sensory ganglia, and craniofacial and trunk

crest derivatives. We focus our analysis on the role of tfap2a NA differentiation in the CNS. In the locus coeruleus, Phox2a and Tfap2a are co-expressed and are both required for NA development. By contrast, in the medulla Phox2a and Tfap2a are expressed in adjacent overlapping domains, but only tfap2a activity is required for NA differentiation, as NA neurons develop normally in soulless/phox2a mutant medulla. phox2a and tfap2a do not appear to affect each others expression. Our studies show that two distinct inductive mechanisms control NA development in the zebrafish hindbrain. For the posterior hindbrain, we identify retinoic acid as an important signal to induce NA differentiation in the medulla oblongata and area postrema, where it expands the tfap2a expression domain and thus acts upstream of tfap2a. By contrast, previous work revealed Fgf8 to be involved in specification of NA neurons in the locus coeruleus. Thus, although the inductive signals may be distinct, hindbrain NA neurons of the locus coeruleus and the posterior groups both require Tfap2a to establish their noradrenergic identity.

Descriptors: zebrafish embryos, adrenergic alpha agonists metabolism, DNA binding proteins genetics, proteins metabolism, embryonic induction , neurons physiology, norepinephrine metabolism, rhombencephalon embryology, transcription factors metabolism, tretinoin metabolism, cell differentiation physiology, molecular cloning, DNA binding proteins genetics, sympathetic ganglia abnormalities, sympathetic ganglia cytology, sympathetic ganglia embryology, homeodomain proteins metabolism, in situ hybridization, nerve tissue nerve tissue proteins metabolism , neurons chemistry, neurons cytology, phenotype, rhombencephalon cytology, serotonin metabolism, signal transduction physiology, tissue distribution, transcription factor ap 2, transcription factors genetics, zebrafish abnormalities, anatomy and histology, genetics, proteins.

Holzschuh, J., N. Wada, C. Wada, A. Schaffer, Y. Javidan, A. Tallafuss, L. Bally Cuif, and T.F. Schilling (2005). **Requirements for endoderm and BMP signaling in sensory neurogenesis in zebrafish.** *Development* 132(16): 3731-42. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Cranial sensory neurons largely derive from neurogenic placodes (epibranchial and dorsolateral), which are ectodermal thickenings that form the sensory ganglia associated with cranial nerves, but the molecular mechanisms of placodal development are unclear. Here, we show that the pharyngeal endoderm induces epibranchial neurogenesis in zebrafish, and that BMP signaling plays a crucial role in this process. Using a her5:egfp transgenic line to follow endodermal movements in living embryos, we show that contact between pharyngeal pouches and the surface ectoderm coincides with the onset of neurogenesis in epibranchial placodes. By genetic ablation and reintroduction of endoderm by cell transplantation, we show that these contacts promote neurogenesis. Using a genetic interference approach we further identify bmp2b and bmp5 as crucial components of the endodermal signals that induce epibranchial neurogenesis. Dorsolateral placodes (trigeminal, auditory, vestibular, lateral line) develop independently of the endoderm and BMP signaling, suggesting that these two sets of placodes are under separate genetic control. Our results show that the endoderm regulates the differentiation of cranial sensory ganglia, which coordinates the cranial nerves with the segments that they innervate.

Descriptors: zebrafish embryo, epibranchial neurogenesis, bone morphogenetic proteins metabolism, endoderm physiology, morphogenesis, afferent neurons physiology, signal transduction physiology, proteins metabolism, genetically modified, biological markers, body patterning, bone morphogenetic embryonic structures anatomy and histology and physiology, ganglia cytology, ganglia metabolism, in situ hybridization, afferent neurons cytology, antisense oligonucleotides genetics, antisense oligonucleotides metabolism.

Hong, E. and R. Brewster (2006). **N-cadherin is required for the polarized cell behaviors that drive neurulation in the zebrafish.** *Development* 133(19): 3895-905. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Through the direct analysis of cell behaviors, we address the mechanisms underlying anterior neural tube morphogenesis in the zebrafish and the role of the cell adhesion molecule N-cadherin (N-cad) in this process. We demonstrate that although the mode of neurulation differs at the morphological level between amphibians and teleosts, the underlying cellular mechanisms are conserved. Contrary to previous reports, the zebrafish neural plate is a multi-layered structure, composed of deep and superficial cells that converge medially while undergoing radial intercalation, to form a single cell-layered neural tube. Time-lapse recording of individual cell behaviors reveals that cells are polarized along the mediolateral axis and exhibit protrusive activity. In N-cad mutants, both convergence and intercalation are blocked. Moreover, although N-cad-depleted cells are not defective in their ability to form protrusions, they are unable to maintain them stably. Taken together, these studies uncover key cellular mechanisms underlying neural tube morphogenesis in teleosts, and reveal a role for cadherins in promoting the polarized cell behaviors that underlie cellular rearrangements and shape the vertebrate embryo.
Descriptors: zebrafish embryos, cadherins physiology, nervous system embryology, cadherins genetics, cell polarity genetics, epithelium metabolism, epithelium ultrastructure, morphogenesis genetics, mutation, nervous system cytology, neural tube defects genetics, neuroepithelial cells cytology, neuroepithelial cells physiology, genetics.

Hong, J.R., G.H. Lin, C.J. Lin, W.P. Wang, C.C. Lee , T.L. Lin, and J.L. Wu (2004). **Phosphatidylserine receptor is required for the engulfment of dead apoptotic cells and for normal embryonic development in zebrafish.** *Development* 131(21): 5417-27. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: During development, the role of the phosphatidylserine receptor (PSR) in the removal of apoptotic cells that have died is poorly understood. We have investigated this role of PSR in developing zebrafish. Programmed cell death began during the shield stage, with dead cells being engulfed by a neighboring cell that showed a normal-looking nucleus and the nuclear condensation multi-micronuclei of an apoptotic cell. The zebrafish PSR engulfing receptor was cloned (zfpsr), and its nucleotide sequence was compared with corresponding sequences in Drosophila melanogaster (76% identity), human (74%), mouse (72%) and Caenorhabditis elegans (60%). The PSR receptor contained a jmjC domain (residues 143-206) that is a member of the cupin metalloenzyme superfamily, but in this case serves an as yet unknown function(s). psr knockdown by a PSR morpholino oligonucleotide led to accumulation of a large number of dead apoptotic cells in whole early embryo. These cells interfered with embryonic cell migration. In addition, normal development of the somite, brain, heart and notochord was sequentially disrupted up to 24 hours post-fertilization. Development could be rescued in defective embryos by injecting psr mRNA. These results are consistent with a PSR-dependent system in zebrafish embryos that engulfs apoptotic cells mediated by PSR-phagocytes during development, with the system assuming an important role in the normal development of tissues such as the brain, heart, notochord and somite.
Descriptors: zebrafish embryos, apoptosis, cytology, metabolism, embryonic development, cell surface receptors metabolism, amino acid sequence, cell movement, molecular cloning, embryology, gene expression profiling, developmental gene expression regulation, electron microscopy, molecular sequence data, organogenesis, phenotype, tertiary protein structure, messenger RNA genetics and metabolism, cell surface receptors chemistry, cell surface receptors genetics, sequence alignment, time factors.

Hong, S.K., C.E. Haldin, P.E. Ulanch, N.D. Lawson, B.M. Weinstein, I.B. Dawid, and N. Hukriede (2005). **The zebrafish kohtalo/trap230 gene is required for the development of the brain, neural crest and pronephric kidney.** *Developmental Biology* 283(2): 611. ISSN: 0012 1606.
NAL Call Number: 442.8 D49
Descriptors: zebrafish, nervous system, neural coordination, molecular genetics, cloning, genetic techniques, morphogenesis, cell migration , kidney, brain.

Honjo, Y. and J.S. Eisen (2005). **Slow muscle regulates the pattern of trunk neural crest migration in zebrafish.** *Development* 132(20): 4461-70. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: In avians and mice, trunk neural crest migration is restricted to the anterior half of each somite. Sclerotome has been shown to play an essential role in this restriction; the potential role of other somite components in specifying neural crest migration is currently unclear. By contrast, in zebrafish trunk neural crest, migration on the medial pathway is restricted to the middle of the medial surface of each somite. Sclerotome comprises only a minor part of zebrafish somites, and the pattern of neural crest migration is established before crest cells contact sclerotome cells, suggesting other somite components regulate the pattern of zebrafish neural crest migration. Here, we use mutants to investigate which components regulate the pattern of zebrafish trunk neural crest migration on the medial pathway. The pattern of trunk neural crest migration is aberrant in spadetail mutants that have very reduced somitic mesoderm, in no tail mutants injected with spadetail morpholino antisense oligonucleotides that entirely lack somitic mesoderm and in somite segmentation mutants that have normal somite components but disrupted segment borders. Fast muscle cells appear dispensable for patterning trunk neural crest migration. However, migration is abnormal in Hedgehog signaling mutants that lack slow muscle cells, providing evidence that slow muscle cells regulate the pattern of trunk neural crest migration. Consistent with this idea, surgical removal of adaxial cells, which are slow muscle precursors, results in abnormal patterning of neural crest migration; normal patterning can be restored by replacing the ablated adaxial cells with ones transplanted from wild-type embryos.
Descriptors: zebrafish embryos, cell movement, muscles cytology, muscles embryology, neural crest cytology, neural crest embryology, body patterning, muscles metabolism, mutation genetics, neural crest metabolism, somites cytology, somites metabolism, T-box domain T-box domain proteins metabolism, time factors, genetics, metabolism.

Horg, E., M. Tsang, M. Halpern, and R. Brewster (2005). **The role of n-cadherin in zebrafish neurulation.** *Developmental Biology* 283(2): 689. ISSN: 0012 1606.
NAL Call Number: 442.8 D49
Descriptors: zebrafish, nervous system, neural coordination, development, molecular genetics, neurulation, n-cadherin.

Horikawa, K., S. Kondo, and H. Takeda (2004). **Cellular mechanism for coordinated oscillation of the segmentation clock.** *Developmental Biology* 271(2): 609-610. ISSN: 0012 1606.
NAL Call Number: 442.8 D49
Descriptors: zebrafish embryos, molecular genetics, development, cell to cell communication, mechanisms.

Hostetter, C.L., J.L. Sullivan Brown, and R.D. Burdine (2003). **Zebrafish pronephros: a model for understanding cystic kidney disease.** *Developmental Dynamics* 228(3): 514-22. ISSN: 1058 8388.

NAL Call Number: QL801.A4
Descriptors: zebrafish embryology, body patterning physiology, kidney embryology, cystic kidney diseases, kidney abnormalities, research models, morphogenesis.

Hou, H.H., M.Y. Kuo, Y.W. Luo, and B.E. Chang (2006). **Recapitulation of human betaB1-crystallin promoter activity in transgenic zebrafish.** *Developmental Dynamics* 235(2): 435-43. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: transgenic zebrafish, promoter regions genetics, and metabolism, beta crystallin B chain genetics, genetically modified, base sequence, binding sites, conserved sequence, molecular sequence data, phylogeny, proto oncogene proteins C maf genetics, sequence alignment and homology, nucleic acid, transcription.

Hove, J.R. (2004). **In vivo blood flow mapping during cardiogenesis**. *Journal of Histochemistry and Cytochemistry* 52(Suppl. 1): S7. ISSN: 0022 1554.
NAL Call Number: 381 J8222
Descriptors: zebrafish embryos, cardiovascular system transport and circulation, development, cardiac malformations, congenital disease, heart disease, cardiovascular disease, vascular disease, embryonic vascular defects, digital particle image velocimetry, fluorescent labeling, spectrum analysis techniques, in vivo blood flow mapping, blood flow characteristics, circulation patterns, dynamic flow, epigenetic factors, fluid forces, genetic factors, hemodynamics, intracardiac flow, velocity vector fields, wall shear stresses.

Hoyle, J., Y.P. Tang, E.L. Wiellette, F.C. Wardle, and H. Sive (2004). **Nlz gene family is required for hindbrain patterning in the zebrafish.** *Developmental Dynamics* 229(4): 835-46. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, DNA binding, repressor, rhombencephalon embryology, amino acid sequence, body patterning, DNA binding proteins genetics, proteins genetics and proteins metabolism, early growth response protein 2, metabolism, ephrin B2 genetics, ephrin B2 metabolism, gene expression, homeodomain proteins genetics and metabolism, in situ hybridization, mafb transcription factor, molecular sequence data, morphogenesis, nerve tissue nerve tissue proteins metabolism, pax2 transcription factor, phylogeny, repressor repressor proteins metabolism, rhombencephalon anatomy and histology, rhombencephalon metabolism, transcription factors genetics and metabolism, zebrafish proteins genetics and metabolism.

Hsiao, C.D., M. Ekker, and H.J. Tsai (2003). **Skin-specific expression of ictacalcin, a homolog of the S100 genes, during zebrafish embryogenesis.** *Developmental Dynamics* 228(4): 745-50. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, calcium binding proteins biosynthesis, metabolism, developmental gene expression regulation, skin metabolism, transcription factors biosynthesis, amino acid sequence, binding sites, complementary DNA metabolism, databases, molecular evolution, keratinocytes metabolism, genetic models, molecular sequence data, olfactory mucosa metabolism, pharynx metabolism, phylogeny, radiation hybrid mapping, s100 proteins genetics and metabolism, amino acid sequence homology, species specificity, time factors.

Hsiao, C.D. and H.J. Tsai (2003). **Transgenic zebrafish with fluorescent germ cell: a useful tool to visualize germ cell proliferation and juvenile hermaphroditism in vivo.** *Developmental Biology* 262(2): 313-23. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Juvenile zebrafish are hermaphroditic; undifferentiated gonads first develop into ovary-like tissues, which then either become ovaries and produce oocytes (female) or degenerate and develop into testes (male). In order to fully capture the dynamic processes of germ cells' proliferation and juvenile hermaphroditism in zebrafish, we established transgenic lines TG(beta-actin:EGFP), harboring an enhanced green fluorescent protein (EGFP) gene driven by a medaka beta-actin promoter. In TG(beta-actin:EGFP), proliferating germ cells and female gonads strongly expressed EGFP, but fluorescence was only dimly detected in male gonads. Based on the fluorescent (+) or nonfluorescent (-) appearance of germ cells seen in living animals, three distinct groups were evident among TG(beta-actin:EGFP). Transgenics in ++ group (44%) were females, had fluorescent germ cells as juveniles, and female gonads continuously fluoresced throughout sexual maturation. Transgenics in +- (23%) and -- (33%) groups were males. Fluorescent germ cells were transiently detected in +- transgenics from 14 to 34 days postfertilization (dpf), but were not detected in -- transgenics throughout their life span. Histological analyses showed that 26-dpf-old transgenics in ++, +-, and -- groups all developed ovary-like tissues: Germ cells in -- group juveniles arrested at the gonocyte stage and accumulated low quantities of EGFP, while those in ++ group juveniles highly proliferated into diplotene to perinucleolar stages and accumulated high quantities of EGFP. In +- group juveniles, degenerating oocytes, gonocytes, and spermatogonia were coexistent in transiently fluorescent gonads. Therefore, the fluorescent appearance of gonads in this study was synchronous with the differentiation of ovary-like tissues. Thus, TG(beta-actin:EGFP) can be used to visualize germ cells' proliferation and juvenile hermaphroditism in living zebrafish for the first time.

Descriptors: juvenile zebrafish, cell division physiology, germ cells physiology, genetically modified reporter genes, hermaphroditism diagnosis, sex determination analysis.

Hsiao, C.D., W.Y. Tsai, L.S. Horng, and H.J. Tsai (2003). **Molecular structure and developmental expression of three muscle-type troponin T genes in zebrafish.** *Developmental Dynamics* 227(2): 266-279. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: Troponin T (Tnnt), a troponin component, interacts with tropomyosin and is crucial to the regulation of striated muscle contraction. To gain insight into the molecular evolution and developmental regulation of Tnnt gene (Tnnt) in lower vertebrates, zebrafish Tnnt1 (slow Tnnt), Tnnt2 (cardiac Tnnt), and Tnnt3b (fast Tnnt isoform b) were characterized. The polypeptides of zebrafish Tnnt1, Tnnt2, and Tnnt3b were conserved in the central tropomyosin- and C-terminal troponin I-binding domains. However, the N-terminal hypervariable regions were highly extended and rich in glutamic acid in polypeptides of Tnnt1 and Tnnt2, but not Tnnt3b. The Tnnt2 and Tnnt3b contain introns, whereas Tnnt1 is intron-free. During development, large to small, alternatively spliced variants were detected in Tnnt2, but not in Tnnt1 or Tnnt3. Whole-mount in situ hybridization showed zebrafish Tnnt1 and Tnnt2 are activated during early somitogenesis (10 hr postfertilization, hpf) and cardiogenesis (14 hpf), respectively, but Tnnt3b is not activated until middle somitogenesis (18 hpf). Tnnt2 and Tnnt3b expression was cardiac- and fast-muscle specific, but Tnnt1 was expressed in both slow and fast muscles. We propose that three, distinct, muscle-type Tnnt evolved after the divergence of fish and deuterostome invertebrates. In zebrafish, the developmental regulation of Tnnt during somitogenesis and cardiogenesis is more restricted and

simpler than in tetrapods. These new findings may provide insight into the developmental regulation and molecular evolution of vertebrate Tnnt.

Descriptors: zebrafish, comprehensive zoology, proteins, troponin T, molecular evolution, implications from gene structures and developmental regulation, musculature , troponin T molecular evolution, development, developmental regulation of troponin T, evolutionary implications, molecular genetics, molecular evolution of troponin T, evolution.

Hsu, H.J., G. Lin, and B.C. Chung (2003). **Parallel early development of zebrafish interrenal glands and pronephros: differential control by Wt1 and ff1b.** *Development* 130(10): 2107-16. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Steroids are synthesized mainly from the adrenal cortex. Adrenal deficiencies are often associated with problems related to its development, which is not fully understood. To better understand adrenocortical development, we studied zebrafish because of the ease of embryo manipulation. The adrenocortical equivalent in zebrafish is called the interrenal, because it is embedded in the kidney. We find that interrenal development parallels that of the embryonic kidney (pronephros). Primordial interrenal cells first appear as bilateral intermediate mesoderm expressing ff1b in a region ventral to the third somite. These cells then migrate toward the axial midline and fuse together. The pronephric primordia are wt1-expressing cells located next to the interrenal. They also migrate to the axial midline and fuse to become glomeruli at later developmental stages. Our gene knockdown experiments indicate that wt1 is required for its initial restricted expression in pronephric primordia, pronephric cell migration and fusion. wt1 also appears to be involved in interrenal development and ff1b expression. Similarly, ff1b is required for interrenal differentiation and activation of the differentiated gene, cyp11a1. Our results show that the zebrafish interrenal and pronephros are situated close together and go through parallel developmental processes but are governed by different signaling events.

Descriptors: zebrafish, interrenal gland physiology, kidney embryology, Wt1 proteins metabolism, proteins metabolism, biological markers, cell movement physiology, cholesterol side chain cleavage enzyme genetics, cholesterol side chain cleavage enzyme metabolism, in situ hybridization, interrenal gland ultrastructure, kidney ultrastructure, antisense oligonucleotides metabolism, organogenesis physiology, phenotype, signal transduction physiology, genetic transcription, Wt1 zebrafish anatomy and histology and physiology.

Huang, C.C., N.D. Lawson, B.M. Weinstein, and S.L. Johnson (2003). **Reg6 is required for branching morphogenesis during blood vessel regeneration in zebrafish caudal fins.** *Developmental Biology* 264(1): 263-74. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Postnatal neovascularization is essential for wound healing, cancer progression, and many other physiological functions. However, its genetic mechanism is largely unknown. In this report, we study neovascularization in regenerating adult zebrafish fins using transgenic fish that express EGFP in blood vessel endothelial cells. We first describe the morphogenesis of regenerating vessels in wild-type animals and then the phenotypic analysis of a genetic mutation that disrupts blood vessel regeneration. In wild-type zebrafish caudal fins, amputated blood vessels heal their ends by 24 h postamputation (hpa) and then reconnect arteries and veins via anastomosis, to resume blood flow at wound sites by 48 hpa. The truncated vessels regenerate by first growing excess vessels to form unstructured plexuses, resembling the primary capillary plexuses formed during embryonic vasculogenesis. Interestingly, this mode of vessel growth switches by 8 days postamputation (dpa) to growth without

a plexus intermediate. During blood vessel regeneration, vessel remodeling begins during early plexus formation and continues until the original vasculature pattern is reestablished at approximately 35 dpa. Temperature-sensitive mutants for reg6 have profound defects in blood vessel regeneration. At the restrictive temperature, reg6 regenerating blood vessels first fail to make reconnections between severed arteries and veins, and then form enlarged vascular sinuses rather than branched vascular plexuses. Reciprocal temperature-shift experiments show that reg6 function is required throughout plexus formation, but not during later growth. Our results suggest that the reg6 mutation causes defects in branch formation and/or angiogenic sprouting.
Descriptors: zebrafish, blood vessels physiology, extremities blood supply, morphogenesis, regeneration, proteins metabolism, genetically modified, blood vessels anatomy and histology, anatomy and histology and physiology, vascular endothelium cytology, vascular endothelium metabolism, anatomic models, physiologic neovascularization.

Huang, C.J., T.S. Jou, Y.L. Ho, W.H. Lee, Y.T. Jeng , F.J. Hsieh, and H.J. Tsai (2005). **Conditional expression of a myocardium-specific transgene in zebrafish transgenic lines.** *Developmental Dynamics* 233(4): 1294-303. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: transgenic zebrafish, gene transfer techniques, myocardium metabolism, transgenes, genetically modified, cardiac myosins genetics, cell line, fibroblasts, reporter genes, plasmids, promoter regions genetics, tetracycline, trans activators genetics, metabolism.

Huang, C.J., C.T. Tu, C.D. Hsiao, F.J. Hsieh, and H.J. Tsai (2003). **Germ-line transmission of a myocardium-specific GFP transgene reveals critical regulatory elements in the cardiac myosin light chain 2 promoter of zebrafish.** *Developmental Dynamics* 228(1): 30-40. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, cardiac myosins genetics, luminescent proteins metabolism, myosin light chains genetics, promoter regions genetics, transgenes, adenoviridae genetics, genetically modified, base sequence, cardiac myosins chemistry, DNA chemistry, exons, developmental gene expression regulation, germ cells, green fluorescent proteins, introns, mutation, myocardium cytology, myocardium metabolism, myosin light chains chemistry, antisense oligonucleotides metabolism, organ specificity, recombinant fusion proteins metabolism, regulatory sequences, nucleic acid, terminal repeat sequences, time factors, genetics proteins chemistry and metabolism.

Huang, H., F.I. Lu, S. Jia, S. Meng, Y. Cao, Y. Wang, W. Ma, K. Yin, Z. Wen, J. Peng, C. Thisse, B. Thisse, and A. Meng (2007). **Amotl2 is essential for cell movements in zebrafish embryo and regulates c-Src translocation.** *Development* 134(5): 979-88. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Angiomotin (Amot), the founding member of the Motin family, is involved in angiogenesis by regulating endothelial cell motility, and is required for visceral endoderm movement in mice. However, little is known about biological functions of the other two members of the Motin family, Angiomotin-like1 (Amotl1) and Angiomotin-like2 (Amotl2). Here, we have identified zebrafish amotl2 as an Fgf-responsive gene. Zebrafish amotl2 is expressed maternally and in restricted cell types zygotically. Knockdown of amotl2 expression delays epiboly and impairs convergence and extension movement, and amotl2-deficient cells in mosaic embryos fail to migrate properly. This coincides with loss of membrane protrusions and disorder of F-actin. Amotl2 partially co-localizes with RhoB-or EEA1-positive

endosomes and the non-receptor tyrosine kinase c-Src. We further demonstrate that Amotl2 interacts preferentially with and facilitates outward translocation of the phosphorylated c-Src, which may in turn regulate the membrane architecture. These data provide the first evidence that amotl2 is essential for cell movements in vertebrate embryos.

Descriptors: zebrafish embryos, membrane, Src family kinases metabolism, actins physiology, amino acid sequence, body patterning, cell movement physiology, physiology, endosomes physiology, Fgf physiology, membrane microfilaments physiology, molecular sequence data, phosphorylation, protein transport, pseudopodia physiology, signal transduction, vesicular transport proteins metabolism, RhoB GTP binding protein metabolism.

Huang, H., B. Zhang, P.A. Hartenstein, J.N. Chen, and S. Lin (2005). **NXT2 is required for embryonic heart development in zebrafish.** *BioMed Central Developmental Biology* 5(1): 7. ISSN: 1471 213X.

NAL Call Number: QH491.B63

Abstract: BACKGROUND: NXT2 is a member of NXT family proteins that are generally involved in exporting nuclear RNA in eukaryotic cells. It is not known if NXT2 has any function in specific biological processes. RESULTS: A zebrafish mutant exhibiting specific heart defects during embryogenesis was generated by animal cloning-mediated retroviral insertions. Molecular analysis indicated that the mutant phenotype was caused by a disruption of NXT2. Whole-mount RNA in situ hybridization showed that NXT2 transcripts were clearly detectable in embryonic heart as well as other tissues. Further analysis revealed that expression level of one form of alternative splicing NXT2 mRNA transcripts was significantly reduced, resulting in deficient myocardial cell differentiation and the malformation of cardiac valve at the atrioventricular boundary. The defects could be reproduced by morpholino antisense oligo knockdown of NXT2. CONCLUSION: NXT2 has a critical role in maintaining morphogenetic integrity of embryonic heart in vertebrate species.

Descriptors: zebrafish , heart embryology, nuclear export signals physiology, active transport, cell nucleus, alternative splicing, cell differentiation genetics, cloning, organism, DNA transposable elements, edema, cardiac genetics, edema, cardiac pathology, heart physiology, congenital heart defects genetics, congenital heart defects pathology, heart valves pathology, myocardium chemistry, myocardium cytology, myocardium pathology, nuclear export signals genetics, phenotype, antisense, messenger RNA genetics and analysis, reverse transcriptase PCR, genetic transcription and physiology.

Huang, X. and J. SaintJeannet (2004). **Induction of the neural crest and the opportunities of life on the edge**. *Developmental Biology* 275(1): 1-11. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The neural crest is a multipotent population of migratory cells unique to the vertebrate embryo. Neural crest arises at the lateral edge of the neural plate and migrates throughout the embryo to give rise to a wide variety of cell types including peripheral and enteric neurons and glia, craniofacial cartilage and bone, smooth muscle, and pigment cells. Here we review recent studies that have addressed the role of several signaling pathways in the induction of the neural crest. Work in the mouse, chick, *Xenopus,* and zebrafish have shown that a complex network of genes is activated at the neural plate border in response to neural crest-inducing signals. We also summarize some of these findings and discuss how the differential activation of these genes may contribute to the establishment of neural crest diversity. Copyright 2004 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, cell biology, neural crest, molecular genetics, *Xenopus*, nervous system, cell migration, transcriptional regulation.

Hughes, I., B. Blasiole, D. Huss, M.E. Warchol, N.P. Rath, B. Hurle, E. Ignatova, J.D. Dickman, R. Thalmann, R. Levenson, and D.M. Ornitz (2004). **Otopetrin 1 is required for otolith formation in the zebrafish *Danio rerio*.** *Developmental Biology* 276(2): 391-402. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Orientation with respect to gravity is essential for the survival of complex organisms. The gravity receptor is one of the phylogenetically oldest sensory systems, and special adaptations that enhance sensitivity to gravity are highly conserved. The fish inner ear contains three large extracellular biomineral particles, otoliths, which have evolved to transduce the force of gravity into neuronal signals. Mammalian ears contain thousands of small particles called otoconia that serve a similar function. Loss or displacement of these structures can be lethal for fish and is responsible for benign paroxysmal positional vertigo (BPPV) in humans. The distinct morphologies of otoconial particles and otoliths suggest divergent developmental mechanisms. Mutations in a novel gene Otopetrin 1 (Otop1), encoding multi-transmembrane domain protein, result in nonsyndromic otoconial agenesis and a severe balance disorder in mice. Here we show that the zebrafish, *Danio rerio*, contains a highly conserved gene, otop1, that is essential for otolith formation. Morpholino-mediated knockdown of zebrafish Otop1 leads to otolith agenesis without affecting the sensory epithelium or other structures within the inner ear. Despite lack of otoliths in early development, otolith formation partially recovers in some fish after 2 days. However, the otoliths are malformed, misplaced, lack an organic matrix, and often consist of inorganic calcite crystals. These studies demonstrate that Otop1 has an essential and conserved role in the timing of formation and the size and shape of the developing otolith.

Descriptors: zebrafish embryology, membrane proteins metabolism, otolithic membrane embryology, proteins metabolism, calcium carbonate metabolism, developmental gene expression regulation, gravitation, in situ hybridization, membrane mice, scanning electron microscopy, morphogenesis, oligonucleotides genetics, oligonucleotides metabolism, otolithic membrane anatomy and histology and chemistry, otolithic membrane metabolism, saccule and utricle anatomy and histology, saccule and utricle embryology, saccule and utricle ultrastructure, x ray diffraction, genetics.

Hutchinson, S.A., S.E. Cheesman, L.A. Hale, J.Q. Boone, and J.S. Eisen (2007). **Nkx6 proteins specify one zebrafish primary motoneuron subtype by regulating late islet1 expression.** *Development* 134(9): 1671-7. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: The ability of animals to carry out their normal behavioral repertoires requires exquisitely precise matching between specific motoneuron subtypes and the muscles they innervate. However, the molecular mechanisms that regulate motoneuron subtype specification remain unclear. Here, we use individually identified zebrafish primary motoneurons to describe a novel role for Nkx6 and Islet1 proteins in the specification of vertebrate motoneuron subtypes. We show that zebrafish primary motoneurons express two related Nkx6 transcription factors. In the absence of both Nkx6 proteins, the CaP motoneuron subtype develops normally, whereas the MiP motoneuron subtype develops a more interneuron-like morphology. In the absence of Nkx6 function, MiPs exhibit normal early expression of islet1, which is required for motoneuron formation; however, they fail to maintain islet1 expression. Misexpression of islet1 RNA can compensate for loss of Nkx6 function, providing evidence

that Islet1 acts downstream of Nkx6. We suggest that Nkx6 proteins regulate MiP development at least in part by maintaining the islet1 expression that is required both to promote the MiP subtype and to suppress interneuron development.
Descriptors: zebrafish, homeodomain proteins metabolism, motor neurons cytology, transcription factors metabolism, interneurons cytology, interneurons metabolism, motor neurons metabolism, transcription factors genetics, zebrafish.

Hutchinson, S.A. and J.S. Eisen (2006). **Islet1 and Islet2 have equivalent abilities to promote motoneuron formation and to specify motoneuron subtype identity**. *Development* 133(11): 2137-2147. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The expression of LIM homeobox genes islet1 and islet2 is tightly regulated during development of zebrafish primary motoneurons. All primary motoneurons express islet1 around the time they exit the cell cycle. By the time primary motoneurons undergo axogenesis, specific subtypes express islet1, whereas other subtypes express islet2, suggesting that these two genes have different functions. Here, we show that Islet1 is required for formation of zebrafish primary motoneurons; in the absence of Islet1, primary motoneurons are missing and there is an apparent increase in some types of ventral interneurons. We also provide evidence that Islet2 can substitute for Islet1 during primary motoneuron formation. Surprisingly, our results demonstrate that despite the motoneuron subtype-specific expression patterns of Islet1 and Islet2, the differences between the Islet1 and Islet2 proteins are not important for specification of the different primary motoneuron subtypes. Thus, primary motoneuron subtypes are likely to be specified by factors that act in parallel to or upstream of islet1 and islet2. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, molecular genetics, motoneuron formation, nervous system, Islet 1.

Hutson, L.D., M.J. Jurynec, S.Y. Yeo, H. Okamoto, and C.B. Chien (2003). **Two divergent slit1 genes in zebrafish.** *Developmental Dynamics* 228(3): 358-69. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, developmental gene expression regulation genetics, nerve tissue variation genetics, amino acid sequence, conserved sequence, molecular sequence data, morphogenesis, protein isoforms genetics, sequence alignment and homology, amino acid, serine proteinase inhibitors chemistry, serine proteinase inhibitors genetics, proteins genetics.

Huxley Jones, J., T.K. Clarke, C. Beck, G. Toubaris, D.L. Robertson, and R.P. Boot Handford (2007). **The evolution of The vertebrate metzincins; insights from *Ciona intestinalis* and *Danio rerio*.** *BioMed Central Evolutionary Biology* 7: 63. ISSN: 1471 2148.
NAL Call Number: QH359.B63
Abstract: The metzincins are a large gene superfamily of proteases characterized by the presence of a zinc protease domain, and include the ADAM, ADAMTS, BMP1/TLL, meprin and MMP genes. Metzincins are involved in the proteolysis of a wide variety of proteins, including those of the extracellular matrix. The metzincin gene superfamily comprises eighty proteins in the human genome and ninety-three in the mouse. When and how the level of complexity apparent in the vertebrate metzincin gene superfamily arose has not been determined in detail. Here we present a comprehensive analysis of vertebrate metzincins using genes from both *Ciona intestinalis* and *Danio rerio* to provide new insights into the complex evolution of this gene superfamily. RESULTS: We have identified 19 metzincin genes in the ciona genome and 83 in the zebrafish genome. Phylogenetic analyses reveal that the

expansion of the metzincin gene superfamily in vertebrates has occurred predominantly by the simple duplication of pre-existing genes rather than by the appearance and subsequent expansion of new metzincin subtypes (the only example of which is the meprin gene family). Despite the number of zebrafish metzincin genes being relatively similar to that of tetrapods (e.g. man and mouse), the pattern of gene retention and loss within these lineages is markedly different. In addition, we have studied the evolution of the related TIMP gene family and identify a single ciona and four zebrafish TIMP genes. CONCLUSION: The complexity seen in the vertebrate metzincin gene families was mainly acquired during vertebrate evolution. The metzincin gene repertoire in protostomes and invertebrate deuterostomes has remained relatively stable. The expanded metzincin gene repertoire of extant tetrapods, such as man, has resulted largely from duplication events associated with early vertebrate evolution, prior to the sarcopterygian-actinopterygian split. The teleost repertoire of metzincin genes in part parallels that of tetrapods but has been significantly modified, perhaps as a consequence of a teleost-specific duplication event.
Descriptors: zebrafish, ADAM *Ciona intestinalis* genetics, molecular evolution, matrix metalloproteinases, metalloendopeptidases genetics, multigene family, phylogeny, species specificity, tissue inhibitor of metalloproteinases genetics.

Huysseune, A. (2006). **Formation of a successional dental lamina in the zebrafish (*Danio rerio*): support for a local control of replacement tooth initiation.** *International Journal of Developmental Biology, The* 50(7): 637-43. ISSN: 0214-6282.
Abstract: In order to test whether the formation of a replacement tooth bud in a continuously replacing dentition is linked to the functional state of the tooth predecessor, I examined the timing of development of replacement teeth with respect to their functional predecessors in the pharyngeal dentition of the zebrafish. Observations based on serial semithin sections of ten specimens, ranging in age from four week old juveniles to adults, indicate that (i) a replacement tooth germ develops at the distal end of an epithelial structure, called the successional dental lamina, budding off from the crypt epithelium surrounding the erupted part of a functional tooth; (ii) there appears to be a developmental link between the eruption of a tooth and the formation of a successional dental lamina and (iii) there can be a time difference between successional lamina formation and initiation of the new tooth germ, i.e., the successional dental lamina can remain quiescent for some time. The data suggest that the formation of a successional lamina and the differentiation of a replacement tooth germ from this lamina, are two distinct phases of a process and possibly under a different control. The strong spatio-temporal coincidence of eruption of a tooth and development of a successional dental lamina is seen as evidence for a local control over tooth replacement.
Descriptors: zebrafish juveniles, adults, odontogenesis, stem cells cytology, tooth growth and development, pharynx, stem cells physiology, time factors, tooth ultrastructure, tooth eruption, tooth germ growth and development, tooth germ ultrastructure, physiology.

Huysseune, A. and J.Y. Sire (2004). **The role of epithelial remodelling in tooth eruption in larval zebrafish.** *Cell and Tissue Research* 315(1): 85-95. ISSN: 0302 766X.
NAL Call Number: QH581.A1Z4
Abstract: Based on light and transmission electron-microscopic observations on erupting first-generation teeth in the zebrafish, *Danio rerio*, we propose a biphasic mechanism for tooth eruption: (1). formation of an epithelial crypt prior to eruption of the tooth, possibly as a result of constraints in the epithelium resulting from the growth of adjacent tooth germs, and (2). detachment of cellular interdigitations both within the pharyngeal epithelium, at the pharyngeal epithelium/enamel organ boundary, and between the outer and inner dental

epithelium, resulting in the exposure of the tooth tip in the crypt, immediately after tooth ankylosis. Later, further detachment of interdigitations between the inner and the outer enamel epithelium unfolds the epithelium even more and leads to a more pronounced exposure of the tooth tip. The presence of small patches of non-collagenous matrix on the outer surface of the tooth close to where it merges with the attachment bone is interpreted as a device to prevent complete detachment of the enamel organ. The biphasic nature of the mechanism for tooth eruption is supported by observations on in vitro cultured heads. First-generation teeth develop normally and crypts are formed, as under in vivo conditions, but the teeth fail to erupt. Taken together, our observations suggest that epithelial remodelling plays a crucial role in eruption of the teeth in this model organism.
Descriptors: zebrafish , branchial region growth and development, branchial region ultrastructure, epithelium growth and development, tooth eruption.

Huysseune, A.N. and I. Thesleff (2004). **Continuous tooth replacement: the possible involvement of epithelial stem cells**. *Bioessays* 26(6): 665-671. ISSN: 0265-9247.
NAL Call Number: QH506.B365
Descriptors: zebrafish, epithelium, freshwater fish, niches, regeneration, stem cells, teeth, *Danio rerio*.

Iovine, M.K., E.P. Higgins, A. Hindes, B. Coblitz, and S.L. Johnson (2005). **Mutations in connexin43 (GJA1) perturb bone growth in zebrafish fins.** *Developmental Biology* 278(1): 208-19. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Mechanisms that regulate the size and shape of bony structures are largely unknown. The molecular identification of the fin length mutant short fin (sof), which causes defects in the length of bony fin ray segments, may provide insights regarding the regulation of bone growth. In this report, we demonstrate that the sof phenotype is caused by mutations in the connexin43 (cx43) gene. This conclusion is supported by genetic mapping, reduced expression of cx43 in the original sof allele (sofb123), identification of missense mutations in three ENU-induced alleles, and by demonstration of partially abrogated cx43 function in sofb123 embryos. Expression of cx43 was identified in cells flanking the germinal region of newly growing segments as well as in the osteoblasts at segment boundaries. This pattern of cx43 expression in cells lateral to new segment growth is consistent with a model where cx43-expressing cells represent a biological ruler that measures segment size. This report identifies the first gene identification for a fin length mutation (sof) as well as the first connexin mutations in zebrafish, and therefore reveals a critical role for local cell-cell communication in the regulation of bone size and growth.
Descriptors: zebrafish, bone development genetics, connexin 43 genetics, mutation, growth and development, alleles, base sequence, bone regeneration genetics, chromosome mapping, connexin 43 physiology, DNA genetics, developmental gene expression regulation, phenotype.

Islam, M.E., H. Kikuta, F. Inoue, M. Kanai, A. Kawakami, M.S. Parvin, H. Takeda, and K. Yamasu (2006). **Three enhancer regions regulate gbx2 gene expression in the isthmic region during zebrafish development.** *Mechanisms of Development* 123(12): 907-24. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: In vertebrate embryos, positioning of the boundary between the midbrain and hindbrain (MHB) and subsequent isthmus formation are dependent upon the interaction

between the Otx2 and Gbx genes. In zebrafish, sequential expression of gbx1 and gbx2 in the anterior hindbrain contributes to this process, whereas in mouse embryos, a single Gbx gene (Gbx2) is responsible for MHB development. In the present study, to investigate the regulatory mechanism of gbx2 in the MHB/isthmic region of zebrafish embryos, we cloned the gene and showed that its organization is conserved among different vertebrates. Promoter analyses revealed three enhancers that direct reporter gene expression after the end of epiboly in the anterior-most hindbrain, which is a feature of the zebrafish gbx2 gene. One of the enhancers is located upstream of gbx2 (AMH1), while the other two enhancers are located downstream of gbx2 (AMH2 and AMH3). Detailed analysis of the AMH1 enhancer showed that it directs expression in the rhombomere 1 (r1) region and the dorsal thalamus, as has been shown for gbx2, whereas no expression was induced by the AMH1 enhancer in other embryonic regions in which gbx2 is expressed. The AMH1 enhancer is composed of multiple regulatory subregions that share the same spatial specificity. The most active of the regulatory subregions is a 291-bp region that contains at least two Pax2-binding sites, both of which are necessary for the function of the main component (PB1-A region) of the AMH1 enhancer. In accordance with these results, enhancer activity in the PB1-A region, as well as gbx2 expression in r1, was missing in no isthmus mutant embryos that lacked functional pax2a. In addition, we identified an upstream conserved sequence of 227bp that suppresses the enhancer activity of AMH1. Taken together, these findings suggest that gbx2 expression during the somitogenesis stage in zebrafish is regulated by a complex mechanism involving Pax2 as well as activators and suppressors in the regions flanking the gene.

Descriptors: zebrafish, enhancer elements genetics physiology, developmental gene expression regulation, homeodomain pax2 transcription factor metabolism, base pairing, binding sites, enhancer elements genetics, reporter genes, genome, insect, green fluorescent proteins analysis, green fluorescent mesencephalon metabolism, molecular sequence data, pax2 transcription factor genetics, rhombencephalon metabolism, transcription factors metabolism, genetic transcription.

Itoh, N. and M. Konishi (2007). **The zebrafish FGF family.** *Zebrafish* 4(3): 179-86. ISSN: 1545-8547.

Abstract: Fibroblast growth factors (FGFs) are secreted polypeptide growth factors. The FGF signaling system plays crucial roles in multiple developmental processes in vertebrates. The human FGF family comprises 22 members. Although 16 zebrafish fgfs have been reported, the zebrafish fgf family has not been well elucidated. We have identified 11 additional zebrafish fgfs by conducting a homology-based search in the zebrafish genome and cDNA databases. The zebrafish fgf family now comprises at least 27 members. By conducting phylogenetic and gene location analyses, we examined relationships of zebrafish fgf genes with human FGF genes. All the zebrafish orthologs of human FGFs except for FGF9 have been identified. Zebrafish fgf9 might have been lost from the genome during evolution. In addition, six paralogs of zebrafish fgf genes have been identified. The phylogenetic analysis suggests that the zebrafish fgf gene family can be divided into seven subfamilies. The zebrafish fgf subfamilies are essentially consistent with the human FGF subfamilies, although some include potential paralogs. As the zebrafish system has proved useful for studying gene functions and genetic diseases, the present findings will be useful for elucidation of roles of FGFs in zebrafish and humans.

Descriptors: zebrafish, Fgf, polypeptide growth factors, developmental processes.

Jackman, W.R., B.W. Draper, and D.W. Stock (2004). **Fgf signaling is required for zebrafish tooth development.** *Developmental Biology* 274(1): 139-57 . ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: We have investigated fibroblast growth factor (FGF) signaling during the development of the zebrafish pharyngeal dentition with the goal of uncovering novel roles for FGFs in tooth development as well as phylogenetic and topographic diversity in the tooth developmental pathway. We found that the tooth-related expression of several zebrafish genes is similar to that of their mouse orthologs, including both epithelial and mesenchymal markers. Additionally, significant differences in gene expression between zebrafish and mouse teeth are indicated by the apparent lack of fgf8 and pax9 expression in zebrafish tooth germs. FGF receptor inhibition with SU5402 at 32 h blocked dental epithelial morphogenesis and tooth mineralization. While the pharyngeal epithelium remained intact as judged by normal pitx2 expression, not only was the mesenchymal expression of lhx6 and lhx7 eliminated as expected from mouse studies, but the epithelial expression of dlx2a, dlx2b, fgf3, and fgf4 was as well. This latter result provides novel evidence that the dental epithelium is a target of FGF signaling. However, the failure of SU5402 to block localized expression of pitx2 suggests that the earliest steps of tooth initiation are FGF-independent. Investigations of specific FGF ligands with morpholino antisense oligonucleotides revealed only a mild tooth shape phenotype following fgf4 knockdown, while fgf8 inhibition revealed only a subtle down-regulation of dental dlx2b expression with no apparent effect on tooth morphology. Our results suggest redundant FGF signals target the dental epithelium and together are required for dental morphogenesis. Further work will be required to elucidate the nature of these signals, particularly with respect to their origins and whether they act through the mesenchyme.

Descriptors: zebrafish embryos, Fgf metabolism, developmental gene expression regulation physiology, signal transduction physiology, tooth embryology, base sequence, cluster analysis, complementary DNA genetics, epithelium metabolism, developmental gene expression regulation drug effects, histological techniques, homeodomain in situ hybridization, ligands, molecular sequence data, morphogenesis, nerve tissue antisense oligonucleotides, pyrroles pharmacology, fibroblast receptorsgrowth factor antagonists and inhibitors, reverse transcriptase PCR , DNA sequence analysis.

Jain, I., C. Stroka, J. Yan, W.M. Huang, and M.K. Iovine (2007). **Bone growth in zebrafish fins occurs via multiple pulses of cell proliferation.** *Developmental Dynamics* 236(9): 2668-74. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: Fin length in the zebrafish is achieved by the distal addition of bony segments of the correct length. Genetic and molecular data provided evidence that segment growth uses a single pulse of growth, followed by a period of stasis. Examination of cell proliferation during segment growth was predicted to expose a graphical model consistent with a single burst of cell division (e.g., constant, parabolic, or exponential decay) during the lengthening of the distal-most segment. Cell proliferation was detected either by labeling animals with bromodeoxyuridine (during S-phase) or monitoring histone3-phosphate (mitosis). Results from both methods revealed that the number of proliferating cells fluctuates in apparent pulses as a segment grows (i.e., during the growth phase). Thus, rather than segment size being the result of a single burst of proliferation, it appears that segment growth is the result of several pulses of cell division that occur approximately every 60 microns (average segment length approximately 250 microns). These results indicate that segment lengthening requires multiple pulses of cell proliferation.

Descriptors: zebrafish fins, bone and bones embryology, extremities embryology, devel-

opmental gene expression regulation, bone and bones physiology, bromodeoxyuridine pharmacology, cell differentiation , cell division, cell proliferation, histones pharmacology, confocal microscopy, mitosis, theoretical models, time factors.

Janicke, M., T.J. Carney, and M. Hammerschmidt (2007). **Foxi3 transcription factors and Notch signaling control the formation of skin ionocytes from epidermal precursors of the zebrafish embryo.** *Developmental Biology* 307(2): 258-71. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Ionocytes are specialized epithelial cell types involved in the maintenance of osmotic homeostasis. In amniotes, they are present in the renal system, while in water-living embryos of lower vertebrates additional ionocytes are found in the skin. Thus far, relatively little has been known about the mechanisms of ionocyte development. Here we demonstrate that skin ionocytes of zebrafish embryos derive from the same precursor cells as keratinocytes. Carrying out various combinations of gain- and loss-of-function studies, we show that the segregation of ionocytes from the epidermal epithelium is governed by an interplay between Notch signaling and two Forkhead-box transcription factors, Foxi3a and Foxi3b. The two foxi3 genes are expressed in ionocyte precursors and are required both for ionocyte-specific expression of the Notch ligand Jagged2a, and for ionocyte differentiation, characterized by the production of particular ATPases. Ionocytic Notch ligands, in turn, signal to neighboring cells, where activated Notch1 leads to a repression of foxi3 expression, allowing those cells to become keratinocytes. A model for ionocyte versus keratinocyte development will be presented, postulating additional thus far unidentified pro-ionocyte factors.
Descriptors: zebrafish embryology, forkhead transcription factors metabolism , Notch receptors metabolism, skin embryology, metabolism, genetically modified, base sequence, calcium binding calcium binding proteins metabolism, cell differentiation, cell proliferation, DNA primers genetics, antisense DNAgenetics, embryonic stem cells cytology, embryonic stem cells metabolism, epistasis, genetic, forkhead transcription factors genetics, in situ hybridization, keratinocytes cytology, keratinocytes metabolism, ligands, Notch receptors genetics, signal transduction, skin cytology, skin metabolism.

Jardine, D. and M.K. Litvak (2003). **Direct yolk sac volume manipulation of zebrafish embryos and the relationship between offspring size and yolk sac volume**. *Journal of Fish Biology* 63(2): 388-397. ISSN: 0022-1112.
NAL Call Number: QL614.J68
Descriptors: zebrafish, biological development, embryos, larvae, life history, marine fish, survival, volume, yolk, *Danio rerio*.

Jasuja, R., N. Voss, G. Ge, G.G. Hoffman, J. Lyman Gingerich, F. Pelegri, and D.S. Greenspan (2006). **Bmp1 and mini fin are functionally redundant in regulating formation of the zebrafish dorsoventral axis.** *Mechanisms of Development* 123(7): 548-58. ISSN: 0925 4773.
NAL Call Number: QH607.A1C4
Abstract: *Drosophila* metalloproteinase Tolloid (TLD) is responsible for cleaving the antagonist Short gastrulation (SOG), thereby regulating signaling by the bone morphogenetic protein (BMP) Decapentaplegic (DPP). In mice there are four TLD-related proteinases, two of which, BMP1 and mammalian Tolloid-like 1 (mTLL1), are responsible for cleaving the SOG orthologue Chordin, thereby regulating signaling by DPP orthologues BMP2 and 4. However, although TLD mutations markedly dorsalize *Drosophila* embryos, mice doubly homozygous null for BMP1 and mTLL1 genes are not dorsalized in early development. Only a single TLD-related proteinase has previously been reported for zebrafish, and mutation of

the zebrafish TLD gene (mini fin) results only in mild dorsalization, manifested by loss of the most ventral cell types of the tail. Here we identify and map the zebrafish BMP1 gene bmp1. Knockdown of BMP1 expression results in a mild tail phenotype. However, simultaneous knockdown of mini fin and bmp1 results in severe dorsalization resembling the Swirl (swr) and Snailhouse (snh) phenotypes; caused by defects in major zebrafish ventralizing genes bmp2b and bmp7, respectively. We conclude that bmp1 and mfn gene products functionally overlap and are together responsible for a key portion of the Chordin processing activity necessary to formation of the zebrafish dorsoventral axis.
Descriptors: zebrafish embryology, body patterning genetics, bone morphogenetic metalloendopeptidases genetics, metalloproteases genetics, genetics, amino acid sequence, bone morphogenetic, glycoproteins metabolism, intercellular signaling peptides and proteins metabolism, metalloendopeptidases physiology, metalloproteases physiology, mice, molecular sequence data.

Jaszai, J., F. Reifers, A. Picker, T. Langenberg, and M. Brand (2003). **Isthmus-to-midbrain transformation in the absence of midbrain-hindbrain organizer activity.** *Development* 130(26): 6611-6623. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Descriptors: zebrafish, nervous system, neural coordination, gene expression, somitogenesis, zebrafish .

Jazwinska, A., R. Badakov, and M.T. Keating (2007). **Activin-betaA signaling is required for zebrafish fin regeneration.** *Current Biology* 17(16): 1390-5. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Abstract: Vertebrate limb regeneration occurs in anamniotes such as newts, salamanders, and zebrafish. After appendage amputation, the resection site is covered by a wound epidermis capping the underlying mature tissues of the stump from which the blastema emerges. The blastema is a mass of progenitor cells that constitute an apical growth zone. During outgrowth formation, the proximal blastemal cells progressively leave the zone and undergo the differentiation that results in the replacement of the amputated structures. Little is known about the mechanisms triggering regenerative events after injury. The zebrafish caudal fin provides a valuable model to study the mechanisms of regeneration. Zebrafish blastemal cells express specific genes, such as the homeobox-containing transcription factors msxB and msxC, and secreted signal FGF20a. In this study, we set out to identify signals that are transcriptionally upregulated after fin amputation and before blastema formation. Accordingly, a gene encoding a TGFbeta-related ligand, activin-betaA (actbetaA), was found to be strongly induced within 6 hr after fin amputation at the wound margin, and later in the blastema. Inhibition of Activin signaling through two specific chemical inhibitors, SB431542 and SB505124, lead to the early and complete block of regeneration. The morpholino knockdown of actbetaA and its receptor alk4 impaired the progression of regeneration. Closer examination of the phenotype revealed that Activin signaling is necessary for cell migration during wound healing and blastemal proliferation. These findings reveal a role of Activin-betaA signaling in the tissue repair after injury and subsequent outgrowth formation during epigenetic regeneration of the vertebrate appendage.
Descriptors: zebrafish, activins metabolism, inhibins metabolism, regeneration, signal transduction, metabolism, activins genetics, inhibins genetics, genetic transcription, up regulation.

Jeong, J.Y., Z. Einhorn, P. Mathur, L. Chen, S. Lee, K. Kawakami, and S. Guo (2007). **Patterning the zebrafish diencephalon by the conserved zinc-finger protein Fezl.** *Development* 134(1): 127-36. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: The forebrain constitutes the most anterior part of the central nervous system, and is functionally crucial and structurally conserved in all vertebrates. It includes the dorsally positioned telencephalon and eyes, the ventrally positioned hypothalamus, and the more caudally located diencephalon [from rostral to caudal: the prethalamus, the zona limitans intrathalamica (ZLI), the thalamus and the pretectum]. Although antagonizing Wnt proteins are known to establish the identity of the telencephalon and eyes, it is unclear how various subdivisions are established within the diencephalon--a complex integration center and relay station of the vertebrate brain. The conserved forebrain-specific zinc-finger-containing protein Fezl plays a crucial role in regulating neuronal differentiation in the vertebrate forebrain. Here, we report a new and essential role of zebrafish Fezl in establishing regional subdivisions within the diencephalon. First, reduced activity of fezl results in a deficit of the prethalamus and a corresponding expansion of the ZLI. Second, Gal4-UAS-mediated fezl overexpression in late gastrula is capable of expanding the prethalamus telencephalon and hypothalamus at the expense of the ZLI and other fore- and/or mid-brain regions. Such altered brain regionalization is preceded by the early downregulation of wnt expression in the prospective diencephalon. Finally, fezl overexpression is able to restore the anterior forebrain and downregulate wnt expression in Headless- and/or Tcf3 (also known as Tcf7l1a)-deficient embryos. Our findings reveal that Fezl is crucial for establishing regional subdivisions within the diencephalon and may also play a role in the development of the telencephalon and hypothalamus.

Descriptors: zebrafish embryos, body patterning genetics, carrier proteins metabolism, diencephalon embryology, proteins metabolism, zinc fingers genetics, genetically modified, diencephalon anatomy and histology and metabolism developmental gene expression regulation, antisense oligonucleotides pharmacology, thalamus embryology, transgenes.

Jin, H., J. Xu, F. Qian, L. Du, C.Y. Tan, Z. Lin, J. Peng, and Z. Wen (2006). **The 5' zebrafish SCL promoter targets transcription to the brain, spinal cord, and hematopoietic and endothelial progenitors.** *Developmental Dynamics* 235(1): 60-7. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryos, basic helix loop helix transcription factors genetics, brain embryology , endothelium embryology, hematopoietic stem cells metabolism, promoter regions genetics, proto oncogene spinal cord embryology, genetics, genetically modified, basic helix loop helix transcription factors biosynthesis, brain and endothelium metabolism, erythrocytes metabolism, reporter genes, mesoderm metabolism, proto oncogene proteins biosynthesis, spinal cord metabolism.

Jin, S.W., D. Beis, T. Mitchell, J.N. Chen, and D.Y. Stainier (2005). **Cellular and molecular analyses of vascular tube and lumen formation in zebrafish.** *Development* 132(23): 5199-209. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Tube and lumen formation are essential steps in forming a functional vasculature. Despite their significance, our understanding of these processes remains limited, especially at the cellular and molecular levels. In this study, we analyze mechanisms of angioblast coalescence in the zebrafish embryonic midline and subsequent vascular tube formation. To facilitate these studies, we generated a transgenic line where EGFP expression is controlled

by the zebrafish flk1 promoter. We find that angioblasts migrate as individual cells to form a vascular cord at the midline. This transient structure is stabilized by endothelial cell-cell junctions, and subsequently undergoes lumen formation to form a fully patent vessel. Down-regulating the VEGF signaling pathway, while affecting the number of angioblasts, does not appear to affect their migratory behavior. Our studies also indicate that the endoderm, a tissue previously implicated in vascular development, provides a substratum for endothelial cell migration and is involved in regulating the timing of this process, but that it is not essential for the direction of migration. In addition, the endothelial cells in endodermless embryos form properly lumenized vessels, contrary to what has been previously reported in Xenopus and avian embryos. These studies provide the tools and a cellular framework for the investigation of mutations affecting vasculogenesis in zebrafish.

Descriptors: zebrafish embryos, blood vessels cytology, blood vessels growth and development, genetically modified, blood vessels embryology, body patterning, cell movement endoderm physiology, endothelial cells cytology, green fluorescent intercellular junctions physiology, promoter regions genetics, stem cells cytology, vascular endothelial growth factor receptor 2 genetics.

Jin, S.-W., W. Herzog, M.M. Santoro, T.S. Mitchell, J. Frantsve, B. Jungblut, D. Beis, I.a.n.C. Scott, L.A. D'Amico, E.A. Ober, H. Verkade, H.A. Field, N.C. Chi, A.n.n.M. Wehman, H. Baier, and D.Y.R. Stainier (2007). **A transgene-assisted genetic screen identifies essential regulators of vascular development in vertebrate embryos**. *Developmental Biology* 307(1): 29-42. ISSN: 0012-1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish, alkaline phosphatase, angiogenesis, axon guidance, blood circulation, blood vessels, differentiation, drug development, embryogenesis, embryonic development, endothelial cells, ethyl nitrosourea, freshwater fish, genetic screening, genomes, mutagenesis, mutation, mutations, neurons, pattern formation, signal transduction, vascular endothelial growth factor, vascular system, *Danio rerio*.

Jing, X.H., S.M. Zhou, W.Q. Wang, and Y. Chen (2006). **Mechanisms underlying long- and short-range nodal signaling in zebrafish.** *Mechanisms of Development* 123(5): 388-94. ISSN: 0925-4773.

NAL Call Number: QH607.A1C4

Abstract: Precise regulation of the signaling range of secreted molecules is essential for proper pattern formation during development. The Nodal family of TGF-beta proteins has been shown to function as both short- and long-range signals. But the underlying mechanisms remain elusive. In this study, we investigated the regulation of the signaling range of zebrafish Nodal proteins Cyclops and Squint, which are short- and long-range signals, respectively. We show that (1) the stability of Cyclops and Squint correlates with the activity range but increasing the stability of the short-range Cyclops does not increase its signaling range; (2) structural differences in the N-terminus region of the mature peptides of Cyclops and Squint determine their differences in the signaling range and swapping the N-terminus region of the Squint mature ligand into that of Cyclops makes the latter function at a distance.

Descriptors: zebrafish, signal transduction, transforming growth factor beta metabolism, amino acid sequence, body patterning green fluorescent proteins genetics and metabolism, intracellular signaling peptides and proteins, molecular sequence data, tertiary protein structure, transforming growth factor beta genetics.

Jopling, C. and J. Hertog (2007). **Essential role for Csk upstream of Fyn and Yes in zebrafish gastrulation.** *Mechanisms of Development* 124(2): 129-36. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: Morphogenetic cell movements during gastrulation shape the vertebrate embryo bodyplan. Non-canonical Wnt signaling has been established to regulate convergence and extension cell movements that mediate anterior-posterior axis elongation. In recent years, many other factors have been implicated in the process by modulation of non-canonical Wnt signaling or by different, unknown mechanisms. We have found that the Src family kinases, Fyn and Yes, are required for normal convergence and extension cell movements in zebrafish embryonic development and they signal in parallel to non-canonical Wnts, eventually converging on a common downstream factor, RhoA. Here, we report that Csk, a negative regulator of Src family kinases has a role in gastrulation cell movements as well. Csk knock down induced a phenotype that was similar to the defects observed after knock down of Fyn and Yes, in that gastrulation cell movements were impaired, without affecting cell fate. The Csk knock down phenotype was rescued by simultaneous partial knock down of Fyn and Yes. We conclude that Csk acts upstream of Fyn and Yes to control vertebrate gastrulation cell movements.
Descriptors: zebrafish embryos, Csk, knock down, phenotype, Fyn, Yes, control, vertebrate, gastrulation, cell movements, Wnts .

Joseph, E.M. (2004). **Zebrafish IRX1b in the embryonic cardiac ventricle.** *Developmental Dynamics* 231(4): 720-6. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish larvae, heart embryology, heart physiology, homeodomain transcription factors genetics, developmental gene expression regulation, heart conduction system cytology, heart conduction system embryology and physiology, heart ventricles cytology, heart ventricles embryology, heart ventricles physiology, receptors, endothelin antagonists and inhibitors, sulfonamides pharmacology.

Ju, B., S.W. Chong, J. He, X. Wang, Y. Xu, H. Wan, Y. Tong, T. Yan, V. Korzh, and Z. Gong (2003). **Recapitulation of fast skeletal muscle development in zebrafish by transgenic expression of GFP under the mylz2 promoter.** *Developmental Dynamics* 227(1): 14-26. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryo, mutants, genetically modified embryology, luminescent proteins metabolism, skeletal muscle growth and development, promoter regions genetics, recombinant fusion proteins metabolism, anatomy and histology, genetically modified genetics , genetically modified growth and development, base sequence, cyclic AMP dependent protein kinases genetics, cyclic AMP dependent protein kinases metabolism, green fluorescent proteins, in situ hybridization, luminescent molecular sequence data, recombinant fusion proteins genetics.

Julich, D., R. Geisler, and S.A. Holley (2005). **Integrinalpha5 and delta/notch signaling have complementary spatiotemporal requirements during zebrafish somitogenesis.** *Developmental Cell* 8(4): 575-86. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Somitogenesis is the process by which the segmented precursors of the skeletal muscle and vertebral column are generated during vertebrate embryogenesis. While somitogenesis appears to be a serially homologous, reiterative process, we find that there are

differences between the genetic control of early/anterior and late/posterior somitogenesis. We demonstrate that point mutations can cause segmentation defects in either the anterior, middle, or posterior somites in the zebrafish. We find that mutations in zebrafish integrinalpha5 disrupt anterior somite formation, giving a phenotype complementary to the posterior defects seen in the notch pathway mutants after eight/deltaD and deadly seven/notch1a. Double mutants between the notch pathway and integrinalpha5 display somite defects along the entire body axis, with a complete loss of the mesenchymal-to-epithelial transition and Fibronectin matrix assembly in the posterior. Our data suggest that notch- and integrinalpha5-dependent cell polarization and Fibronectin matrix assembly occur concomitantly and interdependently during border morphogenesis.

Descriptors: zebrafish embryos, body patterning, integrin alpha5 metabolism, membrane proteins metabolism, signal transduction physiology, somites physiology, amino acid sequence, genetically modified, cell polarity, extracellular matrix chemistry, extracellular matrix metabolism, fibronectins metabolism, developmental gene expression regulation, in situ hybridization, integrin alpha5 genetics, membrane molecular sequence data, morphogenesis, phenotype, point mutation, Notch receptors, recombinant fusion proteins metabolism, somites cytology.

Julich, D., C. Hwee Lim, J. Round, C. Nicolaije, J. Schroeder, A. Davies, R. Geisler, J. Lewis, Y.J. Jiang, and S.A. Holley (2005). **Beamter/deltaC and the role of Notch ligands in the zebrafish somite segmentation, hindbrain neurogenesis and hypochord differentiation.** *Developmental Biology* 286(2): 391-404. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The Tubingen large-scale zebrafish genetic screen completed in 1996 identified a set of five genes required for orderly somite segmentation. Four of them have been molecularly identified and three were found to code for components of the Notch pathway, which are required for the coordinated oscillation of gene expression, known as the segmentation clock, in the presomitic mesoderm (PSM). Here, we show that the final member of the group, beamter (bea), codes for the Notch ligand DeltaC, and we present and characterize two new alleles, including one allele encoding for a protein truncated in the 7th EGF repeat and an allele deleting only the DSL domain which was previously shown to be necessary for ligand function. Interestingly however, when we over-express any of the mutant deltaC mRNAs, we observe antimorphic effects on both hindbrain neurogenesis and hypochord formation. Expression of bea/deltaC oscillates in the PSM, and a triple fluorescent in situ analysis of its oscillation in relation to that of other oscillating genes in the PSM reveals differences in subcellular localization of the oscillating mRNAs in individual cells in different oscillation phases. Mutations in aei/deltaD and bea/deltaC differ in the way they disrupt the oscillating expression of her1 and deltaC. Furthermore, we find that the double mutants have significantly stronger defects in hypochord formation but not in somitogenesis or hindbrain neurogenesis, indicating genetically that the two delta's may function either semi-redundantly or distinctly, depending upon context.

Descriptors: zebrafish embryos, membrane rhombencephalon embryology, somites , alleles, biological clocks physiology, developmental gene expression regulation, ligands, membrane, mutation, messenger RNA analysis, Notch receptors metabolism, rhombencephalon cytology, rhombencephalon growth and development.

Kaji, T. and K.B. Artinger (2004). **Dlx3b and dlx4b function in the development of Rohon-Beard sensory neurons and trigeminal placode in the zebrafish neurula.** *Developmental Biology* 276(2): 523-40. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Rohon-Beard sensory neurons, neural crest cells, and sensory placodes can be distinguished at the boundary of the embryonic epidermis (skin) and the neural plate. The inductive signals at the neural plate border region are likely to involve a gradient of bone morphogenic protein (BMP) in conjunction with FGF and Wnts and other signals. However, how these signals are transduced to produce the final cell fate remains to be determined. Recent evidence from Xenopus and chick suggest that Dlx genes are required for the generation of cell fates at the neural plate border (McLarren, K.W., Litsiou, A., Streit, A., 2003. DLX5 positions the neural crest and preplacode region at the border of the neural plate. Dev. Biol. 259, 34-47; Woda, J.M., Pastagia, J., Mercola, M., Artinger, K.B., 2003. Dlx proteins position the neural plate border and determine adjacent cell fates. Development 130, 331-342). In the present study, we extend these findings to zebrafish, where we unequivocally demonstrate that dlx3b and dlx4b function in a dose-dependent manner to specify cell fates such as Rohon-Beard sensory neurons and trigeminal sensory placodes. dlx function was examined by inhibiting: (1) protein levels with antisense morpholino oligonucleotides (MOs), and (2) activity by repressing the ability of dlx-homeodomain to bind to downstream targets (EnR-dlx3bhd mRNA; dlx3b homeodomain fused to Engrailed transcriptional repressor domain). Inhibition of dlx3b and dlx4b protein and activity resulted in the reduction or complete loss of Rohon-Beard (RB) sensory neurons and trigeminal (TG) sensory placodes. These data suggest that dlx3b and dlx4b function in the specification of RB neurons and trigeminal sensory placodes in zebrafish. Further, we have shown that dlx3b and dlx4b function in a non-cell-autonomous manner for RB neuron development; dlx3b and dlx4b act to regulate bmp2b expression at the non-neural ectodermal border. These data suggest that the contribution of dlx3b and dlx4b to neural plate border formation is partially non-cell-autonomous acting via BMP activity.

Descriptors: zebrafish, sensory ganglia embryology, homeodomain proteins metabolism, morphogenesis, afferent neurons physiology, transcription factors metabolism, proteins metabolism, basic helix loop helix transcription factors, biological markers, bone morphogenetic proteins metabolism, embryonic structures cytology, embryonic structures metabolism, sensory ganglia cytology, sensory ganglia metabolism, homeodomain microinjections, nerve tissue proteins metabolism, afferent neurons cytology , antisense oligonucleotides genetics, antisense oligonucleotides metabolism, phenotype, RNA splicing, messenger RNA metabolism , transcription factors genetics, transforming growth factor beta metabolism, metabolism.

Kalev Zylinska, M.L., J.A. Horsfield, M.V. Flores, J.H. Postlethwait, J.Y. Chau, P.M. Cattin, M.R. Vitas, P.S. Crosier, and K.E. Crosier (2003). **Runx3 is required for hematopoietic development in zebrafish.** *Developmental Dynamics* 228(3): 323-36. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: cloned zebrafish embryos, DNA binding proteins genetics, developmental gene expression regulation genetics, hematopoiesis physiology, transcription factors genetics, molecular cloning, core bindingfactor alpha 3 subunit, DNA binding proteins genetics, proteins metabolism, physiology, hematopoiesis genetics, molecular sequence data, neurons physiology, recombinant proteins metabolism, transcription factors metabolism, genetics and metabolism.

Kalinowski, R.R., C.H. Berlot, T.L.Z. Jones, L.F. Ross, L.A. Jaffe, and L.M. Mehlmann (2004). **Maintenance of meiotic prophase arrest in vertebrate oocytes by a G sub(s) protein-mediated pathway**. *Developmental Biology* 267(1): 1-13. ISSN: 0012-1606.

NAL Call Number: 442.8D49
Descriptors: zebrafish, amphibiotic species, antibodies, cell differentiation, embryology, freshwater fish, gene expression, meiosis, morphogenesis, ontogeny, oocytes, *Danio rerio*, *Xenopus*.

Kallivretaki, E., R.I. Eggen, S.C. Neuhauss, O. Kah, and H. Segner (2007). **The zebrafish, brain-specific, aromatase cyp19a2 is neither expressed nor distributed in a sexually dimorphic manner during sexual differentiation.** *Developmental Dynamics* 236(11): 3155-66. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, brain-specific, aromatase, cyp19a2, sexually dimorphism, sexual differentiation.

Kamei, M., S. Isogai, and B.M. Weinstein (2004). **Imaging blood vessels in the zebrafish.** *Methods in Cell Biology* 76: 51-74. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, blood vessels anatomy and histology, diagnostic imaging methods, acrylic resins chemistry, alkaline phosphatase analysis , genetically modified, blood vessels embryology, growth and development, coloring agents chemistry, embryo anatomy and histology, chemistry, fluorescein angiography methods, fluorescent dyes chemistry, developmental gene expression regulation, green fluorescent proteins analysis and genetics, immunohistochemistry, in situ hybridization, fluorescence microscopy, microspheres, tissue fixation methods, .

Kane, D.A., K.N. Mcfarland, and R.M. Warga (2005). **Mutations in half baked/e-cadherin block cell behaviors that are necessary for teleost epiboly**. *Development* 132(5): 1105-1116. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Epiboly, the spreading of the blastoderm over the large yolk cell, is the first morphogenetic movement of the teleost embryo. Examining this movement as a paradigm of vertebrate morphogenesis, we have focused on the epiboly arrest mutant half baked (hab), which segregates as a recessive lethal, including alleles expressing zygotic-maternal dominant (ZMD) effects. Here we show that hab is a mutation in the zebrafish homolog of the adhesion protein E-cadherin. Whereas exclusively recessive alleles of hab produce truncated proteins, dominant alleles all contain transversions in highly conserved amino acids of the extracellular domains, suggesting these alleles produce dominant-negative effects. Antisense oligonucleotides that create specific splicing defects in the hab mRNA phenocopy the recessive phenotypes and, surprisingly, some of the. p ZMD phenotypes as well. In situ analyses show that during late epiboly hab is expressed in a radial gradient in the non axial epiblast, from high concentrations in the exterior layer of the epiblast to low concentrations in the interior layer of the epiblast. During epiboly, using an asymmetric variant of radial intercalation, epiblast cells from the interior layer sequentially move into the exterior layer and become restricted to that layer; there they participate in subtle cell shape changes that further expand the blastoderm. In hab mutants, when cells intercalate into the exterior layer, they tend to neither change cell shape nor become restricted, and many of these cells 'de-intercalate' and move back into the interior layer. Cell transplantation showed all these defects to be cell-autonomous. Hence, as for the expansion of the mammalian trophoblast at a similar developmental stage, hab/E-cadherin is necessary for the cell rearrangements that spread the teleost blastoderm over the yolk. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, molecular genetics, development, zygotic maternal dominant effect, epiboly, e-cadherin.

Kang, J.S., T. Oohashi, Y. Kawakami, Y. Bekku, J.C. Izpisua Belmonte, and Y. Ninomiya (2004). **Characterization of dermacan, a novel zebrafish lectican gene, expressed in dermal bones.** *Mechanisms of Development* 121(3): 301-12. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: We report here the isolation and characterization of a cDNA encoding zebrafish dermacan, a novel member of hyaluronan (HA)-binding proteoglycans, which was termed after its characteristic expression in the zebrafish dermal bones. The deduced protein sequence shares the typical modular elements of lecticans. Sequence comparison covering the C-terminal globular domain demonstrated that dermacan shows high homology with zebrafish versican but is distinct from any other identified lecticans. Genomic DNA analysis demonstrated that dermacan and versican were encoded by distinct genes in the zebrafish genome. The expression of dermacan is initiated in the sclerotome and cephalic paraxial mesoderm at 16 h postfertilization. During the pharyngular period, dermacan transcripts were detected in the sclerotome, tail fin bud, pharyngular arch primordial region, and otic vesicle. In the development of craniofacial bones, dermacan expression was detected typically in the opercle and dentary. These regions belong to the craniofacial dermal bones. aggrecan expression, in contrast, was observed in the elements of craniofacial cartilage bones. In the dermacan-morpholino-injected embryos, dermal bones, e.g. opercle, dentary, and branchiostegal rays, as well as axial skeleton in the trunk, showed decreased ossification. We conclude that dermacan is a novel lectican gene, and that zebrafish lectican genes have genetically diverged. In addition, our data suggest the involvement of dermacan in zebrafish dermal bone development.
Descriptors: zebrafish embryology, bone development genetics, proteoglycans genetics, proteoglycans physiology, aggrecans, amino acid and base sequence, extracellular matrix proteins metabolism, facial bones embryology, facial bones growth and development, facial bones metabolism, gene expression, in situ hybridization, fluorescence, larva drug effects, C type lectins, molecular sequence data, phylogeny, proteochondroitin sulfates metabolism, proteoglycans metabolism, sequence alignment, versicans.

Kapsimali, M., L. Caneparo, C. Houart, and S.W. Wilson (2004). **Inhibition of Wnt/Axin/beta-catenin pathway activity promotes ventral CNS midline tissue to adopt hypothalamic rather than floorplate identity**. *Development* 131(23): 5923-5933. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Ventral midline cells in the neural tube form floorplate throughout most of the central nervous system (CNS) but in the anterior forebrain, they differentiate with hypothalamic identity. The signalling pathways responsible for subdivision of midline neural tissue into hypothalamic and floorplate domains are uncertain, and in this study, we have explored the role of the Wnt/Axin/beta-catenin pathway in this process. This pathway has been implicated in anteroposterior regionalisation of the dorsal neural tube but its role in patterning ventral midline tissue has not been rigorously assessed. We find that masterblind zebrafish embryos that carry a mutation in Axin1, an intracellular negative regulator of Wnt pathway activity, show an expansion of prospective floorplate coupled with a reduction of prospective hypothalamic tissue. Complementing this observation, transplantation of cells overexpressing axin1 into the prospective floorplate leads to induction of hypothalamic gene expression and suppression of floorplate marker gene expression. Axin1 is more efficient at inducing hypothalamic markers than several other Wnt pathway antagonists, and we present

data suggesting that this may be due to an ability to promote Nodal signalling in addition to suppressing Wnt activity. Indeed, extracellular Wnt antagonists can promote hypothalamic gene expression when co-expressed with a modified form of Madh2 that activates Nodal signalling. These results suggest that Nodal signalling promotes the ability of cells to incorporate into ventral midline tissue, and within this tissue, antagonism of Wnt signalling promotes the acquisition of hypothalamic identity. Wnt signalling also affects patterning within the hypothalamus, suggesting that this pathway is involved in both the initial anteroposterior subdivision of ventral CNS midline fates and in the subsequent regionalisation of the hypothalamus. We suggest that by regulating the response of midline cells to signals that induce ventral fates, Axin1 and other modulators of Wnt pathway activity provide a mechanism by which cells can integrate dorsoventral and anteroposterior patterning information. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, development, molecular genetics, nervous system, Wnt, Axin, beta catenin signaling pathway, central nervous system, neural tube form floorplate.

Karima, K., E. Murayama, and P. Herbomel (2007). **Le danio zebre revele l'odyssee des precurseurs hematopoietiques au cours du developpement des embryons de vertebres. [The zebrafish reveals the odyssey of hematopoietic precursors in developing vertebrate embryos]**. *Medecine Sciences* 23(8-9): 698-700. ISSN: 0767-0974.
Descriptors: zebrafish embryos, hematopoietic stem cells physiology, embryonic development, hematopoiesis, hematopoietic stem cells cytology, vertebrates, zebrafish growth, development and physiology.
Language of Text: French.

Karlstrom, R.O., O.V. Tyurina, A. Kawakami, N. Nishioka, W.S. Talbot, H. Sasaki, and A.F. Schier (2003). **Genetic analysis of zebrafish gli1 and gli2 reveals divergent requirements for gli genes in vertebrate development.** *Development* 130(8): 1549-64. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Gli proteins regulate the transcription of Hedgehog (Hh) target genes. Genetic studies in mouse have shown that Gli1 is not essential for embryogenesis, whereas Gli2 acts as an activator of Hh target genes. In contrast, misexpression studies in Xenopus and cultured cells have suggested that Gli1 can act as an activator of Hh-regulated genes, whereas Gli2 might function as a repressor of a subset of Hh targets. To clarify the roles of gli genes during vertebrate development, we have analyzed the requirements for gli1 and gli2 during zebrafish embryogenesis. We report that detour (dtr) mutations encode loss-of-function alleles of gli1. In contrast to mouse Gli1 mutants, dtr mutants and embryos injected with gli1 antisense morpholino oligonucleotides display defects in the activation of Hh target genes in the ventral neuroectoderm. Mutations in you-too (yot) encode C-terminally truncated Gli2. We find that these truncated proteins act as dominant repressors of Hh signaling, in part by blocking Gli1 function. In contrast, blocking Gli2 function by eliminating full-length Gli2 results in minor Hh signaling defects and uncovers a repressor function of Gli2 in the telencephalon. In addition, we find that Gli1 and Gli2 have activator functions during somite and neural development. These results reveal divergent requirements for Gli1 and Gli2 in mouse and zebrafish and indicate that zebrafish Gli1 is an activator of Hh-regulated genes, while zebrafish Gli2 has minor roles as a repressor or activator of Hh targets.
Descriptors: zebrafish embryos, developmental gene expression regulation, oncogene trans activators metabolism, transcription factors genetics, amino acid sequence, cell line, molecular cloning, Hedgehog proteins, in situ hybridization, mice, molecular sequence data, mutation, antisense oligonucleotides genetics, antisense oligonucleotides metabolism,

oncogene proteins Classification, oncogene proteins metabolism, phylogeny, rats, sequence alignment, signal transduction physiology, trans activators genetics, transcription factors classification and metabolism, veratrum alkaloids metabolism, proteins classification and metabolism.

Katsuyama, Y., Y. Oomiya, H. Dekimoto, E. Motooka, A. Takano, S. Kikkawa, M. Hibi, and T. Terashima (2007). **Expression of zebrafish ROR alpha gene in cerebellar-like structures.** *Developmental Dynamics* 236(9): 2694-701. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, developmental gene expression regulation, cytoplasmic receptors and nuclear biosynthesis, proteins biosynthesis, amino acid and base sequence, cerebellum embryology, molecular cloning, complementary DNA metabolism, exons, gene expression profiling, molecular sequence data, phylogeny, glutamate receptors metabolism, amino acid sequence homology.

Kawakami, A., T. Fukazawa, and H. Takeda (2004). **Early fin primordia of zebrafish larvae regenerate by a similar growth control mechanism with adult regeneration.** *Developmental Dynamics* 231(4): 693-9. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos and larvae, extremities embryology and physiology, regeneration physiology, age factors, cell division physiology, ectoderm physiology, embryology, epithelial cells physiology, Fgf physiology, signal transduction physiology.

Kawakami, K. (2005). **Transposon tools and methods in zebrafish** . *Developmental Dynamics* 234(2): 244-254. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Zebrafish is an excellent model animal to study vertebrate development by genetic approaches. Hundreds of mutations affecting various processes of development have been isolated by chemical mutagenesis and insertional mutagenesis using a pseudotyped retrovirus. However, useful transposon tools and methods had not been available in zebrafish. This is mainly because no active transposable element has been found from the zebrafish genome. Recently, efficient transgenesis, gene trap, and enhancer trap methods have been developed in zebrafish by using the Tol2 and the Sleeping Beauty transposon systems. These methods should increase the usefulness of zebrafish as a model vertebrate and facilitate the study of developmental biology, genetics, and genomics. Copyright © 2008 CABI
Descriptors: zebrafish embryos research, animal models, genes, genetic models, genomes, mutagenesis, mutations, transposable elements, *Danio rerio*, fishes.

Kawakami, K., H. Takeda, N. Kawakami, M. Kobayashi, N. Matsuda, and M. Mishina (2004). **A transposon-mediated gene trap approach identifies developmentally regulated genes in zebrafish.** *Developmental Cell* 7(1): 133-44. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: We report here development of a novel gene trap method in zebrafish using the Tol2 transposon system. First, we established a highly efficient transgenesis method in which a plasmid DNA containing the Tol2 transposon vector and the transposase mRNA synthesized in vitro were coinjected into one-cell stage embryos. The transposon vector inserted in the genome could be transmitted to the F1 progeny at high frequencies, and regulated gene expression by a specific promoter could be recapitulated in transgenic fish. Then we constructed a transposon-based gene trap vector containing a splice acceptor and the GFP gene,

performed a pilot screen for gene trapping, and obtained fish expressing GFP in temporally and spatially restricted patterns. We confirmed the endogenous transcripts were indeed trapped by the insertions, and the insertion could interfere with expression of the trapped gene. We propose our gene trap approach should facilitate studies of vertebrate development and organogenesis.

Descriptors: zebrafish embryos, DNA transposable elements genetics, developmental gene expression regulation genetics and metabolism, genetically modified, base sequence genetics, gene transfer techniques, genetic vectors genetics, genome, green fluorescent proteins, luminescent molecular sequence data, plasmids genetics, RNA splicing genetics, messenger RNA pharmacology, genetic transcription, trans transposases genetics.

Kawakami, Y., C.R. Esteban, T. Matsui, J. Rodriguez-Leonz, S. Kato, and J.C.I. Belmonte (2004). **Sp8 and Sp9, two closely related buttonhead-like transcription factors, regulate Fgf8 expression and limb outgrowth in vertebrate embryos**. *Development* 131(19): 4763-4774. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Initiation and maintenance of signaling centers is a key issue during embryonic development. The apical ectodermal ridge, a specialized epithelial structure and source of Fgf8, is a pivotal signaling center for limb outgrowth. We show that two closely related buttonhead-like zinc-finger transcription factors, Sp8 and Sp9, are expressed in the AER, and regulate Fgf8 expression and limb outgrowth. Embryological and genetic analyses have revealed that Sp8 and Sp9 are ectodermal targets of Fgf10 signaling from the mesenchyme. We also found that Wnt/beta-catenin signaling positively regulates Sp8, but not Sp9. Overexpression functional analyses in chick unveiled their role as positive regulators of Fgf8 expression. Moreover, a dominant-negative approach in chick and knockdown analysis with morpholinos in zebrafish revealed their requirement for Fgf8 expression and limb outgrowth, and further indicate that they have a coordinated action on Fgf8 expression. Our study demonstrates that Sp8 and Sp9, via Fgf8, are involved in mediating the actions of Fgf10 and Wnt/beta-catenin signaling during vertebrate limb outgrowth. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, development, molecular genetics, embryological analysis, genetic analysis, genetic techniques, knockdown analysis, overexpression functional analysis, zebrafish embryonic development, limb outgrowth.

Kay, J.N., B.A. Link, and H. Baier (2005). **Staggered cell-intrinsic timing of ath5 expression underlies the wave of ganglion cell neurogenesis in the zebrafish retina.** *Development* 132(11): 2573-85. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: In the developing nervous system, progenitor cells must decide when to withdraw from the cell cycle and commence differentiation. There is considerable debate whether cell-extrinsic or cell-intrinsic factors are most important for triggering this switch. In the vertebrate retina, initiation of neurogenesis has recently been explained by a 'sequential-induction' model--signals from newly differentiated neurons are thought to trigger neurogenesis in adjacent progenitors, creating a wave of neurogenesis that spreads across the retina in a stereotypical manner. We show here, however, that the wave of neurogenesis in the zebrafish retina can emerge through the independent action of progenitor cells--progenitors in different parts of the retina appear pre-specified to initiate neurogenesis at different times. We provide evidence that midline Sonic hedgehog signals, acting before the onset of neurogenesis, are part of the mechanism that sets the neurogenic timer in these cells. Our results

highlight the importance of intrinsic factors for triggering neurogenesis, but they also suggest that early signals can modulate these intrinsic factors to influence the timing of neurogenesis many cell cycles later, thereby potentially coordinating axial patterning with control of neuron number and cell fate.

Descriptors: zebrafish embryos retina, cell differentiation physiology, DNA binding proteins genetics, proteins metabolism, sensory ganglia embryology, developmental gene expression regulation, growth substances metabolism, retinal ganglion cells physiology, proteins metabolism, chimera physiology, DNA primers, Hedgehog proteins, lasers, micromanipulation, retinal ganglion cells transplantation, reverse transcriptase PCR, signal transduction genetics, stem cells physiology , time factors, trans activators metabolism, veratrum alkaloids.

Kay, J.N., T. Roeser, J.S. Mumm, L. Godinho, A. Mrejeru, R.O.l. Wong, and H. Baier (2004). **Transient requirement for ganglion cells during assembly of retinal synaptic layers**. *Development* 131(6): 1331-1342. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: The inner plexiform layer (IPL) of the vertebrate retina comprises functionally specialized sublaminae, representing connections between bipolar, amacrine and ganglion cells with distinct visual functions. Developmental mechanisms that target neurites to the correct synaptic sublaminae are largely unknown. Using expressing GFP in subsets of amacrine cells, we imaged IPL formation and sublamination. in vivo and asked whether the major postsynaptic cells in this circuit, the ganglion cells, organize the presynaptic inputs. We found that in the lak/ath5 mutant retina, where ganglion cells are never born, formation of the IPL is delayed, with initial neurite outgrowth ectopically located and grossly disorganized. Over time, the majority of early neurite projection errors are corrected, and major ON and OFF sublaminae do form. However, focal regions of disarray persist where sublaminae do not form properly. Bipolar axons, which arrive later, are targeted correctly, except at places where amacrine stratification is disrupted. The lak mutant phenotype reveals that ganglion cells have a transient role organizing the earliest amacrine projections to the IPL. However, it also suggests that amacrine cells interact with each other during IPL formation; these interactions alone appear sufficient to form the IPL. Furthermore, our results suggest that amacrines may guide IPL sublamination by providing stratification cues for other cell types. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryo retina, development, sense organs, neurite projection errors, presynaptic inputs, synaptic sublaminae, transgenic zebrafish, retinal synaptic layers, ganglion cells.

Kazakova, N., H. Li, A. Mora, K.R. Jessen, R. Mirsky, W.D. Richardson, and H.K. Smith (2006). **A screen for mutations in zebrafish that affect myelin gene expression in Schwann cells and oligodendrocytes.** *Developmental Biology* 297(1): 1-13. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Myelin is the multi-layered glial sheath around axons in the vertebrate nervous system. Myelinating glia develop and function in intimate association with neurons and neuron-glial interactions control much of the life history of these cells. However, many of the factors that regulate key aspects of myelin development and maintenance remain unknown. To discover new molecules that are important for glial development and myelination, we undertook a screen of zebrafish mutants with previously characterized neural defects. We screened for myelin basic protein (mbp) mRNA by in situ hybridization and identified four mutants (neckless, motionless, iguana and doc) that lacked mbp expression in parts of the peripheral and central nervous systems (PNS or CNS), despite the presence of axons. In all

four mutants electron microscopy revealed that myelin-forming glia were present and had formed loose wraps around axons but did not form compact myelin. We found that addition of exogenous retinoic acid (RA) rescued mbp expression in neckless mutant embryos, which lack endogenous RA synthesis. Timed application of the RA synthesis inhibitor DEAB to wild type embryos showed that RA signalling is required at least 48 h before the onset of myelin protein synthesis in both CNS and PNS.

Descriptors: zebrafish embryos, mutation, myelin sheath genetics, oligodendroglia physiology, Schwann cells physiology, genetics, carrier proteins genetics developmental gene expression regulation, myelin basic nervous system pathology, neuroglia pathology, oligodendroglia pathology , Schwann cells pathology, tretinoin metabolism and pharmacology, growth and development, proteins drug effects, P aminoazobenzene analogs and derivatives, P aminoazobenzene pharmacology.

Ke, Z., A. Emelyanov, S.E. Lim, V. Korzh, and Z. Gong (2005). **Expression of a novel zebrafish zinc finger gene, gli2b, is affected in Hedgehog and Notch signaling related mutants during embryonic development.** *Developmental Dynamics* 232(2): 479-86. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish, mutation, trans activators biosynthesis, transcription factors biosynthesis, transcription factors genetics, proteins biosynthesis and genetics, amino acid sequence, chromosome mapping, complementary DNA metabolism, genetic complementation test, Hedgehog proteins, immunohistochemistry, in situ hybridization, genetic models, molecular sequence data, phosphorylation, phylogeny, tertiary protein structure, messenger RNA metabolism, reverse transcriptase PCR, amino acid sequence homology, signal transduction, time factors, trans activators genetics, zinc fingers.

Keegan, B.R., D. Meyer, and D. Yelon (2004). **Organization of cardiac chamber progenitors in the zebrafish blastula.** *Development* 131(13): 3081-91. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Organogenesis requires the specification of a variety of cell types and the organization of these cells into a particular three-dimensional configuration. The embryonic vertebrate heart is organized into two major chambers, the ventricle and atrium, each consisting of two tissue layers, the myocardium and endocardium. The cellular and molecular mechanisms responsible for the separation of ventricular and atrial lineages are not well understood. To test models of cardiac chamber specification, we generated a high-resolution fate map of cardiac chamber progenitors in the zebrafish embryo at 40% epiboly, a stage prior to the initiation of gastrulation. Our map reveals a distinct spatial organization of myocardial progenitors: ventricular myocardial progenitors are positioned closer to the margin and to the dorsal midline than are atrial myocardial progenitors. By contrast, ventricular and atrial endocardial progenitors are not spatially organized at this stage. The relative orientations of ventricular and atrial myocardial progenitors before and after gastrulation suggest orderly movements of these populations. Furthermore, the initial positions of myocardial progenitors at 40% epiboly indicate that signals residing at the embryonic margin could influence chamber fate assignment. Indeed, via fate mapping, we demonstrate that Nodal signaling promotes ventricular fate specification near the margin, thereby playing an important early role during myocardial patterning.

Descriptors: zebrafish embryo, blastula metabolism, heart embryology, myocardium metabolism, stem cells metabolism, blastomeres metabolism, cell lineage, endocardium metabolism, fluorescein pharmacology, heart ventricles metabolism, in situ hybridization,

RNA metabolism, signal transduction, time factors, transforming growth factor beta metabolism.

Keller, M.J. and A.B. Chitnis (2006). **Her9-dependent regulation of neurogenesis by ZIC family proteins**. *Developmental Biology* 295(1): 441. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, development, molecular genetics, nervous system, cell differentiation, phenotype, neurogenesis.

Kennedy, B.N., G.W. Stearns, V.A. Smyth, V. Ramamurthy, F. van Eeden, I. Ankoudinova, D. Raible, J.B. Hurley, and S.E. Brockerhoff (2004). **Zebrafish rx3 and mab21l2 are required during eye morphogenesis.** *Developmental Biology* 270(2): 336-49. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Two alleles of an eyeless mutant, chokh (chk), were identified in ongoing zebrafish F(3) mutagenesis screens. Morphologically, chk mutants can be identified at 15 h post-fertilization by the failure of optic primordia to evaginate from the forebrain. The chk phenotype appears specific, as marker genes in the forebrain, midbrain, and pineal are expressed in normal temporal, spatial, and circadian patterns. Sequence analysis of the chk alleles revealed nonsense or missense mutations in the rx3 homeobox. Rx genes encode paired-type homeodomain transcription factors known to be key regulators of eye development in mouse, medaka, Xenopus, and zebrafish. To uncover novel Rx targets, we analyzed the expression of multiple eye development genes in chk. We find that expression of mab21l2, mab21l1 and rx2 are specifically absent in the eye field of chk embryos. Knockdown of Mab21l2 by antisense morpholino microinjections partially phenocopies the rx3 mutation, leading to microphthalmia, incomplete eye maturation, and dramatic increases in apoptotic eye progenitors. We propose that mab21l2 is an early downstream effector of rx3 and is critical for survival of eye progenitors.
Descriptors: zebrafish, eye embryology, developmental gene expression regulation, homeodomain phenotype, chromosome mapping, genetic crosses, complementary DNA genetics, homeodomain proteins metabolism, immunohistochemistry, in situ hybridization, in situ nick end labeling, microinjections, morphogenesis, mutation genetics, oligonucleotides, polymorphism, single stranded conformational, reverse transcriptase PCR, DNA sequence analysis, transcription factors genetics and metabolism, proteins.

Kerr, T., E.H. Roalson, and B.D. Rodgers (2005). **Phylogenetic analysis of the myostatin gene sub-family and the differential expression of a novel member in zebrafish.** *Evolution and Development* 7(5): 390-400. ISSN: 1520 541X.
NAL Call Number: QH359
Abstract: The myostatin (MSTN)-null phenotype in mammals is characterized by extreme gains in skeletal muscle mass or "double muscling" as the cytokine negatively regulates skeletal muscle growth. Recent attempts, however, to reproduce a comparable phenotype in zebrafish have failed. Several aspects of MSTN biology in the fishes differ significantly from those in mammals and at least two distinct paralogs have been identified in some species, which possibly suggests functional divergence between the different vertebrate classes or between fish paralogs. We therefore conducted a phylogenetic analysis of the entire MSTN gene sub-family. Maximum likelihood, Bayesian inference, and bootstrap analyses indicated a monophyletic distribution of all MSTN genes with two distinct fish clades: MSTN-1 and -2. These analyses further indicated that all Salmonid genes described are actually MSTN-1 orthologs and that additional MSTN-2 paralogs may be present in most, if not all, teleosts.

An additional zebrafish homolog was identified by BLAST searches of the zebrafish Hierarchical Tets Generation System database and was subsequently cloned. Comparative sequence analysis of both genes (zebrafish MSTN (zfMSTN)-1 and -2) revealed many differences, primarily within the latency-associated peptide regions, but also within the bioactive domains. The 2-kb promoter region of zfMSTN-2 contained many putative cis regulatory elements that are active during myogenesis, but are lacking in the zfMSTN-1 promoter. In fact, zfMSTN-2 expression was limited to the early stages of somitogenesis, whereas zfMSTN-1 was expressed throughout embryogenesis. These data suggest that zfMSTN-2 may be more closely associated with skeletal muscle growth and development. They also resolve the previous ambiguity in classification of fish MSTN genes.

Descriptors: zebrafish embryos, muscle development genetics, skeletal muscle embryology, phylogeny, transforming growth factor beta genetics, nucleic acid databases, embryonic development genetics, mice, rats, sequence alignment methods, DNA sequence analysis.

Kerstetter, A.E., E. Azodi, J.A. Marrs, and Q. Liu (2004). **Cadherin-2 function in the cranial ganglia and lateral line system of developing zebrafish.** *Developmental Dynamics* 230(1): 137-43. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: Cadherins are cell surface molecules that mediate cell-cell adhesion through homophilic interactions. Cadherin-2 (also called N-cadherin), a member of classic cadherin subfamily, has been shown to play important roles in development of a variety of tissues and organs, including the nervous system. We recently reported that cadherin-2 was strongly expressed by the majority of cranial ganglia and lateral line system of developing zebrafish. To gain insight into cadherin-2 role in the formation of these structures, we have used several markers to analyze zebrafish embryos injected with a specific cadherin-2 antisense morpholino oligonucleotide (cdh2MO). We find that development of several cranial ganglia, including the trigeminal, facial, and vagal ganglia, and the lateral line ganglia and neuromasts of the cdh2MO-injected embryos are severely disrupted. These phenotypes were confirmed by analyzing a cadherin-2 mutant, glass onion. Our results suggest that cadherin-2 function is crucial for the normal formation of the zebrafish lateral line system and a subset of cranial ganglia.

Descriptors: zebrafish brain embryology, cell adhesion molecules biosynthesis, developmental gene expression regulation, basal ganglia embryology, cadherins biosynthesis, cell adhesion, cell membrane metabolism, immunohistochemistry, in situ hybridization, anatomic models, neurons metabolism, antisense oligonucleotides metabolism, proteins chemistry.

Kida, Y., T. Sato, and T. Ogura (2005). **Novel Dvl2 interacting protein Crip2 regulates notochord cell intercalation of zebrafish embryo.** *Developmental Biology* 283(2): 619. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish, development, phenotype, cell intercalation, gastrulation, signal transduction cascade, morphogenetic cell movement, Wnt, pcp signaling pathway, convergent and extension movemen, zebrafish.

Kikuchi, Y., H. Verkade, J.F. Reiter, C.H. Kim, A.B. Chitnis, A. Kuroiwa, and D.Y.R. Stainier (2004). **Notch signalling can regulate endoderm formation in zebrafish**. *Developmental Dynamics* 229(4): 756-762. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: Early in vertebrate development, the processes of gastrulation lead to the formation of the three germ layers: ectoderm, mesoderm, and endoderm. The mechanisms leading to the segregation of the endoderm and mesoderm are not well understood. In mid-blastula stage zebrafish embryos, single marginal cells can give rise to both endoderm and mesoderm (reviewed by Warga and Stainier (2002) The guts of endoderm formation. In: Solnica-Krezel L, editor. Pattern formation in zebrafish. Berlin: Springer-Veriag. p 28-47). By the late blastula stage, however, single marginal cells generally give rise to either endoderm or mesoderm. To investigate this segregation of the blastoderm into cells with either endodermal or mesodermal fates, we analyzed the role of Notch signaling in this process. We show that deltaC, deltaD, and notch1 are expressed in the marginal domain of blastula stage embryos and that this expression is dependent on Nodal signaling. Activation of Notch signaling from an early stage leads to a reduction of endodermal cells, as assessed by sox17 and foxA2 expression. We further find that this reduction in endoderm formation by the activation of Notch signaling is preceded by a reduction in the expression of bonnie and clyde (bon) and faust/gata5, two genes necessary for endoderm formation (Reiter et al. (1999) Genes Dev 13:2983-2995; Reiter et al. (2001) Development 128:125-135; Kikuchi et al. (2001) Genes Dev 14:1279-1289). However, activation of Notch signaling in bon mutant embryos leads to a further reduction in endodermal cells, also arguing for a bon-independent role for Notch signaling in endoderm formation. Altogether, these results suggest that Notch signaling plays a role in the formation of the endoderm, possibly in its segregation from the mesoderm.
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Descriptors: zebrafish embryo, development, molecular genetics, veterinary medicine, medical sciences, Notch signaling, activation, bon independence, role, bon mutant embryos, endoderm formation, reduction, late blastula stage, mid blastula stage, vertebrate development.

Kikuta, H., M. Kanai, Y. Ito, and K. Yamasu (2003). **Gbx2 Homeobox gene is required for the maintenance of the isthmic region in the zebrafish embryonic brain.** *Developmental Dynamics* 228(3): 433-50. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish brain embryology, cerebellum embryology, homeobox genes, homeodomain amino acid sequence, molecular cloning, conserved sequence, complementary DNA genetics, developmental gene expression regulation genetics, homeodomain proteins chemistry, molecular sequence data, morphogenesis genetics, sequence alignment, amino acid sequence homology, proteins chemistry and genetics.

Kilian, B., H. Mansukoski, F.C. Barbosa, F. Ulrich, M. Tada, and C.P. Heisenberg (2003). **The role of Ppt/Wnt5 in regulating cell shape and movement during zebrafish gastrulation.** *Mechanisms of Development* 120(4): 467-76. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: Wnt genes play important roles in regulating patterning and morphogenesis during vertebrate gastrulation. In zebrafish, slb/wnt11 is required for convergence and extension movements, but not cell fate specification during gastrulation. To determine if other Wnt genes functionally interact with slb/wnt11, we analysed the role of ppt/wnt5 during zebrafish gastrulation. ppt/wnt5 is maternally provided and zygotically expressed at all stages during gastrulation. The analysis of ppt mutant embryos reveals that Ppt/Wnt5 regulates cell elongation and convergent extension movements in posterior regions of the gastrula, while its function in more anterior regions is largely redundant to that of Slb/Wnt11. Frizzled-2 functions downstream of ppt/wnt5, indicating that it might act as a receptor for Ppt/Wnt5 in

this process. The characterisation of the role of Ppt/Wnt5 provides insight into the functional diversity of Wnt genes in regulating vertebrate gastrulation movements.
Descriptors: zebrafish embryos, gastrula physiology, developmental gene expression regulation, proto oncogene, transcription factors physiology, cell lineage, gastrula metabolism, glycoglyco, in situ hybridization, confocal microscopy, mutation, antisense oligonucleotides pharmacology, phenotype, receptors, neurotransmitter metabolism, signal transduction, time factors, transcription factors genetics, Wnt proteins.

Kim, D.H., Y. Sun, S. Yun, B. Kim, C.N. Hwang, B. Nelson, and S.H. Lee (2004). **Mechanical property characterization of the zebrafish embryo chorion.** *Engineering in Medicine and Biology Magazine* 7: 5061-4. ISSN: 0739 5175.
Abstract: A microrobotic force sensing system is used to characterize the mechanical properties of the chorion of zebrafish embryos and quantitate the mechanical property differences of the chorion at different developmental stages. Quantitative relationships between applied forces and chorion structural deformations are established for various developmental stages. The measured penetration forces for puncturing chorion at the blastula stage are 1.3 times as large as those at the pre-hatching stage. Through the use of an analytical biomembrane elastic model, chorion elastic modulus values are determined. The modulus at the blastula stage is 1.66 times as large as that at the pre-hatching stage. The experimental results quantitatively describe "chorion softening," which is mostly due to the proteolytic activity at the pre-hatching stage. This study also reveals the effect of pronase treatment on zebrafish chorion, which produces an "artificial chorion softening" effect. This draws an analogy between pronase treatment and proteolytic activities at the pre-hatching stage.
Descriptors: zebrafish embryo, pronase, treatment, chorion, softening, proteolytic activities, pre-hatching stage, blastula.

Kim, F.A., A. Sing, T. Kaneko, M. Bieman, N. Stallwood, V.S. Sadl, and S.P. Cordes (2005). **The vHNF1 homeodomain protein establishes early rhombomere identity by direct regulation of Kreisler expression**. *Mechanisms of Development* 122(12): 1300-1309. ISSN: 0925 4773.
NAL Call Number: QH607.A1C4
Abstract: The early transcriptional hierarchy that subdivides the vertebrate hindbrain into seven to eight segments, the rhombomeres (r1-r8), is largely unknown. The Kreisler (MafB, Krm11, Val) gene is earliest gene expressed in an r5/r6-restricted manner and is essential for r5 and r6 development. We have identified the S5 regulatory element that directs early Kreisler expression in the future r5/r6 domain in 0-10 somite stage embryos. variant Hepatocyte Nuclear Factor 1 (vHNF1/HNF1 beta/LF-3B) is transiently expressed in the r5/r6 domain of 0-10 somite stage embryos and a vHNF1 binding site within this element is essential but not sufficient for r5/r6-specific expression. Thus, early inductive events that initiate Kreisler expression are clearly distinct from later-acting ones that modulate its expression levels. This site and some of the surrounding sequences are evolutionarily conserved in the genomic DNA upstream of the Kreisler gene among species as divergent as mouse, humans, and chickens. This provides the first evidence of a direct requirement for vHNF1 in initiation of Kreisler expression, suggests that the role of vHNF1 is evolutionarily conserved, and indicates that vHNF1 collaborates with other transcription factors, which independently bind to the S5 regulatory region, to establish the r5/r6 domain. (c) 2005 Elsevier Ireland Ltd. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, nervous system, molecular genetics, genbank sequence data, transcriptional hierarchy.

Kim, K.H., D.S. Antkiewicz, L. Yan, K.W. Eliceiri, W. Heideman, R.E. Peterson, and Y. Lee (2007). **Lrrc10 is required for early heart development and function in zebrafish.** *Developmental Biology* 308(2): 494-506. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Leucine-rich Repeat Containing protein 10 (LRRC10) has recently been identified as a cardiac-specific factor in mice. However, the function of this factor remains to be elucidated. In this study, we investigated the developmental roles of Lrrc10 using zebrafish as an animal model. Knockdown of Lrrc10 in zebrafish embryos (morphants) using morpholinos caused severe cardiac morphogenic defects including a cardiac looping failure accompanied by a large pericardial edema, and embryonic lethality between day 6 and 7 post fertilization. The Lrrc10 morphants exhibited cardiac functional defects as evidenced by a decrease in ejection fraction and cardiac output. Further investigations into the underlying mechanisms of the cardiac defects revealed that the number of cardiomyocyte was reduced in the morphants. Expression of two cardiac genes was deregulated in the morphants including an increase in atrial natriuretic factor, a hallmark for cardiac hypertrophy and failure, and a decrease in cardiac myosin light chain 2, an essential protein for cardiac contractility in zebrafish. Moreover, a reduced fluorescence intensity from NADH in the morphant heart was observed in live zebrafish embryos as compared to control. Taken together, the present study demonstrates that Lrrc10 is necessary for normal cardiac development and cardiac function in zebrafish embryos, which will enhance our understanding of congenital heart defects and heart disease.

Descriptors: zebrafish embryos, heart embryology, muscle, amino acid sequence, genetically modified, molecular cloning, DNA primers genetics, complementary DNA genetics, developmental gene expression regulation, molecular sequence data, muscle proteins antagonists, inhibitors and genetics, oligodeoxyribonucleotides, antisense genetics, organ specificity, proteins antagonists and inhibitors genetics, amino acid sequence homology, proteins antagonists, inhibitors and genetics.

Kimelman, D. (2006). **Squinting at the zebrafish axis.** *Developmental Cell* 10(1): 6-7. ISSN: 1534 5807.

NAL Call Number: QH573.C42

Abstract: The Nodal family of signaling molecules includes critical intercellular regulators of early vertebrate development. In a recent issue of Nature, maternal transcripts encoding the zebrafish nodal squint were shown to be localized to the future organizer region by the four-cell stage, providing the earliest evidence of embryonic axis asymmetry in the zebrafish embryo.

Descriptors: zebrafish embryos, body patterning physiology, signal transduction physiology endoderm, developmental gene expression regulation, models.

Kimmel, C.B., B. Ullmann, M. Walker, C.T. Miller, and J.G. Crump (2003). **Endothelin 1-mediated regulation of pharyngeal bone development in zebrafish.** *Development* 130(7): 1339-51. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Endothelin 1 (Edn1), a secreted peptide expressed ventrally in the primordia of the zebrafish pharyngeal arches, is required for correct patterning of pharyngeal cartilage development. We have studied mutants and morpholino-injected larvae to examine the role of the Edn1 signal in patterning anterior pharyngeal arch bone development during the first week after fertilization. We observe a remarkable variety of phenotypic changes in dermal bones of the anterior arches after Edn1 reduction, including loss, size reduction and expan-

sion, fusion and shape change. Notably, the changes that occur appear to relate to the level of residual Edn1. Mandibular arch dermal bone fusions occur with severe Edn1 loss. In the dorsal hyoid arch, the dermal opercle bone is usually absent when Edn1 is severely reduced and is usually enlarged when Edn1 is only mildly reduced, suggesting that the same signal can act both positively and negatively in controlling development of a single bone. Position also appears to influence the changes: a branchiostegal ray, a dermal hyoid bone normally ventral to the opercle, can be missing in the same arch where the opercle is enlarged. We propose that Edn1 acts as a morphogen; different levels pattern specific positions, shapes and sizes of bones along the dorso-ventral axis. Changes involving Edn1 may have occurred during actinopterygian evolution to produce the efficient gill-pumping opercular apparatus of teleosts.

Descriptors: zebrafish embryos, bone and bones embryology, branchial region embryology, endothelin 1 genetics and metabolism, proteins, cartilage embryology, gene expression regulation, head development embryology, hyoid bone embryology, mandible embryology, mutation.

Kinkel, M.D., M. Alonzo, D. Stafford, and V. Prince (2005). **Cdx4 is required to correctly position the zebrafish pancreas.** *Developmental Biology* 283(2): 685-686. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish embryology, molecular genetics: biochemistry, digestive system, endocrine system, homeostasis, pancreas.

Kinna, G., G. Kolle, A. Carter, B. Key, G.J. Lieschke, A. Perkins, and M.H. Little (2006). **Knockdown of zebrafish crim1 results in a bent tail phenotype with defects in somite and vascular development.** *Mechanisms of Development* 123(4): 277-87. ISSN: 0925-4773.

NAL Call Number: QH607.A1C4

Abstract: The Crim1 gene encodes a transmembrane protein containing six cysteine-rich repeats similar to those found in the BMP antagonist, chordin (chd). To investigate its physiological role, zebrafish crim1 was cloned and shown to be both maternally and zygotically expressed during zebrafish development in sites including the vasculature, intermediate cell mass, notochord, and otic vesicle. Bent or hooked tails with U-shaped somites were observed in 85% of morphants from 12 hpf. This was accompanied by a loss of muscle pioneer cells. While morpholino knockdown of crim1 showed some evidence of ventralisation, including expansion of the intermediate cell mass (ICM), reduction in head size bent tails and disruption to the somites and notochord, this did not mimic the classically ventralised phenotype, as assessed by the pattern of expression of the dorsal markers chordin, otx2 and the ventral markers eve1, pax2.1, tal1 and gata1 between 75% epiboly and six-somites. From 24 hpf, morphants displayed an expansion of the ventral mesoderm-derived ICM, as evidenced by expansion of tal1, lmo2 and crim1 itself. Analysis of the crim1 morphant phenotype in Tg(fli:EGFP) fish showed a clear reduction in the endothelial cells forming the intersegmental vessels and a loss of the dorsal longitudinal anastomotic vessel (DLAV). Hence, the primary role of zebrafish crim1 is likely to be the regulation of somitic and vascular development.

Descriptors: zebrafish embryos, blood vessels embryology, bone morphogenetic protein receptors physiology, membrane, somites cytology, tail embryology, genetically modified, base sequence, body patterning genetics, bone morphogenetic protein receptors genetics, molecular cloning, developmental gene expression regulation, green fluorescent proteins, membrane molecular sequence data, physiologic neovascularization genetics, antisense oligonucleotides, phenotype, sequence homology, zebrafish proteins genetics.

Kinsey, W.H., W. Wu, and E. Macgregor (2003). **Activation of Src-family PTK activity at fertilization: role of the SH2 domain**. *Developmental Biology* 264(1): 255-262. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish fertilization, metabolism, calcium , embryology, embryonic development, gametogenesis, inhibitors, marine invertebrates, polyspermy, spermatogenesis, tyrosine, *Danio rerio*.

Kishimoto, Y., S. Koshida, M. Furutani Seiki, and H. Kondoh (2004). **Zebrafish maternal-effect mutations causing cytokinesis defect without affecting mitosis or equatorial vasa deposition.** *Mechanisms of Development* 121(1): 79-89. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: Maternal-effect genes play essential roles in early embryogenesis particularly before activation of the zygotic genes. A genetic screen for mutations affecting such maternal-effect genes was carried out employing an F3 screen strategy, identifying six recessive mutations out of 60 mutagenized genomes. Three of the mutations (acytokinesis mutations: ackkt5, ackkt62 and ackkt119) caused absence of cell cleavage in the embryos derived from homozygous females regardless of the paternal genotype, without affecting nuclear divisions. These embryos are defective in generating contractile rings, ackkt62 mutation abolishing reactions to organize cortical F-actin, while other mutations causing abortive contractile ring-like structures at ectopic sites. Defect of contractile ring formation in the affected embryos leads to the absence of microtubule arrays at the prospective cleavage plane. Thus, these mutations reveal the sequence of events associated with cytokinesis, in particular, the cortical actin dynamics. It is remarkable that in all acytokinetic embryos, daughter nuclei after mitosis are arranged in spatially normal positions, and maternal vasa mRNAs accumulate in the prospective planes of the first and second cell cleavages in the total absence of cytokinesis. This indicates that the basic cell architectures of early embryos are largely established by the autonomous activities of the mitotic apparatus, without much dependence on the cell cleavage machinery.
Descriptors: acytokinetic embryo zebrafish, mitosis physiology, mutation, genetic crosses, phenotype, mitotic apparatus.

Kleinjan, D.A., A. Seawright, A.J. Childs, and H.V. Van (2004). **Conserved elements in Pax 6 intron 7 involved in (auto)regulation and alternative transcription.** *Developmental Biology* 265(2): 462-477. ISSN: 0012 1606.
NAL Call Number: 442.8 D49
Abstract: Pax6 is a transcription factor with an essential role in eye, central nervous system, and pancreas development. Its expression pattern is restricted to these specific domains within the developing embryo. Here four conserved elements are identified in Pax6 intron 7, showing a high level of sequence conservation between human, mouse, pufferfish, and zebrafish. Three of these are shown to act as cis-regulatory elements, directing expression of a reporter gene to distinct subsets of the Pax6 expression domain. CE1 regulates gene expression in late eye development, CE2 drives expression in the diencephalon and in the developing heart tube where Pax6 is not normally expressed, while CE3 directs expression in rhombencephalon. CE2 is shown to be autoregulated in the diencephalon, responding to absence of Pax6. We identify a highly conserved Pax6 recognition site and demonstrate its ability to bind Pax6 specifically. CE1 is embedded in a CpG island, and we identify a novel Pax6 transcript which initiates from this region. Functional analysis of evolutionary conserved sequences pinpoints novel cis-acting elements that govern the regulation of the complex spatio-temporal and quantitative expression of Pax6. Copyright©Thomson Reuters

2009
Descriptors: zebrafish, human, pufferfish, mouse, evolution and adaptation, molecular genetics, biochemistry, alternative transcription, autoregulation, evolutionary conservation, transcriptional regulation, Pax 6 intron 7 transcription factor , sequence conservation.

Knight, R.D., Y. Javidan, S. Nelson, T. Zhang, and T. Schilling (2004). **Skeletal and pigment cell defects in the lockjaw mutant reveal multiple roles for zebrafish tfap2a in neural crest development.** *Developmental Dynamics* 229(1): 87-98. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, tfap2a function, neural crest cytology, neural crest embryology, body patterning genetics, bone development genetics, branchial region cytology, branchial region embryology and metabolism, cell movement genetics, evolution, developmental gene expression regulation, homeobox genes, in situ hybridization, melanocytes cytology, melanocytes metabolism, mutation, neural crest metabolism, metabolism.

Knight, R.D., Y. Javidan, T. Zhang, S. Nelson, and T.F. Schilling (2005). **AP2-dependent signals from the ectoderm regulate craniofacial development in the zebrafish embryo.** *Development* 132(13): 3127-38. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: AP2 transcription factors regulate many aspects of embryonic development. Studies of AP2a (Tfap2a) function in mice and zebrafish have demonstrated a role in patterning mesenchymal cells of neural crest origin that form the craniofacial skeleton, while the mammalian Tfap2b is required in both the facial skeleton and kidney. Here, we show essential functions for zebrafish tfap2a and tfap2b in development of the facial ectoderm, and for signals from this epithelium that induce skeletogenesis in neural crest cells (NCCs). Zebrafish embryos deficient for both tfap2a and tfap2b show defects in epidermal cell survival and lack NCC-derived cartilages. We show that cartilage defects arise after NCC migration during skeletal differentiation, and that they can be rescued by transplantation of wild-type ectoderm. We propose a model in which AP2 proteins play two distinct roles in cranial NCCs: an early cell-autonomous function in cell specification and survival, and a later non-autonomous function regulating ectodermal signals that induce skeletogenesis.
Descriptors: zebrafish embryos, DNA binding, facial bones embryology, neural crest physiology, skull embryology, transcription factors physiology, cartilage metabolism, molecular cloning, DNA binding proteins genetics, ectoderm metabolism, Fgf metabolism, mesoderm metabolism, neural crest embryology and metabolism, signal transduction genetics, transcription factor AP2, transcription factors genetics.

Knight, R.D., S. Nair, S.S. Nelson, A. Afshar, Y. Javidan, R. Geisler, G.J. Rauch, and T.F. Schilling (2003). **Lockjaw encodes a zebrafish tfap2a required for early neural crest development.** *Development* 130(23): 5755-68. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The neural crest is a uniquely vertebrate cell type that gives rise to much of the craniofacial skeleton, pigment cells and peripheral nervous system, yet its specification and diversification during embryogenesis are poorly understood. Zebrafish homozygous for the lockjaw (low) mutation show defects in all of these derivatives and we show that low (allelic with montblanc) encodes a zebrafish tfap2a, one of a small family of transcription factors implicated in epidermal and neural crest development. A point mutation in low truncates the DNA binding and dimerization domains of tfap2a, causing a loss of function. Consistent with this, injection of antisense morpholino oligonucleotides directed against splice sites in

tfap2a into wild-type embryos produces a phenotype identical to low. Analysis of early ectodermal markers revealed that neural crest specification and migration are disrupted in low mutant embryos. TUNEL labeling of dying cells in mutants revealed a transient period of apoptosis in crest cells prior to and during their migration. In the cranial neural crest, gene expression in the mandibular arch is unaffected in low mutants, in contrast to the hyoid arch, which shows severe reductions in dlx2 and hoxa2 expression. Mosaic analysis, using cell transplantation, demonstrated that neural crest defects in low are cell autonomous and secondarily cause disruptions in surrounding mesoderm. These studies demonstrate that low is required for early steps in neural crest development and suggest that tfap2a is essential for the survival of a subset of neural crest derivatives.

Descriptors: zebrafish embryos, DNA binding proteins genetics, proteins metabolism, neural crest growth and development, transcription factors metabolism, amino acid sequence, cell movement, cell survival, cell transplantation, molecular cloning, craniofacial abnormalities, DNA binding proteins genetics, in situ hybridization, molecular sequence data, neural crest cytology, neural crest metabolism, neuroglia cytology, neuroglia metabolism, neurons cytology, neurons metabolism, pigmentation, sequence alignment, transcription factor ap 2, transcription factors genetics, zebrafish abnormalities, zebrafish anatomy, histology and genetics.

Knoll Gellida, A., M. Andre, T. Gattegno, J. Forgue, A. Admon, and P.J. Babin (2006). **Molecular phenotype of zebrafish ovarian follicle by serial analysis of gene expression and proteomic profiling, and comparison with the transcriptomes of other animals**. *Bio Med Central Genomics* 7(46) ISSN: 1471 2164.

Online: http://www.biomedcentral.com/content/pdf/1471-2164-7-46.pdf

NAL Call Number: QH447.B63

Abstract: Background: The ability of an oocyte to develop into a viable embryo depends on the accumulation of specific maternal information and molecules, such as RNAs and proteins. A serial analysis of gene expression (SAGE) was carried out in parallel with proteomic analysis on fully-grown ovarian follicles from zebrafish (*Danio rerio)*. The data obtained were compared with ovary/follicle/egg molecular phenotypes of other animals, published or available in public sequence databases. Results: Sequencing of 27 486 SAGE tags identified 11 399 different ones, including 3329 tags with an occurrence superior to one. 58 genes were expressed at over 0.15% of the total population and represented 17.34% of the mRNA population identified. The three most expressed transcripts were a rhamnose-binding lectin, beta-actin 2, and a transcribed locus similar to the H2B histone family. Comparison with the large-scale expressed sequence tags sequencing approach revealed highly expressed transcripts that were not previously known to be expressed at high levels in fish ovaries, like the short-sized polarized metallothionein 2 transcript. A higher sensitivity for the detection of transcripts with a characterized maternal genetic contribution was also demonstrated compared to large-scale sequencing of cDNA libraries. Ferritin heavy polypeptide 1, heat shock protein 90-beta, lactate dehydrogenase B4, beta-actin isoforms, tubulin beta 2, ATP synthase subunit 9, together with 40 S ribosomal protein S27a, were common highly-expressed transcripts of vertebrate ovary/unfertilized egg. Comparison of transcriptome and proteome data revealed that transcript levels provide little predictive value with respect to the extent of protein abundance. All the proteins identified by proteomic analysis of fully-grown zebrafish follicles had at least one transcript counterpart, with two exceptions: eosinophil chemotactic cytokine and nothepsin. Conclusion: This study provides a complete sequence data set of maternal mRNA stored in zebrafish germ cells at the end of oogenesis. This catalogue contains highly-expressed transcripts that are part of a vertebrate ovarian expressed

gene signature. Comparison of transcriptome and proteome data identified downregulated transcripts or proteins potentially incorporated in the oocyte by endocytosis. The molecular phenotype described provides groundwork for future experimental approaches aimed at identifying functionally important stored maternal transcripts and proteins involved in oogenesis and early stages of embryo development. Copyright © 2008 CABI

Descriptors: zebrafish embryos, complementary DNA, DNA libraries, embryonic development, gene expression, genes, maternal effects, messenger RNA, nucleotide sequences, oocytes, oogenesis, ovarian follicles, phenotypes, rhamnose, transcription, *Danio rerio*.

Knowlton, M.N., B.M. Chan, and G.M. Kelly (2003). **The zebrafish band 4.1 member Mir is involved in cell movements associated with gastrulation.** *Developmental Biology* 264(2): 407-29. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Cellular processes rely on dynamic events occurring between the cortical cytoskeleton and plasma membrane. Members of the Band 4.1 superfamily, which are best known for their ability to tether the cytoskeleton to the plasma membrane, play prominent structural and regulatory roles that influence cell-cell and cell-substrate interactions, endo- and exocytosis, cell polarity, migration, proliferation, and differentiation. We have identified a new member of the zebrafish Band 4.1 superfamily, which is the homolog of human myosin regulatory light chain interacting protein (MIR), and have examined its role in embryonic development. Zebrafish Mir contains the conserved amino-terminal plasma membrane-binding FERM (Band 4.1/ezrin/radixin/moesin) domain as well as other putative protein-protein interacting domains, including a RING finger. Overall, zebrafish Mir is 71% identical to human MIR located at chromosome 6p23-p22.3, and maps on linkage group 19 to a region of synteny with human chromosome 6. In situ hybridization and RT-PCR revealed that mir is expressed maternally and ubiquitously throughout development. Blocking Mir translation using a mir-specific, morpholino-based, knock-down strategy or expressing Mir constructs lacking the RING finger domain disrupts gastrulation and leads to subsequent trunk and tail defects. In severe cases, morphants exogastrulate. The synergistic effect seen when two mir-specific morpholinos are used in conjunction reflects the specific knock-down of mir. In addition, morphant phenotypes induced by mir-specific morpholinos are rescued by overexpression of the full-length Mir. In situ hybridization analysis with mesodermal- and neural-specific markers shows that morphants exhibit a delay in cell movements associated with gastrulation, epiboly, convergence, and extension. A yeast two-hybrid analysis was performed to identify binding partners that may participate with Mir during gastrulation, and Annexin V, a calcium channel protein, was isolated. At early developmental stages, annexin V transcripts colocalize with mir, but after gastrulation, annexin V mRNA becomes localized to the distal tail region and an area in the olfactory placode. At the protein level, Mir colocalizes with Annexin V when expressed in COS cells. Together, these results indicate that Mir is essential for embryonic development and that its role in early embryonic development likely involves calcium-dependent mechanisms essential during the extensive cell movements associated with gastrulation.

Descriptors: zebrafish embryos, cytoskeletal proteins, gastrula physiology, membrane, neuropeptides , amino acid sequence, annexin a5 physiology, base sequence, cell movement physiology, developmental gene expression regulation, membrane molecular sequence data.

Ko, C.S., H.S. Liu, W.H. Chen, T. Yan, and T.M. Lim (2003). **Effects of retinoid acid and thyroid hormone on the development of zebrafish embryo.** *Fertility and Sterility* 80(Suppl. 3): S269. ISSN: 0015 0282.

NAL Call Number: 448.8 F41
Descriptors: zebrafish embryos, biochemistry, development, endocrine system, skeletal system, movement and support, thyroid hormone, retinoid acid.

Koester, R.W. and S.E. Fraser (2004). **Time-lapse microscopy of brain development**. *Methods in Cell Biology* 76: 207-235. ISSN: 0091 679X.
NAL Call Number: 442P92
Descriptors: zebrafish embryology, nervous system, neural coordination, time lapse microscopy, brain development.

Kok, F.O., E. Oster, L. Mentzer, J.C. Hsieh, C.A. Henry, and H.I. Sirotkin (2007). **The role of The SPT6 chromatin remodeling factor in zebrafish embryogenesis.** *Developmental Biology* 307(2): 214-26. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Somitogenesis is a highly controlled process that results in segmentation of the paraxial mesoderm. Notch pathway activity in the presomitic mesoderm is fundamental for management of synchronized gene expression which is necessary for regulation of somitogenesis. We have isolated an embryonic lethal mutation, SBU2, that causes somite formation defects very similar to Notch pathway mutants. SBU2 mutants generate only 6-7 asymmetrically arranged somites. However, in contrast to Notch pathway mutants, these mutants do not maintain previously formed somite boundaries and by 24 hpf, almost no somite boundaries remain. Other developmental processes disrupted in SBU2 mutants include tail morphogenesis, muscle fiber elongation, pigmentation, circulatory system development and neural differentiation. We demonstrated that these defects are the result of a nonsense mutation within the spt6 gene. spt6 encodes a transcription elongation factor that genetically interacts with the Paf-1 chromatin remodeling complex. SBU2 mutant phenotypes could be rescued by microinjection of spt6 mRNA and microinjection of spt6 morpholinos phenocopied the mutation. Our real-time PCR analysis revealed that Spt6 is essential for the transcriptional response to activation of the Notch pathway. Analysis of sbu2;mib double mutants indicates that Spt6 deficiency suppresses the neurogenic effects of the mib. Altogether, these results demonstrate that Spt6 is critical for somite formation in zebrafish and suggest that some defects observed in spt6 mutants result from alterations in Notch signaling. However, additional Spt6 mutant phenotypes are likely caused by vital functions of Spt6 in other pathways.
Descriptors: zebrafish embryos, chromatin assembly and disassembly physiology, transcription factors metabolism, base sequence, chromatin assembly and disassembly genetics, chromosome mapping, DNA genetics, epistasis, developmental gene expression regulation, in situ hybridization, mutation, Notch receptors genetics, Notch receptors metabolism, signal transduction, somites cytology, somites metabolism, transcription factors genetics.

Kopinke, D., J. Sasine, J. Swift, W.Z. Stephens, and T. Piotrowski (2006). **Retinoic acid is required for endodermal pouch morphogenesis and not for pharyngeal endoderm specification**. *Developmental Dynamics* 235(10): 2695-2709. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Because tissues from all three germ layers contribute to the pharyngeal arches, it is not surprising that all major signaling pathways are involved in their development. We focus on the role of retinoic acid (RA) signaling because it has been recognized for quite some time that alterations in this pathway lead to craniofacial malformations. Several studies exist that describe phenotypes observed upon RA perturbations in pharyngeal arch develop-

ment; however, these studies did not address whether RA plays multiple roles at distinct time points during development. Here, we report the resulting phenotypes in the hindbrain, the neural crest-derived tissues, and the pharyngeal endoderm when RA synthesis is disrupted during zebrafish gastrulation and pharyngeal arch morphogenesis. Our results demonstrate that RA is required for the post-gastrulation morphogenesis and segmentation of endodermal pouches, and that loss of RA does not affect the length of the pharyngeal ectoderm or medial endoderm along the anterior-posterior axis. We also provide evidence that RA is not required for the specification of pharyngeal pouch endoderm and that the pharyngeal endoderm consists of at least two different cell populations, of which the pouch endoderm is sensitive to RA and the more medial pharyngeal endoderm is not. These results demonstrate that the developmental processes underlying pharyngeal arch defects differ depending on when RA signaling is disturbed during development. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, biochemistry, nervous system, development, phenotype, craniofacial development, post gastrulation morphogenesis, embryonic specification, retinoic acid effects, post-gastrulation morphogenesis, segmentation of endodermal pouches.

Koppen, M., B.G. Fernandez, L. Carvalho, A. Jacinto, and C.P. Heisenberg (2006). **Coordinated cell-shape changes control epithelial movement in zebrafish and *Drosophila*.** *Development* 133(14): 2671-81. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Epithelial morphogenesis depends on coordinated changes in cell shape, a process that is still poorly understood. During zebrafish epiboly and *Drosophila* dorsal closure, cell-shape changes at the epithelial margin are of critical importance. Here evidence is provided for a conserved mechanism of local actin and myosin 2 recruitment during theses events. It was found that during epiboly of the zebrafish embryo, the movement of the outer epithelium (enveloping layer) over the yolk cell surface involves the constriction of marginal cells. This process depends on the recruitment of actin and myosin 2 within the yolk cytoplasm along the margin of the enveloping layer. Actin and myosin 2 recruitment within the yolk cytoplasm requires the Ste20-like kinase Msn1, an orthologue of *Drosophila* Misshapen. Similarly, in *Drosophila*, actin and myosin 2 localization and cell constriction at the margin of the epidermis mediate dorsal closure and are controlled by Misshapen. Thus, this study has characterized a conserved mechanism underlying coordinated cell-shape changes during epithelial morphogenesis.
Descriptors: zebrafish embryos, cell movement physiology, cell shape, *Drosophila melanogaster* anatomy, histology and embryology, epithelial cells physiology, morphogenesis, actins metabolism, *Drosophila* proteins genetics and metabolism, epithelial cells cytology, in situ hybridization, membrane membrane proteins metabolism, molecular sequence data, myosins metabolism, phylogeny, protein serine threonine kinases genetics, protein serine threonine kinases metabolism, zebrafish proteins genetics and metabolism.

Korzh, S.N., A.V. Emelyanov, Z. Li, V.P. Korzh, and Z. Gong (2004). **Ceruloplasmin and liver development in zebrafish.** *Developmental Biology* 271(2): 622. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryology, molecular genetics, digestive system, enzymology, development, ceruloplasmin.

Kosaka, K., K. Kawakami, H. Sakamoto, and K. Inoue (2007). **Spatiotemporal localization of germ plasm RNAs during zebrafish oogenesis.** *Mechanisms of Development* 124(4): 279-89. ISSN: 0925-4773.

NAL Call Number: QH607.A1C4
Abstract: In zebrafish, primordial germ cells (PGCs) are determined by a specialized maternal cytoplasm, the germ plasm, which forms at the distal ends of the cleavage furrows in 4-cell embryos. The germ plasm includes maternal mRNAs from the germline-specific genes such as vasa and nanos1, and vegetally localized dazl RNA is also incorporated into the germ plasm. However, little is known about the distributions and assembly mechanisms of germ plasm components, especially during oogenesis. Here we report that the germ plasm RNAs vasa, nanos1, and dazl co-localize with the mitochondrial cloud (MC) and are transported to the vegetal cortex during early oogenesis. We found that a mitochondrial cloud localization element (MCLE) previously identified in the 3' untranslated region (3'UTR) of *Xenopus* Xcat2 gene can direct RNA localization to the vegetal cortex via the MC in zebrafish oocytes. In addition, the RNA-binding protein Hermes is a component of the MC in zebrafish oocytes, as is the case in *Xenopus*. Moreover, we provide evidence that the dazl 3'UTR possesses at least three types of cis-acting elements that direct multiple steps in the localization process: MC localization, anchorage at the vegetal cortex, and localization at the cleavage furrows. Taken together, the data show that the MC functions as a conserved feature that participates in transport of the germ plasm RNAs in *Xenopus* and zebrafish oocytes. Furthermore, we propose that the germ plasm components are assembled in a stepwise and spatiotemporally-regulated manner during oogenesis and early embryogenesis in zebrafish.
Descriptors: zebrafish egg development, oogenesis physiology, ovum metabolism, RNA metabolism, dead box RNA helicases genetics, molecular sequence data, proteins genetics.

Koshida, S., Y. Kishimoto, H. Ustumi, T. Shimizu, M. Furutani Seiki, H. Kondoh, and S. Takada (2005). **Integrinalpha5-dependent fibronectin accumulation for maintenance of somite boundaries in zebrafish embryos.** *Developmental Cell* 8(4): 587-98. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Boundary formation and epithelialization are crucial processes in the morphological segmentation of vertebrate somites. By a genetic screening procedure with zebrafish, we identified two genes, integrinalpha5 (itga5) and fibronectin (fn), required for these processes. Fibronectin proteins accumulate at somite boundaries in accordance with epithelialization of the somites. Both Fibronectin accumulation and the epithelialization are dependent on itga5, which is expressed in the most medial part of somites. Although somite boundaries are initially formed, but not maintained, in the anterior trunk of the mutant embryos deficient in either gene, their maintenance is defective at all axial levels of embryos deficient for both of these genes. Therefore, Integrinalpha5-directed assembly of Fibronectin appears critical for epithelialization and boundary maintenance of somites. Furthermore, with an additional deficiency in ephrin-B2a, the segmental defect in itga5 or fn mutant embryos is expanded posteriorly, indicating that both Integrin-Fibronectin and Eph-Ephrin systems function cooperatively in maintaining somite boundaries.
Descriptors: zebrafish embryos, body patterning, fibronectins metabolism, integrin alpha5 metabolism, somites cytology, somites physiology, cell polarity, ephrin B2 genetics, ephrin B2 metabolism, epithelium embryology, fibronectins genetics, developmental gene expression regulation, in situ hybridization, integrin alpha5 genetics, morphogenesis, antisense oligonucleotides genetics and metabolism, zebrafish anatomy, histology, genetics and metabolism.

Kozlowski, D.J., T.T. Whitfield, N.A. Hukriede, W.K. Lam, and E.S. Weinberg (2005). **The zebrafish dog-eared mutation disrupts Eya1, a gene required for cell survival and differentiation in the inner ear and lateral line.** *Developmental Biology* 277(1): 27-41. ISSN: 0012 1606.

NAL Call Number: 442.8D49
Abstract: To understand the molecular basis of sensory organ development and disease, we have cloned and characterized the zebrafish mutation dog-eared (dog) that is defective in formation of the inner ear and lateral line sensory systems. The dog locus encodes the eyes absent-1 (eya1) gene and single point mutations were found in three independent dog alleles, each prematurely truncating the expressed protein within the Eya domain. Moreover, morpholino-mediated knockdown of eya1 gene function phenocopies the dog-eared mutation. In zebrafish, the eya1 gene is widely expressed in placode-derived sensory organs during embryogenesis but Eya1 function appears to be primarily required for survival of sensory hair cells in the developing ear and lateral line neuromasts. Increased levels of apoptosis occur in the migrating primordia of the posterior lateral line in dog embryos and as well as in regions of the developing otocyst that are mainly fated to give rise to sensory cells of the cristae. Importantly, mutation of the EYA1 or EYA4 gene causes hereditary syndromic deafness in humans. Determination of eya gene function during zebrafish organogenesis will facilitate understanding the molecular etiology of human vestibular and hearing disorders.
Descriptors: zebrafish embryos, inner ear embryology, trans activators physiology, apoptosis, bone morphogenetic chromosome mapping, hair cells, vestibular embryology, intracellular signaling peptides and proteins, mutation, nuclear proteins, protein tyrosine phosphatase, messenger RNA analysis, trans activators genetics.

Kramer Zucker, A.G., F. Olale, C.J. Haycraft, B.K. Yoder, A.F. Schier, and I.A. Drummond (2005). **Cilia-driven fluid flow in the zebrafish pronephros, brain and Kupffer's vesicle is required for normal organogenesis.** *Development* 132(8): 1907-21. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Cilia, as motile and sensory organelles, have been implicated in normal development, as well as diseases including cystic kidney disease, hydrocephalus and situs inversus. In kidney epithelia, cilia are proposed to be non-motile sensory organelles, while in the mouse node, two cilia populations, motile and non-motile have been proposed to regulate situs. We show that cilia in the zebrafish larval kidney, the spinal cord and Kupffer's vesicle are motile, suggesting that fluid flow is a common feature of each of these organs. Disruption of cilia structure or motility resulted in pronephric cyst formation, hydrocephalus and left-right asymmetry defects. The data show that loss of fluid flow leads to fluid accumulation, which can account for organ distension pathologies in the kidney and brain. In Kupffer's vesicle, loss of flow is associated with loss of left-right patterning, indicating that the 'nodal flow' mechanism of generating situs is conserved in non-mammalian vertebrates.
Descriptors: zebrafish, body fluids physiology, central nervous system embryology, cilia physiology, kidney embryology, organizers, organogenesis physiology, base sequence, body patterning physiology, molecular cloning, DNA primers, dynein ATPase genetics, embryo ultrastructure, immunohistochemistry, in situ hybridization, electron microscopy, fluorescence microscopy, video microscopy, molecular sequence data, nerve tissue oligonucleotides, DNA sequence analysis.

Kramer Zucker, A.G., S. Wiessner, A.M. Jensen, and I.A. Drummond (2005). **Organization of the pronephric filtration apparatus in zebrafish requires Nephrin, Podocin and the FERM domain protein Mosaic eyes.** *Developmental Biology* 285(2): 316-29. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Podocytes are specialized cells of the kidney that form the blood filtration barrier in the kidney glomerulus. The barrier function of podocytes depends upon the development of specialized cell-cell adhesion complexes called slit-diaphragms that form between podo-

cyte foot processes surrounding glomerular blood vessels. Failure of the slit-diaphragm to form results in leakage of high molecular weight proteins into the blood filtrate and urine, a condition called proteinuria. In this work, we test whether the zebrafish pronephros can be used as an assay system for the development of glomerular function with the goal of identifying novel components of the slit-diaphragm. We first characterized the function of the zebrafish homolog of Nephrin, the disease gene associated with the congenital nephritic syndrome of the Finnish type, and Podocin, the gene mutated in autosomal recessive steroid-resistant nephrotic syndrome. Zebrafish nephrin and podocin were specifically expressed in pronephric podocytes and required for the development of pronephric podocyte cell structure. Ultrastructurally, disruption of nephrin or podocin expression resulted in a loss of slit-diaphragms at 72 and 96 h post-fertilization and failure to form normal podocyte foot processes. We also find that expression of the band 4.1/FERM domain gene mosaic eyes in podocytes is required for proper formation of slit-diaphragm cell-cell junctions. A functional assay of glomerular filtration barrier revealed that absence of normal nephrin, podocin or mosaic eyes expression results in loss of glomerular filtration discrimination and aberrant passage of high molecular weight substances into the glomerular filtrate.
Descriptors: zebrafish embryology and ultrastructure, cell adhesion genetics, eye proteins metabolism, kidney glomerulus embryology, membrane proteins metabolism, podocytes metabolism, proteins metabolism, amino acid sequence, molecular cloning, DNA primers, histocytochemistry, in situ hybridization, intracellular signaling peptides and proteins, membrane electron transmission microscopy, molecular sequence data, oligonucleotides.

Kranenbarg, S., J.G. Van Den Boogaart, and J.L. Van Leeuwen (2003). **Oxygen profile in zebra fish embryo (*Danio rerio*) elucidated by theory and experiment.** *Animal Biology (Leiden)* 53(4): 339-346. ISSN: 1568 542X.
Abstract: We present a numerical-experimental diffusion study in which we elucidate the spatial oxygen profile around and inside a zebra fish embryo in the pre-circulation stage (24-28 hpf). Lowest oxygen partial pressures are found in the head with a gradient of posteriorly increasing pressure along the midline of the embryo. Furthermore, this study shows the yolk mass to have a relatively high oxygen permeability as compared to the surrounding medium and the respiring tissue. The oxygen permeability of the respiring tissue in this stage is close to that of water. Knowledge of the details of the oxygen distribution are important for an understanding of vasculogenesis and angiogenesis, since oxygen levels influence the expression of endothelial growth factors. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryo, cardiovascular system, development, respiration, numerical experimental diffusion model, angiogenesis, oxygen partial pressure, oxygen permeability, vasculogenesis.

Kreiling, J.A., G. Williams, and R. Creton (2007). **Analysis of Kupffer's vesicle in zebrafish embryos using a cave automated virtual environment.** *Developmental Dynamics* 236(7): 1963-9. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryo, Kupffer's vesicle, cilia physiology, cytology .

Krone, P.H., T.G. Evans, and S.R. Blechinger (2003). **Heat shock gene expression and function during zebrafish embryogenesis.** *Seminars in Cell and Developmental Biology* 14(5): 267-74. ISSN: 1084-9521.
NAL Call Number: QH573
Abstract: Recent work in the zebrafish, *Danio rerio*, indicates that heat shock genes are

expressed in unique spatial patterns under non-stress conditions. In particular, hsp90alpha is expressed during the normal differentiation of striated muscle fibres, and hsp70-4 is expressed during normal lens development in the eye. Furthermore, disruption of the activity of either of these genes or their protein products gives rise to unique embryonic phenotypes that result from failures in proper somitic muscle development and lens development, respectively. Embryonic hsp70-4 expression is also activated in a cell-specific manner following heavy metal exposure. This has allowed for the development of a hsp70-4/eGFP reporter gene system in stable transgenic zebrafish that serves as a reliable yet extremely quick indicator of cell-specific toxicity in the context of the multicellular, living embryo.
Descriptors: zebrafish embryos, HSP70 heat shock proteins metabolism and genetics, HSP90 heat shock proteins metabolism and genetics, heat shock response physiology, skeletal muscle metabolism, molecular cloning, metabolism, embryonic development, developmental gene expression regulation physiology, heat shock response genetics, skeletal muscle embryology, somites metabolism.

Krovel, A.V. and L.C. Olsen (2004). **Sexual dimorphic expression pattern of a splice variant of zebrafish vasa during gonadal development.** *Developmental Biology* 271(1): 190-7. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: In *Drosophila*, the RNA helicase VASA (VAS) is required for both germ line formation and oocyte differentiation. While the murine VAS homologue is required for spermatogenesis, it is dispensable for germ line formation. The molecular basis for this apparently dual role of VAS in germ line ontogeny is, however, unclear. Recent evidence indicates that fish, like flies, employs VAS both in early and late stages of the germ line development and that there is a sex-linked differential expression of splice variants. We show here that the longer of two splice variants of zebrafish vas is transiently downregulated in the germ line around the time when the germ cells reach the developing gonad. Using transgenic vas::EGFP fish lines, which allow us to distinguish between male and female individuals, we show that the long splice variant reappears in both sexes at around day 25 and is subsequently downregulated during male gonadal development. Our data further suggest that there is a switch from maternal to zygotic expression of the long splice variant of vas as sexual dimorphic development commences.
Descriptors: zebrafish embryos, alternative splicing genetics, developmental gene expression regulation, germ cells growth and development, gonads embryology, sex characteristics, *Xenopus* proteins metabolism, DNA primers, fluorescence microscopy, reverse transcriptase PCR, transgenes.

Kubota, F., T. Murakami, K. Mogi, and H. Yorifuji (2007). **Cadherin-6 is required for zebrafish nephrogenesis during early development**. *International Journal of Developmental Biology* 51(2): 123-129. ISSN: 0214-6282.
Descriptors: zebrafish embryos, amphibiotic species, antisense oligonucleotides, biological fertilization, edema, embryonic development, kidney development, glomerulus, nephrons, oligonucleotides, peripheral nervous system, primordia, tails, thorax, tubules, yolk, *Danio rerio*, *Xenopus*, clawed frogs.

Kuchler, A.M., E. Gjini, J. Peterson Maduro, B. Cancilla, H. Wolburg, and S. Schulte Merker (2006). **Development of the zebrafish lymphatic system requires VEGFC signaling.** *Current Biology* 16(12): 1244-8. ISSN: 0960 9822.
NAL Call Number: QH301.C85

Abstract: Lymphangiogenesis results in the formation of a vascular network distinct from arteries and veins that serves to drain interstitial fluid from surrounding tissues and plays a pivotal role in the immune defense of vertebrates as well as in the progression of cancer and other diseases . In mammals, lymph vessels are lined by endothelial cells possibly sprouting from embryonic veins, and their development appears to be critically dependent on the function of PROX1 and VEGFC signaling . The existence of a lymphatic system in teleosts has been a matter of debate for decades. Here we show on the morphological, molecular, and functional levels that zebrafish embryos develop a lymphatic vasculature that serves to retrieve components of the interstitium to the lymph system. We demonstrate the existence of vessels that are molecularly and functionally distinct from blood vessels and show that the development of these vessels depends on Vegfc and VEGFR-3/Flt4 signaling. These findings imply that the molecular components controlling lymphangiogenesis in zebrafish and mammals are conserved and that the zebrafish lymphatic system develops early enough to allow in vivo observations, lineage tracing, and genetic as well as pharmacological screens.
Descriptors: zebrafish embryos, lymphatic vessels embryology, signal transduction, vascular endothelial growth factor C metabolism, proteins metabolism, angiopoietin 2 metabolism, genetically modified anatomy and histology, genetically modified metabolism, biological transport, cytology, metabolism, endothelium, lymphatic cytology, endothelium, lymphatic metabolism, extracellular fluid metabolism, green fluorescent proteins analysis, lymphatic vessels metabolism, vascular endothelial growth factor receptor 3 metabolism, .

Kudo, H., N. Amizuka, K. Araki, K. Inohaya, and A. Kudo (2004). **Zebrafish periostin is required for the adhesion of muscle fiber bundles to the myoseptum and for the differentiation of muscle fibers.** *Developmental Biology* 267(2): 473-87. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The myoseptum of fishes, composed of dense collagen, is a connective tissue layer that forms in the embryo, dividing somites from the trunk, and its structure and function are similar to those of the mammalian tendon. Both the myoseptum and tendon serve as the transmitter of muscular contractility to bones and adjoining muscles, and their structure is indispensable for movement of vertebrate animals. We cloned the zebrafish periostin gene and examined its expression and function in the myoseptum. The expression in embryos started in the rostral part of each segmented somite in the early segmentation stage; and consequently, metameric stripes were observed. At the end of segmentation, the expression region shifted to the transverse myoseptum and the myotome-epidermis boundary, and each myotome was surrounded by periostin. Using a polyclonal antibody, we found that the periostin protein was localized to the transverse myoseptum. Consistently, periostin morpholino antisense oligonucleotide led to defects in myoseptum formation, a delay in the differentiation of myofibers, and disorder of connection between myofibrils and myoseptum. We demonstrated here that periostin is the first molecule involved in myoseptum formation and propose that periostin secretion on the surface of the myoseptum is required for the adhesion of muscle fiber bundles to the myoseptum and the differentiation of muscle fibers.
Descriptors: zebrafish, cell adhesion molecules genetics, cell adhesion molecules physiology, cell differentiation physiology, connective tissue embryology, muscle fibers physiology, amino acid sequence, Western blotting, cell adhesion molecules metabolism, connective tissue metabolism, DNA primers, complementary DNA genetics, ELISA, gene expression profiling, histological techniques, immunohistochemistry, in situ hybridization , electron microscopy, molecular sequence data, morphogenesis, muscle fibers cytology and metabolism, phylogeny, somites ultrastructure, proteins.

Kulesa, P., D.L. Ellies, and P. Trainor (2004). **Comparative analysis of neural crest cell death, migration, and function during vertebrate embryogenesis.** *Developmental Dynamics* 229(1): 14-29. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Cranial neural crest cells are a multipotent, migratory population that generates most of the cartilage, bone, connective tissue and peripheral nervous system in the vertebrate head. Proper neural crest cell patterning is essential for normal craniofacial morphogenesis and is highly conserved among vertebrates. Neural crest cell patterning is intimately connected to the early segmentation of the neural tube, such that neural crest cells migrate in discrete segregated streams. Recent advances in live embryo imaging have begun to reveal the complex behaviour of neural crest cells which involve intricate cell-cell and cell-environment interactions. Despite the overall similarity in neural crest cell migration between distinct vertebrates species there are important mechanistic differences. Apoptosis for example, is important for neural crest cell patterning in chick embryos but not in mouse, frog or fish embryos. In this paper we highlight the potential evolutionary significance of such interspecies differences in jaw development and evolution. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, chicken, mouse, frog development, nervous system, comparative analysis, live embryo imaging, imaging and microscopy techniques, apoptosis, apoptotic cell death, cell migratory behaviors, cell patterning, cell -cell interactions, cell environment interactions, cellular differentiation, craniofacial morphogenesis, embyrogenesis, segmental patterning.

Kunwar, P.S., S. Zimmerman, J.T. Bennett, Y. Chen, M. Whitman, and A.F. Schier (2003). **Mixer/bon and foxh1/sur have overlapping and divergent roles in nodal signaling and mesendoderm induction.** *Development* 130(23): 5589-5599. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Descriptors: zebrafish embryo, biochemistry, development, Nodal signaling, embryogenesis, mesendoderm induction.

Kurita, R., H. Sagara, Y. Aoki, B.A. Link, K. Arai, and S. Watanabe (2003). **Suppression of lens growth by alphaA-crystallin promoter-driven expression of diphtheria toxin results in disruption of retinal cell organization in zebrafish.** *Developmental Biology* 255(1): 113-27. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: In order to study lens-retina relationships during development, we cloned the zebrafish alphaA-crystallin cDNA and its promoter region. Using a 2.8-kb fragment of the zebrafish alphaA-crystallin promoter (z(alpha)Acry), we expressed the diphtheria toxin A fragment (DTA) in zebrafish embryos in a lens-specific manner. Injection of the z(alpha) Acry-DTA plasmid into eggs at the one-or two-cell stage resulted in the formation of small eyes, in which both lens and retina were reduced in size. In the DTA-expressing lenses, their fiber structure was disorganized, indicating that normal lens development had been abrogated. The neural retina also showed abnormal development, although this tissue did not express DTA. Lamination in the retina did not develop well, and molecular markers for the outer and inner plexiform layers were either abnormally expressed or absent. However, cell type-specific markers of ganglion and bipolar cells, as well as photoreceptors, were expressed in appropriate positions, indicating that initial differentiation of these retinal subpopulations occurred in the DTA-expressing embryos. Cell proliferation also proceeded normally in these embryos, although apoptosis was enhanced. These results suggest that the differentiated lens plays a critical role in the morphogenetic organization of retinal cells during eye development

in zebrafish embryos.
Descriptors: zebrafish, diphtheria toxin genetics, crystalline lens embryology, retina embryology, alpha crystallins genetics, apoptosis, base sequence, cell differentiation, cell division, ultrastructure, gene deletion, gene expression, mutation, promoter regions genetics , messenger RNA genetics, retina cytology, retina ultrastructure, sequence alignment, transgenes, alpha crystallins metabolism.

Kwak, S.J., S. Vemaraju, S.J. Moorman, D. Zeddies, A.N. Popper, and B.B. Riley (2006). **Zebrafish pax5 regulates development of the utricular macula and vestibular function.** *Developmental Dynamics* 235(11): 3026-38. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryo, acoustic maculae growth and development, B cell specific activator protein physiology, hair cells, vestibular growth and development and physiology, growth and development, acoustic maculae chemistry and cytology, amino acid sequence, B cell specific activator protein analysis and genetics, base sequence, molecular cloning, fibroblast growth factor 3 analysis and genetics and metabolism, vestibular chemistry, hair cells, vestibular metabolism, larva chemistry, larva cytology, larva growth and development, molecular sequence data, mutation, antisense oligonucleotides pharmacology, pax2 transcription factor analysis, pax2 transcription factor genetics and metabolism , messenger RNA analysis and metabolism, saccule and utricle chemistry and cytology and growth and development, vestibule chemistry and cytology, proteins analysis and genetics.

Kwan, K.M., E. Fujimoto, C. Grabher, B. Mangum, M.E. Hardy, D.S. Campbell, J.M. Parant, H.J. Yost, J.P. Kanki, and C.-B. Chien (2007). **The Tol2kit: A multisite gateway-based construction kit for Tol2 transposon transgenesis constructs**. *Developmental Dynamics* 236(11): 3088-3099. ISSN: 1058-8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryo, actin, construction, expression vectors, fluorescent indicators, green fluorescent protein, HSP70 protein, integration, mosaicism, promoters, ribosomes, transposons, *Danio rerio*.

Kwok, C.W. and S.H. Cheng (2004). **Effects of ethanol on craniofacial development of zebrafish embryos.** *Developmental Biology* 271(2): 590-591. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, toxicology, nervous system, development, skeletal system, molecular genetics, holoprosencephaly, nervous system disease, congenital disease, cyclopia, congenital disease, craniofacial development, ethanol effects.

Lacosta, A.M., J. Canudas, C. Gonzalez, P. Muniesa, M. Sarasa, and L. Dominguez (2007). **Pax7 identifies neural crest, chromatophore lineages and pigment stem cells during zebrafish development**. *International Journal of Developmental Biology* 51(4): 327-331. ISSN: 0214-6282.
Descriptors: zebrafish, cell migration, chromatophores, cytology, embryogenesis, embryonic development, freshwater fish, larval development, melanin, melanocytes, neural crest, neural stem cells, Pax3 protein, pigments, skull, stem cells, *Danio rerio*.

Laguerre, L., F. Soubiran, A. Ghysen, N. Konig, and C. Dambly Chaudiere (2005). **Cell proliferation in the developing lateral line system of zebrafish embryos.** *Developmental Dynamics* 233(2): 466-72. ISSN: 1058 8388.

NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, body patterning physiology, cytology, embryology, bromodeoxyuridine, cell movement, cell proliferation, functional laterality.

Lakowski, J., A. Majumder, and J.D. Lauderdale (2007). **Mechanisms controlling Pax6 isoform expression in the retina have been conserved between teleosts and mammals**. *Developmental Biology* 307(2): 498-520. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, amacrine cells, bacterial artificial chromosomes, cell fate, cell proliferation, chromosomes, conservation, freshwater fish, gene expression, Pax6 protein, phylogenetics, promoters, retina, retinas, stem cells, transcription, transgenic mice, *Danio rerio*.

Lamoreux, M.L., R.N. Kelsh, Y. Wakamatsu, and K. Ozato (2005). **Pigment pattern formation in the medaka embryo**. *Pigment Cell Research* 18(2): 64-73. ISSN: 0893 5785.
Abstract: For the study of development of pigmentation, compared with mammalian models, fish offer the advantage of multiple chromatophore types and ready access to the developing embryo for observation and experimental manipulation. Compared with zebrafish embryos, *medaka* embryos have an additional unique chromatophore-type and superb properties for conditional mutation studies. The rich resources of medaka mutants, combined with data obtainable from other species, potentially offer information not otherwise readily available regarding chromatophore lineage. Here we summarize the embryonic development of normal medaka pigment pattern, based on observations using embryos of a panel of wild type and mutant fish. A more detailed description of development is available in the appendix of the on-line version of this paper (see Supplementary Material). We make some comparisons with zebrafish development to emphasize the increased power of both systems when utilized together. These two models will, in combination, be a powerful system for studies of the embryogenesis and evolution of pigmentation. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, embryonic development, embryogenesis, pigmentation, pigment pattern, chromatophore lineage, *medaka*.

Lampert, J.M., J. Holzschuh, S. Hessel, W. Driever, K. Vogt, and J. von Lintig (2003). **Provitamin A conversion to retinal via the beta,beta-carotene-15,15'-oxygenase (bcox) is essential for pattern formation and differentiation during zebrafish embryogenesis.** *Development* 130(10): 2173-86. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The egg yolk of vertebrates contains carotenoids, which account for its characteristic yellow color in some species. Such plant-derived compounds, e.g. beta-carotene, serve as the natural precursors (provitamins) of vitamin A, which is indispensable for chordate development. As egg yolk also contains stored vitamin A, carotenoids have so far been solely discussed as pigments for the coloration of the offspring. Based on our recent molecular identification of the enzyme catalyzing provitamin A conversion to vitamin A, we address a possible role of provitamin A during zebrafish (*Danio rerio*) development. We cloned the zebrafish gene encoding the vitamin A-forming enzyme, a beta,beta-carotene-15,15'-oxygenase. Analysis of its mRNA expression revealed that it is under complex spatial and temporal control during development. Targeted gene knockdown using the morpholino antisense oligonucleotide technique indicated a vital role of the provitamin A-converting enzyme. Morpholino-injected embryos developed a morphological phenotype that included

severe malformation of the eyes, the craniofacial skeleton and pectoral fins, as well as reduced pigmentation. Analyses of gene expression changes in the morphants revealed that distinct retinoic acid-dependent developmental processes are impaired, such as patterning of the hindbrain and differentiation of hindbrain neurons, differentiation of neural crest derivatives (including the craniofacial skeleton), and the establishment of the ventral retina. Our data provide strong evidence that, for several developmental processes, retinoic acid generation depends on local de novo formation of retinal from provitamin A via the carotene oxygenase, revealing an unexpected, essential role for carotenoids in embryonic development.
Descriptors: zebrafish embryo, body patterning physiology, cell differentiation physiology, oxygenases metabolism, retinaldehyde metabolism, vitamin A metabolism, amino acid sequence, molecular cloning, developmental gene expression regulation, in situ hybridization, molecular sequence data, monoterpenes metabolism, morphogenesis physiology, neural crest embryology, antisense oligonucleotides metabolism, ovum chemistry, oxygenases genetics, pharynx anatomy and histology and embryology, phenotype, retina embryology, rhombencephalon growth and development, sequence alignment, tretinoin metabolism, vitamin A chemistry , beta carotene 15,15' monooxygenase.

Langenberg, T. and M. Brand (2005). **Lineage restriction maintains a stable organizer cell population at the zebrafish midbrain-hindbrain boundary.** *Development* 132(14): 3209-16. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The vertebrate hindbrain is subdivided into segments, termed neuromeres, that are units of gene expression, cell differentiation and behavior. A key property of such segments is that cells show a restricted ability to mix across segment borders -- termed lineage restriction. In order to address segmentation in the midbrain-hindbrain boundary (mhb) region, we have analyzed single cell behavior in the living embryo by acquiring time-lapse movies of the developing mhb region in a transgenic zebrafish line. We traced the movement of hundreds of nuclei, and by matching their position with the expression of a midbrain marker, we demonstrate that midbrain and hindbrain cells arise from two distinct cell populations. Single cell labeling and analysis of the distribution of their progeny shows that lineage restriction is probably established during late gastrulation stages. Our findings suggest that segmentation as an organizing principle in early brain development can be extended to the mhb region. We argue that lineage restriction serves to constrain the position of the mhb organizer cell population.
Descriptors: zebrafish, cell lineage physiology, mesencephalon embryology, organizers, embryonic embryology, rhombencephalon embryology, cell lineage genetics, gene expression physiology, reporter genes, iontophoresis, mesencephalon cytology, organizers, embryonic cytology, rhombencephalon cytology, time factors.

Langenberg, T., M. Brand, and M.S. Cooper (2003). **Imaging brain development and organogenesis in zebrafish using immobilized embryonic explants.** *Developmental Dynamics* 228(3): 464-74. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, cytology, physiology, morphogenesis physiology, organogenesis physiology, body patterning physiology, cell movement physiology, culture media, organ culture techniques methods.

Langenberg, T., T. Dracz, A.C. Oates, C.P. Heisenberg, and M. Brand (2006). **Analysis and visualization of cell movement in the developing zebrafish brain.** *Developmental Dynamics* 235(4): 928-33. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, cell movement, mesencephalon cytology, mesencephalon embryology and cytology, metencephalon embryology, cell lineage, computer graphics, statistical data interpretation, mesencephalon physiology, metencephalon physiology, video microscopy, software, time factors.

Larson, J.D., S.A. Wadman, E. Chen, L. Kerley, K.J. Clark, M. Eide, S. Lippert, A. Nasevicius, S.C. Ekker, P.B. Hackett, and J.J. Essner (2004). **Expression of VE-cadherin in zebrafish embryos: a new tool to evaluate vascular development.** *Developmental Dynamics* 231(1): 204-13. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: We have identified the zebrafish homologue of VE-cadherin and documented its expression in the developing vascular system. The zebrafish VE-cadherin gene is specifically expressed in the vascular endothelial cell lineage beginning with the differentiation and migration of angioblasts and persists throughout vasculogenesis, angiogenesis, and endocardium development. Staining zebrafish embryos by whole-mount in situ hybridization with the VE-cadherin probe provides a method to screen embryos for vascular defects. To illustrate this utility, we used VE-cadherin expression to demonstrate a conservation of vascular endothelial growth factor-A (VEGF-A) function. The morpholino antisense oligonucleotide knockdown of VEGF-A function in zebrafish embryos results in a loss of angiogenic blood vessels, as indicated by the lack of VE-cadherin expression in the intersegmental vasculature. This loss can be restored in embryos supplemented with either zebrafish or human VEGF-A, the latter indicating that genes crucial to angiogenesis have highly conserved functional activities in vertebrates.
Descriptors: zebrafish embryos, blood vessels metabolism, cadherins metabolism, endothelial cells metabolism, physiologic neovascularization physiology, amino acid sequence, antigens, cd, blood vessels cytology, metabolism, endothelial cells cytology, molecular sequence data, amino acid sequence homology, metabolism.

Latimer, A.J. and B. Appel (2006). **Notch signaling regulates midline cell specification and proliferation in zebrafish.** *Developmental Biology* 298(2): 392-402. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Notochord and floor plate cells are sources of molecules that pattern tissues near the midline, including the spinal cord. Hypochord cells are also found at the midline of anamniote embryos and are important for aorta development. Delta-Notch signaling regulates midline patterning in the dorsal organizer by inhibiting notochord formation and promoting hypochord and possibly floor plate development, but the precise mechanisms by which this regulation occurs are unknown. We demonstrate here that floor plate and hypochord cells arise from distinct regions of the zebrafish shield. Blocking Notch signaling during gastrulation entirely prevented hypochord specification but only reduced the number of floor plate cells that developed compared to control embryos. In contrast, elevation of Notch signaling at the beginning of gastrulation caused expansion of hypochord at the expense of notochord, but floor plate was not affected. A cell proliferation assay revealed that Notch signaling maintains dividing floor plate progenitors. Together, our results indicate that Notch signaling regulates allocation of appropriate numbers of different midline cells by different mechanisms.

Descriptors: zebrafish embryos, body patterning, Notch receptors physiology, genetically modified, cell differentiation, cell proliferation gastrula metabolism, gastrula physiology, developmental gene expression regulation, notochord embryology, Notch receptors genetics and metabolism, signal transduction, stem cells physiology, T-box domain proteins.

Latimer, A.J., J. Shin, and B. Appel (2005). **Her9 promotes floor plate development in zebrafish.** *Developmental Dynamics* 232(4): 1098-104. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, developmental gene expression regulation physiology, notochord embryology, signal transduction physiology, proteins metabolism, basic helix loop helix transcription factors, membrane proteins metabolism, notochord cytology, Notch receptors, signal transduction genetics, genetics.

Lau, G.T.C., W.H. Tsang, Y.W. Lee, and K.L. Chow (2004). **Role of MAB2112 in vertebrate development.** *Developmental Biology* 271(2): 589-590. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, nervous system, development, molecular genetics, MAB2112.

Laurenti, P., C. Thaeron, F. Allizard, A. Huysseune, and J.Y. Sire (2004). **Cellular expression of eve1 suggests its requirement for the differentiation of the ameloblasts and for the initiation and morphogenesis of the first tooth in the zebrafish (*Danio rerio*).** *Developmental Dynamics* 230(4): 727-33. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos and larvae, ameloblasts metabolism, carrier proteins biosynthesis, carrier, muscle proteins biosynthesis, muscle, odontogenesis, tooth embryology, body patterning, cell differentiation, cell lineage, enamel organ physiology, in situ hybridization, time factors.

Lawrence, N.A. and V. Morel (2003). **Dorsal closure and convergent extension: two polarised morphogenetic movements controlled by similar mechanisms?** *Mechanisms of Development* 120(11): 1385-1393. ISSN: 0925 4773.
NAL Call Number: QH607.A1C4
Descriptors: zebrafish, development, dorsal closure, morphogenetic movements, zebrafish.

Le Guellec, D., G. Morvan Dubois, and J.Y. Sire (2004). **Skin development in bony fish with particular emphasis on collagen deposition in the dermis of the zebrafish (*Danio rerio*).** *International Journal of Developmental Biology, The* 48(2-3): 217-31. ISSN: 0214-6282.
Abstract: The first part of this article is a review of the current status of knowledge of the fish skin, with particular attention to its development. In the second part we present original results obtained in zebrafish (*Danio rerio*), with particular emphasis on the deposition and organisation of the dermal collagenous stroma. Using a series of zebrafish specimens aged between 15 hours postfertilization (hpf) and 4.5 years old, we have combined Transmission Electron Microscopy (TEM) observations and in situ hybridisation using type I collagen a2 chain (Col1a2) probe. Collagen fibrils, with a diameter of 22 nm, appear first in an acellular subepidermal space at 24 hpf, are first all oriented in the same direction, and form the primary dermal stroma. Subsequently, three events occur. (1) From 5-7 days pf (dpf) onwards the collagen fibrils self-organise into several lamellae arranged in a plywood-like structure, starting in the upper layers and progressing throughout the entire thickness of the

dermis. (2) At 20-26 dpf, fibroblasts of unknown origin progressively invade the acellular collagenous stroma, some of them accumulating below the epidermis. (3) Concomitant with the invasion of fibroblasts, the collagen fibrils increase progressively in diameter to reach 160 nm towards the end of the fish life. In situ hybridisation experiments reveal that, between 24 and 48 hpf, the collagen matrix is produced by the epidermis only. From 72 hpf to 20-26 dpf, both the basal epidermal cells and the dermal cells bordering the deep region of the dermis are involved in the production of collagen. When the fibroblasts invade the plywood-like structure, the epidermal cells progressively cease to synthesise collagen, which from this point is produced only by the fibroblasts. This suggests that the fibroblasts secrete a still unidentified signalling molecule that downregulates collagen production by the epidermis.
Descriptors: zebrafish embryos, collagen Type I metabolism, dermis metabolism, skin metabolism, growth and development, cell movement, collagen biosynthesis and ultrastructure, collagen Type I ultrastructure, dermis chemistry and ultrastructure, down regulation, embryo metabolism and ultrastructure, epidermis metabolism, epidermis ultrastructure, extracellular matrix metabolism and ultrastructure, fibroblasts metabolism, physiology and ultrastructure, developmental gene expression regulation, in situ hybridization, larva, messenger RNA metabolism, messenger RNAultrastructure, skin embryology, skin ultrastructure, time factors.

Le Guyader, S., J. Maier, and S. Jesuthasan (2005). **Esrom, an ortholog of PAM (protein associated with c-myc), regulates pteridine synthesis in the zebrafish.** *Developmental Biology* 277(2): 378-86. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Zebrafish esrom mutants have an unusual combination of phenotypes: in addition to a defect in the projection of retinal axons, they have reduced yellow pigmentation. Here, we investigate the pigment phenotype and, from this, provide evidence for an unexpected defect in retinal neurons. Esrom is not required for the differentiation of neural crest precursors into pigment cells, nor is it essential for cell migration, pigment granule biogenesis, or translocation. Instead, loss of yellow color is caused by a deficiency of sepiapterin, a yellow pteridine. The level of several other pteridines is also affected in mutants. Importantly, the cofactor tetrahydrobiopterin (BH4) is drastically reduced in esrom mutants. Mutant retinal neurons also appear deficient in this pteridine. BH4-synthesizing enzymes are active in mutants, indicating a defect in the regulation rather than production of enzymes. Esrom has recently been identified as an ortholog of PAM (protein associated with c-myc), a very large protein involved in synaptogenesis in Drosophila and C. elegans. These data thus introduce a new regulator of pteridine synthesis in a vertebrate and establish a function for the Esrom protein family outside synaptogenesis. They also raise the possibility that neuronal defects are due in part to an abnormality in pteridine synthesis.
Descriptors: zebrafish embryos, signal transducing adaptor proteins metabolism, biopterin analogs and derivatives, enzymologic gene expression regulation, mixed function oxygenases metabolism, pigmentation physiology, pterins metabolism, retina metabolism, proteins metabolism, signal transducing adaptor biopterin metabolism, thin layer chromatography, embryology and enzymology, ultrastructure, electron microscopy, mixed function oxygenases genetics.

Lecaudey, V., I. Anselme, F. Rosa, and S. Schneider Maunoury (2004). **The zebrafish Iroquois gene iro7 positions the r4/r5 boundary and controls neurogenesis in the rostral hindbrain.** *Development* 131(13): 3121-31. ISSN: 0950 1991.
NAL Call Number: QL951.D38

Abstract: Early brain regionalisation involves the activation of genes coding for transcription factors in distinct domains of the neural plate. The limits of these domains often prefigure morphological boundaries. In the hindbrain, anteroposterior patterning depends on a segmentation process that leads to the formation of seven bulges called rhombomeres (r). The molecular cues involved in the early subdivision of the hindbrain and in rhombomere formation are not well understood. We show that iro7, a zebrafish gene coding for a transcription factor of the Iroquois family, is expressed at the end of gastrulation in the future midbrain and hindbrain territories up to the prospective r4/r5 boundary. This territory is strictly complementary to the expression domain of another homeobox gene, vhnf1, in the caudal neural plate. We demonstrate that Iro7 represses vhnf1 expression anterior to their common border and that, conversely, vHnf1 represses iro7 expression caudal to it. This suggests that the r4/r5 boundary is positioned by mutual repression between these two transcription factors. In addition, iro7 is involved in the specification of primary neurons in the rostral hindbrain. In particular, it is essential for the formation of the Mauthner neurons in r4. We propose that iro7 has a dual function in the hindbrain of the zebrafish embryo: it is required for the proper positioning of the prospective r4/r5 boundary and it promotes neurogenesis in the anterior hindbrain.
Descriptors: zebrafish embryos, developmental gene expression regulation, homeodomain, neurons metabolism, rhombencephalon embryology, complementary DNA metabolism, DNA binding proteins genetics, proteins biosynthesis, gene library, hepatocyte nuclear factor 1 beta, immunohistochemistry, in situ hybridization, protein biosynthesis, RNA metabolism, signal transduction, time factors, transcription factors biosynthesis, genetic transcription.

Lecaudey, V., E. Ulloa, I. Anselme, A. Stedman, S. Schneider Maunoury, and C. Pujades (2007).
Role of the hindbrain in patterning the otic vesicle: a study of the zebrafish vhnf1 mutant. *Developmental Biology* 303(1): 134-43. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The vertebrate inner ear develops from an ectodermal placode adjacent to rhombomeres 4 to 6 of the segmented hindbrain. The placode then transforms into a vesicle and becomes regionalised along its anteroposterior, dorsoventral and mediolateral axes. To investigate the role of hindbrain signals in instructing otic vesicle regionalisation, we analysed ear development in zebrafish mutants for vhnf1, a gene expressed in the caudal hindbrain during otic induction and regionalisation. We show that, in vhnf1 homozygous embryos, the patterning of the otic vesicle is affected along both the anteroposterior and dorsoventral axes. First, anterior gene expression domains are either expanded along the whole anteroposterior axis of the vesicle or duplicated in the posterior region. Second, the dorsal domain is severely reduced, and cell groups normally located ventrally are shifted dorsally, sometimes forming a single dorsal patch along the whole AP extent of the otic vesicle. Third, and probably as a consequence, the size and organization of the sensory and neurogenic epithelia are disturbed. These results demonstrate that, in zebrafish, signals from the hindbrain control the patterning of the otic vesicle, not only along the anteroposterior axis, but also, as in amniotes, along the dorsoventral axis. They suggest that, despite the evolution of inner ear structure and function, some of the mechanisms underlying the regionalisation of the otic vesicle in fish and amniotes have been conserved.
Descriptors: zebrafish, inner ear embryology, hepatocyte nuclear factor 1 metabolism, morphogenesis physiology, rhombencephalon physiology, signal transduction physiology, inner ear metabolism, in situ hybridization, phalloidine, rhombencephalon metabolism.

Lee, B.C. and S. Roy (2006). **Blimp-1 is an essential component of the genetic program controlling development of the pectoral limb bud**. *Developmental Biology* 300(2): 623-634. ISSN: 0012-1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish, BLIMp-1 protein, BLIMP-1 gene, ectoderm, embryonic development, embryos, fibroblast growth factor 10, fins, freshwater fish, gene expression, Hedgehog protein, limb buds, limbs, mesenchyme, pattern formation, primordia, skin, transcription, zinc , amniota, *Danio rerio*.

Lee, H.C., H.Y. Huang, C.Y. Lin, Y.H. Chen, and H.J. Tsai (2006). **Foxd3 mediates zebrafish myf5 expression during early somitogenesis.** *Developmental Biology* 290(2): 359-72. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Myf5, one of the basic helix-loop-helix transcription factors, controls muscle differentiation and is expressed in somites during early embryogenesis. However, the transcription factors bound to the cis-elements of myf5 are poorly understood. In this study, we used the yeast one-hybrid assay and found that Forkhead box d3 (Foxd3) interacted specifically with the -82/-62 cassette, a key element directing somite-specific expression of myf5. The dual-luciferase assay revealed that the expression of Foxd3 potently transactivated the myf5 promoter. Knocking down foxd3 with morpholino oligonucleotide (MO) resulted in a dramatic down-regulation of myf5 in somites and adaxial cells but not in the presomitic mesoderm. On the other hand, myod expression remained unchanged in foxd3 morphants. Foxd3 mediation of myf5 expression is stage-dependent, maintaining myf5 expression in the somites and adaxial cells during the 7- to 18-somite stage. Furthermore, in the pax3 morphant, the expression of foxd3 was down-regulated greatly and the expression of myf5 was similar to that of the foxd3 morphant. Co-injection of foxd3 mRNA and pax3-MO1 greatly restored the expression of myf5 in the somites and adaxial cells, suggesting that pax3 induces foxd3 expression, which then induces the expression of myf5. This report is the first study to show that Foxd3, a well-known regulator in neural crest development, is also involved in myf5 regulation.

Descriptors: zebrafish, forkhead transcription factors physiology, developmental gene expression regulation, myogenic regulatory factor 5 biosynthesis, somites metabolism, COS cells, cultured cells, cercopithecus aethiops, molecular cloning, complementary DNA metabolism, drug dose response relationship, down regulation, forkhead transcription factors metabolism, in situ hybridization, luciferases metabolism, mesoderm metabolism, neural crest embryology, oligonucleotides chemistry, paired box transcription factors metabolism, plasmids metabolism, protein binding, messenger RNA metabolism, recombinant proteins chemistry, time factors, trans activation genetics, two hybrid system techniques, zebrafish.

Lee, H.C., J.N. Tsai, P.Y. Liao, W.Y. Tsai, K.Y. Lin, C.C. Chuang, C.K. Sun, W.C. Chang, and H.J. Tsai (2007). **Glycogen synthase kinase 3 alpha and 3 beta have distinct functions during cardiogenesis of zebrafish embryo.** *BioMed Central Developmental Biology* 7: 93. ISSN: 1471 213X.

NAL Call Number: QH491.B63

Abstract: BACKGROUND: Glycogen synthase kinase 3 (GSK3) encodes a serine/threonine protein kinase, is known to play roles in many biological processes. Two closely related GSK3 isoforms encoded by distinct genes: GSK3alpha (51 kDa) and GSK3beta (47 kDa). In previously studies, most GSK3 inhibitors are not only inhibiting GSK3, but are also affecting many other kinases. In addition, because of highly similarity in amino acid sequence between

GSK3alpha and GSK3beta, making it difficult to identify an inhibitor that can be selective against GSK3alpha or GSK3beta. Thus, it is relatively difficult to address the functions of GSK3 isoforms during embryogenesis. At this study, we attempt to specifically inhibit either GSK3alpha or GSK3beta and uncover the isoform-specific roles that GSK3 plays during cardiogenesis. RESULTS: We blocked gsk3alpha and gsk3beta translations by injection of morpholino antisense oligonucleotides (MO). Both gsk3alpha- and gsk3beta-MO-injected embryos displayed similar morphological defects, with a thin, string-like shaped heart and pericardial edema at 72 hours post-fertilization. However, when detailed analysis of the gsk3alpha- and gsk3beta-MO-induced heart defects, we found that the reduced number of cardiomyocytes in gsk3alpha morphants during the heart-ring stage was due to apoptosis. On the contrary, gsk3beta morphants did not exhibit significant apoptosis in the cardiomyocytes, and the heart developed normally during the heart-ring stage. Later, however, the heart positioning was severely disrupted in gsk3beta morphants. bmp4 expression in gsk3beta morphants was up-regulated and disrupted the asymmetry pattern in the heart. The cardiac valve defects in gsk3beta morphants were similar to those observed in axin1 and apcmcr mutants, suggesting that GSK3beta might play a role in cardiac valve development through the Wnt/beta-catenin pathway. Finally, the phenotypes of gsk3alpha mutant embryos cannot be rescued by gsk3beta mRNA, and vice versa, demonstrating that GSK3alpha and GSK3beta are not functionally redundant. CONCLUSION: We conclude that (1) GSK3alpha, but not GSK3beta, is necessary in cardiomyocyte survival; (2) the GSK3beta plays important roles in modulating the left-right asymmetry and affecting heart positioning; and (3) GSK3alpha and GSK3beta play distinct roles during zebrafish cardiogenesis.
Descriptors: zebrafish, glycogen synthase kinase 3 genetics, genetically modified, apoptosis, Western blotting glycogen synthase kinase 3 antagonists and inhibitors, heart embryology, isoenzymes antagonists and inhibitors, isoenzymes genetics, cardiac myocytes cytology, cardiac myocytes enzymology, messenger RNA .

Lee, J.R., Y.C. Tseng, and P.P. Hwang (2005). **Glycogen distribution patterns in developing zebrafish (*Danio rerio*)**. *Journal of the Fisheries Society of Taiwan* 32(1): 102-103. ISSN: 0379-4180.
NAL Call Number: SH135.T3
Descriptors: zebrafish embryos, central nervous system, developmental stages, embryonic development, energy resources, freshwater fish, glycogen, monoclonal antibodies, *Danio rerio*.

Lee, J.E., S.F. Wu, L.M. Goering, and R.I. Dorsky (2006). **Canonical Wnt signaling through Lef1 is required for hypothalamic neurogenesis**. *Development* 133(22): 4451-4461. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Although the functional importance of the hypothalamus has been demonstrated throughout vertebrates, the mechanisms controlling neurogenesis in this forebrain structure are poorly understood. We report that canonical Wnt signaling acts through Lef1 to regulate neurogenesis in the zebrafish hypothalamus. We show that Lef1 is required for proneural and neuronal gene expression, and for neuronal differentiation in the posterior hypothalamus. Furthermore, we find that this process is dependent on Wnt8b, a ligand of the canonical pathway expressed in the posterior hypothalamus, and that both Wnt8b and Lef1 act to mediate beta-catenin-dependent transcription in this region. Finally, we show that Lef1 associates in vivo with the promoter of sox3, which depends on Lef1 for its expression and can rescue neurogenesis in the absence of Lef1. The conserved presence of this pathway in

other vertebrates suggests a common mechanism for regulating hypothalamic neurogenesis. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, nervous system, neural coordination, molecular genetics, biochemistry and molecular biophysics, neurogenesis, Wnt signaling, Lef1, neural gene expression.

Lee, K.W., S.E. Webb, and A.L. Miller (2003). **Ca super(2+) released via IP sub(3) receptors is required for furrow deepening during cytokinesis in zebrafish embryos**. *International Journal of Developmental Biology* 47(6): 411-421. ISSN: 0214-6282.
Descriptors: zebrafish embryos, calcium, cell differentiation, cell division, embryonic development, embryos, freshwater fish, gene expression, heparin, receptors, release mechanisms, *Danio rerio*.

Lee, K.W., S.E. Webb, and A.L. Miller (2003). **Ca2+ released via IP3 receptors is required for furrow deepening during cytokinesis in zebrafish embryos.** *International Journal of Developmental Biology, The* 47(6): 411-21. ISSN: 0214-6282.
Abstract: We have previously visualized three Ca2+ transients, generated by release from intracellular stores, which are associated with cytokinesis during the early cell division cycles of zebrafish embryos: the furrow positioning, propagation and deepening transients. Here we demonstrate the requirement of the latter for furrow deepening, and identify the Ca2+ release channels responsible for generating the deepening transient. The introduction of the Ca2+ buffer 5,5'-dibromo-BAPTA, at an appropriate time to challenge only the deepening transient, resulted in the dissipation of this transient and an inhibition of furrow deepening. Introduction of antagonists of the inositol 1,4,5-trisphosphate (IP3) receptor (heparin and 2-aminoethoxydiphenylborate; 2-APB) at the appropriate time, blocked the furrow deepening transient and resulted in an inhibition of furrow deepening. In contrast, antagonists of the ryanodine receptor and the NAADP-sensitive channel had no effect on either the furrow deepening transient or on furrow deepening. In addition, microinjection of IP3 led to the release of calcium from IP3-sensitive stores, whereas the introduction of caffeine or cADPR failed to induce any increase in intracellular Ca2+. Our new data thus support the idea that Ca2+ released via IP3 receptors is essential for generating the furrow deepening transient and demonstrate a requirement for a localized cytosolic Ca2+ riseforthe furrow deepening process. We also present data to show that the endoplasmic reticulum and IP3 receptors are localized on either side of the cleavage furrow, thus providing the intracellular Ca2+ store and release mechanism for generating the deepening transient.
Descriptors: zebrafish embryos, calcium metabolism, calcium channels metabolism, egtazic acid analogs and derivatives, cytology, cytoplasmic receptors and nuclear metabolism, buffers, cell division drug effects, cell division physiology, egtazic acid pharmacology, physiology, endoplasmic reticulum metabolism, heparin pharmacology, cytoplasmic receptors and nuclear antagonists and inhibitors.

Lee, S.A., E.L. Shen, A. Fiser, A. Sali, and S. Guo (2003). **The zebrafish forkhead transcription factor Foxi1 specifies epibranchial placode-derived sensory neurons.** *Development* 130(12): 2669-79. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Vertebrate epibranchial placodes give rise to visceral sensory neurons that transmit vital information such as heart rate, blood pressure and visceral distension. Despite the pivotal roles they play, the molecular program underlying their development is not well understood. Here we report that the zebrafish mutation no soul, in which epibranchial pla-

codes are defective, disrupts the fork headrelated, winged helix domain-containing protein Foxi1. Foxi1 is expressed in lateral placodal progenitor cells. In the absence of foxi1 activity, progenitor cells fail to express the basic helix-loop-helix gene neurogenin that is essential for the formation of neuronal precursors, and the paired homeodomain containing gene phox2a that is essential for neuronal differentiation and maintenance. Consequently, increased cell death is detected indicating that the placodal progenitor cells take on an apoptotic pathway. Furthermore, ectopic expression of foxi1 is sufficient to induce phox2a-positive and neurogenin-positive cells. Taken together, these findings suggest that Foxi1 is an important determination factor for epibranchial placodal progenitor cells to acquire both neuronal fate and subtype visceral sensory identity.

Descriptors: zebrafish , DNA binding proteins genetics, proteins metabolism, afferent neurons metabolism, transcription factors metabolism, proteins metabolism, amino acid sequence, DNA binding proteins genetics, forkhead transcription factors, developmental gene expression regulation physiology, homeodomain molecular sequence data, mutation, nerve tissue proteins, transcription factors genetics.

Lee, Y., S. Grill, A. Sanchez, M. Murphy Ryan, and K.D. Poss (2005). **Fgf signaling instructs position-dependent growth rate during zebrafish fin regeneration.** *Development* 132(23): 5173-83. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: During appendage regeneration in urodeles and teleosts, tissue replacement is precisely regulated such that only the appropriate structures are recovered, a phenomenon referred to as positional memory. It is believed that there exists, or is quickly established after amputation, a dynamic gradient of positional information along the proximodistal (PD) axis of the appendage that assigns region-specific instructions to injured tissue. These instructions specify the amount of tissue to regenerate, as well as the rate at which regenerative growth is to occur. A striking theme among many species is that the rate of regeneration is more rapid in proximally amputated appendages compared with distal amputations. However, the underlying molecular regulation is unclear. Here, we identify position-dependent differences in the rate of growth during zebrafish caudal fin regeneration. These growth rates correlate with position-dependent differences in blastemal length, mitotic index and expression of the Fgf target genes mkp3, sef and spry4. To address whether PD differences in amounts of Fgf signaling are responsible for position-dependent blastemal function, we have generated transgenic fish in which Fgf receptor activity can be experimentally manipulated. We find that the level of Fgf signaling exhibits strict control over target gene expression, blastemal proliferation and regenerative growth rate. Our results demonstrate that Fgf signaling defines position-dependent blastemal properties and growth rates for the regenerating zebrafish appendage.

Descriptors: zebrafish embryos, Fgf physiology, regeneration physiology, signal transduction, genetically modified, developmental gene expression regulation, growth, membrane nerve tissue protein tyrosine phosphatase genetics, time factors.

Lee, Y.M., J.J. Cope, G.E. Ackermann, K. Goishi, E.J. Armstrong, B.H. Paw, and J. Bischoff (2006). **Vascular endothelial growth factor receptor signaling is required for cardiac valve formation in zebrafish.** *Developmental Dynamics* 235(1): 29-37. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryos, heart valves embryology, receptors, vascular endothelial growth factor physiology, signal transduction physiology, bone morphogenetic proteins biosynthesis and genetics, cell nucleus metabolism, cultured cells, developmental gene expres-

sion regulation drug effects, heart valves abnormalities, heart valves drug effects, heart valves physiology, nfatc transcription factors metabolism, phthalazines pharmacology, pyridines pharmacology, Notch1 receptor biosynthesis, Notch1 receptor genetics, signal transduction drug effects.

Lekven, A.C., G.R. Buckles, N. Kostakis, and R.T. Moon (2003). **Wnt1 and wnt10b function redundantly at the zebrafish midbrain-hindbrain boundary.** *Developmental Biology* 254(2): 172-87. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, mesencephalon physiology, proto oncogene, rhombencephalon physiology, proteins, alleles, amino acid sequence, chromosome mapping, DNA binding proteins genetics, proteins analysis, enhancer elements genetics, fibroblast growth factor 8, Fgf analysis, molecular sequence data, pax2 transcription factor , proto oncogene proteins chemistry and genetics, transcription factors analysis, Wnt proteins, Wnt1 protein.

Lemus, C.G., S. Hartel, J. Regan, C. Russel, S. Wilson, and M.L. Concha (2007). **Asymmetric morphogenesis of the parapineal organ in the developing zebrafish brain**. *Developmental Biology* 306(1): 302. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, brain, developmental stages, freshwater fish, morphogenesis, *Danio rerio*, *embryology*.

Lessman, C.A., R. Nathani, R. Uddin, J. Walker, and J. Liu (2007). **Computer-Aided Meiotic Maturation Assay (CAMMA) of zebrafish (*Danio rerio*) oocytes in vitro.** *Molecular Reproduction and Development* 74(1): 97-107. ISSN: 1040-452X.
NAL Call Number: QP251.M64
Abstract: We have developed a new technique called Computer-Aided Meiotic Maturation Assay (CAMMA) for imaging large arrays of zebrafish oocytes and automatically collecting image files at regular intervals during meiotic maturation. This novel method uses a transparency scanner interfaced to a computer with macro programming that automatically scans and archives the image files. Images are stacked and analyzed with ImageJ to quantify changes in optical density characteristic of zebrafish oocyte maturation. Major advantages of CAMMA include (1) ability to image very large arrays of oocytes and follow individual cells over time, (2) simultaneously image many treatment groups, (3) digitized images may be stacked, animated, and analyzed in programs such as ImageJ, NIH-Image, or ScionImage, and (4) CAMMA system is inexpensive, costing less than most microscopes used in traditional assays. We have used CAMMA to determine the dose response and time course of oocyte maturation induced by 17alpha-hydroxyprogesterone (HP). Maximal decrease in optical density occurs around 5 hr after 0.1 micro g/ml HP (28.5 degrees C), approximately 3 hr after germinal vesicle migration (GVM) and dissolution (GVD). In addition to changes in optical density, GVD is accompanied by streaming of ooplasm to the animal pole to form a blastodisc. These dynamic changes are readily visualized by animating image stacks from CAMMA; thus, CAMMA provides a valuable source of time-lapse movies for those studying zebrafish oocyte maturation. The oocyte clearing documented by CAMMA is correlated to changes in size distribution of major yolk proteins upon SDS-PAGE, and, this in turn, is related to increased cyclin B(1) protein.
Descriptors: zebrafish oocytes, cytogenetic analysis methods, computer assisted image processing methods, oocytes cytology, tissue array analysis methods, meiosis, meiotic maturation.

Leung, A.Y., E.M. Mendenhall, T.T. Kwan, R. Liang, C. Eckfeldt, E. Chen, M. Hammerschmidt, S. Grindley, S.C. Ekker, and C.M. Verfaillie (2005). **Characterization of expanded intermediate cell mass in zebrafish chordin morphant embryos.** *Developmental Biology* 277(1): 235-54. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: We investigated the mechanisms of intermediate cell mass (ICM) expansion in zebrafish chordin (Chd) morphant embryos and examined the role of BMPs in relation to this phenotype. At 24 h post-fertilization (hpf), the expanded ICM of embryos injected with chd morpholino (MO) (ChdMO embryos) contained a monotonous population of hematopoietic progenitors. In situ hybridization showed that hematopoietic transcription factors were ubiquitously expressed in the ICM whereas vascular gene expression was confined to the periphery. BMP4 (but not BMP2b or 7) and smad5 mRNA were ectopically expressed in the ChdMO ICM. At 48 hpf, monocytic cells were evident in both the ICM and circulation of ChdMO but not WT embryos. While injection of BMP4 MO had no effect on WT hematopoiesis, co-injecting BMP4 with chd MOs significantly reduced ICM expansion. Microarray studies revealed a number of genes that were differentially expressed in ChdMO and WT embryos and their roles in hematopoiesis has yet to be determined. In conclusion, the expanded ICM in ChdMO embryos represented an expansion of embryonic hematopoiesis that was skewed towards a monocytic lineage. BMP4, but not BMP2b or 7, was involved in this process. The results provide ground for further research into the mechanisms of embryonic hematopoietic cell expansion.

Descriptors: zebrafish embryos, bone morphogenetic, glyco, hematopoiesis, intercellular signaling peptides and, cell division, drug dose response relationship, gene expression profiling, oligonucleotide array sequence analysis, signal transduction.

Leung, T., J. Bischof, I. Soll, D. Niessing, D. Zhang, J. Ma, H. Jackle, and W. Driever (2003). **Bozozok directly represses bmp2b transcription and mediates the earliest dorsoventral asymmetry of bmp2b expression in zebrafish.** *Development* 130(16): 3639-49. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Formation of the gastrula organizer requires suppression of ventralizing signals and, in fish and frog, the need to counteract the effect of ubiquitously present maternal factors that activate the expression of Bmps. How the balance between dorsalizing and ventralizing factors is shifted towards organizer establishment at late blastula stages is not well understood. Mutations in zebrafish bozozok (boz) cause severe defects in axial mesoderm and anterior neurectoderm and affect organizer formation. The boz gene encodes the homeodomain protein Bozozok/Dharma and its expression in the region of the organizer is activated through beta-catenin signaling. Here, we investigate the molecular mechanism by which boz contributes to the establishment of the organizer. We demonstrate that the homeodomain protein Boz acts as a transcriptional repressor in zebrafish: overexpression of an En-Boz fusion protein can rescue the boz phenotype, whereas a VP16-Boz fusion protein acts as an antimorph. Expression analysis of bmp2b indicates that Boz negatively regulates bmp2b in the prospective organizer. We demonstrate that this Boz activity is independent of that of other zygotic genes, because it also occurs when translation of zygotic genes is suppressed by cycloheximide (CHX). We identify two high-affinity binding sites for Boz within the first intron of the bmp2b gene. Deletion of these control elements abolishes Boz-dependent repression of bmp2b in the early blastula. Thus, Boz directly represses bmp2b by binding to control elements in the bmp2b locus. We propose that early transcriptional repression of bmp2b by Boz is one of the first steps toward formation of a stable organizer, whereas the

later-acting Bmp antagonists (e.g. Chordin, Noggin) modulate Bmp activity in the gastrula to induce patterning along the dorsoventral axis. Thus, similar to *Drosophila* Dpp, asymmetry of Bmp expression in zebrafish is initiated at the transcriptional level, and the shape of the gradient and its function as a morphogen are later modulated by post-transcriptional mechanisms.

Descriptors: zebrafish, body patterning, bone morphogenetic proteins metabolism, homeodomain proteins metabolism, repressor proteins metabolism, genetic transcription, bone morphogenetic developmental gene expression regulation, herpes simplex virus protein vmw65 genetics, herpes simplex virus protein vmw65 metabolism, homeodomain organizers, embryonic physiology, phenotype, promoter regions genetics, recombinant fusion proteins metabolism, repressor metabolism.

Leung, T., I. Soell, S.J. Arnold, R. Kemler, and W. Driever (2003). **Direct binding of lef1 to sites in the boz promoter may mediate pre-midblastula-transition activation of boz expression.** *Developmental Dynamics* 228(3): 424-432. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryos, biochemistry, development, genetics , pre midblastula transition.

Lewis, J.L., J. Bonner, M. Modrell, J.W. Ragland, R.T. Moon, R.I. Dorsky, and D.W. Raible (2004). **Reiterated Wnt signaling during zebrafish neural crest development.** *Development* 131(6): 1299-308. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: While Wnt/beta-catenin signaling is known to be involved in the development of neural crest cells in zebrafish, it is unclear which Wnts are involved, and when they are required. To address these issues we employed a zebrafish line that was transgenic for an inducible inhibitor of Wnt/beta-catenin signaling, and inhibited endogenous Wnt/beta-catenin signaling at discrete times in development. Using this approach, we defined a critical period for Wnt signaling in the initial induction of neural crest, which is distinct from the later period of development when pigment cells are specified from neural crest. Blocking Wnt signaling during this early period interfered with neural crest formation without blocking development of dorsal spinal neurons. Transplantation experiments suggest that neural crest precursors must directly transduce a Wnt signal. With regard to identifying which endogenous Wnt is responsible for this initial critical period, we established that wnt8 is expressed in the appropriate time and place to participate in this process. Supporting a role for Wnt8, blocking its function with antisense morpholino oligonucleotides eliminates initial expression of neural crest markers. Taken together, these results demonstrate that Wnt signals are critical for the initial induction of zebrafish neural crest and suggest that this signaling pathway plays reiterated roles in its development.

Descriptors: zebrafish embryos, neural crest embryology, proto oncogene proteins metabolism, signal transduction physiology, cytoskeletal proteins, embryonic induction physiology, hmgb hmgb proteins metabolism, mutation, neural crest metabolism, Tcf transcription factors, transcription factors genetics and metabolism, Wnt proteins, metabolism.

Lewis, K.E., J. Bates, and J.S. Eisen (2005). **Regulation of iro3 expression in the zebrafish spinal cord.** *Developmental Dynamics* 232(1): 140-8. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: Specification of spinal cord neurons is regulated by several different transcription factors. In this study, we analyze expression and regulation of the transcription factor iro3 in

zebrafish spinal cord. In addition to its broad expression in the progenitor domain of intermediate spinal cord, iro3 is also expressed in postmitotic ventral neurons, starting at early somitogenesis stages. Initially, this expression is only in two primary motoneurons, CaP and VaP, but by 24 hr postfertilization, iro3 is expressed by all classes of zebrafish spinal motoneurons as well as by a ventral interneuron called VeLD. iro3 expression in the progenitor domain of intermediate spinal cord is regulated independently from its expression in ventral neurons. Hedgehog (Hh) signaling is unnecessary for iro3 expression in intermediate spinal cord, but it is required to repress iro3 expression in the progenitor domain of ventral spinal cord. We also show that the basic helix-loop-helix transcription factor Olig2 is required for repression of iro3 expression in the progenitor domain of ventral spinal cord. We discuss our findings in the context of previous studies, suggesting that iro3 represses formation of motoneurons and promotes formation of interneurons.

Descriptors: zebrafish embryos, developmental gene expression regulation, homeodomain proteins genetics and physiology, nerve tissue nerve tissue, spinal cord embryology and physiology, alleles, basic helix loop helix transcription factors, Hedgehog proteins, heterozygote, homeodomain proteins biosynthesis, in situ hybridization, anatomic models, mutation, nerve tissue proteins biosynthesis, neurons metabolism, neurons physiology, RNA metabolism, stem cells physiology, time factors, trans activators physiology, proteins biosynthesis.

Lewis, K.E. and J.S. Eisen (2004). **Paraxial mesoderm specifies zebrafish primary motoneuron subtype identity.** *Development* 131(4): 891-902. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: We provide the first analysis of how a segmentally reiterated pattern of neurons is specified along the anteroposterior axis of the vertebrate spinal cord by investigating how zebrafish primary motoneurons are patterned. Two identified primary motoneuron subtypes, MiP and CaP, occupy distinct locations within the ventral neural tube relative to overlying somites, express different genes and innervate different muscle territories. In all vertebrates examined so far, paraxial mesoderm-derived signals specify distinct motoneuron subpopulations in specific anteroposterior regions of the spinal cord. We show that signals from paraxial mesoderm also control the much finer-grained segmental patterning of zebrafish primary motoneurons. We examined primary motoneuron specification in several zebrafish mutants that have distinct effects on paraxial mesoderm development. Our findings suggest that in the absence of signals from paraxial mesoderm, primary motoneurons have a hybrid identity with respect to gene expression, and that under these conditions the CaP axon trajectory may be dominant.

Descriptors: zebrafish embryos, cell differentiation physiology, mesoderm physiology, motor neurons physiology, heparan sulfate proteoglycans genetics and metabolism, mutation, signal transduction physiology, somites.

Li, G., T. Liu, J. Nie, L. Guo, J. Malicki, A. Mara, S.A. Holley, W. Xia, and S.T. Wong (2007). **Detection of blob objects in microscopic zebrafish images based on gradient vector diffusion.** *Cytometry. Part A* 71(10): 835-45. ISSN: 1552 4922.

Abstract: The zebrafish has become an important vertebrate animal model for the study of developmental biology, functional genomics, and disease mechanisms. It is also being used for drug discovery. Computerized detection of blob objects has been one of the important tasks in quantitative phenotyping of zebrafish. We present a new automated method that is able to detect blob objects, such as nuclei or cells in microscopic zebrafish images. This method is composed of three key steps. The first step is to produce a diffused gradient vector field by a physical elastic deformable model. In the second step, the flux image is computed

on the diffused gradient vector field. The third step performs thresholding and nonmaximum suppression based on the flux image. We report the validation and experimental results of this method using zebrafish image datasets from three independent research labs. Both sensitivity and specificity of this method are over 90%. This method is able to differentiate closely juxtaposed or connected blob objects, with high sensitivity and specificity in different situations. It is characterized by a good, consistent performance in blob object detection.
Descriptors: zebrafish, microscopy methods, cell nucleus metabolism, cell shape, cell size, diffusion, computer assisted image processing, light, mesoderm cytology, afferent neurons cytology, reproducibility of results, retina cytology.

Li Li, Zhang Shicui, Yuan Jinduo, and Li Hongyan (2003). **Development of inter-family nuclear transplant embryos by transplanting the nuclei from the loach blastulae into the non-enucleated zebrafish eggs.** *Chinese Journal of Oceanology and Limnology* 21(1): 64-71. ISSN: 0254 4059.
Abstract: The developmental fate of the pronuclei in recombined embryos obtained by transplanting the donor nuclei into the non-enucleated eggs remains controversial in the case of fish. In the present study, the nuclei from the loach blastulae were transplanted into non-enucleated zebrafish eggs, the resulting 9 inter-family nuclear transplant embryos developed to larval stages. Although the development timing of the nuclear transplants resembled that of zebrafish, chromosome examination revealed that most of the recombined embryos were diploids with karyotype characteristic of loach, which was also proved by RAPD analysis. Moreover, 3 out of the 9 larval fish formed barb rudiments specific to loach. It was therefore concluded that the nuclear transplant larval fish were inter-family nucleo-cytoplasmic hybrids; and that only the donor nuclei were involved in the development of the nuclear transplant embryos, while the pronuclei in the non-enucleated eggs were likely automatically eliminated during the development.
Descriptors: zebrafish, *brachyDanio rerio*, *misgurnus anguillicaudatus*, artificial hybridization techniques, nucleus , pronucleus , embryo development, inter family nuclear transplant embryos, developmental fate of pronuclei, hybridization.

Li, P., S. Shah, L. Huang, A.L. Carr, Y. Gao, C. Thisse, B. Thisse, and L. Li (2007). **Cloning and spatial and temporal expression of the zebrafish dopamine D1 receptor.** *Developmental Dynamics* 236(5): 1339-46. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, dopamine D1 receptors genetics, amino acid and base sequence, central nervous system embryology, central nervous system growth and development, central nervous system metabolism, chromosome mapping, molecular cloning, complementary DNA genetics, developmental gene expression regulation, in situ hybridization, molecular sequence data, phylogeny, dopamine D2 receptors genetics, retina embryology, retina growth and development, retina metabolism, amino acid sequence homology, zebrafish growth and development.

Li, Q., K. Shirabe, and J.Y. Kuwada (2004). **Chemokine signaling regulates sensory cell migration in zebrafish.** *Developmental Biology* 269(1): 123-36. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Chemokines play an important role in the migration of a variety of cells during development. Recent investigations have begun to elucidate the importance of chemokine signaling within the developing nervous system. To better appreciate the neural function of chemokines in vivo, the role of signaling by SDF-1 through its CXCR4 receptor was ana-

lyzed in zebrafish. The SDF-1-CXCR4 expression pattern suggested that SDF-1-CXCR4 signaling was important for guiding migration by sensory cells known as the migrating primordium of the posterior lateral line. Ubiquitous induction of the ligand in transgenic embryos, antisense knockdown of the ligand or receptor, and a genetic receptor mutation all disrupted migration by the primordium. Furthermore, in embryos in which endogenous SDF-1 was knocked down, the primordium migrated towards exogenous sources of SDF-1. These data demonstrate that SDF-1 signaling mediated via CXCR4 functions as a chemoattractant for the migrating primordium and that chemokine signaling is both necessary and sufficient for directing primordium migration.
Descriptors: zebrafish embryos, cell movement physiology, Cxc chemokines genetics, sensory receptors embryology, signal transduction physiology, genetically modified, Cxc chemokines metabolism, antisense oligonucleotides, Cxcr4 receptors genetics, Cxcr4 receptors metabolism, sensory receptors metabolism, physiology.

Li, W. and R.A. Cornell (2007). **Redundant activities of Tfap2a and Tfap2c are required for neural crest induction and development of other non-neural ectoderm derivatives in zebrafish embryos.** *Developmental Biology* 304(1): 338-54. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: A knockdown study suggested that transcription factor AP-2 alpha (Tfap2a) is required for neural crest induction in frog embryos. However, because Tfap2a is expressed in neural crest and in presumptive epidermis, a source of signals that induce neural crest, it was unclear whether this requirement is cell autonomous. Moreover, neural crest induction occurs normally in zebrafish tfap2a and mouse Tcfap2a mutant embryos, so it was unclear if a requirement for Tfap2a in this process has been evolutionarily conserved. Here we show that zebrafish tfap2c, encoding AP-2 gamma (Tfap2c), is expressed in non-neural ectoderm including transiently in neural crest. Inhibition of tfap2c with antisense oligonucleotides does not visibly perturb development. However, simultaneous inhibition of tfap2a and tfap2c utterly prevents neural crest induction, supporting a conserved role for Tfap2-type activity in neural crest induction. Transplant studies suggest that this role is cell-autonomous. In addition, in tfap2a/tfap2c doubly deficient embryos cranial placode derivatives are reduced, although gene expression characteristic of pre-placodal domain is normal. Unexpectedly, Rohon-Beard sensory neurons, which previous studies indicated are derived from the same precursor population as neural crest, are reduced by less than half in such embryos, implying a non-neural crest origin for a subset of them.
Descriptors: zebrafish, embryonic induction genetics, neural crest embryology, transcription factor AP 2 metabolism, proteins metabolism, apoptosis genetics, cell proliferation, DNA primers, reverse transcriptase PCR, transcription factor AP 2 genetics.

Li, W., F. Deng, H. Wang, Y. Zhen, F. Xiang, Y. Sui, and J. Li (2006). **Germ cell-less expression in zebrafish embryos.** *Development Growth and Differentiation* 48(5): 333-8. ISSN: 0012 1592.
NAL Call Number: QL951.E4
Abstract: The gene of germ cell-less (gcl) has been shown to be important in early differentiation of germ cells in *Drosophila*. Although the gcl homologue genes have been identified in some organisms, there is little data on the expression pattern and functional analysis of the gcl gene in zebrafish. In this research, real-time quantitative reverse transcription-polymerase chain reaction showed that the level of gcl mRNA expression rapidly decreases from the 4-cell stage to the sphere stage at which it reaches a minimum, gradually increases from the 50%-epiboly stage, and then remains stable during the posterior stages. Results of in situ hybridization indicated that the transcripts of zebrafish gcl are evenly distributed in all

blastomeres from the 2-cell stage to the blastula period, different from that of vasa, nonas1 and dead end mRNA, and condense into some clusters of cells located along the blastoderm margin from the gastrulation period. During subsequent development, the transcripts are segregated as subcellular clumps to a small number of cells that would migrate to the position of the gonad in the dorsal side. In the adult, gcl mRNA was widely expressed in developing germ cells of both ovary and testis. These data suggest that zebrafish gcl have potentially important roles in the formation of primordial germ cells.
Descriptors: zebrafish embryos, germ cells physiology, messenger RNA metabolism, cell lineage, cleavage stage, ovum, dead box RNA helicases, physiology, embryonic development, in situ hybridization, ovary embryology, RNA helicases genetics, RNA stability, messenger RNA genetics, reverse transcriptase PCR, testis embryology.

Li, W. and R.A. Cornell (2005). **Ap-2 transcription factors function at multiple steps of zebrafish neural crest.** *Developmental Biology* 283(2): 659-660. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, biochemistry and molecular biophysics, embryogenesis, neural crest development.

Li, W. and R.A. Cornell (2006). **Transcription factor ap-2alpha and ap-2gamma act redundantly in zebrafish neural crest specification**. *Developmental Biology* 295(1): 410. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryo, nervous system, neural coordination, molecular genetics, development.

Li, X., J.W. Xiong, C.S. Shelley, H. Park, and M.A. Arnaout (2006). **The transcription factor ZBP-89 controls generation of the hematopoietic lineage in zebrafish and mouse embryonic stem cells.** *Development* 133(18): 3641-50. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Hematopoietic development is closely linked to that of blood vessels and the two processes are regulated in large part by transcription factors that control cell fate decisions and cellular differentiation. Both blood and blood vessels derive from a common progenitor, termed the hemangioblast, but the factor(s) specifying the development and differentiation of this stem cell population into the hematopoietic and vascular lineages remain ill defined. Here, we report that knockdown of the Kruppel-like transcription factor ZBP-89 in zebrafish embryos results in a bloodless phenotype, caused by disruption of both primitive and definitive hematopoiesis, while leaving primary blood vessel formation intact. Injection of ZBP-89 mRNA into cloche zebrafish embryos, which lack both the hematopoietic and endothelial lineages, rescues hematopoiesis but not vasculogenesis. Injection of mRNA for Stem Cell Leukemia (SCL), a transcription factor that directs hemangioblast development into blood cell precursors, rescues the bloodless phenotype in ZBP-89 zebrafish morphants. Forced expression of ZBP-89 induces the expansion of hematopoietic progenitors in wild-type zebrafish and in mouse embryonic stem cell cultures but inhibits angiogenesis in vivo and in vitro. These findings establish a unique regulatory role for ZBP-89, positioned at the interface between early blood and blood vessel development.
Descriptors: zebrafish embryos, transcription factors genetics, Western blotting, cultured cells, DNA binding proteins genetics, proteins genetics and metabolism, DNA binding, embryonic stem cells cytology, embryonic stem cells metabolism, flow cytometry, developmental gene expression regulation genetics , hematopoiesis genetics and physiology,

hematopoietic stem cells cytology, metabolism and physiology, in situ hybridization, in situ nick end labeling, mice, mutation genetics, physiologic neovascularization genetics, reverse transcriptase PCR, transcription factors metabolism, and physiology, metabolism.

Liebling, M., A.S. Forouhar, R. Wolleschensky, B. Zimmermann, R. Ankerhold, S.E. Fraser, M.A. Gharib, and M.E. Dickinson (2006). **Rapid three-dimensional imaging and analysis of the beating embryonic heart reveals functional changes during development**. *Developmental Dynamics* 235(11): 2940-2948. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: We report an accurate method for studying the functional dynamics of the beating embryonic zebrafish heart. The fast cardiac contraction rate and the high velocity of blood cells have made it difficult to study cellular and subcellular events relating to heart function in vivo. We have devised a dynamic three-dimensional acquisition, reconstruction, and analysis procedure by combining (1) a newly developed confocal slit-scanning microscope, (2) novel strategies for collecting and synchronizing cyclic image sequences to build volumes with high temporal and spatial resolution over the entire depth of the beating heart, and (3) data analysis and reduction protocols for the systematic extraction of quantitative information to describe phenotype and function. We have used this approach to characterize blood flow and heart efficiency by imaging fluorescent protein-expressing blood and endocardial cells as the heart develops from a tube to a multichambered organ. The methods are sufficiently robust to image tissues within the heart at cellular resolution over a wide range of ages, even when motion patterns are only quasiperiodic. These tools are generalizable to imaging and analyzing other cyclically moving structures at microscopic scales. Copyright©Thomson Reuters 2009
Descriptors: beating embryonic zebrafish heart, methods and techniques, development, devices and instrumentation, cardiovascular system, transport and circulation, rapid three dimensional imaging, imaging and microscopy techniques, confocal slit scanning microscope, cardiac contraction, functional dynamics.

Lillesaar, C., B. Tannhauser, C. Stigloher, E. Kremmer, and L. Bally Cuif (2007). **The serotonergic phenotype is acquired by converging genetic mechanisms within the zebrafish central nervous system.** *Developmental Dynamics* 236(4): 1072-84. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: To gain knowledge about the developmental origin of serotonergic precursors and the regulatory cascades of serotonergic differentiation in vertebrates, we determined the spatiotemporal expression profile of the Ets-domain transcription factor-encoding gene pet1 in developing and adult zebrafish. We show that it is an early, specific marker of raphe serotonergic neurons, but not of other serotonergic populations. We then use pet1 expression together with tracing techniques to demonstrate that serotonergic neurons of rhombomeres (r) 1-2 largely originate from a progenitor pool at the midbrain-hindbrain boundary. Furthermore, by combining expression analyses of pet1 and the raphe tryptophan hydroxylase (Tph2) with rhombomere identity markers, we show that anterior and posterior hindbrain clusters of serotonergic precursors are separated by r3, rather than r4 as in other vertebrates. Our findings establish the origin of r1-2 serotonergic precursors, and strengthen the evidence for molecular, ontogenic and phylogenic heterogeneities among the vertebrate brain serotonergic cell populations.
Descriptors: zebrafish embryos, central nervous system embryology, neurons physiology and secretion, phenotype, serotonin secretion, amino acid and base sequence, cell differentiation, central nervous system growth and development developmental gene expression regulation,

molecular sequence data, phylogeny, amino acid sequence homology, transcription factors genetics, genetics, growth and development.

Lin, C.Y., R.F. Yung, H.C. Lee, W.T. Chen, Y.H. Chen, and H.J. Tsai (2006). **Myogenic regulatory factors Myf5 and Myod function distinctly during craniofacial myogenesis of zebrafish.** *Developmental Biology* 299(2): 594-608. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The functions of Myf5 and Myod are well known in trunk myogenesis. However, the roles that Myf5 and Myod play during craniofacial myogenesis are far from well known. We observed that zebrafish myf5 was detected in the primordia of the obliques, lateral rectus, sternohyoideus, and pharyngeal mesoderm cores. In contrast, myod transcripts were expressed in all head muscle precursors at later stages. Knockdown of myf5 revealed that Myf5 was required for the development of the obliques, lateral rectus, sternohyoideus, and all pharyngeal muscles, whereas knockdown of myod proved that Myod was required for the development of superior rectus, medial rectus, inferior rectus, lateral rectus, and the ventral pharyngeal muscles. myod mRNA did not rescue the loss of the cranial muscle caused by injecting myf5-morpholino, or vice versa, suggesting that the functions of Myf5 and Myod were not redundant in head paraxial mesoderm, a finding different from their functions in trunk myogenesis. Myf5, but not Myod, was required for the forward migration of myf5-positive oblique precursors. All evidences reveal that Myf5 and Myod function independently during cranial myogenesis. On the basis of the expression patterns of myf5 and myod, we propose a model to present how Myf5 and Myod are involved in head myogenesis of zebrafish.
Descriptors: zebrafish, skeletal muscle embryology, Myod protein metabolism, myogenic regulatory factor 5 metabolism, proteins metabolism, genetically modified, branchial region metabolism, cell movement, developmental gene expression regulation, head embryology and physiology, mesoderm metabolism, developmental research models, muscle development, skeletal muscle metabolism.

Lin, J.W., A.V. Biankin, M.E. Horb, B. Ghosh, N.B. Prasad, N.S. Yee, M.A. Pack, and S.D. Leach (2004). **Differential requirement for ptf1a in endocrine and exocrine lineages of developing zebrafish pancreas.** *Developmental Biology* 270(2): 474-86. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Mammalian studies have implicated important roles for the basic helix-loop-helix transcription factor Ptf1a-p48 in the development of both exocrine and endocrine pancreas. We have cloned the Ptf1a-p48 ortholog in *Danio rerio*. Early zebrafish ptf1a expression is observed in developing hindbrain and in endodermal pancreatic precursors. Analysis of ptf1a and insulin expression reveals a population of exocrine precursors that, throughout early development, are temporally and spatially segregated from endocrine elements. Morpholino-mediated knockdown of ptf1a confirms early divergence of these endocrine and exocrine lineages. Ptf1a morphants lack differentiated exocrine pancreas, but maintain normal differentiation and organization of the principal islet. In addition to the exocrine phenotype, ptf1a knockdown also reduces the prevalence of a small population of anterior endocrine cells normally found outside the principal islet. Together, these findings suggest the presence of distinct ptf1a-dependent and ptf1a-independent precursor populations in developing zebrafish pancreas.
Descriptors: zebrafish , cell differentiation physiology, developmental gene expression regulation, homeodomain proteins, pancreas embryology, transcription factors genetics and metabolism, amino acid and base sequence, cell lineage physiology, DNA primers, comple-

mentary DNA genetics, fluorescent antibody technique, helix loop helix motifs genetics, histological techniques, in situ hybridization, insulin metabolism, molecular sequence data, morphogenesis, pancreas cytology, pancreas metabolism, sequence alignment, DNA sequence analysis, trans activators metabolism, metabolism.
Notes:
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Lin, X., L. Rinaldo, A.F. Fazly, and X. Xu (2007). **Depletion of Med10 enhances Wnt and suppresses Nodal signaling during zebrafish embryogenesis.** *Developmental Biology* 303(2): 536-48. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The transcriptional Mediator (MED) is a multiprotein complex that transmits information from transcription factors to RNA polymerase II (PolII) to regulate transcription. At present, the role of distinct MED subunits in general transcription versus transcription stimulated by specific signaling pathways is unclear. By means of positional cloning, we reveal that the zebrafish mutant tennismatch is a hypomorphic allele of Med10, a conserved MED middle domain subunit. Using morpholino antisense oligonucleotides, we further demonstrate that reduction of Med10 levels led to an enhancement of the Wnt signaling pathway, while also suggesting a role for Med10 in mediating the Nodal signaling pathway. In contrast to the dual roles of Med10, reduction of Med12 and Med13 levels, two MED subunits in the regulatory domain, led to an enhancement of the Wnt signaling pathway but not the Nodal pathway, while reduction of Med15 levels, a MED subunit in the tail domain, suppressed the Nodal signaling pathway but not the Wnt signaling pathway. Thus, Med10 appears to be a unique MED subunit that differentially transduces information from distinct signaling pathways during zebrafish embryogenesis.
Descriptors: zebrafish embryology, transforming growth factor beta metabolism, Wnt proteins metabolism, proteins deficiency, amino acid and base sequence, molecular cloning, DNA primers genetics, molecular sequence data, mutation, amino acid sequence homology, signal transduction, genetics and metabolism.

Link, V., A. Shevchenko, and C.P. Heisenberg (2006). **Proteomics of early zebrafish embryos.** *BioMed Central Developmental Biology* 6: 1. ISSN: 1471 213X.
NAL Call Number: QH491.B63
Abstract: BACKGROUND: Zebrafish *(D. rerio*) has become a powerful and widely used model system for the analysis of vertebrate embryogenesis and organ development. While genetic methods are readily available in zebrafish, protocols for two dimensional (2D) gel electrophoresis and proteomics have yet to be developed. RESULTS: As a prerequisite to carry out proteomic experiments with early zebrafish embryos, we developed a method to efficiently remove the yolk from large batches of embryos. This method enabled high resolution 2D gel electrophoresis and improved Western blotting considerably. Here, we provide detailed protocols for proteomics in zebrafish from sample preparation to mass spectrometry (MS), including a comparison of databases for MS identification of zebrafish proteins. CONCLUSION: The provided protocols for proteomic analysis of early embryos enable research to be taken in novel directions in embryogenesis.
Descriptors: zebrafish embryos, proteomics methods, proteins analysis, Western blotting, databases, protein, egg yolk chemistry, two dimensional gel electrophoresis, embryo chemistry and metabolism, mass spectrometry.

Linville, A., E. Gumusaneli, R.A. Chandraratna, and T.F. Schilling (2004). **Independent roles for retinoic acid in segmentation and neuronal differentiation in the zebrafish hindbrain.** *Developmental Biology* 270(1): 186-99. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Segmentation of the vertebrate hindbrain into rhombomeres is essential for the anterior-posterior patterning of cranial motor nuclei and their associated nerves. The vitamin A derivative, retinoic acid (RA), is an early embryonic signal that specifies rhombomeres, but its roles in neuronal differentiation within the hindbrain remain unclear. Here we have analyzed the formation of primary and secondary hindbrain neurons in the zebrafish mutant neckless (nls), which disrupts retinaldehyde dehydrogenase 2 (raldh2), and in embryos treated with retinoid receptor (RAR) antagonists. Mutation of nls disrupts secondary, branchiomotor neurons of the facial and vagal nerves, but not the segmental pattern of primary, reticulospinal neurons, suggesting that RA acts on branchiomotor neurons independent of its role in hindbrain segmentation. Very few vagal motor neurons form in nls mutants and many facial motor neurons do not migrate out of rhombomere 4 into more posterior segments. When embryos are treated with RAR antagonists during gastrulation, we observe more severe patterning defects than seen in nls. These include duplicated reticulospinal neurons and posterior expansions of rhombomere 4, as well as defects in branchiomotor neurons. However, later antagonist treatments after rhombomeres are established still disrupt branchiomotor development, suggesting that requirements for RARs in these neurons occur later and independent of segmental patterning. We also show that RA produced by the paraxial mesoderm controls branchiomotor differentiation, since we can rescue the entire motor innervation pattern by transplanting wild-type cells into the somites of nls mutants. Thus, in addition to its role in determining rhombomere identities, RA plays a more direct role in the differentiation of subsets of branchiomotor neurons within the hindbrain.

Descriptors: zebrafish embryos, body patterning, cell differentiation physiology, neurons physiology, rhombencephalon embryology, tretinoin metabolism, aldehyde oxidoreductases genetics and metabolism, branchial region cytology, branchial region embryology, cell movement physiology, neurons cytology, retinoic acid receptors antagonists and inhibitors, retinal dehydrogenase, rhombencephalon cytology and metabolism, signal transduction physiology, zebrafish anatomy and histology and genetics.

Lister, J.A., C. Cooper, K. Nguyen, M. Modrell, K. Grant, and D.W. Raible (2006). **Zebrafish Foxd3 is required for development of a subset of neural crest derivatives.** *Developmental Biology* 290(1): 92-104. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: foxd3 encodes a winged helix/forkhead class transcription factor expressed in the premigratory neural crest cells of many vertebrates. We have investigated the function of this gene in zebrafish neural crest by a loss of function approach using antisense morpholino oligonucleotides and immunostaining for Foxd3 protein. Knockdown of Foxd3 expression produces deficits in several differentiated neural crest derivatives, including jaw cartilage, peripheral neurons, and glia, and iridophore pigment cells. Other derivatives, such as melanophore and xanthophore pigment cells are not affected. Reduction in the expression of several lineage-specific markers becomes evident soon after the onset of neural crest migration, suggesting that Foxd3 knockdown affects these lineages at early stages in their development. In contrast, analysis of the expression of early neural crest markers indicates little effect on neural crest induction or initial emigration. Finally, cell transplantation suggests that with respect to dorsal root ganglia neurons the Foxd3 requirement is cell autonomous, although Foxd3 itself is not detectable in differentiated DRG neurons. These results

suggest that in zebrafish Foxd3 may not be required for induction of neural crest identity but is necessary for the differentiation of a subset of neural crest cell fates, perhaps in precursors of particular neural crest lineages.
Descriptors: zebrafish embryos, body patterning, cell differentiation, forkhead transcription factors metabolism, neural crest embryology, proteins metabolism, cartilage embryology, cartilage metabolism, cell lineage, cell movement, cell transplantation, forkhead transcription factors genetics, spinal ganglia embryology and metabolism, jaw embryology and metabolism, melanophores cytology and metabolism, neural crest metabolism, neuroglia cytology, neuroglia metabolism, neurons cytology and metabolism, antisense oligonucleotides genetics, pigment epithelium of eye embryology and metabolism.

Little, S.C. and M.C. Mullins (2004). **Twisted gastrulation promotes BMP signaling in zebrafish dorsal-ventral axial patterning.** *Development* 131(23): 5825-35. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: In vertebrates and invertebrates, the bone morphogenetic protein (BMP) signaling pathway patterns cell fates along the dorsoventral (DV) axis. In vertebrates, BMP signaling specifies ventral cell fates, whereas restriction of BMP signaling by extracellular antagonists allows specification of dorsal fates. In misexpression assays, the conserved extracellular factor Twisted gastrulation (Tsg) is reported to both promote and antagonize BMP signaling in DV patterning. To investigate the role of endogenous Tsg in early DV patterning, we performed morpholino (MO)-based knockdown studies of Tsg1 in zebrafish. We found that loss of tsg1 results in a moderately strong dorsalization of the embryonic axis, suggesting that Tsg1 promotes ventral fates. Knockdown of tsg1 combined with loss of function of the BMP agonist tolloid (mini fin) or heterozygosity for the ligand bmp2b (swirl) enhanced dorsalization, supporting a role for Tsg1 in specifying ventral cell fates as a BMP signaling agonist. Moreover, loss of tsg1 partially suppressed the ventralized phenotypes of mutants of the BMP antagonists Chordin or Sizzled (Ogon). Our results support a model in which zebrafish Tsg1 promotes BMP signaling, and thus ventral cell fates, during DV axial patterning.
Descriptors: zebrafish, bone morphogenetic proteins metabolism, gastrula metabolism, signal transduction, Western blotting, cell lineage, *Drosophila* genotype, glycoproteins metabolism, heterozygote, in situ hybridization, intercellular signaling peptides and proteins metabolism, phenotype, RNA metabolism, messenger RNA metabolism, reverse transcriptase PCR, time factors, transgenes.

Little, S.C. and M.C. Mullins (2005). **Twisted gastrulation promotes BMP signaling in zebrafish DV axial patterning.** *Developmental Biology* 283(2): 620. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, molecular genetics, phenotype, signaling pathway, gastrulation, axial patterning.

Liu, E., E.B. Friedrich, S. Sinha, R. St.Arnaud, T. Force, A. Rosenzweig, S. Dedhar, and R.E. Gerszten (2004). **Targeted deletion of ILK reveals an essential role in vertebrate development**. *Circulation* 110(17, Suppl. S): 145. ISSN: 0009 7322.
NAL Call Number: RC681.A1C8
Abstract: Integrin-Linked Kinase (ILK) is a 59-kDa PI 3-Kinase-dependent serine/threonine kinase that interacts with beta integrins. We have previously used adenoviral manipulation of primary endothelial cells to demonstrate a role for ILK in beta 1 integrin activation, focal adhesion formation and endothelial cell apoptosis in vitro. Here we tested the hypothesis that ILK is necessary for vertebrate vascular development in vivo. We crossed recently gener-

ated ILKflox/flox mice with Tie2-Cre transgenic mice to achieve endothelial-specific ILK deletion. Subsequent intercrosses yielded no viable Tie2-Cre(tg/+) ILKflox/flox mice among 132 offspring (p<0.0001 by (X)2 analysis). Timed matings revealed gross retardation of ILK deficient embryos by E10.5. Because vascularization of the placental labyrinth is a critical determinant of development at this stage, we first examined the chorioallantoic placental circulation. Tie2-Cre(tg/+) ILKflox/flox E1.05 embryos had ineffective allantoic vessel extension into the chorion, and markedly diminished endothelial cell lined vascular networks, as opposed to Tie2-Cre(tg/+) ILK+/+ littermates (2.5 +/- 0.9 vs. 25.5 +/- 7.6 per hpf, n = 5, p<0.02). There was also striking failure of yolk sac vascularization, in which the fine network of branching capillaries was entirely absent. To complement the analysis in the mice and circumvent placental confounders, we used antisense morpholino oligonucleotides to knockdown ILK in zebrafish. ILK knockdown fish (>95% decrease in mRNA as compared to wild type) also exhibited dramatic vascular abnormalities, with marked patterning defects of the intersegmental vessels. Taken together, these results confirm an important role for ILK in endothelial cell survival and angiogenesis. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, mice, development, cardiovascular system, transport and circulation, angiogenesis, endothelial cell survival, Integrin-Linked kinase.

Liu, N.A., H. Huang, Z. Yang, W. Herzog, M. Hammerschmidt, S. Lin, and S. Melmed (2003). **Pituitary corticotroph ontogeny and regulation in transgenic zebrafish.** *Molecular Endocrinology* 17(5): 959-66. ISSN: 0888-8809.
NAL Call Number: QP187.3M64M6
Abstract: We characterized zebrafish proopiomelanocortin (POMC) gene promoter, and sequence analysis revealed that the promoter contains regulatory elements conserved among vertebrate species. To monitor the ontogeny of the pituitary POMC lineage in living vertebrates, we generated transgenic zebrafish expressing green fluorescent protein (GFP) driven by the POMC promoter. Zebrafish POMC-GFP is first expressed asymmetrically as two bilateral groups of cells most anterior to the neural ridge midline at 18-20 h post fertilization (hpf). POMC-GFP-positive cells then fuse into a single-cell mass within the pituitary anlage after 24 hpf and subsequently organize as distinct anterior and posterior domains between 48 and 64 hpf. Immunohistochemical studies with ACTH and alphaMSH antisera showed that POMC-GFP was mainly targeted to both anterior and posterior pituitary corticotrophs, whereas posterior pituitary region melanotrophs did not express GFP. To determine in vivo zebrafish corticotroph responses, dexamethasone (10(-5) m) was added to live embryos, which selectively suppressed POMC-GFP expression in the anterior group of corticotrophs, suggesting a distinct domain that is responsive to glucocorticoid feedback. Transgenic zebrafish with specific POMC-GFP expression in pituitary corticotrophs offers a powerful genetic system for in vivo study of vertebrate corticotroph lineage development.
Descriptors: zebrafish embryos, developmental gene expression regulation, pituitary gland physiology, pro opiomelanocortin genetics, genetically modified, base sequence, cell lineage, conserved sequence, dexamethasone pharmacology, down regulation drug effects, drug effects, green fluorescent proteins, luminescent proteins genetics and metabolism, molecular sequence data, pituitary gland cytology, drug effects and embryology, pro opiomelanocortin metabolism, promoter regions genetics, vertebrates genetics, proteins metabolism.

Liu, Q., A.E. Kerstetter, E. Azodi, and J.A. Marrs (2003). **Cadherin 1, 2 and 11 expression and cadherin-2 function in the pectoral limb bud and fin of the developing zebrafish.** *Developmental Dynamics* 228(4): 734-9. ISSN: 1058 8388.
NAL Call Number: QL801.A4

Descriptors: zebrafish embryos, cadherins biosynthesis and physiology, extremities embryology, developmental gene expression regulation, limb bud embryology, immunohistochemistry, in situ hybridization, antisense oligonucleotides pharmacology, messenger RNA metabolism, pectoral fins.

Liu, Q., B. Liu, A.L. Wilson, and J. Rostedt (2006). **Cadherin-6 message expression in the nervous system of developing zebrafish.** *Developmental Dynamics* 235(1): 272-8. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, cadherins biosynthesis and genetics, central nervous system metabolism, peripheral nervous system metabolism, messenger RNAbiosynthesis, central nervous system chemistry, peripheral nervous system chemistry, messenger RNA metabolism.

Liu, W.Y., Y. Wang, Y.H. Sun, Y. Wang, Y.P. Wang, S.P. Chen, and Z.Y. Zhu (2005). **Efficient RNA interference in zebrafish embryos using siRNA synthesized with SP6 RNA polymerase.** *Development Growth and Differentiation* 47(5): 323-31 . ISSN: 0012 1592.
NAL Call Number: QL951.E4
Abstract: Double-stranded RNA (dsRNA) has been shown to be a useful tool for silencing genes in zebrafish (*Danio rerio*), while the blocking specificity of dsRNA is still of major concern for application. It was reported that siRNA (small interfering RNA) prepared by endoribonuclease digestion (esiRNA) could efficiently silence endogenous gene expression in mammalian embryos. To test whether esiRNA could work in zebrafish, we utilized *Escherichia coli* RNaseIII to digest dsRNA of zebrafish no tail (ntl), a mesoderm determinant in zebrafish and found that esi-ntl could lead to developmental defects, however, the effective dose was so close to the toxic dose that esi-ntl often led to non-specific developmental defects. Consequently, we utilized SP6 RNA polymerase to produce si-ntl, siRNA designed against ntl, by in vitro transcription. By injecting in vitro synthesized si-ntl into zebrafish zygotes, we obtained specific phenocopies of reported mutants of ntl. We achieved up to a 59%no tail phenotype when the injection concentration was as high as 4 microg/microL. Quantitative reverse transcription-polymerase chain reaction (RT-PCR) and whole-mount in situ hybridization analysis showed that si-ntl could largely and specifically reduce mRNA levels of the ntl gene. As a result, our data indicate that esiRNA is unable to cause specific developmental defects in zebrafish, while siRNA should be an alternative for downregulation of specific gene expression in zebrafish in cases where RNAi techniques are applied to zebrafish reverse genetics.
Descriptors: zebrafish, DNA directed RNA polymerases metabolism, RNA interference, double stranded RNA genetics, small interfering RNA biosynthesis, base sequence, metabolism, GTP binding developmental gene expression regulation, microinjections, molecular sequence data, phenotype, small interfering RNA genetics, reverse transcriptase PCR, T-box domain proteins genetics and metabolism, proteins genetics and metabolism.

Liu, Y., N. Pathak, A. Kramer Zucker, and I.A. Drummond (2007). **Notch signaling controls the differentiation of transporting epithelia and multiciliated cells in the zebrafish pronephros.** *Development* 134(6): 1111-22. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Epithelial tubules consist of multiple cell types that are specialized for specific aspects of organ function. In the zebrafish pronephros, multiciliated cells (MCCs) are specialized for fluid propulsion, whereas transporting epithelial cells recover filtered-blood solutes. These cell types are distributed in a ;salt-and-pepper' fashion in the pronephros, suggesting that a lateral inhibition mechanism may play a role in their differentiation. We find that the

Notch ligand Jagged 2 is expressed in MCCs and that notch3 is expressed in pronephric epithelial cells. Morpholino knockdown of either jagged 2 or notch3, or mutation in mind bomb (in which Notch signaling is impaired), dramatically expands ciliogenic gene expression, whereas ion transporter expression is lost, indicating that pronephric cells are transfated to MCCs. Conversely, ectopic expression of the Notch1a intracellular domain represses MCC differentiation. Gamma-secretase inhibition using DAPT demonstrated a requirement for Notch signaling early in pronephric development, before the pattern of MCC differentiation is apparent. Strikingly, we find that jagged 2 knockdown generates extra cilia and is sufficient to rescue the kidney cilia mutant double bubble. Our results indicate that Jagged 2/Notch signaling modulates the number of multiciliated versus transporting epithelial cells in the pronephros by way of a genetic pathway involving repression of rfx2, a key transcriptional regulator of the ciliogenesis program.

Descriptors: zebrafish embryos, homeodomain proteins metabolism, nephrons embryology , nerve tissue proteins metabolism, Notch1 receptor metabolism, Notch receptors physiology, proteins metabolism and physiology, amyloid precursor protein secretases antagonists and inhibitors, amyloid precursor protein secretases metabolism, calcium binding proteins antagonists and inhibitors, calcium binding proteins metabolism and genetics, cell differentiation, cilia metabolism, cilia physiology, cytology, metabolism, epithelium metabolism, homeodomain nephrons cytology and metabolism, nerve tissue proteins genetics, Notch1 receptor genetics, Notch receptors genetics, signal transduction, ubiquitin protein ligases genetics, ubiquitin protein ligases metabolism, proteins antagonists and inhibitors.

Liu, Y.W. and L. Guo (2006). **Endothelium is required for the promotion of interrenal morphogenetic movement during early zebrafish development.** *Developmental Biology* 297(1): 44-58. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The adrenal cortex has a complex vasculature that is essential for growth, tissue maintenance, and access of secreted steroids to the bloodstream. However, the interaction between vasculature and adrenal cortex during early organogenesis remains largely unclear. In this study, we focused on the zebrafish counterpart of adrenal cortex, interrenal tissue, to explore the possible role of endothelium in the development of steroidogenic tissues. The ontogeny of interrenal tissue was found to be tightly associated with the endothelial cells (ECs) that constitute the axial vessels. The early interrenal primordia emerge as two clusters of cells that migrate centrally and converge at the midline, whereas the central convergence was abrogated in the avascular cloche (clo) mutant. Neither loss of blood circulation nor perturbations of vessel assembly could account for the interrenal convergence defect, implying a role of endothelial signaling prior to the formation of axial blood vessels. Moreover, as the absence of trunk endothelium in clo mutant was rescued by the forced expression of SCL, the interrenal fusion defect could be alleviated. We thus conclude that endothelial signaling is involved in the morphogenetic movement of early interrenal tissue.

Descriptors: zebrafish embryos, endothelium embryology, kidney embryology, 3 hydroxysteroid dehydrogenases metabolism, basic helix loop helix transcription factors genetics, cell movement endothelial cells cytology, developmental gene expression regulation, kidney blood supply, kidney cytology, mutation, proto oncogene renal circulation, steroids metabolism, transcription factors genetics.

Logan, M. (2003). **Finger or toe: the molecular basis of limb identity.** *Development* 130(26): 6401-6410. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Descriptors: zebrafish embryo, development, molecular genetics, whole mount in situ hybridization, genetic techniques, gene expression.

Lom, B., D.C. Chemotti, L.W. Cook, S.N. Davis, I.R. Willoughby, C. Morales, and C.J. Paradise (2004). **The pesticide malathion disrupts *xenopus* and zebrafish development: an investigative undergraduate laboratory in developmental toxicology.** *Developmental Biology* 271(2): 594-595. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, *Xenopus*, toxicology, development, education , malathion, pesticide, disruption.

Londin, E.R., J. Niemiec, and H.I. Sirotkin (2005). **Chordin, FGF signaling, and mesodermal factors cooperate in zebrafish neural induction.** *Developmental Biology* 279(1): 1-19. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The ectoderm gives rise to both neural tissue and epidermis. In vertebrates, specification of the neural plate requires repression of bone morphogenetic protein (BMP) signaling in the dorsal ectoderm. The extracellular BMP antagonist Chordin and other signals from the dorsal mesoderm play important roles in this process. We utilized zebrafish mutant combinations that disrupt Chordin and mesoderm formation to reveal additional signals that contribute to the establishment of the neural domain. We demonstrate that fibroblast growth factor (FGF) signaling accounts for the additional activity in neural specification. Impeding FGF signaling results in a shift of ectodermal markers from neural to epidermal. However, following inhibition of FGF signaling, expression of anterior neural markers recovers in a Nodal-dependent fashion. Simultaneously blocking, Chordin, mesoderm formation, and FGF signaling eliminates neural marker expression during gastrula stages. We observed that FGF signaling is required for chordin expression but that it also acts via other mechanisms to repress BMP transcription during late blastula stages. Activation of FGF signaling was also able to repress BMP transcription in the absence of protein synthesis. Our results support a model in which specification of anterior neural tissue requires early FGF-mediated repression of BMP transcript levels and later activities of Chordin and mesodermal factors.
Descriptors: zebrafish embryos, bone morphogenetic, Fgf physiology, glyco, intercellular signaling peptides and, mesoderm cytology, nervous system embryology , signal transduction physiology, ectoderm physiology morphogenesis.

Long, S., N. Ahmad, and M. Rebagliati (2003). **The zebrafish nodal-related gene southpaw is required for visceral and diencephalic left-right asymmetry.** *Development* 130(11): 2303-16. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: We have identified and characterized a new zebrafish gene, southpaw, that is required for visceral and diencephalic left-right asymmetry. southpaw encodes a new member of the nodal-related class of proteins, a subfamily within the transforming growth factor beta superfamily of secreted factors. southpaw is expressed bilaterally in paraxial mesoderm precursors and then within the left lateral plate mesoderm. At late somite stages, left-sided southpaw expression transiently overlaps the left-sided expression domains of other genes that mark the developing heart, such as lefty2. We have injected morpholinos to block the translation of the southpaw mRNA or to block splicing of the southpaw pre-mRNA. These morpholinos cause a severe disruption of early (cardiac jogging) and late (cardiac looping) aspects of cardiac left-right asymmetry. As the left-right asymmetry of the pancreas is also

affected, southpaw appears to regulate left-right asymmetry throughout a large part of the embryo. Consistent with the morphological changes, the left-sided expression domains of downstream genes (cyclops, pitx2, lefty1 and lefty2) are severely downregulated or abolished within the lateral plate mesoderm of Southpaw-deficient embryos. Surprisingly, despite the absence of southpaw expression in the brain, we find that early diencephalic left-right asymmetry also requires Southpaw activity. These observations lead to a model of how visceral organ and brain left-right asymmetry are coordinated during embryogenesis.
Descriptors: zebrafish embryo, diencephalon embryology, amino acid and base sequence, body patterning genetics, complementary DNA genetics, developmental gene expression regulation, congenital heart defects embryology , congenital heart defects genetics, in situ hybridization, mesoderm cytology, molecular sequence data, mutation, oligodeoxyribonucleotides, antisense genetics, oligodeoxyribonucleotides, antisense pharmacology, organizers, embryonic embryology, pancreas embryology, amino acid sequence homology, transforming growth factor beta genetics.

Lopez Schier, H., C.J. Starr, J.A. Kappler, R. Kollmar, and A.J. Hudspeth (2004). **Directional cell migration establishes the axes of planar polarity in the posterior lateral-line organ of the zebrafish.** *Developmental Cell* 7(3): 401-12. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: The proper orientation of mechanosensory hair cells along the lateral-line organ of a fish or amphibian is essential for the animal's ability to sense directional water movements. Within the sensory epithelium, hair cells are polarized in a stereotyped manner, but the mechanisms that control their alignment relative to the body axes are unknown. We have found, however, that neuromasts can be oriented either parallel or perpendicular to the anteroposterior body axis. By characterizing the strauss mutant zebrafish line and by tracking labeled cells, we have demonstrated that neuromasts of these two orientations originate from, respectively, the first and second primordia. Furthermore, altering the migratory pathway of a primordium reorients a neuromast's axis of planar polarity. We propose that the global orientation of hair cells relative to the body axes is established through an interaction between directional movement by primordial cells and the timing of neuromast maturation.
Descriptors: zebrafish embryos, epithelium embryology, hair cells embryology, body patterning, cell division, cell line, cell lineage, cell movement, DNA chemistry, fishes, immunohistochemistry, lasers, confocal microscopy, fluorescence microscopy, mutation, oligonucleotides chemistry, sense organs embryology.

Lowery, L.A., J. Rubin, and H. Sive (2007). **Whitesnake/sfpq is required for cell survival and neuronal development in the zebrafish.** *Developmental Dynamics* 236(5): 1347-57. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, brain embryology, brain growth and development, brain metabolism, cell death genetics, cell survival genetics, developmental gene expression regulation, in situ hybridization, mutation, neurons cytology, phenotype, messenger RNA genetics and metabolism, proteins genetics.

Lowery, L.A. and H. Sive (2005). **Initial formation of zebrafish brain ventricles occurs independently of circulation and requires the nagie oko and snakehead/atp1a1a.1 gene products.** *Development* 132(9): 2057-67. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The mechanisms by which the vertebrate brain develops its characteristic three-

dimensional structure are poorly understood. The brain ventricles are a highly conserved system of cavities that form very early during brain morphogenesis and that are required for normal brain function. We have initiated a study of zebrafish brain ventricle development and show here that the neural tube expands into primary forebrain, midbrain and hindbrain ventricles rapidly, over a 4-hour window during mid-somitogenesis. Circulation is not required for initial ventricle formation, only for later expansion. Cell division rates in the neural tube surrounding the ventricles are higher than between ventricles and, consistently, cell division is required for normal ventricle development. Two zebrafish mutants that do not develop brain ventricles are snakehead and nagie oko. We show that snakehead is allelic to small heart, which has a mutation in the Na+K+ ATPase gene atp1a1a.1. The snakehead neural tube undergoes normal ventricle morphogenesis; however, the ventricles do not inflate, probably owing to impaired ion transport. By contrast, mutants in nagie oko, which was previously shown to encode a MAGUK family protein, fail to undergo ventricle morphogenesis. This correlates with an abnormal brain neuroepithelium, with no clear midline and disrupted junctional protein expression. This study defines three steps that are required for brain ventricle development and that occur independently of circulation: (1) morphogenesis of the neural tube, requiring nok function; (2) lumen inflation requiring atp1a1a.1 function; and (3) localized cell proliferation. We suggest that mechanisms of brain ventricle development are conserved throughout the vertebrates.
Descriptors: zebrafish embryos, blood circulation physiology, cerebral ventricles embryology, guanylate cyclase genetics, Na+ K+ exchanging ATPase genetics, apoptosis physiology, cell proliferation, cerebral ventricles physiology, epistasis, genetic, guanylate cyclase metabolism, Na+ K+ exchanging ATPase metabolism.

Lucas, M.E. and P.D. Henion (2006). **Characterization of neural crest and lateral plate mesoderm development in zebrafish hatchback**. *Developmental Biology* 295(1): 410-411. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryology, nervous system, neural coordination, molecular genetics, development.

Lucas, M.E., F. Mueller, R. Ruediger, P.D. Henion, and H. Rohrer (2006). **The bhlh transcription factor hand2 is essential for noradrenergic differentiation of sympathetic neurons**. *Development* 133(20): 4015-4024. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The basic helix-loop-helix transcription factor Hand2, together with Ascl1, Phox2a, Phox2b and Gata2/Gata3, is induced by bone morphogenetic proteins in neural crest-derived precursor cells during sympathetic neuron generation. Hand2 overexpression experiments and the analysis of its function at the Dbh promotor implicated Hand2 in the control of noradrenergic gene expression. Using the zebrafish hand2 deletion mutant hands off, we have now investigated the physiological role of hand2 in the development of sympathetic ganglia. In hands off mutant embryos, sympathetic precursor cells aggregate to form normal sympathetic ganglion primordia characterized by the expression of phox2b, phox2a and the achaete-scute family member zash1a/ascl1. The expression of the noradrenergic marker genes th and dbh is strongly reduced, as well as the transcription factors gata2 and tfap2a (Ap-2 alpha). By contrast, generic neuronal differentiation seems to be unaffected, as the expression of elavl3 (HuC) is not reduced in hands off sympathetic ganglia. These results demonstrate in vivo an essential and selective function of hand2 for the noradrenergic differentiation of sympathetic neurons, and implicates tfap2a and gata2 as downstream effectors.

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Descriptors: zebrafish, molecular genetics, nervous system, neural coordination, cell differentiation, Hand 2 physiological role, development of sympathetic ganglia.

Lunde, K., G. Belting-Heinz, and W. Driever (2004). **Zebrafish pou5f/pou2, homolog of mammalian Oct4, functions in the endoderm specification cascade.** *Developmental Biology* 271(2): 572. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, development, molecular genetics, endoderm cascade, Oct4.

Lyman Gingerich, J., T.A. Westfall, D.C. Slusarski, and F. Pelegri (2005). **Hecate, a zebrafish maternal effect gene, affects dorsal organizer induction and intracellular calcium transient frequency.** *Developmental Biology* 286(2): 427-39. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: A zebrafish maternal effect mutation, in the gene hecate, results in embryos that have defects in the formation of dorsoanterior structures and altered calcium release. hecate mutant embryos lack nuclear accumulation of beta-catenin and have reduced expression of genes specific to the dorsal organizer. We found that hecate mutant embryos exhibit a nearly 10-fold increase in the frequency of intracellular Ca2+ transients normally present in the enveloping layer during the blastula stages. Inhibition of Ca2+ release leads to ectopic expression of dorsal genes in mutant embryos suggesting that Ca2+ transients are important in mediating dorsal gene expression. Inhibition of Ca2+ release also results in the expression of dorsal-specific genes in the enveloping layer in a beta-catenin-independent manner, which suggests an additional function for the Ca2+ transients in this cellular layer. The mutant phenotype can be reversed by the expression of factors that activate Wnt/beta-catenin signaling, suggesting that the Wnt/beta-catenin pathway, at least as activated by an exogenous Wnt ligand, is intact in hec mutant embryos. Our results are consistent with a role for the hecate gene in the regulation of Ca2+ release during the cleavage stages, which in turn influences dorsal gene expression in both marginal cells along the dorsoventral axis and in the enveloping layer.
Descriptors: zebrafish, calcium metabolism, choristoma genetics, mutation, organizers, embryonic embryology, developmental gene expression regulation, Wnt proteins metabolism, beta catenin metabolism.

Ma, A., R. Lin, P.K. Chan, J.C. Leung, L.Y. Chan, A. Meng, C.M. Verfaillie, R. Liang, and A.Y. Leung (2007). **The role of survivin in angiogenesis during zebrafish embryonic development.** *BioMed Central Developmental Biology* 7: 50. ISSN: 1471 213X.
NAL Call Number: QH491.B63
Abstract: BACKGROUND: Survivin is the smallest member of the inhibitor of apoptosis (IAP) gene family. Recently, the zebrafish survivin-1 gene has been cloned, showing remarkable sequence identity and similarity over the BIR domain compared with human and mouse survivin gene. Here we investigated the role of survivin in angiogenesis during zebrafish development. Morpholinos (MOs) targeting the 5' untranslated region (UTR) (SurUTR) and sequences flanking the initiation codon (SurATG) of zebrafish survivin-1 gene were injected into embryos at 1-4 cell stage. Vasculature was examined by microangiography and GFP expression in Tg(fli1:EGFP)y1 embryos. RESULTS: In embryos co-injected with SurUTR and SurATG-MOs, vasculogenesis was intact but angiogenesis was markedly perturbed, especially in the inter-segmental vessels (ISV) and dorsal longitudinal anastomotic vessels (DLAV) of the trunk, the inner optic circle and optic veins of developing eyes and the

sub-intestinal vessels. Apoptosis was increased, as shown by TUNEL staining and increase in caspase-3 activity. Efficacy of SurUTR and SurATG-MOs was demonstrated by translation inhibition of co-injected 5'UTR survivin:GFP plasmids. The phenotypes could be recapitulated by splice-site MO targeting the exon2-intron junction of survivin gene and rescued by survivin mRNA. Injection of human vascular endothelial growth factor (VEGF) protein induced ectopic angiogenesis and increased survivin expression, whereas treatment with a VEGF receptor inhibitor markedly reduced angiogenesis and suppressed survivin expression. CONCLUSION: Survivin is involved in angiogenesis during zebrafish development and may be under VEGF regulation.

Descriptors: zebrafish, metabolism, developmental gene expression regulation, inhibitor of apoptosis proteins genetics, physiologic neovascularization genetics, reverse transcriptase PCR, vascular endothelialgrowth factor A genetics, physiology.

Ma, O.L., S.E. Webb, and A.L. Miller (2004). **Imaging patterns of Ca2+ transients during the blastula period in zebrafish embryos**. *Biological Bulletin (Woods Hole)* 207(2): 165. ISSN: 0006 3185.

NAL Call Number: 442.8B52

Descriptors: zebrafish, cell biology, development, marine ecology, environmental sciences, two photon confocal microscopy, imaging and microscopy techniques, photon imaging microscope, laboratory equipment, cell to cell adhesion, dorsoventral patterning.

Maack, G. and H. Segner (2003). **Morphological development of the gonads in zebrafish.** *Journal of Fish Biology* 62(4): 895-906. ISSN: 0022 1112.

NAL Call Number: QL614.J68

Abstract: Gonadogenesis of zebrafish *Danio rerio* was investigated by means of light microscopy to test the suitability of gonad histology as an endpoint in hazard assessment of endocrine-active compounds. At age 2 weeks post-fertilization (pf), primordial germ cells were found in a dorsocaudal position in the body cavity. At 4 weeks pf, the majority of the fish (86%) possessed paired gonads with meiotic germ cells; these gonads represented presumptive ovaries. At week 5 pf, 87% of the fish examined had ovaries with perinucleolar oocytes. Further development of the gonads in female zebrafish up to week 11 pf was characterized by an increase in gonad size as well as in the number and size of perinucleolar oocytes. Starting with week 5, some fish showed alterations of gonad morphology, including a decrease in the number and size of the oocytes, an enhanced basophilia and irregular shape of the oocytes, and finally their degeneration into residual bodies. With the decline in oocyte number, stromal cells became more numerous and they infiltrated the gonadal matrix. In several 7 week-old zebrafish with altered gonadal morphology, enhanced numbers of gonial cells arranged in cyst-like groups appeared. These gonads were interpreted as presumptive testes. In one fish out of 32 individuals examined, spermatocytes were detected, in addition to the gonial cells. During the subsequent weeks, the percentage of fish showing early testes with spermatogonia, spermatocytes and spermatids increased and reached 40% at 11 weeks pf. The sequence of gonadal alterations taking place in some of the individuals from week 5 pf onwards was interpreted to reflect the transition of protogynic ovaries into testes. The developmental pattern described identifies zebrafish to be a juvenile hermaphrodite. The results of this study are of relevance for the use of gonadal histopathology as endpoint in endocrine disruption testing, particularly in order to avoid false diagnoses of 'intersex gonads' in zebrafish.

Descriptors: zebrafish, *Danio rerio*, gonad morphological development, endocrine disruption testing.

Mably, J.D., L.P. Chuang, F.C. Serluca, M.A. Mohideen, J.N. Chen, and M.C. Fishman (2006). **Santa and valentine pattern concentric growth of cardiac myocardium in the zebrafish.** *Development* 133(16): 3139-46. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: During embryogenesis, the myocardial layer of the primitive heart tube grows outward from the endocardial-lined lumen, with new cells added to generate concentric thickness to the wall. This is a key evolutionary step, demarcating vertebrates from more primitive chordates, and is essential for normal cardiac function. Zebrafish embryos with the recessive lethal mutations santa (san) and valentine (vtn) do not thicken, but do add the proper number of cells to the myocardium. Consequently, the heart chambers are huge, constituted of a monolayered myocardium lined by endocardium. This phenotype is similar to that of the heart of glass (heg) mutation, which we described previously as a novel endocardial expressed gene. By positional cloning, we here identify san as the zebrafish homolog of human CCM1, and vtn as the homolog of human CCM2. Dominant mutations of either in humans cause vascular anomalies in the brain, known as cerebral cavernous malformations. The synergistic effects of morpholino pairs indicate that san, vtn and heg are in a genetic pathway, and san and vtn contain protein motifs, NPxY and PTB domain, respectively, known to interact. This suggests that concentric growth of the myocardium, crucial for blood pressure generation, is dictated by a heg-san-vtn signaling pathway.

Descriptors: zebrafish embryo, congenital heart defects embryology, membrane glycoproteins metabolism, muscle proteins metabolism, proteins metabolism, amino acid sequence, body patterning genetics, cell count, cytology, metabolism, embryonic development genetics, endocardium cytology, endocardium embryology, gene expression regulation, lethal genes, heart embryology, congenital heart defects genetics and metabolism, molecular sequence data, muscle mutation, myocardium cytology, myocardium metabolism, messenger RNA metabolism, proteins genetics.

Macatee, T.L., B.P. Hammond, B.R. Arenkiel, L. Francis, D.U. Frank, and A.M. Moon (2003). **Ablation of specific expression domains reveals discrete functions of ectoderm- and endoderm-derived fgf8 during cardiovascular and pharyngeal development.** *Development* 130(25): 6361-6374. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Fibroblast growth factor 8 (Fgf8) is expressed in many domains of the developing embryo. Globally decreased FGF8 signaling during murine embryogenesis results in a hypomorphic phenotype with a constellation of heart, outflow tract, great vessel and pharyngeal gland defects that phenocopies human deletion 22q11 syndromes, such as DiGeorge. We postulate that these Fgf8 hypomorphic phenotypes result from disruption of local FGF8 signaling from pharyngeal arch epithelia to mesenchymal cells populating and migrating through the third and fourth pharyngeal arches. To test our hypothesis, and to determine whether the pharyngeal ectoderm and endoderm Fgf8 expression domains have discrete functional roles, we performed conditional mutagenesis of Fgf8 using novel Cre-recombinase drivers to achieve domain-specific ablation of Fgf8 gene function in the pharyngeal arch ectoderm and endoderm. Remarkably, ablating FGF8 protein in the pharyngeal arch ectoderm causes failure of formation of the fourth pharyngeal arch artery that results in aortic arch and subclavian artery anomalies in 95% of mutants; these defects recapitulate the spectrum and frequency of vascular defects reported in Fgf8 hypomorphs. Surprisingly, no cardiac, outflow tract or glandular defects were found in ectodermal-domain mutants, indicating that ectodermally derived FGF8 has essential roles during pharyngeal arch vascular development distinct from those in cardiac, outflow tract and pharyngeal gland morphogenesis. By contrast, abla-

tion of FGF8 in the third and fourth pharyngeal endoderm and ectoderm caused glandular defects and bicuspid aortic valve, which indicates that the FGF8 endodermal domain has discrete roles in pharyngeal and valvar development. These results support our hypotheses that local FGF8 signaling from the pharyngeal epithelia is required for pharyngeal vascular and glandular development, and that the pharyngeal ectodermal and endodermal domains of FGF8 have separate functions. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, development, molecular genetics, DiGeorge syndrome, immune system disease, congenital heart disease, congenital disease, heart disease, cardiovascular development, murine embryogenesis, pharyngeal development , specific expression domain ablation, vasculogenesis, zebrafish .

Machingo, Q.J., A. Fritz, and B.D. Shur (2006). **A beta1,4-galactosyltransferase is required for Bmp2-dependent patterning of the dorsoventral axis during zebrafish embryogenesis.** *Development* 133(11): 2233-41. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Complex carbohydrates are highly polymorphic macromolecules that are involved in diverse biological processes; however, a detailed understanding of their function remains obscure. To better define the roles of complex carbohydrates during vertebrate embryogenesis, we have initiated an analysis of glycosyltransferase function using the zebrafish system. In this study, we report the characterization of a zebrafish beta1,4-galactosyltransferase (GalT), which has substantial homology with mammalian beta4GalT5 and is expressed zygotically throughout the zebrafish embryo. Downregulating the expression of beta4GalT5 by injection of specific morpholino oligonucleotides results in dorsalized zebrafish embryos, suggesting a role of beta4GalT5 in Bmp2-mediated specification of the dorsoventral axis. Consistent with this, morpholino-injected embryos have ventrally expanded chordin expression and reduced activation of the Bmp-dependent transcription factors Smad1/5/8. Because other growth factors, such as Egf and Fgf, require binding to extracellular proteoglycans for delivery and/or binding to their cognate receptors, we examined whether proteoglycans isolated from control and morpholino-injected embryos show differential binding affinities for Bmp2. In this regard, proteoglycans isolated from beta4GalT5 morphant embryos are underglycosylated and are unable to bind recombinant Bmp2 as efficiently as proteoglycans from control-injected embryos, whereas the binding of Bmp7 is relatively unaffected. These results suggest that beta4GalT5 is a previously unidentified zebrafish galactosyltransferase that is essential for proper patterning of the dorsoventral axis by regulating Bmp2 signaling. Furthermore, this work demonstrates that a relatively simple carbohydrate modification to endogenous proteoglycans can modulate the specificity of cytokine signaling.
Descriptors: zebrafish, body patterning, bone morphogenetic proteins metabolism, embryology, enzymology, galactosyltransferases metabolism, transforming growth factor beta metabolism, amino acid sequence, genetically modified, bone morphogenetic molecular cloning, metabolism, galactosyltransferases chemistry, deficiency and genetics, developmental gene expression regulation, glycoproteins metabolism, glycosylation, intercellular signaling peptides and proteins metabolism, molecular sequence data, molecular weight, phenotype, phylogeny, protein binding, proteoglycans metabolism, sequence alignment, amino acid sequence homology, Smad proteins, transforming growth factor beta genetics.

Machingo, Q.J., A. Fritz, and B.D. Shur (2006). **A beta1,4-galactosyltransferase is required for convergent extension movements in zebrafish.** *Developmental Biology* 297(2): 471-82. ISSN: 0012 1606.
NAL Call Number: 442.8D49

Abstract: Our understanding of how complex carbohydrates function during embryonic development is still very limited, primarily due to the large number of glycosyltransferases now known to be involved in their synthesis. To overcome these limitations, we have taken advantage of the zebrafish system to analyze the function of complex carbohydrates during development by down-regulating the expression of specific glycosyltransferases. Herein, we report the identification of the zebrafish ortholog of mammalian beta1,4-galactosyltransferase I, beta4GalT1, and its requirement for proper convergent extension movements during gastrulation. beta4GalT1 is expressed in the oocyte and throughout the embryo during the first 24 h of development. Knockdown of zebrafish beta4GalT1 by two independent morpholino oligonucleotides results in embryos with a truncated anterior-posterior axis, as well as elongated somites and moderate defects in the patterning of the head mesenchyme. Co-injection of zebrafish beta4GalT1 mRNA returns galactosyltransferase activity to control levels and rescues the defects produced by morpholino oligonucleotides. In situ hybridizations of various molecular markers reveal that the axial mesoderm of epiboly stage embryos is abnormally widened in beta4GalT1 morphants, indicative of abnormal convergent extension. Consistent with this, the rate of anterior-posterior axis elongation is reduced relative to control-injected embryos, similar to that seen in known convergent extension mutants. Among the many potential substrates for beta4GalT1 is laminin, a principle component of the extracellular matrix that supports cell movements such as those that occur during convergent extension. Previous in vitro studies have shown that the galactosylation status of laminin directly influences its ability to support cell spreading and migration. In this regard, laminin isolated from beta4GalT1 morphant embryos is poorly galactosylated, which may contribute to defective cell migration during convergent extension movements. This work demonstrates that zebrafish can be used to identify critical developmental roles for specific glycosyltransferases that would not be obvious otherwise, such as an absolute requirement for beta4GalT1 during convergent extension movements.
Descriptors: zebrafish, developmental model, developmental gene expression regulation, n acetyllactosamine synthase physiology, amino acid sequence, extracellular matrix metabolism, galactose metabolism, laminin metabolism, genetic models, molecular sequence data, mutation, n acetyllactosamine synthase chemistry, messenger RNA metabolism, amino acid sequence homology.

Mackay, A.B., A. Mhanni, and P.H. Krone (2005). **DNA methylation reprogramming during embryonic development in zebrafish.** *Developmental Biology* 283(2): 606. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryo, immunohistochemistry, immunologic techniques, South-Western dot blot analysis, spectrum analysis techniques, DNA methylation reprogramming.

Mackereth, M.D., S.J. Kwak, A. Fritz, and B.B. Riley (2005). **Zebrafish pax8 is required for otic placode induction and plays a redundant role with Pax2 genes in the maintenance of the otic placode.** *Development* 132(2): 371-82. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Vertebrate Pax2 and Pax8 proteins are closely related transcription factors hypothesized to regulate early aspects of inner ear development. In zebrafish and mouse, Pax8 expression is the earliest known marker of otic induction, and Pax2 homologs are expressed at slightly later stages of placodal development. Analysis of compound mutants has not been reported. To facilitate analysis of zebrafish pax8, we completed sequencing of the entire gene, including the 5' and 3' UTRs. pax8 transcripts undergo complex alternative splicing

to generate at least ten distinct isoforms. Two different subclasses of pax8 splice isoforms encode different translation initiation sites. Antisense morpholinos (MOs) were designed to block translation from both start sites, and four additional MOs were designed to target different exon-intron boundaries to block splicing. Injection of MOs, individually and in various combinations, generated similar phenotypes. Otic induction was impaired, and otic vesicles were small. Regional ear markers were expressed correctly, but hair cell production was significantly reduced. This phenotype was strongly enhanced by simultaneously disrupting either of the co-inducers fgf3 or fgf8, or another early regulator, dlx3b, which is thought to act in a parallel pathway. In contrast, the phenotype caused by disrupting foxi1, which is required for pax8 expression, was not enhanced by simultaneously disrupting pax8. Disrupting pax8, pax2a and pax2b did not further impair otic induction relative to loss of pax8 alone. However, the amount of otic tissue gradually decreased in pax8-pax2a-pax2b-deficient embryos such that no otic tissue was detectable by 24 hours post-fertilization. Loss of otic tissue did not correlate with increased cell death, suggesting that otic cells dedifferentiate or redifferentiate as other cell type(s). These data show that pax8 is initially required for normal otic induction, and subsequently pax8, pax2a and pax2b act redundantly to maintain otic fate.

Descriptors: zebrafish embryos, DNA binding proteins genetics, proteins genetics and physiology, ear embryology, nuclear proteins genetics and physiology, trans activators genetics, trans activators physiology, 3' untranslated regions, 5' untranslated regions, amino acid and base sequence, cell death, cell lineage, molecular cloning, DNA binding proteins genetics, proteins metabolism, exons, fibroblast growth factor 3, fibroblast growth factor 8, Fgf metabolism, forkhead transcription factors, homeodomain proteins metabolism, in situ hybridization, fluorescence microscopy, molecular sequence data, nuclear proteins metabolism, antisense oligonucleotides chemistry, pax2 transcription factor, paired box transcription factors, phenotype, protein isoforms, DNA sequence analysis, time factors, transcription factors metabolism, genetic transcription.

Maderspacher, F. and C. Nusslein Volhard (2003). **Formation of the adult pigment pattern in zebrafish requires leopard and obelix dependent cell interactions.** *Development* 130(15): 3447-57. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Colour patterns are a prominent feature of many animals and are of high evolutionary relevance. In zebrafish, the adult pigment pattern comprises alternating stripes of two pigment cell types, melanophores and xanthophores. How the stripes are defined and a straight boundary is formed remains elusive. We find that mutants lacking one pigment cell type lack a striped pattern. Instead, cells of one type form characteristic patterns by homotypic interactions. Using mosaic analysis, we show that juxtaposition of melanophores and xanthophores suffices to restore stripe formation locally. Based on this, we have analysed the pigment pattern of two adult specific mutants: leopard and obelix. We demonstrate that obelix is required in melanophores to promote their aggregation and controls boundary integrity. By contrast, leopard regulates homotypic interaction within both melanophores and xanthophores, and interaction between the two, thus controlling boundary shape. These findings support a view in which cell-cell interactions among pigment cells are the major driving force for adult pigment pattern formation.

Descriptors: zebrafish, color pattern, cell communication physiology, skin pigmentation physiology, blastula cytology, cell transplantation, melanophores physiology, xanthophores, leopard and obelix patterns.

Maeda, A. and T. Mizuno (2005). **The role of tailbud in the tail formation in zebrafish**. *Zoological Science* 22(12): 1465. ISSN: 0289 0003.
NAL Call Number: 442P92
Descriptors: zebrafish embryology, development, somitogenesis, gastrulation, tail development.

Maegawa, S., M. Varga, and E.S. Weinberg (2006). **FGF signaling is required for {beta}-catenin-mediated induction of the zebrafish organizer.** *Development* 133(16): 3265-76. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: We have used the maternal effect mutant ichabod, which is deficient in maternal beta-catenin signaling, to test for the epistatic relationship between beta-catenin activation, FGF signaling and bozozok, squint and chordin expression. Injection of beta-catenin RNA into ichabod embryos can completely rescue normal development. By contrast, when FGF signaling is inhibited, beta-catenin did not induce goosecoid and chordin, repress bmp4 expression or induce a dorsal axis. These results demonstrate that FGF signaling is necessary for beta-catenin induction of the zebrafish organizer. We show that FGFs function downstream of squint and bozozok to turn on chordin expression. Full rescue of ichabod by Squint is dependent on FGF signaling, and partial rescue by FGFs is completely dependent on chordin. By contrast, Bozozok can rescue the complete anteroposterior axis, but not notochord, in embryos blocked in FGF signaling. Surprisingly, accumulation of bozozok transcript in beta-catenin RNA-injected ichabod embryos is also dependent on FGF signaling, indicating a role of FGFs in maintenance of bozozok RNA. These experiments show that FGF-dependent organizer function operates through both bozozok RNA accumulation and a pathway consisting of beta-catenin-->Squint-->FGF-->Chordin, in which each component is sufficient for expression of the downstream factors of the pathway, and in which Nodal signaling is required for FGF gene expression and FGF signaling is required for Squint induction of chordin.
Descriptors: zebrafish embryos, fibroblast growth factor 3 metabolism, fibroblast growth factor 8 metabolism and genetics, organizers, embryonic metabolism, beta catenin metabolism, body patterning, bone morphogenetic proteins metabolism, fibroblast growth factor 3 antagonists and inhibitors, fibroblast growth factor 3 genetics , fibroblast growth factor 8 antagonists and inhibitors, glycoproteins metabolism, homeodomain proteins genetics and metabolism, intercellular signaling peptides and proteins metabolism, signal transduction , genetic transcription, beta catenin agonists.

Mahler, J. and W. Driever (2007). **Expression of the zebrafish intermediate neurofilament Nestin in the developing nervous system and in neural proliferation zones at postembryonic stages.** *BioMed Central Developmental Biology* 7: 89. ISSN: 1471 213X.
NAL Call Number: QH491.B63
Abstract: BACKGROUND: The intermediate filament Nestin has been reported as a marker for stem cells and specific precursor cell populations in the developing mammalian central nervous system (CNS). Nestin expressing precursors may give rise to neurons and glia. Mouse nestin expression starts at the onset of neurulation in the neuroectodermal cells and is dramatically down regulated when progenitor cells differentiate and become post-mitotic. It has been reported that in the adult zebrafish (Danio rerio) active neurogenesis continues in all major subdivisions of the CNS, however few markers for zebrafish precursors cells are known, and Nestin has not been described in zebrafish. RESULTS: We cloned a zebrafish nestin gDNA fragment in order to find a marker for precursor cells in the develop-

ing and postembryonic brain. Phylogenetic tree analysis reveals that this zebrafish ortholog clusters with Nestin sequences from other vertebrates but not with other intermediate filament proteins. We analyzed nestin expression from gastrula stage to 4 day larvae, and in post-embryonic brains. We found broad expression in the neuroectoderm during somitogenesis. In the larvae, nestin expression progressively becomes restricted to all previously described proliferative zones of the developing and postembryonic central nervous system. nestin expressing cells of the forebrain also express PCNA during late embryogenesis, identifying them as proliferating precursor or neural stem cells. nestin is also expressed in the cranial ganglia, in mesodermal precursors of muscle cells, and in cranial mesenchymal tissue. CONCLUSION: Our data demonstrate that in zebrafish, like in mammals, the expression of the intermediated neurofilament nestin gene may serve as a marker for stem cells and proliferating precursors in the developing embryonic nervous system as well as in the postembryonic brain.
Descriptors: zebrafish embryology, cell proliferation, intermediate filament nerve tissue nervous system embryology, nervous system growth and development, growth and development, cell movement developmental gene expression regulation, intermediate filament proteins metabolism, intermediate filaments genetics and metabolism, nerve tissue proteins metabolism, nervous system metabolism, neurons physiology, phylogeny, proliferating cell nuclear antigen genetics, proliferating cell nuclear antigen metabolism, somites metabolism, stem cells metabolism and physiology.

Majumder, A., A. Sornborger, C. Keith, and J. Lauderdale (2006). **Zebrafish lateral line neuromasts respond to chemical stimulation: activation can be optically imaged in the embryo**. *Developmental Biology* 295(1): 456-457. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, nervous system, neural coordination, development, sense organs, sensory reception, optical imaging, chemical stimulation, fluorescence resonance energy transfer signal, structure function relationship, behavioral response, predator avoidance, zebrafish.

Makky, K. and A.N. Mayer (2005). **Target of rapamycin (tor) mediates the endoderm-intestine transition in zebrafish**. *Journal of Pediatric Gastroenterology and Nutrition* 41(4): 514. ISSN: 0277 2116.
NAL Call Number: RJ446.J68
Descriptors: zebrafish, development, molecular genetics, digestive system, ingestion and assimilation, endoderm intestine transition.

Makky, K., J. Tekiela, and A.N. Mayer (2007). **Target of rapamycin (TOR) signaling controls epithelial morphogenesis in the vertebrate intestine**. *Developmental Biology* 303(2): 501-513. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, cytology, developmental stages, digestive system, epithelial morphogenesis, embryonic development, freshwater fish, morphogenesis, organogenesis, *Danio rerio*.

Maldonado, E. and R.E. Navarro (2007). **An insertional mutation in the vesicle traffic vps18 gene produces visual acuity loss in zebrafish embryos**. *Developmental Biology* 306(1): 316. ISSN: 0012-1606.
NAL Call Number: 442.8D49

Descriptors: zebrafish embryos, embryonic development, freshwater fish, genes, mutations, *Danio rerio*.

Malicki, J., H. Jo, and Z. Pujic (2003). **Zebrafish N-cadherin, encoded by the glass onion locus, plays an essential role in retinal patterning.** *Developmental Biology* 259(1): 95-108. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Genetic screens in zebrafish identified several loci that play essential roles in the patterning of retinal architecture. Here, we show that one of them, glass onion, encodes the N-cadherin gene. The glo(m117) mutant allele contains a substitution of the Trp2 residue known for its essential role in the adhesive properties of classic cadherins. Both the glo(m117) and pac(tm101b) mutant N-cadherin alleles affect the polarity of the retinal neuroepithelial sheet and, unexpectedly, both result in cell-nonautonomous phenotypes in retinal patterning. The late onset of mutant N-cadherin phenotypes may be due to the ability of classic cadherins to substitute each other's function.

Descriptors: zebrafish embryos, cadherins physiology, retina embryology, amino acid sequence, body patterning, cadherins chemistry, cadherins genetics, chromosome mapping, molecular sequence data, mutation.

Marlow, F., E.M. Gonzalez, C. Yin, C. Rojo, and L. Solnica Krezel (2004). **No tail co-operates with non-canonical Wnt signaling to regulate posterior body morphogenesis in zebrafish.** *Development* 131(1): 203-16. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: The vertebrate posterior body is formed by a combination of the gastrulation movements that shape the head and anterior trunk and posterior specific cell behaviors. Here, we investigated whether genes that regulate cell movements during gastrulation [no tail (ntl)/brachyury, knypek (kny) and pipetail (ppt)/wnt5] interact to regulate posterior body morphogenesis. Both kny;ntl and ppt;ntl double mutant embryos exhibit synergistic trunk and tail shortening by early segmentation. Gene expression analysis in the compound mutants indicates that anteroposterior germ-layer patterning is largely normal and that the tail elongation defects are not due to failure to specify or maintain posterior tissues. Moreover, ntl interacts with ppt and kny to synergistically regulate the posterior expression of the gene encoding bone morphogenetic protein 4 (bmp4) but not of other known T-box genes, fibroblast growth factor genes or caudal genes. Examination of mitotic and apoptotic cells indicates that impaired tail elongation is not simply due to decreased cell proliferation or increased cell death. Cell tracing in ppt;ntl and kny;ntl mutants demonstrates that the ventral derived posterior tailbud progenitors move into the tailbud. However, gastrulation-like convergence and extension movements and cell movements within the posterior tailbud are impaired. Furthermore, subduction movements of cells into the mesendoderm are reduced in kny;ntl and ppt;ntl mutants. We propose that Ntl and the non-canonical Wnt pathway components Ppt and Kny function in parallel, partially redundant pathways to regulate posterior body development. Our work initiates the genetic dissection of posterior body morphogenesis and links genes to specific tail-forming movements. Moreover, we provide genetic evidence for the notion that tail development entails a continuation of mechanisms regulating gastrulation together with mechanisms unique to the posterior body.

Descriptors: zebrafish embryo, physiology, gastrula physiology, developmental gene expression regulation genetics, morphogenesis physiology, proto oncogene, tail embryology, proteins, body patterning physiology, genotype, movement physiology, mutation, phenotype, signal transduction, tail abnormalities, Wnt proteins.

Martin, E.D. and M. Grealy (2005). **Knockdown of plakoglobin in zebrafish development.** *Developmental Biology* 283(2): 673. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, cardiovascular system, transport and circulation, biochemistry and molecular biophysics, pericardial edema, heart disease.

Martinez, S., V. Robles, L. Anel, P. DePaz, and M.P. Herraez (2005). **Cell viability after vitrification of zebrafish embryos**. *Reproduction in Domestic Animals* 40(4): 402. ISSN: 0936 6768.
NAL Call Number: SF105.A1Z8
Descriptors: zebrafish embryos, methods and techniques, metabolism, development, reproductive system, reproduction, freeze damage, injury, cryopreservation, vitrification, applied and field techniques, sybr14, ip live, dead test.

Martyn, U. and S. Schulte Merker (2003). **The ventralized ogon mutant phenotype is caused by a mutation in the zebrafish homologue of Sizzled, a secreted Frizzled-related protein.** *Developmental Biology* 260(1): 58-67. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The BMP signaling pathway plays a key role during dorsoventral pattern formation of vertebrate embryos. In zebrafish, all cloned mutants affecting this process are deficient in members of the BMP pathway. In a search for factors differentially expressed in swirl/bmp2b mutants compared with wild type, we isolated zebrafish Sizzled, a member of the secreted Frizzled-related protein family and putative Wnt inhibitor. The knockdown of sizzled using antisense morpholino phenocopied the ventralized mutant ogon (formerly also known as mercedes and short tail). By sequencing and rescue experiments, we demonstrate that ogon encodes sizzled. Overexpression of sizzled, resulting in strongly dorsalized phenotypes, and the expression domains of sizzled in wild type embryos, localized in the ventral side during gastrulation and restricted to the posterior end during segmentation stages, correlate with its role in dorsoventral patterning. The expanded expression domain of sizzled in ogon and chordino together with its downregulation in swirl suggests a BMP2b-dependent negative autoregulation of sizzled. Indicating a novel role for a secreted Frizzled-related protein, we show that, in addition to the BMP pathway, a component of the Wnt signaling pathway is required for dorsoventral pattern formation in zebrafish.
Descriptors: zebrafish embryos amino acid and base sequence, body patterning genetics, bone morphogenetic proteins deficiency, bone morphogenetic proteins genetics and molecular evolution, gene expression regulation, molecular sequence data, open reading frames, phenotype, phylogeny, point mutation, tertiary protein structure, proteins chemistry and metabolism, amino acid sequence homology, signal transduction, proteins chemistry and metabolism.

Martyn, U. and S. Schulte Merker (2004). **Zebrafish neuropilins are differentially expressed and interact with vascular endothelial growth factor during embryonic vascular development.** *Developmental Dynamics* 231(1): 33-42. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Neuropilin1 (Nrp1) and Neuropilin2 (Nrp2) are nonkinase vascular endothelial growth factor receptors (VEGFR) identified in several vertebrates, which function as coreceptors for the receptor tyrosine kinases VEGFR1 and VEGFR2. We identified four zebrafish nrp genes, nrp1a, nrp1b, nrp2a, and nrp2b, and characterized their function in vascular development. We show that all nrp genes display distinct expression patterns and that nrp1a and nrp1b are expressed in the dorsal aorta, while nrp2a and nrp2b transcripts could be

detected in the region of the posterior cardinal vein. Knockdown of nrp1a, nrp1b, and nrp2a resulted in improper arteriovenous connections and irregular intersegmental vessel patterning, indicating that these Nrps function in the same process. Nrp2b knockdown also caused vessel malformations and a pericardial defect. In addition, we provide evidence that the newly identified Nrps synergistically interact with VEGF in vivo. Taken together, our results show that, in zebrafish, all four neuropilins are involved in VEGF-mediated vessel development.

Descriptors: zebrafish embryos, blood vessels embryology, neuropilins metabolism, phylogeny, receptors, vascular endothelial growth factor metabolism, blood vessels metabolism, cardiovascular abnormalities embryology, metabolism, in situ hybridization, neuropilins genetics, receptors, vascular endothelial growth factor genetics and metabolism.

Marx, M., E. Rivera-Milla, K. Stummeyer, R. Gerardy-Schahn, and M. Bastmeyer (2007). **Divergent evolution of the vertebrate polysialyltransferase Stx and Pst genes revealed by fish-to-mammal comparison**. *Developmental Biology* 306(2): 560-571. ISSN: 0012-1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish, abnormalities, axon guidance, axonogenesis, carbohydrates, cell migration, developmental stages, enzymes, evolution, evolutionary conservation, evolutionary genetics, freshwater fish, genes, growth, neural cell adhesion molecule, phylogenetics, plasticity (neural), synteny, polysialic acid, polysialylation, polysialyltransferase, *Danio rerio*.

Marza, E., C. Barthe, M. Andre, L. Villeneuve, C. Helou, and P.J. Babin (2005). **Developmental expression and nutritional regulation of a zebrafish gene homologous to mammalian microsomal triglyceride transfer protein large subunit.** *Developmental Dynamics* 232(2): 506-18. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish, carrier proteins biosynthesis, carrier, developmental gene expression regulation, amino acid sequence, nutrition physiology, molecular cloning, conserved sequence, enterocytes metabolism, in situ hybridization, intestines metabolism, lipid metabolism, lipoproteins chemistry, genetic models, molecular sequence data, PCR, tertiary protein structure, RNA metabolism, messenger RNA metabolism, reverse transcriptase PCR, amino acid sequence homology, species specificity, time factors, tissue distribution, up regulation.

Mathieu, M., L. Girosi, M. Vallarino, and G. Tagliafierro (2005). **PACAP in developing sensory and peripheral organs of the zebrafish, *Danio rerio.*** *European Journal of Histochemistry* 49(2): 167-78. ISSN: 1121 760X.

Abstract: The anatomical distribution of PACAP-like immunoreactivity was investigated in sensory and peripheral organs of the zebrafish, *Danio rerio*, during the pharyngula, hatching and larval periods, by using indirect immunofluorescence methods. First PACAP-like immunoreactive (ir) elements appeared during the pharyngula period, at 24 hours post fertilization (hpf), within the most superficial layer of the retina and the dorsal aorta. At 48 hpf, additional ir cells were found in the olfactory placode and esophagus. At 72 hpf (hatching period), PACAP-like immunoreactivity was first detected in the ganglion cell layer of the retina, the otic sensory epithelium, pharyngeal arches, swim bladder and pancreatic progenitor cells. During day 5 of larval development, new groups of ir cells appeared in the liver, whereas no ir elements were observed in the olfactory placode. Subsequently, at day 13 of larval development, additional ir elements were found for the first time in some gut epithelial cells while those previously observed in the retina and otic sensory epithelium were absent. The transient expression of PACAP-like ir material in sensory organs suggests that

the peptide could be implicated in neurotrophic activities and neurosensorial connections in the migration and/or differentiation processes. The appearance of PACAP-like ir elements in peripheral organs at different developmental stages, indicates that this peptide could be involved in the control of more specific functions as soon as these peripheral structures begin to operate.

Descriptors: zebrafish embryos, zebrafish growth and development and metabolism, fluorescence microscopy, embryology.

Matsuda, N. and M. Mishina (2004). **Identification of chaperonin CCT gamma subunit as a determinant of retinotectal development by whole-genome subtraction cloning from zebrafish no tectal neuron mutant.** *Development* 131(9): 1913-25. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Zebrafish no tectal neuron (ntn) mutant obtained by trimethylpsoralen (TMP) mutagenesis showed defects in tectal neuropil formation and small eyes. We carried out whole-genome subtraction between wild-type and mutant zebrafish embryos using the representational difference analysis (RDA) method. Nineteen subtraction products enabled us to construct genetic and physical maps of the ntn region. Direct selection of cDNAs using a YAC clone encompassing the ntn locus and RT-PCR analysis of transcripts identified a 143 bp deletion in the cct3 gene encoding the gamma subunit of chaperonin containing TCP-1 (CCT). Injection of antisense cct3 morpholino oligonucleotides into zebrafish embryos induced characteristic ntn phenotypes including defects in retinal ganglion cell (RGC) differentiation and tectal neuropil formation. Moreover, injection of cct3 mRNA successfully rescued ntn mutant embryos. Our results suggest that RDA is an efficient and widely applicable cloning strategy in zebrafish genetics. The strong expression of the cct3 mRNA started in the entire embryos by 12 hpf and was sustained thereafter, but there were no detectable abnormalities in body patterning and neurogenesis in ntn mutant embryos at 30 hpf. The expression patterns of transcription factor genes ath5 and brn3b that are essential for the development and maintenance of RGCs were indistinguishable between wild-type and ntn mutant embryos, but those of early and late differentiation markers of RGCs, nicotinic acetylcholine receptor beta 3 and zn5, were diminished in mutant embryos. Immunostaining of acetylated tubulin also revealed the impairment of RGC neurite extension. Thus, the ntn mutation of the cct3 gene impaired the differentiation of retinal neuroepithelial cells to RGCs. Similarly, the expression of brn3b was normal in the tectum of ntn mutants, but tectal neuropil formation was abolished. These results suggest that the gamma subunit of chaperonin CCT plays an essential role in retinotectal development.

Descriptors: zebrafish embryos, chaperonins metabolism, genome, protein subunits metabolism, retina embryology, superior colliculus embryology, amino acid and base sequence, molecular cloning, in situ hybridization, molecular sequence data, morphogenesis, mutation, neurons physiology, antisense oligonucleotides genetics, antisense oligonucleotides metabolism, photosensitizing agents pharmacology, retina cytology, retina drug effects, retina physiology, superior colliculus cytology, superior colliculus drug effects, superior colliculus physiology, trioxsalen pharmacology, zebrafish abnormalities.

Matsui, J.I., A.L. Egana, T.R. Sponholtz, A.R. Adolph, and J.E. Dowling (2004). **Effects of ethanol on the development of photoreceptors in embryonic zebrafish**. *Investigative Ophthalmology & Visual Science* 45(Suppl. 2): U628. ISSN: 0146 0404.

Abstract: Purpose: Some children who are born to mothers who have consumed alcohol during pregnancy present a number of morphological, cognitive, and sensory abnormalities, including vision deficits, collectively known as fetal alcohol syndrome(FAS). The retina

is one of the organs affected in children with PAS. The goal of this study is to determine if the zebrafish retina is a good model systemto study retinal and visual function abnormalities caused by exposure to ethanol during embryogenesis. Methods: Photoreceptor differentiation commences at 2 days post-fertilization (dpf) and by 5 dpf, the differentiation of the photoreceptor layer is easily observable by light microscopy. Zebrafish embryos were raised in fish water supplemented with varying concentrations of ethanol (1.5%-2% per volume) from 2 dpf through 5 dpf. Photoreceptor morphology was examined using light- and transmission electron microscopy. Visual thresholds from treated and untreated animals were measured using an optokinetic response (OKR) behavioral assay. To assess retinal function, full-field electroretinograms (ERGs) were performed under photopic conditions. Results: Ethanol treatment resulted in a significant reduction in the size of the retina. Furthermore, electron microscopy showed morphological changes in the photoreceptor outer segments. Ethanol-treated animals also exhibited a consistent increase in visual threshold as determined by OKR behavioral testing and they showed a significant decrease in the amplitude of both the b- and d-wave of the ERG. Conclusions: Ethanol treatment of zebrafish embryos during the period of photoreceptor differentiation results in decreased eye size and inhibition of outer segment differentiation, which could explain the deficits in visual function. These results mirror those obtained by investigators who have analyzed the effect of ethanolin visual function in mammalian systems. Zebrafish may prove to be a useful model system to study the molecular mechanisms underlying the development of FAS. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, fetal alcohol syndrome(FAS), disease model, sensory reception, cell differentiation, embryogenesis, visual threshold, ethanol.

Matthews, R.P., K. Lorent, P. Russo, and M. Pack (2004). **The zebrafish onecut gene hnf-6 functions in an evolutionarily conserved genetic pathway that regulates vertebrate biliary development.** *Developmental Biology* 274(2): 245-59. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Targeted disruption of the onecut transcription factor, hnf-6, alters mammalian biliary system development. We have identified a related zebrafish cDNA expressed in the developing liver that is a functional ortholog of mammalian hnf-6. Antisense-mediated knockdown of zebrafish hnf-6 perturbs development of the intrahepatic biliary system. Knockdown of zebrafish hnf-6 alters expression of vhnf1 and the zebrafish orthologs of other mammalian genes regulated by hnf-6. Coinjection of mRNA encoding zebrafish vhnf1 rescues the biliary phenotype of hnf-6 morphants. These experiments strongly suggest that hnf-6 and vhnf1 function within an evolutionarily conserved pathway that regulates biliary development. Forced expression of either hnf-6 or vhnf1 also produces biliary phenotypes. Altered bile duct development in both loss- and gain-of-function experiments suggests that zebrafish biliary cells are sensitive to the dosage of hnf-6-mediated gene transcription.
Descriptors: zebrafish embryos, evolution, homeodomain proteins genetics and metabolism, liver embryology, trans activators genetics and metabolism, amino acid and base sequence, bile ducts embryology, bile ducts ultrastructure, DNA binding proteins genetics, proteins genetics and metabolism, developmental gene expression regulation, hepatocyte nuclear factor 1 beta, hepatocyte nuclear factor 6, in situ hybridization, lipids secretion, liver metabolism, liver physiology and ultrastructure, molecular sequence data, antisense oligonucleotides genetics, antisense oligonucleotides metabolism, pancreas cytology and embryology, pancreas metabolism, messenger RNA metabolism, sequence alignment, transcription factors genetics and metabolism.

Matthews, R.P., N. Plumb Rudewiez, K. Lorent, P. Gissen, C.A. Johnson, F. Lemaigre, and M. Pack (2005). **Zebrafish vps33b, an ortholog of the gene responsible for human arthrogryposis-renal dysfunction-cholestasis syndrome, regulates biliary development downstream of the onecut transcription factor hnf6.** *Development* 132(23): 5295-306. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Arthrogryposis-renal dysfunction-cholestasis syndrome (ARC) is a rare cause of cholestasis in infants. Causative mutations in VPS33B, a gene that encodes a Class C vacuolar sorting protein, have recently been reported in individuals with ARC. We have identified a zebrafish vps33b-ortholog that is expressed in developing liver and intestine. Knockdown of vps33b causes bile duct paucity and impairs intestinal lipid absorption, thus phenocopying digestive defects characteristic of ARC. By contrast, neither motor axon nor kidney epithelial defects typically seen in ARC could be identified in vps33b-deficient larvae. Biliary defects in vps33b-deficient zebrafish larvae closely resemble the bile duct paucity associated with knockdown of the onecut transcription factor hnf6. Consistent with this, reduced vps33b expression was evident in hnf6-deficient larvae and in larvae with mutation of vhnf1, a downstream target of hnf6. Zebrafish vhnf1, but not hnf6, increases vps33b expression in zebrafish embryos and in mammalian liver cells. Electrophoretic mobility shift assays suggest that this regulation occurs through direct binding of vHnf1 to the vps33b promoter. These findings identify vps33b as a novel downstream target gene of the hnf6/vhnf1 pathway that regulates bile duct development in zebrafish. Furthermore, they show that tissue-specific roles for genes that regulate trafficking of intracellular proteins have been modified during vertebrate evolution.

Descriptors: zebrafish, biliary tract growth and development, hepatocyte nuclear factor 6 physiology, membrane, genetically modified, cholestasis etiology, developmental gene expression regulation, hepatocyte nuclear factor 1 beta metabolism, hepatocyte nuclear factor 6 deficiency and metabolism, larva growth and development, membrane proteins deficiency, membrane mutation, promoter regions genetics, protein transport genetics, zebrafish, proteins deficiency.

Maves, L. and C.B. Kimmel (2005). **Dynamic and sequential patterning of the zebrafish posterior hindbrain by retinoic acid.** *Developmental Biology* 285(2): 593-605. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: A prominent region of the vertebrate hindbrain is subdivided along the anterior-posterior axis into a series of seven segments, or rhombomeres. The identity of each rhombomere is specified by the expression of conserved transcription factors, including Krox-20, vHnf1, Val (Kreisler, Mafb) and several Hox proteins. Previous work has shown that retinoic acid (RA) signaling plays a critical role in regulating the expression of these factors and that more posterior rhombomeres require higher levels of RA than more anterior rhombomeres. Models to account for RA concentration dependency have proposed either a static RA gradient or increasing time periods of RA exposure. Here, we provide evidence against both of these models. We show that early zebrafish rhombomere-specification genes, including vhnf1 in r5-r6 and hoxd4a in r7, initiate expression sequentially in the hindbrain, each adjacent to the source of RA synthesis in paraxial mesoderm. By knocking down RA signaling, we show that progressively more posterior rhombomeres require increasingly higher levels of RA signaling, and vhnf1 and hoxd4a expression are particularly RA-dependent. RA synthesis is required just at the time of initiation, but not for maintenance, of vhnf1 and hoxd4a expression. Furthermore, a premature RA increase causes premature activation of vhnf1 and hoxd4a expression. Our results support a new model of dynamic RA action in the

hindbrain, in which a temporally increasing source of RA is required to sequentially initiate progressively more posterior rhombomere identities.

Descriptors: zebrafish embryos, body patterning physiology, developmental gene expression regulation physiology, biological research models, rhombencephalon embryology, signal transduction physiology, tretinoin metabolism, developmental gene expression regulation drug effects, in situ hybridization, microspheres, rhombencephalon metabolism, signal transduction drug effects, transcription factors metabolism, tretinoin pharmacology, metabolism, P aminoazobenzene analogs and derivatives, P aminoazobenzene pharmacology.

Mavropoulos, A., N. Devos, F. Biemar, E. Zecchin, F. Argenton, H. Edlund, P. Motte, J.A. Martial, and B. Peers (2005). **Sox4b is a key player of pancreatic alpha cell differentiation in zebrafish.** *Developmental Biology* 285(1): 211-23. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Pancreas development relies on a network of transcription factors belonging mainly to the Homeodomain and basic Helix-Loop-Helix families. We show in this study that, in zebrafish, sox4, a member of the SRY-like HMG-box (SOX) family, is required for proper endocrine cell differentiation. We found that two genes orthologous to mammalian Sox4 are present in zebrafish and that only one of them, sox4b, is strongly expressed in the pancreatic anlage. Transcripts of sox4b were detected in mid-trunk endoderm from the 5-somite stage, well before the onset of expression of the early pancreatic gene pdx-1. Furthermore, by fluorescent double in situ hybridization, we found that expression of sox4b is mostly restricted to precursors of the endocrine compartment. This expression is not maintained in differentiated cells although transient expression can be detected in alpha cells and some beta cells. That sox4b-expressing cells belong to the endocrine lineage is further illustrated by their absence from the pancreata of slow-muscle-omitted mutant embryos, which specifically lack all early endocrine markers while retaining expression of exocrine markers. The involvement of sox4b in cell differentiation is suggested firstly by its up-regulation in mind bomb mutant embryos displaying accelerated pancreatic cell differentiation. In addition, sox4b knock-down leads to a drastic reduction in glucagon expression, while other pancreatic markers including insulin, somatostatin, and trypsin are not significantly affected. This disruption of alpha cell differentiation is due to down-regulation of the homeobox arx gene specifically in the pancreas. Taken together, these data demonstrate that, in zebrafish, sox4b is expressed transiently during endocrine cell differentiation and plays a crucial role in the generation of alpha endocrine cells.

Descriptors: zebrafish embryos, high mobility group, Islets of Langerhans cytology and physiology, trans activators physiology, amino acid and base sequence, cell differentiation, DNA genetics, developmental gene expression regulation, glucagon metabolism, high mobility group in situ hybridization, fluorescence, Islets of Langerhans embryology, molecular sequence data , phylogeny, antisense RNA administration and dosage, antisense RNA genetics, amino acid sequence homology, signal transduction, trans activators genetics, proteins genetics.

Mawdsley, D.J., H.M. Cooper, B.M. Hogan, S.H. Cody, G.J. Lieschke, and J.K. Heath (2004). **The Netrin receptor Neogenin is required for neural tube formation and somitogenesis in zebrafish.** *Developmental Biology* 269(1): 302-15. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The Netrin receptor Deleted in colon cancer (Dcc) has been shown to play a pivotal role in the guidance of nascent axons towards the ventral midline in the developing nervous systems of both vertebrates and invertebrates. In contrast, the function during

embryogenesis of a second Dcc-like Netrin receptor Neogenin has not yet been defined. We used antisense morpholino oligonucleotides to knockdown Neogenin activity in zebrafish embryos and demonstrate that Neogenin plays an important role in neural tube formation and somitogenesis. In Neogenin knockdown embryos, cavitation within the neural rod failed to occur, producing a neural tube lacking a lumen. Somite formation was also defective, implicating Neogenin in the migration events underlying convergent extension during gastrulation. These observations suggest a role for Neogenin in determining cell polarity or migrational directionality of both neuroectodermal and mesodermal cells during early embryonic development.
Descriptors: zebrafish embryos, central nervous system embryology, membrane proteins metabolism, cell surface receptors metabolism, somites metabolism, central nervous system abnormalities and metabolism, Hedgehog proteins, trans activators metabolism.

Mayer, A.N. and M.C. Fishman (2003). **Nil per os encodes a conserved RNA recognition motif protein required for morphogenesis and cytodifferentiation of digestive organs in zebrafish.** *Development* 130(17): 3917-28. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Digestive organ development occurs through a sequence of morphologically distinct stages, from overtly featureless endoderm, through organ primordia to, ultimately, adult form. The developmental controls that govern progression from one stage to the next are not well understood. To identify genes required for the formation of vertebrate digestive organs we performed a genetic screen in zebrafish. We isolated the nil per os (npo) mutation, which arrests morphogenesis and cytodifferentiation of the gut and exocrine pancreas in a primordial state. We identified the npo gene by positional cloning. It encodes a conserved protein, with multiple RNA recognition motifs, that is related to the yeast protein Mrd1p. During development npo is expressed in a dynamic fashion, functioning cell autonomously to promote organ cytodifferentiation. Antisense-mediated knockdown of npo results in organ hypoplasia, and overexpression of npo causes an overgrowth of gastrointestinal organs. Thus, npo is a gene essential for a key step in the gut morphogenetic sequence.
Descriptors: zebrafish, digestive system embryology, RNA metabolism, RNA binding amino acid motifs genetics, amino acid motifs physiology, amino acid sequence, cell differentiation genetics, cell differentiation physiology, molecular cloning, endoderm physiology, epithelium embryology, liver embryology, molecular sequence data, pancreas embryology, RNA binding proteins metabolism.

McCarthy, K.A., C. Glynn, L. Hartnett, C. Nolan, and L. Byrnes (2005). **The role of insulin-like growth factor-2 (igf-2) in zebrafish embryonic development.** *Developmental Biology* 283(2): 620-621. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryo, cardiovascular system, transport and circulation, pericardial edema, heart disease, phenotype.

McClure, M. and A.R. McCune (2003). **Evidence for developmental Linkage of Pigment Patterns with body size and shape in danios (Teleostei: Cyprinidae)**. *Evolution* 57(8): 1863-1875. ISSN: 0014-3820.
Descriptors: zebrafish, body size, color, developmental genetics, evolution, freshwater fish, growth rate, morphology, pattern formation, pigmentation, sexual reproduction, sexual selection, *Danio browni*, *Danio malabaricus*, *Danio rerio*, *Malabar Danio*.

McCollum, C.W., S.R. Amin, P. Pauerstein, and M.E. Lane (2007). **A zebrafish LMO4 ortholog limits the size of the forebrain and eyes through negative regulation of six3b and rx3.** *Developmental Biology* 309(2): 373-85. ISSN: 0012-1606.

NAL Call Number: 442.8D49

Abstract: The Six3 and Rx3 homeodomain proteins are essential for the specification and proliferation of forebrain and retinal precursor cells of the vertebrate brain, and the regulatory networks that control their expression are beginning to be elucidated. We identify the zebrafish lmo4b gene as a negative regulator of forebrain growth that acts via restriction of six3 and rx3 expression during early segmentation stages. Loss of lmo4b by morpholino knockdown results in enlargement of the presumptive telencephalon and optic vesicles and an expansion of the post-gastrula expression domains of six3 and rx3. Overexpression of lmo4b by mRNA injection causes complementary phenotypes, including a reduction in the amount of anterior neural tissue, especially in the telencephalic, optic and hypothalamic primordia, and a dosage-sensitive reduction in six3 and rx3 expression. We suggest that lmo4b activity is required at the neural boundary to restrict six3b expression, and later within the neural plate to for attenuation of rx3 expression independently of its effect on six3 transcription. We propose that lmo4b has an essential role in forebrain development as a modulator of six3 and rx3 expression, and thus indirectly influences neural cell fate commitment, cell proliferation and tissue growth in the anterior CNS.

Descriptors: zebrafish, LMO4, ortholog, forebrain, eyes, negative regulation, six3b, rx3.

McCune, A.R. and R.L. Carlson (2004). **Twenty ways to lose your bladder: common natural mutants in zebrafish and widespread convergence of swim bladder loss among teleost fishes.** *Evolution and Development* 6(4): 246-59. ISSN: 1520 541X.

NAL Call Number: QH359

Abstract: Convergence is an important evolutionary phenomenon often attributed solely to natural selection acting in similar environments. The frequency of mutation and number of ways a phenotypic trait can be generated genetically, however, may also affect the probability of convergence. Here we report both a high frequency of loss of gas bladder (swim bladder) mutations in zebrafish and widespread convergent loss of gas bladders among teleost fishes. The phenotypes of 22 of 27 recessive lethal mutations, carried by a sample of 26 wild-caught zebrafish, involve loss or noninflation of the gas bladder. Nine of these bladderless mutations showed no other obvious phenotypic abnormalities other than the lack of an inflated gas bladder. At least 19 of the 22 bladderless mutations are genetically distinct, as shown by unique morphology or complementation. Although we were not able to obtain eggs for all 21 required crosses to demonstrate definitively that the remaining three mutations are different from all other bladderless mutations, all available evidence suggests that these mutants are also distinct. At least 79 of 425 families of extant teleosts include one or more species lacking a gas bladder as adults. Analysis of the trait's phylogenetic distribution shows that the gas bladder has been lost at least 30-32 times independently. Although adaptive explanations for gas bladder loss are convincing, a developmental bias toward bladderless phenotypes may also have contributed to the widespread convergence of this trait among teleosts. If gas bladder development in teleosts is as vulnerable to genetic perturbation as it is in zebrafish, then perhaps a supply of bladderless phenotypes has been readily available to natural selection under conditions for which it is advantageous not to have a gas bladder. In this way, developmental bias and selection can work together to produce widespread convergence.

Descriptors: zebrafish, air sacs anatomy and histology, mutation genetics, phenotype, phylogeny, selection genetics, genetic crosses, genetic complementation test, histological techniques, India.

McCune, A.R., D. Houle, K. McMillan, R. Annable, and A.S. Kondrashov (2004). **Two classes of deleterious recessive alleles in a natural population of zebrafish, *Danio rerio*.** *Proceedings of the Royal Society Biological Sciences Series B* 271(1552): 2025-2033. ISSN: 0962 8452.
NAL Call Number: 501 L84B
Abstract: Natural populations carry deleterious recessive alleles which cause inbreeding depression. We compared mortality and growth of inbred and outbred zebrafish, *Danio rerio*, between 6 and 48 days of age. Grandparents of the studied fish were caught in the wild. Inbred fish were generated by brother-sister mating. Mortality was 9% in outbred fish, and 42% in inbred fish, which implies at least 3.6 lethal equivalents of deleterious recessive alleles per zygote. There was no significant inbreeding depression in the growth, perhaps because the surviving inbred fish lived under less crowded conditions. In contrast to alleles that cause embryonic and early larval mortality in the same population, alleles responsible for late larval and early juvenile mortality did not result in any gross morphological abnormalities. Thus, deleterious recessive alleles that segregate in a wild zebrafish population belong to two sharply distinct classes: early-acting, morphologically overt, unconditional lethals; and later-acting, morphologically cryptic, and presumably milder alleles.
Descriptors: zebrafish, *Danio rerio*, growth rate, genotype, evidence for two classes of deleterious recessive alleles in natural population, population genetics, diseases and disorders, morphological abnormalities, mortality.

McDearmid, J.R., M. Liao, and P. Drapeau (2006). **Glycine receptors regulate interneuron differentiation during spinal network development**. *Proceedings of the National Academy of Sciences, USA* 103(25): 9679-9684. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, differentiation, embryonic development, freshwater fish, glutamatergic transmission, glycine, glycine receptors, interneurons, mitosis, motor neurons, nervous system, neural networks, neurons, neurotransmission, receptors, sensory neurons, spinal cord, gamma -aminobutyric acid, *Danio rerio*.

McFarland, K.N., R.M. Warga, and D.A. Kane (2005). **Genetic locus half baked is necessary for morphogenesis of the ectoderm**. *Developmental Dynamics* 233(2): 390-406. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: The zebrafish epiboly mutants partially block epiboly, the vegetalward movement of the blastoderm around the giant yolk cell. Here, we show that the epiboly mutations are located near the centromere of Linkage Group 7 in a single locus, termed the half baked locus. Nevertheless, except for the similar mutants lawine and avalanche, we find the epiboly traits of each of the alleles to be distinguishable, forming an allelic series. Using in situ analysis, we show that the specification and the formation of the germ layers is unaffected. However, during early gastrulation, convergence movements are slowed in homozygous and zygotic maternal dominant (ZMD) heterozygous mutants, especially in the epiblast layer of the blastoderm. Using triple-mutant analysis with squint and cyclops, we show that ablating involution and hypoblast formation in hab has no effect on the epiboly phenotype on the ventral and lateral sides of the embryo, suggesting that the hypoblast has no role in epiboly. Moreover, the triple mutant enhances the depletion of cells on the dorsal side of the embryo, consistent with the idea that convergence movements are defective. Double-mutant analysis with one-eyed pinhead reveals that hab is necessary in the ectodermal portion of the hatching gland. In ZMD heterozygotes, in addition to the slowing of epiboly, morphogenesis of the neural tube is abnormal, with gaps forming in the midline during segmentation stages; later,

ectopic rows of neurons form in the widened spinal cord and hindbrain. Cell transplantation reveals that half baked acts both autonomously and nonautonomously in interactions among cells of the forming neural tube. Together, these results suggest that half baked is necessary within the epiblast for morphogenesis during both epiboly and neurulation and suggest that the mechanisms that drive epiboly possess common elements with those that underlie convergence and extension. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryo, nervous system, neural coordination, development, mutation screening, genetic techniques, ectoderm morphogenesis, half baked locus .

McHenry, M.J. and G.V. Lauder (2006). **Ontogeny of form and function: locomotor morphology and drag in zebrafish (*Danio rerio*).** *Journal of Morphology* 267(9): 1099-109. ISSN: 0362-2525.
NAL Call Number: 444.8J826
Descriptors: extremities physiology, swimming physiology, biomechanics, anatomy and histology, embryology, extremities anatomy and histology.

McHenry, M.J. and G.V. Lauder (2005). **The mechanical scaling of coasting in zebrafish (*Danio rerio*).** *Journal of Experimental Biology* 208(12): 2289-2301. ISSN: 0022 0949.
NAL Call Number: 442.8B77
Abstract: Many fish species span two or three orders of magnitude in length during the growth from larvae to adults, and this change may have dramatic consequences for locomotor performance. We measured how the performance of coasting changes over the life history of zebrafish (*Danio rerio*) and examined the scaling of mechanics underlying this change. Adult zebrafish coast disproportionately further and faster and maintain their speed for a longer duration than do larvae and juveniles. Measurements of drag on tethered dead fish suggest that adult fish operate in an inertial regime by coasting at relatively high Reynolds numbers (Re>1000), and in vivo drag measurements showed adults to operate with a drag coefficient (Cinert[approximately equal]0.024) that was consistent with previously published estimates. However, drag scaled differently at lower Re values than those assumed in previous studies. We found a viscous regime at Re<300, which corresponds to the routine coasting of larvae and juveniles. Despite these changes in hydrodynamics overgrowth, a mathematical model of coasting mechanics suggests that the disproportionately longer coasting of adults is caused primarily by their large body mass and high speed at the beginning of coasting. We therefore propose that changes in coasting performance withgrowth are dictated primarily by the scaling of momentum rather than resulting from hydrodynamic changes. These results provide an opportunity for new interpretations of function in the growth and evolution of fish.
Descriptors: zebrafish, *Danio rerio*, age, biomechanics, swimming, coasting performance, age related changes and scaling of mechanics underlying change.

McManus, C. (2005). **Reversed bodies, reversed brains, and (some) reversed behaviors: of zebrafish and men.** *Developmental Cell* 8(6): 796-7. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Although zebrafish with situs inversus show complete reversal of normal visceral and cerebral asymmetries, they show left-right reversal of only some behaviors, with others continuing to show species-typical lateralization. The implication is that, as in humans, there are at least two independent mechanisms for generating asymmetry.
Descriptors: zebrafish embryos, behavior physiology, body patterning physiology, central

nervous system embryology, physiology, cytology, functional laterality physiology, situs inversus metabolism.

Megason, S.G. and S.E. Fraser (2003). **Digitizing life at the level of the cell: high-performance laser-scanning microscopy and image analysis for in toto imaging of development.** *Mechanisms of Development* 120(11): 1407-1420. ISSN: 0925 4773.
NAL Call Number: QH607.A1C4
Descriptors: zebrafish, cell biology, development, morphology, high performance laser scanning microscopy, zebrafish.

Megason, S.G. and S.E. Fraser (2005). **In toto imaging of organogenesis in zebrafish.** *Developmental Biology* 283(2): 688. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryo, development, image analysis, imaging and microscopy techniques, in toto imaging, imaging and microscopy techniques, gofigure, computer software, organogenesis.

Megason, S.G. and S. Fraser (2005). **In toto imaging of zebrafish development**. *Federation of American Societies for Experimental Biology Journal* 19(4, Suppl. S, Part 1): A216-A217. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: We are studding zebrafish development using in toto imaging, a novel technology we are developing whose goal is to track nearly every single cell through space and time in a developing tissue or whole embryo. There are several technical challenges to in toto imaging. Firstly, embryos must be labeled so that allof the cells are individually distinguishable so they can be segmented. We have developed a labeling method using Histone2B-EGFP and membrane localized mRFP1 to label all the nuclei green and cell membranes red. This labeling technique allows each cell in a tissue to be segmented and reveals tissue morphology,very nicely. The next challenge is to image embryos at sufficient spatial and temporal resolution to allow every cell to be tracked during development without damaging the embryo. We have developed mounting, confocal imaging, and datastorage techniques that make it possible to continuously image a zebrafish embryo at very high spatial and temporal resolution for 48 hours and to successfully archive the gigabytes of images. The final and most difficult challenge is to segment and track all the cells in the image set. We have developed a software package called GoFigure that can automatically segment cells in image sets and connect the segmented cells to form cell tracks and cell lineages. We are first applying in toto imaging to determine the complete lineage of the inner ear of zebrafish. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryo, development, in toto imaging, embryo inner ear development, tissue morphology.

Meier, N., S. Krpic, P. Rodriguez, J. Strouboulis, M. Monti, J. Krijgsveld, M. Gering, R. Patient, A. Hostert, and F. Grosveld (2006). **Novel binding partners of ldb1 are required for haematopoietic development**. *Development* 133(24): 4913-4924. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Ldb1, a ubiquitously expressed LIM domain binding protein, is essential in a number of tissues during development. It interacts with Gata1, Tal1, E2A and Lmo2 to form a transcription factor complex regulating late erythroid genes. We identify a number of novel Ldb1 interacting proteins in erythroleukaemic cells, in particular the repressor protein Eto-2

(and its family member Mtgr1), the cyclin-dependent kinase Cdk9, and the bridging factor Lmo4. MO-mediated knockdowns in zebrafish show these factors to be essential for definitive haematopoiesis. In accordance with the zebrafish results these factors are coexpressed in prehaematopoietic cells of the early mouse embryo, although we originally identified the complex in late erythroid cells. Based on the change in subcellullar localisation of Eto-2 we postulate that it plays a central role in the transition from the migration and expansion phase of the prehaematopoietic cells to the establishment of definitive haematopoietic stem cells. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, biochemistry, erythroleukaemic cells, development, blood and lymphatics, cell migration, hematopoiesis.

Mercader, N., S. Fischer, and C.J. Neumann (2006). **Prdm1 acts downstream of a sequential RA, Wnt and Fgf signaling cascade during zebrafish forelimb induction.** *Development* 133(15): 2805-15. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Vertebrate limb induction is triggered in the lateral plate mesoderm (LPM) by a cascade of signaling events originating in the axial mesoderm. While it is known that Fgf, Wnt and retinoic acid (RA) signals are involved in this cascade, their precise regulatory hierarchy has not been determined in any species. tbx5 is the earliest gene expressed in the limb bud mesenchyme. Recently, another transcription factor, Prdm1, has been shown to be crucial for zebrafish forelimb development. Here, we show that Prdm1 is downstream of RA, Wnt2b and Tbx5 activity. We find that RA activity, but not Fgf signaling, is necessary for wnt2b expression. Fgf signaling is required for prdm1 expression in the fin bud, but is not necessary for the initiation of tbx5 expression. We propose a model in which RA signaling from the somitic mesoderm leads to activation of wnt2b expression in the intermediate mesoderm, which then signals to the LPM to trigger tbx5 expression. tbx5 is required for Fgf signaling in the limb bud leading to activation of prdm1 expression, which in turn is required for downstream activation of fgf10 expression.

Descriptors: zebrafish forelimb development, Fgf physiology, forelimb embryology, limb bud physiology, mesoderm physiology , repressor, transcription factors physiology, tretinoin physiology, Wnt, DNA binding proteins genetics, proteins, physiology, glyco, morphogenesis, mutation, nuclear proteins, signal transduction.

Notes: Comments:Erratum In: *Development*.2006 Oct;133(19):3949

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Mercader, N. (2007). **Early steps of paired fin development in zebrafish compared with tetrapod limb development.** *Development Growth and Differentiation* 49(6): 421-437. ISSN: 0012 1592.

NAL Call Number: QL951.E4

Abstract: The development of zebrafish paired fins and tetrapod forelimbs and hindlimbs show striking similarities at the molecular level. In recent years, the zebrafish, *Danio rerio* has become a valuable model for the study of the development of vertebrate paired appendages and several large-scale mutagenesis screens have identified novel fin mutants. This review summarizes recent advances in research into zebrafish paired fin development and highlights features that are shared with and distinct from limb development in other main animal models.

Descriptors: zebrafish embryos, comparison, limb development, tetrapod-limb, zebrafish-pectoral-fin.

Notes: A review.

Miano, J.M. (2006). **Fishing for function in zebrafish.** *Circulation Research* 98(6): 723-6. ISSN: 0009 7330.
Descriptors: zebrafish embryos, acetyltransferases physiology, embryonic development, smooth muscle, vascular embryology, myocytes, smooth muscle cytology, acetylation, cell proliferation, protein kinases physiology, protein subunits, signal transduction.

Miller, A.L., C. Leclerc, M. Moreau, and S.E. Webb (2004). **Role of Ca2+ signaling during early pronephric development in zebrafish and *xenopus* embryos**. *Biological Bulletin (Woods Hole)* 207(2): 165. ISSN: 0006 3185.
NAL Call Number: 442.8B52
Descriptors: zebrafish embryo, development, urinary system, chemical coordination and homeostasis, marine ecology, ecology, environmental sciences, polycystic kidney disease, urologic disease, genetic disease, congenital disease, etiology, photon imaging microscope, organogenesis, tubulogenesis, early pronephric development.

Miller, C.T., L. Maves, and C.B. Kimmel (2004). **MOZ regulates Hox expression and pharyngeal segmental identity in zebrafish.** *Development* 131(10): 2443-61. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: In vertebrate embryos, streams of cranial neural crest (CNC) cells migrate to form segmental pharyngeal arches and differentiate into segment-specific parts of the facial skeleton. To identify genes involved in specifying segmental identity in the vertebrate head, we screened for mutations affecting cartilage patterning in the zebrafish larval pharynx. We present the positional cloning and initial phenotypic characterization of a homeotic locus discovered in this screen. We show that a zebrafish ortholog of the human oncogenic histone acetyltransferase MOZ (monocytic leukemia zinc finger) is required for specifying segmental identity in the second through fourth pharyngeal arches. In moz mutant zebrafish, the second pharyngeal arch is dramatically transformed into a mirror-image duplicated jaw. This phenotype resembles a similar but stronger transformation than that seen in hox2 morpholino oligo (hox2-MO) injected animals. In addition, mild anterior homeotic transformations are seen in the third and fourth pharyngeal arches of moz mutants. moz is required for maintenance of most hox1-4 expression domains and this requirement probably at least partially accounts for the moz mutant homeotic phenotypes. Homeosis and defective Hox gene expression in moz mutants is rescued by inhibiting histone deacetylase activity with Trichostatin A. Although we find early patterning of the moz mutant hindbrain to be normal, we find a late defect in facial motoneuron migration in moz mutants. Pharyngeal musculature is transformed late, but not early, in moz mutants. We detect relatively minor defects in arch epithelia of moz mutants. Vital labeling of arch development reveals no detectable changes in CNC generation in moz mutants, but later prechondrogenic condensations are mispositioned and misshapen. Mirror-image hox2-dependent gene expression changes in postmigratory CNC prefigure the homeotic phenotype in moz mutants. Early second arch ventral expression of goosecoid (gsc) in moz mutants and in animals injected with hox2-MOs shifts from lateral to medial, mirroring the first arch pattern. bapx1, which is normally expressed in first arch postmigratory CNC prefiguring the jaw joint, is ectopically expressed in second arch CNC of moz mutants and hox2-MO injected animals. Reduction of bapx1 function in wild types causes loss of the jaw joint. Reduction of bapx1 function in moz mutants causes loss of both first and second arch joints, providing functional genetic evidence that bapx1 contributes to the moz-deficient homeotic pattern. Together, our results reveal an essential embryonic role and a crucial histone acetyltransferase activity for Moz in regulating Hox expression and segmental identity, and provide two early targets, bapx1 and

gsc, of moz and hox2 signaling in the second pharyngeal arch.
Descriptors: zebrafish embryos cartilage patterning, acetyltransferases genetics, developmental gene expression regulation genetics, histone acetyltransferases genetics, homeodomain pharynx embryology, base sequence, body patterning genetics, molecular cloning, homeobox morphogenesis, morpholines pharmacology, mutagenesis, oligodeoxyribonucleotides pharmacology, recombinant proteins metabolism, restriction mapping.

Miller, C.T., M.E. Swartz, P.A. Khuu, M.B. Walker, J.K. Eberhart, and C.B. Kimmel (2007). **Mef2ca is required in cranial neural crest to effect Endothelin1 signaling in zebrafish.** *Developmental Biology* 308(1): 144-57. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Mef2 genes encode highly conserved transcription factors involved in somitic and cardiac mesoderm development in diverse bilaterians. Vertebrates have multiple mef2 genes. In mice, mef2c is required for heart and vascular development. We show that a zebrafish mef2c gene (mef2ca) is required in cranial neural crest (CNC) for proper head skeletal patterning. mef2ca mutants have head skeletal phenotypes resembling those seen upon partial loss-of-function of endothelin1 (edn1). Furthermore, mef2ca interacts genetically with edn1, arguing that mef2ca functions within the edn1 pathway. mef2ca is expressed in CNC and this expression does not require edn1 signaling. Mosaic analyses reveal that mef2ca is required in CNC for pharyngeal skeletal morphogenesis. Proper expression of many edn1-dependent target genes including hand2, bapx1, and gsc, depends upon mef2ca function. mef2ca plays a critical role in establishing the proper nested expression patterns of dlx genes. dlx5a and dlx6a, known Edn1 targets, are downregulated in mef2ca mutant pharyngeal arch CNC. Surprisingly, dlx4b and dlx3b are oppositely affected in mef2ca mutants. dlx4b expression is abolished while the edn1-dependent dlx3b is ectopically expressed in more dorsal CNC. Together our results support a model in which CNC cells require mef2ca downstream of edn1 signaling for proper craniofacial development.
Descriptors: zebrafish embryology, endothelin 1 metabolism, myogenic regulatory factors metabolism, neural crest metabolism, genetically modified, base sequence, body patterning, branchial region embryology, branchial region metabolism, DNA primers genetics , endothelin 1 genetics, developmental gene expression regulation, genetic models, mutation, myogenic regulatory factors genetics, neural crest embryology, phenotype, signal transduction.

Miller, C.T., D. Yelon, D.Y.R. Stainier, and C.B. Kimmel (2003). **Two Endothelin 1 effectors, hand2 and bapx1, pattern ventral pharyngeal cartilage and the jaw joint**. *Development* 130(7): 1353-1365. ISSN: 0950-1991.
NAL Call Number: QL951.D38
Descriptors: zebrafish, biological development, cartilage, developmental genetics, endothelin 1, genes, homeobox, Joints, mandible, marine fish, mouth parts, mutations, osteogenesis, pattern formation, pharynx, signal transduction, Skeleton, *Danio rerio*.

Millimaki, B.B., E.M. Sweet, M.S. Dhason, and B.B. Riley (2007). **Zebrafish atoh1 genes: classic proneural activity in the inner ear and regulation by Fgf and Notch.** *Development* 134(2): 295-305. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Hair cells of the inner ear develop from an equivalence group marked by expression of the proneural gene Atoh1. In mouse, Atoh1 is necessary for hair cell differentiation, but its role in specifying the equivalence group (proneural function) has been questioned and

little is known about its upstream activators. We have addressed these issues in zebrafish. Two zebrafish homologs, atoh1a and atoh1b, are together necessary for hair cell development. These genes crossregulate each other but are differentially required during distinct developmental periods, first in the preotic placode and later in the otic vesicle. Interactions with the Notch pathway confirm that atoh1 genes have early proneural function. Fgf3 and Fgf8 are upstream activators of atoh1 genes during both phases, and foxi1, pax8 and dlx genes regulate atoh1b in the preplacode. A model is presented in which zebrafish atoh1 genes operate in a complex network leading to hair cell development.

Descriptors: zebrafish embryo, basic helix loop helix transcription factors genetics, inner ear embryology and metabolism, Fgf metabolism, Notch receptors metabolism, transcription factors genetics, base sequence, basic helix loop helix transcription factors metabolism, DNA genetics, developmental gene expression regulation, hair cells embryology and metabolism, membrane proteins metabolism, mice, mutation, pax2 transcription factor genetics and metabolism, paired box transcription factors genetics, paired box transcription factors metabolism, signal transduction, transcription factors metabolism, metabolism.

Miyachi, Y., T. Kanao, and T. Okamoto (2003). **Marked depression of time interval between fertilization period and hatching period following exposure to low-dose X-rays in zebrafish.** *Environmental Research* 93(2): 216-9. ISSN: 0013 9351.

NAL Call Number: RA565.A1E5

Abstract: In recent years there has been growing concern over the stimulating effects of very low-dose X-rays. Our laboratory had observed that zebrafish irradiated with low-dose X-rays tended to emerge earlier than sham controls. This observation led us to quantitatively examine the effects of low-dose X irradiation on a series of stages of development in the zebrafish. The embryos were fertilized simultaneously in vitro and incubated at an optimal temperature without crowding. Following exposure of the cleavage period (1.5 h after fertilization) to 0.025-Gy X-rays, the duration to hatching was slightly shorter than that of the sham controls. This tendency was increased when the X-ray exposure occurred during the blastula period (3.5 h). In these embryos, the duration to hatching decreased significantly by an average of 6 h sooner than for sham controls. No differences in duration to hatching were seen when irradiation was given during either the zygote period (45 min) or the segmentation period (12 h). On the contrary, upon exposure to 0.5-Gy X-rays during the blastula period, the duration to hatching increased significantly relative to that of sham controls. These results suggest that the radiation-induced early hatching effect is observed for low doses of X-rays.

Descriptors: zebrafish embryology, fertilization, ovum growth and development, radiation dose response relationship, embryonic development, ovum radiation effects, time factors, X rays adverse effects.

Miyagi, C., S. Yamashita, Y. Ohba, H. Yoshizaki, M. Matsuda, and T. Hirano (2004). **STAT3 noncell-autonomously controls planar cell polarity during zebrafish convergence and extension.** *Journal of Cell Biology* 166(7): 975-81. ISSN: 0021-9525.

NAL Call Number: 442.8J828

Abstract: Zebrafish signal transducer and activator of transcription 3 (STAT3) controls the cell movements during gastrulation. Here, we show that noncell-autonomous activity of STAT3 signaling in gastrula organizer cells controls the polarity of neighboring cells through Dishevelled-RhoA signaling in the Wnt-planar cell polarity (Wnt-PCP) pathway. In STAT3-depleted embryos, although all the known molecules in the Wnt-PCP pathway were expressed normally, the RhoA activity in lateral mesendodermal cells was down-regulated,

resulting in severe cell polarization defects in convergence and extension movements identical to Strabismus-depleted embryos. Cell-autonomous activation of Wnt-PCP signaling by DeltaN-dishevelled rescued the defect in cell elongation, but not the orientation of lateral mesendodermal cells in STAT3-depleted embryos. The defect in the orientation could be rescued by transplantation of shield cells having noncell-autonomous activity of STAT3 signaling. These results suggest that the cells undergoing convergence and extension movement may sense the gradient of signaling molecules, which are expressed in gastrula organizer by STAT3 and noncell-autonomously activate PCP signaling in neighboring cells during zebrafish gastrulation.

Descriptors: zebrafish embryos, cell movement genetics, cell polarity genetics , DNA binding proteins genetics, proteins metabolism, gastrula metabolism, trans activators metabolism, signal transducing adaptor proteins, cell communication genetics, DNA binding proteins genetics, down regulation genetics, embryo cytology, embryology , metabolism, gastrula cytology, developmental gene expression regulation genetics, mesoderm cytology and metabolism, phosphoproteins genetics and metabolism, proto oncogene proto oncogene proteins metabolism, STAT3 transcription factor, signal transduction genetics, trans activators genetics, Wnt proteins, metabolism, proteins, rhoa GTP binding protein genetics and metabolism.

Miyake, A., Y. Nakayama, M. Konishi, and N. Itoh (2005). **Fgf19 regulated by Hh signaling is required for zebrafish forebrain development.** *Developmental Biology* 288(1): 259-75. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Fibroblast growth factor (Fgf) signaling plays important roles in brain development. Fgf3 and Fgf8 are crucial for the formation of the forebrain and hindbrain. Fgf8 is also required for the midbrain to form. Here, we identified zebrafish Fgf19 and examined its roles in brain development by knocking down Fgf19 function. We found that Fgf19 expressed in the forebrain, midbrain and hindbrain was involved in cell proliferation and cell survival during embryonic brain development. Fgf19 was also essential for development of the ventral telencephalon and diencephalon. Regional specification is linked to cell type specification. Fgf19 was also essential for the specification of gamma-aminobutyric acid (GABA)ergic interneurons and oligodendrocytes generated in the ventral telencephalon and diencephalon. The cross talk between Fgf and Hh signaling is critical for brain development. In the forebrain, Fgf19 expression was down-regulated on inhibition of Hh but not of Fgf3/Fgf8, and overexpression of Fgf19 rescued partially the phenotype on inhibition of Hh. The present findings indicate that Fgf19 signaling is crucial for forebrain development by interacting with Hh and provide new insights into the roles of Fgf signaling in brain development.

Descriptors: zebrafish embryos, Fgf physiology, prosencephalon metabolism, signal transduction physiology, trans activators physiology, amino acid sequence, cell differentiation physiology, cell proliferation, cell survival physiology, cerebellum embryology, Fgf deficiency and metabolism, GABA agents metabolism, gene expression profiling, Hedgehog proteins, interneurons cytology, interneurons metabolism, mesencephalon embryology, mesencephalon metabolism, molecular sequence data, oligodendroglia cytology and metabolism, prosencephalon abnormalities, prosencephalon embryology, rhombencephalon embryology and metabolism, protein sequence analysis, signal transduction genetics, thalamus embryology and physiology, up regulation, metabolism, proteins deficiency.

Miyake, A., T. Saito, T. Kashiwagi, D. Ando, A. Yamamoto, T. Suzuki, N. Nakatsuji, and T. Nakatsuji (2006). **Cloning and pattern of expression of the shiro-uo VASA gene during embryogenesis and its roles in PGC development**. *International Journal of Developmental Biology* 50(7): 619-625. ISSN: 0214 6282.

Abstract: The vasa genes are expressed in the germ cell lineage in many organisms, but their expression patterns show large variations. Recent studies suggest that vasa transcripts are involved in germ cell lineage development. In this paper, we isolated the vasa cDNA clone from a teleost, shiro-uo, Leucopsarion petersii and examined its expression pattern during embryogenesis. Then, we examined the functional significance of vasa mRNA during the formation of primordial germ cells (PGCs). The amino acid sequence of shiro-uo VASA is 61.1% identical to that of zebrafish. In whole-mount in situ hybridization, vasa transcripts appeared at the 4- and 8-cell stages as four spots at both ends of two cleavage planes between the lower tier of blastomeres and the yolk cell mass. At the 16-cell stage, eight spots were observed. After the blastula stage, shiro-uo vasa transcripts showed similar localization as in the zeblafish. Ultrastructural analysis of 4-cell stage embryos revealed the presence of a subcellular organelle that resembled "nuage" in the germ cell lineage observed in the embryos of various organisms. We carried out micromanipulation of 4- or 8-cell stage embryos to remove the vasa mRNA-containig spots and then measured the number of the vasa-expressing PGCs in the genital ridge of the manipulated embryos. The numbers decreased when all of the four spots were removed, indicating that the vasa-containing spots at early cleavage stages have important functions in the development of PGCs. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryo, development, molecular genetics, reproductive system, reproduction, molecular cloning, genetic techniques, embryogenesis.

Miyasaka, N., H. Knaut, and Y. Yoshihara (2007). **Cxcl12/Cxcr4 chemokine signaling is required for placode assembly and sensory axon pathfinding in the zebrafish olfactory system.** *Development* 134(13): 2459-68. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Positioning neurons in the right places and wiring axons to the appropriate targets are essential events for establishment of neural circuits. In the zebrafish olfactory system, precursors of olfactory sensory neurons (OSNs) assemble into a compact cluster to form the olfactory placode. Subsequently, OSNs differentiate and extend their axons to the presumptive olfactory bulb with high precision. In this study, we aim to elucidate the molecular mechanism underlying these two developmental processes. cxcr4b, encoding a chemokine receptor, is expressed in the migrating olfactory placodal precursors, and cxcl12a (SDF-1a), encoding a ligand for Cxcr4b, is expressed in the abutting anterior neural plate. The expression of cxcr4b persists in the olfactory placode at the initial phase of OSN axon pathfinding. At this time, cxcl12a is expressed along the placode-telencephalon border and at the anterior tip of the telencephalon, prefiguring the route and target of OSN axons, respectively. Interfering with Cxcl12a/Cxcr4b signaling perturbs the assembly of the olfactory placode, resulting in the appearance of ventrally displaced olfactory neurons. Moreover, OSN axons frequently fail to exit the olfactory placode and accumulate near the placode-telencephalon border in the absence of Cxcr4b-mediated signaling. These data indicate that chemokine signaling contributes to both the olfactory placode assembly and the OSN axon pathfinding in zebrafish.

Descriptors: zebrafish, axons metabolism, Cxc chemokines metabolism, afferent neurons metabolism, olfactory pathways metabolism, Cxcr4 receptors metabolism, signal transduction, metabolism, genetically modified, Cxc chemokines genetics, developmental gene

expression regulation, mutation genetics, olfactory pathways cytology and embryology, phenotype, Cxcr4 receptors genetics and metabolism.

Miyasaka, N., Y. Sato, S.Y. Yeo, L.D. Hutson, C.B. Chien, H. Okamoto, and Y. Yoshihara (2005). **Robo2 is required for establishment of a precise glomerular map in the zebrafish olfactory system.** *Development* 132(6): 1283-93. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Olfactory sensory neurons (OSNs) expressing a given odorant receptor project their axons to specific glomeruli, creating a topographic odor map in the olfactory bulb (OB). The mechanisms underlying axonal pathfinding of OSNs to their precise targets are not fully understood. Here, we demonstrate that Robo2/Slit signaling functions to guide nascent olfactory axons to the OB primordium in zebrafish. robo2 is transiently expressed in the olfactory placode during the initial phase of olfactory axon pathfinding. In the robo2 mutant, astray (ast), early growing olfactory axons misroute ventromedially or posteriorly, and often penetrate into the diencephalon without reaching the OB primordium. Four zebrafish Slit homologs are expressed in regions adjacent to the olfactory axon trajectory, consistent with their role as repulsive ligands for Robo2. Masking of endogenous Slit gradients by ubiquitous misexpression of Slit2 in transgenic fish causes posterior pathfinding errors that resemble the ast phenotype. We also found that the spatial arrangement of glomeruli in OB is perturbed in ast adults, suggesting an essential role for the initial olfactory axon scaffold in determining a topographic glomerular map. These data provide functional evidence for Robo2/Slit signaling in the establishment of olfactory neural circuitry in zebrafish.
Descriptors: zebrafish embryos, olfactory pathways embryology, immunologic receptors metabolism, axons metabolism, gene expression profiling, glycoproteins genetics and metabolism, mutation, nerve tissue nerve tissue proteins metabolism, olfactory pathways metabolism, proteins.

Miyashita, T., S.B. Yeo, Y. Hirate, H. Segawa, H. Wada, M.H. Little, T. Yamada, N. Takahashi, and H. Okamoto (2004). **Plexina4 is necessary as a downstream target of islet2 to mediate slit signaling for promotion of sensory axon branching**. *Development* 131(15): 3705-3715. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Slit is a secreted protein known to repulse the growth cones of commissural neurons. By contrast, Slit also promotes elongation and branching of axons of sensory neurons. The reason why different neurons respond to Slit in different ways is largely unknown. Islet2 is a LIM/homeodomaintype transcription factor that specifically regulates elongation and branching of the peripheral axons of the primary sensory neurons in zebrafish embryos. We found that PlexinA4, a transmembrane protein known to be a coreceptor for class III semaphorins, acts downstream of Islet2 to promote branching of the peripheral axons of the primary sensory neurons. Intriguingly, repression of PlexinA4 function by injection of the antisense morpholino oligonucleotide specific to PlexinA4 or by overexpression of the dominant-negative variant of PlexinA4 counteracted the effects of overexpression of Slit2 to induce branching of the peripheral axons of the primary sensory neurons in zebrafish embryos, suggesting involvement of PlexinA4 in the Slit signaling cascades for promotion of axonal branching of the sensory neurons. Colocalized expression of Robo, a receptor for Slit2, and PlexinA4 is observed not only in the primary sensory neurons of zebrafish embryos but also in the dendrites of the pyramidal neurons of the cortex of the mammals, and may be important for promoting the branching of either axons or dendrites in response to Slit, as opposed to the growth cone collapse. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, development, nervous system, neural coordination, immunohistochemistry , immunologic techniques, in situ hybridization, genetic techniques.

Mizoguchi, T., T. Izawa, A. Kuroiwa, and Y. Kikuchi (2006). **Fgf signaling negatively regulates Nodal-dependent endoderm induction in zebrafish.** *Developmental Biology* 300(2): 612-22. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: In zebrafish development, Nodal signaling is critical for the induction of endoderm and mesoderm. Three transcription factors downstream of Nodal, Bonnie and Clyde (Bon), Faust (Fau)/Gata5 and Casanova (Cas), are required for endoderm induction. However, it is not yet fully understood how the Nodal signaling pathway regulates the decision process of endoderm and mesoderm induction. In this study, we focused on Fgf signaling, downstream of Nodal signaling, during endoderm induction. We found that activation of Fgf signaling decreases the number of cas-expressing endodermal cells. Conversely, inhibition of this signaling increases the number of endodermal cells without affecting the expression of Nodal, Nodal antagonists, bon or fau/gata5. Inhibition of Fgf signaling in endoderm mutants suggests that this signaling negatively regulates cas expression by a pathway parallel to Bon and Fau/Gata5 in the molecular cascade leading to endoderm. Furthermore, activation of Fgf signaling can overcome Cas-mediated abrogation of mesodermal gene expression. Altogether, these results suggest that Fgf signaling negatively regulates endoderm induction, possibly through repression of cas expression and down-regulation of Cas function.
Descriptors: zebrafish embryos, down regulation physiology, embryonic induction physiology, endoderm physiology, Fgf physiology, transforming growth factor beta physiology, cell division physiology, endoderm cytology, Fgf antagonists and inhibitors, high mobility group proteins genetics and metabolism, transcription factors genetics and metabolism.

Mohideen, M.A., L.G. Beckwith, G.S. Tsao Wu, J.L. Moore, A.C. Wong, M.R. Chinoy, and K.C. Cheng (2003). **Histology-based screen for zebrafish mutants with abnormal cell differentiation.** *Developmental Dynamics* 228(3): 414-23. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish larvae, mutant selection, cell differentiation genetics, mutagenesis, chromosome mapping, eye cytology, eye embryology, morphogenesis, parthenogenesis, vertebrate photoreceptors cytology.

Molina, G.A., S.C. Watkins, and M. Tsang (2007). **Generation of FGF reporter transgenic zebrafish and their utility in chemical screens.** *BioMed Central Developmental Biology* 7: 62. ISSN: 1471 213X.
NAL Call Number: QH491.B63
Abstract: BACKGROUND: Fibroblast Growth Factors (FGFs) represent a large family of secreted proteins that are required for proper development and physiological processes. Mutations in mouse and zebrafish FGFs result in abnormal embryogenesis and lethality. A key to understanding the precise role for these factors is to determine their spatial and temporal activity during embryogenesis. RESULTS: Expression of Dual Specificity Phosphatase 6 (dusp6, also known as Mkp3) is controlled by FGF signalling throughout development. The Dusp6 promoter was isolated from zebrafish and used to drive expression of destabilized green fluorescent protein (d2EGFP) in transgenic embryos (Tg(Dusp6:d2EGFP)). Expression of d2EGFP is initiated as early as 4 hours post-fertilization (hpf) within the future dorsal region of the embryo, where fgf3 and fgf8 are initially expressed. At later stages,

d2EGFP is detected within structures that correlate with the expression of Fgf ligands and their receptors. This includes the mid-hindbrain boundary (MHB), pharyngeal endoderm, otic vesicle, hindbrain, and Kupffer's vesicle. The expression of d2EGFP is under the control of FGF signalling as treatment with FGF Receptor (FGFR) inhibitors results in the suppression of d2EGFP expression. In a pilot screen of commercially available small molecules we have evaluated the effectiveness of the transgenic lines to identify specific FGF inhibitors within the class of indolinones. These compounds were counter screened with the transgenic line Tg(Fli1:EGFP)y1, that serves as an indirect read-out for Vascular Endothelial Growth Factor (VEGF) signalling in order to determine the specificity between related receptor tyrosine kinases (RTKs). From these assays it is possible to determine the specificity of these indolinones towards specific RTK signalling pathways. This has enabled the identification of compounds that can block specifically the VEGFR or the FGFR signalling pathway. CONCLUSION: The generation of transgenic reporter zebrafish lines has allowed direct visualization of FGF signalling within the developing embryo. These FGF reporter transgenic lines provide a tool to screen for specific compounds that can distinguish between two conserved members of the RTK family.

Descriptors: zebrafish embryology, Fgf genetics, developmental gene expression regulation, reporter genes, genetically modified, embryology, protein tyrosine phosphatase genetics, signal transduction .

Montero Balaguer, M., M.R. Lang, S.W. Sachdev, C. Knappmeyer, R.A. Stewart, A. De La Guardia, A.K. Hatzopoulos, and E.W. Knapik (2006). **The mother superior mutation ablates foxd3 activity in neural crest progenitor cells and depletes neural crest derivatives in zebrafish.** *Developmental Dynamics* 235(12): 3199-212. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryos, forkhead transcription factors genetics, neural crest metabolism, proto oncogene proteins C mos genetics, *Xenopus* base sequence, body patterning genetics, chondrogenesis genetics, chromosome mapping , embryonic stem cells cytology and metabolism, forkhead transcription factors metabolism, developmental gene expression regulation, in situ hybridization, mutation, neural crest cytology, oligodeoxyribonucleotides, antisense genetics, phenotype, pigmentation genetics, *Xenopus* proteins metabolism, metabolism.

Montero, J.A., L. Carvalho, M. Wilsch Brauninger , B. Kilian, C. Mustafa, and C.P. Heisenberg (2005). **Shield formation at the onset of zebrafish gastrulation.** *Development* 132(6): 1187-98. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: During vertebrate gastrulation, the three germ layers, ectoderm, mesoderm and endoderm are formed, and the resulting progenitor cells are brought into the positions from which they will later contribute more complex tissues and organs. A core element in this process is the internalization of mesodermal and endodermal progenitors at the onset of gastrulation. Although many of the molecules that induce mesendoderm have been identified, much less is known about the cellular mechanisms underlying mesendodermal cell internalization and germ layer formation. Here we show that at the onset of zebrafish gastrulation, mesendodermal progenitors in dorsal/axial regions of the germ ring internalize by single cell delamination. Once internalized, mesendodermal progenitors upregulate E-Cadherin (Cadherin 1) expression, become increasingly motile and eventually migrate along the overlying epiblast (ectodermal) cell layer towards the animal pole of the gastrula. When E-Cadherin function is compromised, mesendodermal progenitors still internalize, but, with gastrula-

tion proceeding, fail to elongate and efficiently migrate along the epiblast, whereas epiblast cells themselves exhibit reduced radial cell intercalation movements. This indicates that cadherin-mediated cell-cell adhesion is needed within the forming shield for both epiblast cell intercalation, and mesendodermal progenitor cell elongation and migration during zebrafish gastrulation. Our data provide insight into the cellular mechanisms underlying mesendodermal progenitor cell internalization and subsequent migration during zebrafish gastrulation, and the role of cadherin-mediated cell-cell adhesion in these processes.
Descriptors: zebrafish embryology, gastrula physiology, body patterning physiology, cadherins physiology, cell adhesion physiology, cell movement physiology, endoderm physiology, endoderm ultrastructure, gastrula ultrastructure, mesoderm physiology, mesoderm ultrastructure, electron microscopy.

Montero, J.A., B. Kilian, J. Chan, P.E. Bayliss, and C.P. Heisenberg (2003). **Phosphoinositide 3-Kinase is required for process outgrowth and cell polarization of gastrulating mesendodermal cells**. *Current Biology* 13(15): 1279-1289. ISSN: 0960-9822.
NAL Call Number: QH301.C85
Descriptors: zebrafish, cell morphology, embryonic development, enzymes, freshwater fish, reproduction, skin, *Danio rerio*.

Moore, C.A., C.A. Parkin, Y. Bidet, and P.W. Ingham (2007). **A role for the Myoblast city homologues Dock1 and Dock5 and the adaptor proteins Crk and Crk-like in zebrafish myoblast fusion.** *Development* 134(17): 3145-53. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Myoblast fusion follows a defined sequence of events that is strikingly similar in vertebrates and invertebrates. Genetic analysis in Drosophila has identified many of the molecules that mediate the different steps in the fusion process; by contrast, the molecular basis of myoblast fusion during vertebrate embryogenesis remains poorly characterised. A key component of the intracellular fusion pathway in Drosophila is the protein encoded by the myoblast city (mbc) gene, a close homologue of the vertebrate protein dedicator of cytokinesis 1 (DOCK1, formerly DOCK180). Using morpholino antisense-oligonucleotide-mediated knockdown of gene activity in the zebrafish embryo, we show that the fusion of embryonic fast-twitch myoblasts requires the activities of Dock1 and the closely related Dock5 protein. In addition, we show that the adaptor proteins Crk and Crk-like (Crkl), with which Dock proteins are known to interact physically, are also required for myoblast fusion.
Descriptors: zebrafish embryo cloning, signal transducing adaptor, muscle development genetics, nuclear, oncogene protein v Crk physiology, cell fusion, molecular cloning, cytoskeletal *Drosophila* proteins genetics fast twitch muscle fibers cytology, myoblasts, skeletal physiology, amino acid sequence homology, proteins genetics.

Moreira, J. and A. Deutsch (2005). **Pigment pattern formation in zebrafish during late larval stages: a model based on local interactions.** *Developmental Dynamics* 232(1): 33-42. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: We present a model to explain pigment cell pattern formation in the late larval stages of zebrafish, Danio rerio, and its mutants, based on new time sequence experimental data. The model stresses the importance of differential intercellular adhesion and the mechanisms of stem cell regulation to explain pigment pattern formation in zebrafish. All interactions included in the model are local in contrast to the previous models for pattern formation, which are based on long range interactions. The hypotheses of the model are

tested through a mathematical (cellular automaton) model. Simulations show that differential cellular adhesion together with an appropriate mechanism of stem cell regulation are able to reproduce the main characteristics of pattern formation in the wild-type zebrafish and mutants. The results obtained with the simulations are compared with experimental data and specific experimental tests to the predictions of the model are provided.
Descriptors: zebrafish embryonic development, developmental gene expression regulation, algorithms, body patterning, cell cycle, mice, anatomic models, statistical models, theoretical models, mutation, phenotype, stem cells cytology, stem cells metabolism, time factors.

Morillo, J.R., S.L. Johnson, K. Kawakami, and R. McMahan (2004). **Transposon-mediated gene-trap: a screen for live markers in the zebrafish, *danio rerio.*** *Developmental Biology* 271(2): 599. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, development, molecular genetics, transposon mediated gene trapping, genetic techniques, zebrafish.

Moro, E., C. Maran, M.L. Slongo, F. Argenton, S. Toppo, and M. Onisto (2007). **Zebrafish spata2 is expressed at early developmental stages**. *International Journal of Developmental Biology* 51(3): 241-246. ISSN: 0214 6282.
Abstract: Spata2 (spermatogenesis-associated protein 2) was originally described as a novel gene involved in the spermatogenic process. In this study, we cloned a potential zebrafish spata2 orthologue. The consensus open reading frame (1650 bp) encodes a polypeptide of 550 amino acids which shares 37% identity with the human SPATA2. Bioinformatic analysis reveals a small pattern PW [KR] KE [YF][RK] which seems to be of particular interest in the light of its strong conservation between SPATA2 and the recently discovered TAMO protein of D. melanogaster. RT-PCR analysis in adult zebrafish tissues revealed that spata2 mRNA has a broad distribution. Whole-mount in situ hybridization demonstrated that spata2 transcripts are maternally derived and becomes strongly localized in the central nervous system at early developmental stages. From 5 dpf, spata2 expression becomes detectable in the gut and pronephric duct epithelium, suggesting a wide tissue function during vertebrate development. Copyright © 2008 CABI
Descriptors: zebrafish embryo, amino acid sequences, biological development, gene expression, genes, messenger RNA, morphogenesis, ontogeny, open reading frames, spermatogenesis, spermatozoa, tissue distribution, transcription, *Danio rerio*.

Morris, A.C. and J.M. Fadool (2005). **Studying rod photoreceptor development in zebrafish.** *Physiology and Behavior* 86(3): 306-13. ISSN: 0031-9384.
NAL Call Number: QP1.P4
Abstract: The zebrafish has rapidly become a favored model vertebrate organism, well suited for studies of developmental processes using large-scale genetic screens. In particular, zebrafish morphological and behavioral genetic screens have led to the identification of genes important for development of the retinal photoreceptors. This may help clarify the genetic mechanisms underlying human photoreceptor development and dysfunction in retinal diseases. In this review, we present the advantages of zebrafish as a vertebrate model organism, summarize retinal and photoreceptor cell development in zebrafish, with emphasis on the rod photoreceptors, and describe zebrafish visual behaviors that can be used for genetic screens. We then describe some of the photoreceptor cell mutants that have been isolated in morphological and behavioral screens and discuss the limitations of current screening methods for uncovering mutations that specifically affect rod function. Finally, we present some alterna-

tive strategies to target the rod developmental pathway in zebrafish.
Descriptors: zebrafish screening for mutants, retinal rodsgrowth and development, genetically modified animals, behavior physiology, animal models.

Morris, J.K., B.B. Willard, X. Yin, G. Jeserich, M. Kinter, and B.D. Trapp (2004). **The 36K protein of zebrafish CNS myelin is a short-chain dehydrogenase.** *Glia* 45(4): 378-91. ISSN: 0894-1491.
Descriptors: zebrafish 36K protein, central nervous system enzymology, myelin proteins biosynthesis, nerve tissue proteins biosynthesis, oxidoreductases biosynthesis, proteins biosynthesis, amino acid and base sequence, central nervous system embryology, growth and development, developmental gene expression regulation physiology, molecular sequence data, molecular weight, myelin nerve tissue oxidoreductases genetics, amino acid sequence homology, zebrafish.

Mudumana, S.P., H. Wan, M. Singh, V. Korzh, and Z. Gong (2004). **Expression analyses of zebrafish transferrin, ifabp, and elastaseB mRNAs as differentiation markers for the three major endodermal organs: liver, intestine, and exocrine pancreas.** *Developmental Dynamics* 230(1): 165-73. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish and cDNA, carrier proteins biosynthesis, developmental gene expression regulation, serine endopeptidases biosynthesis, transferrin biosynthesis, amino acid sequence, Northern blotting, carps, carrier carrier proteins metabolism, cell differentiation, cell nucleus metabolism, complementary DNA metabolism, fatty acid binding proteins, in situ hybridization, intestines metabolism, iron metabolism, liver metabolism, molecular sequence data, pancreas metabolism, messenger RNA metabolism, DNA sequence analysis, amino acid sequence homology, serine endopeptidases genetics, time factors, transferrin genetics, yolk sac metabolism.

Mueller, I.I., E.W. Knapik, and A.K. Hatzopoulos (2006). **Expression of the protein related to dan and cerberus gene- PRDC during eye, pharyngeal arch, somite, and swim bladder in zebrafish**. *Developmental Dynamics* 235(10): 2881-2888. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: The protein related to Dan and Cerberus, or PRDC, is a secreted glycoprotein, which belongs to the DAN subfamily of bone morphogenetic protein (BMP) antagonists. In zebrafish, PRDC is expressed initially around 17 hours postfertilization in the developing eyes and the first two pharyngeal arches. Expression in the eye starts in the outer layers of the optic cup. Later, PRDC expression domains are juxtaposed at the edges of the optic cup surrounding the choroid fissure, then gradually becoming restricted to a small site in the ventral marginal zone. PRDC expression in the arch mesenchyme expands stepwise to the remaining posterior arches. PRDC is also detectable in the ventral part of the somites and the mesenchyme of the swim bladder. The relatively late appearance during development is a unique feature of PRDC among BMP antagonists. Moreover, the complexity of the PRDC expression pattern suggests possible roles in eye development, pharyngeal arch remodeling, somitogenesis, and swim bladder organogenesis. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, biochemistry, development, organogenesis, expression pattern, somitogenesis.

Muller, I.I., E.W. Knapik, and A.K. Hatzopoulos (2006). **Expression of the protein related to Dan and Cerberus gene PRDC during eye, pharyngeal arch, somite, and swim bladder development in zebrafish.** *Developmental Dynamics* 235(10): 2881-8. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, air sacs metabolism, branchial region metabolism, eye metabolism, intercellular signaling peptides and somites metabolism, air sacs embryology, amino acid sequence, branchial region embryology, eye embryology, developmental gene expression regulation genetics, in situ hybridization, molecular sequence data, phylogeny, reverse transcriptase PCR, amino acid sequence homology, somites enzymology .

Muller, U.K. and J.L. van Leeuwen (2004). **Swimming of larval zebrafish: Ontogeny of body waves and implications for locomotory development.** *Journal of Experimental Biology* 207(5): 853-868. ISSN: 0022 0949.
NAL Call Number: 442.8B77
Abstract: Fish larvae, like most adult fish, undulate their bodies to propel themselves. A detailed kinematic study of the larval body wave is a prerequisite to formulate a set of functional requirements that the locomotor system must fulfil to generate the observed swimming kinematics. Lateral displacement and curvature profiles were obtained for zebrafish (*Danio rerio*) larvae at 2-21 days post-fertilisation for three swimming behaviours (cyclic swimming, slow starts and fast startle responses) using high-speed video. During cyclic swimming, fish larvae maintain tail beat frequencies of up to 100 Hz. The corresponding longitudinal strains, estimated from the peak curvatures of the midline, reach up to 0.19 in superficial tissue. The strain rate can reach 120 g-1. The wave of curvature travels along the body at a near-constant rate. Posterior to the stiff head, body-length-specific curvature is high and rises gently along the entire trunk to a maximum value of 6. Burst-and-coast swimming generates similar peak curvatures to cyclic swimming, but curvature rises more steeply from head to tail. Fish larvae exhibit phase shifts of 57-63[degree] between the wave of lateral displacement and the wave of curvature, resulting in a 1:1.2 ratio of body wave length to curvature wave length. During C-starts, muscle strain can reach 0.19 and superficial longitudinal strain rates approach 30 s-1. Fish larvae do not initiate their escape response with a standing wave of curvature, although their C-starts approach a standing wave as the larvae grow older. The performance demands derived from swimming kinematics suggest that larval axial muscles have very short contraction cycles (10 ms), experience considerable strains (up to 0.2) and strain rates (up to 30 s-1 in white muscle fibres) yet are able to power swimming for several seconds.
Descriptors: zebrafish larvae, *Danio rerio*, biomechanics, swimming kinematics, larval development, larval locomotory development, swimming, swimming behavior, body wave characteristics and ontogenetic changes, locomotory development implications.

Murakami, T., T. Hijikata, M. Matsukawa, H. Ishikawa, and H. Yorifuji (2006). **Zebrafish protocadherin 10 is involved in paraxial mesoderm development and somitogenesis.** *Developmental Dynamics* 235(2): 506-14. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, segmental plate, molecular cloning, cadherins metabolism, mesoderm metabolism, somites metabolism, cadherins genetics, cell adhesion, central nervous system embryology and metabolism, molecular cloning, complementary DNA genetics, cytology, embryology, metabolism, developmental gene expression regulation, head embryology, mesoderm cytology, zebrafish proteins.

Murayama, E., P. Herbomel, A. Kawakami, H. Takeda, and H. Nagasawa (2005). **Otolith matrix proteins omp-1 and otolin-1 are necessary for normal otolith growth and their correct anchoring onto the sensory maculae**. *Mechanisms of Development* 122(6): 791-803. ISSN: 0925 4773.

NAL Call Number: QH607.A1C4

Abstract: Fish otoliths are highly calcified concretions deposited in the inner ear and serve as a part of the hearing and balance systems. They consist mainly of calcium carbonate and a small amount of organic matrix. The latter component is considered to play important roles in otolith formation. Previously, we identified two major otolith matrix proteins, OMP-1 (otolith matrix protein-1) and Otolin-1, from salmonid species. To assess the function of these proteins in otolith formation, we performed antisense morpholino oligonucleotide (MO)-mediated knockdown of omp-1 and otolin-1 in zebrafish embryos. We first identified zebrafish cDNA homologs of omp-1 (zomp-1) and otolin-1 (zotolin-1). Whole-mount in situ hybridization then revealed that the expression of both zomp-1 and zotolin-1 mRNAs is restricted to the otic vesicles. zomp-1 mRNA was expressed from the 14-somite stage in the otic placode, but the zOMP-1 protein was detected only from 26-somite stage onwards. In contrast, zotolin-1 mRNA expression became clear around 72 hpf. MOs designed to inhibit zomp-1 and zotolin-1 mRNA translation, respectively, were injected into 1-2 cell stage embryos. zomp-1 MO caused a reduction in otolith size and an absence of zOtolin-1 deposition, while zotolin-1 MO caused a fusion of the two otoliths, and an increased instability of otoliths after fixation. We conclude that zOMP-1 is required for normal otolith growth and deposition of zOtolin-1 in the otolith, while zOtolin-1, a collagenous protein, is involved in the correct arrangement of the otoliths onto the sensory epithelium of the inner ear and probably in stabilization of the otolith matrix. (c) 2005 Elsevier Ireland Ltd. All rights reserved. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, development, molecular genetics, sense organs, sensory reception, otolithic matrix.

Murciano, C., J. Perez Claros, A. Smith, F. Avaron, T.D. Fernandez, I. Duran, J. Ruiz Sanchez, F. Garcia, J. Becerra, M.A. Akimenko, and M. Mari Beffa (2007). **Position dependence of hemiray morphogenesis during tail fin regeneration in *Danio rerio*.** *Developmental Biology* 312(1): 272-83. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The fins of actinopterygian can regenerate following amputation. Classical papers have shown that the ray, a structural unit of these fins, might regenerate independent of this appendage. Each fin ray is formed by two apposed contralateral hemirays. A hemiray may autonomously regenerate and segmentate in a position-independent manner. This is observed when heterotopically grafted into an interray space, after amputation following extirpation of the contralateral hemiray or when simply ablated. During this process, a proliferating hemiblastema is formed, as shown by bromodeoxyuridine incorporation, from which the complete structure will regenerate. This hemiblastema shows a patterning of gene expression domain similar to half ray blastema. Interactions between contralateral hemiblastema have been studied by recombinant rays composed of hemirays from different origins on the proximo-distal or dorso-ventral axis of the caudal fin. Dye 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocianine perchlorate labeling of grafted tissues was used as tissular marker. Our results suggest both that there are contralateral interactions between hemiblastema of each ray, and that hemiblastema may vary its morphogenesis, always differentiating as their host region. These non-autonomous, position-dependent interactions control coordinated bifurcations, segment joints and ray length independently. A morphological study of the

developing and regenerating fin of another long fin mutant zebrafish suggests that contralateral hemiblastema interactions are perturbed in this mutant.
Descriptors: zebrafish, animal structures growth and development, body patterning, regeneration, tail growth and development, structures cytology, bromodeoxyuridine metabolism, cell proliferation, developmental gene expression regulation, mutation genetics, phenotype, tail anatomy and histology.

Muto, A., K. Arai, and S. Watanabe (2006). **Rab11-FIP4 is predominantly expressed in neural tissues and involved in proliferation as well as in differentiation during zebrafish retinal development.** *Developmental Biology* 292(1): 90-102. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Rab11 family interacting protein 4 (Rab11-FIP4) was initially identified in humans as an Rab11-binding protein, but its biological function has remained unknown. We cloned the zebrafish orthologue of Rab11-FIP4 (zRab11-FIP4) and analyzed its function in vivo by using antisense morpholino. zRab11-FIP4 was expressed as 2 alternative transcripts, i.e., the longer A-form predominantly expressed in neural tissues and the shorter B-form expressed ubiquitously; and in situ hybridization revealed that the A-form was the dominant form. In the developing retina, zRab11-FIP4 was expressed in progenitors throughout the retina at early stages; and then, along with the differentiation, the expression became gradually restricted to the ganglion cell layer and ciliary marginal zone. zRab11-FIP4A knockdown embryos exhibited eye phenotypes similar to those of the shh mutant, such as a small eye with impaired cell proliferation and the delay in cell-cycle exit and differentiation of retinal progenitors. The lack of induction of p57kip2 and enhanced expression of cyclin D1 were observed in the morphant retina. Importantly, the delay in cell-cycle exit was rescued by ectopic expression of either p57Kip2 or dominant-negative PKA, suggesting that Rab11-FIP4A plays pivotal roles in retinal development by regulating Shh signaling and a mechanism acting in parallel with Shh signaling in the control of cell-cycle exit.
Descriptors: zebrafish embryo, carrier proteins biosynthesis, cell differentiation physiology, cell proliferation, nerve tissue proteins biosynthesis, retina embryology, proteins biosynthesis, rab GTP binding proteins biosynthesis, brain cytology, brain embryology, brain metabolism, carrier proteins genetics and physiology, cell count, cell cycle genetics, cell cycle physiology, cell differentiation genetics, cyclic AMP dependent protein kinases biosynthesis and genetics, cyclin dependent kinase inhibitor p57 biosynthesis, cyclin dependent kinase inhibitor p57 genetics, Hedgehog proteins, nerve tissue nerve tissue, antisense oligonucleotides, retina cytology, retina metabolism, signal transduction physiology, stem cells cytology, stem cells metabolism, trans activators physiology, Rab GTP binding proteins genetics and physiology.

Muyskens, J.B. and C.B. Kimmel (2007). **Tbx16 cooperates with Wnt11 in assembling the zebrafish organizer.** *Mechanisms of Development* 124(1): 35-42. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: The organizer, the signaling center that specifies vertebrate axial polarity and the nervous system, is a dorsal midline mesodermal domain in the gastrula that will form prechordal plate and anterior notochord. We show that in zebrafish the organizer is not a single domain when it first arises in the nascent mesoderm at the onset of gastrulation. Rather, in the presumptive prechordal plate region, the organizer is subdivided into two side-by-side cellular fields. Within minutes, concurrent medial and anterior cellular movements merge, or 'coalesce', the two fields to form the well-known singular midline field. Coalescence forms a symmetrical domain because the cell movements on the left and right sides initiate simultaneously and occur synchronously. However, in embryos with reduced function of the T-box

transcription factor Tbx16 (Spadetail) or its genetic target paraxial protocadherin (Papc), synchrony is lost, coalesence is disrupted, and the midline domain is misshaped. Furthermore, with combined loss of Tbx16 and Wnt11 (Silberblick), coalesence is essentially absent. Possibly as a consequence, both the anterior movement of presumptive prechordal plate and organizer function, as assayed by eye-field separation, are disrupted. Our findings thus reveal that Tbx16, in combination with Wnt11, are critical components not only in morphogenesis but also in initial assembly of the organizer.

Descriptors: zebrafish, organizers, embryonic embryology, T-box domain proteins metabolism, Wnt proteins metabolism, base sequence, body patterning, cadherins genetics and metabolism, developmental gene expression regulation, oligodeoxyribonucleotides, antisense genetics, oligodeoxyribonucleotides, antisense pharmacology, organizers, embryonic metabolism, messenger RNA genetics and metabolism, signal transduction, T-box domain proteins deficiency and genetics, Wnt proteins deficiency and genetics, proteins deficiency.

Nair, S., W. Li, R. Cornell, and T.F. Schilling (2007). **Requirements for Endothelin type-A receptors and Endothelin-1 signaling in the facial ectoderm for the patterning of skeletogenic neural crest cells in zebrafish.** *Development* 134(2): 335-45. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Genetic studies in mice and zebrafish have revealed conserved requirements for Endothelin 1 (Edn1) signaling in craniofacial development. Edn1 acts through its cognate type-A receptor (Ednra) to promote ventral skeletal fates and lower-jaw formation. Here, we describe the isolation and characterization of two zebrafish ednra genes - ednra1 and ednra2 - both of which are expressed in skeletal progenitors in the embryonic neural crest. We show that they play partially redundant roles in lower-jaw formation and development of the jaw joint. Knockdown of Ednra1 leads to fusions between upper- and lower-jaw cartilages, whereas the combined loss of Ednra1 and Ednra2 eliminates the lower jaw, similar to edn1-/- mutants. edn1 is expressed in pharyngeal arch ectoderm, mesoderm and endoderm. Tissue-mosaic studies indicate that, among these tissues, a crucial source of Edn1 is the surface ectoderm. This ectoderm also expresses ednrA1 in an edn1-dependent manner, suggesting that edn1 autoregulates its own expression. Collectively, our results indicate that Edn1 from the pharyngeal ectoderm signals through Ednra proteins to direct early dorsoventral patterning of the skeletogenic neural crest.

Descriptors: zebrafish, endothelin 1 genetics, receptor, endothelin A genetics, amino acid and base sequence, body patterning, complementary DNA genetics, endothelin 1 metabolism, joints embryology, joints metabolism, mice, molecular sequence data, mutation, neural crest embryology and metabolism, phylogeny, receptor, endothelin a metabolism, amino acid sequence homology, signal transduction, skull embryology, skull metabolism, metabolism.

Nakada, Y., P. Parab, A. Simmons, A. Omer-Abdalla, and J.E. Johnson (2004). **Separable enhancer sequences regulate the expression of the neural bHLH transcription factor neurogenin 1**. *Developmental Biology* 271(2): 479-487. ISSN: 0012-1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish, brain, enhancers, freshwater fish, ganglia, gene expression, helix-loop-helix proteins, neural tube, olfactory organs, reporter gene, spinal cord, transcription, transgenic mice, neurogenin 1, *Danio rerio*, gene regulation, neural bHLH transcription factor neurogenin 1, neurogenin 1, zebra fish, chickens, man, mice, ngn1 gene.

Nakano, Y., H.R. Kim, A. Kawakami, S. Roy, A.F. Schier, and P.W. Ingham (2004). **Inactivation of dispatched 1 by the chameleon mutation disrupts Hedgehog signalling in the zebrafish embryo.** *Developmental Biology* 269(2): 381-92. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Searches of zebrafish EST and whole genome shotgun sequence databases for sequences encoding the sterol-sensing domain (SSD) protein motif identified two sets of DNA sequences with significant homology to the Drosophila dispatched gene required for release of secreted Hedgehog protein. Using morpholino antisense oligonucleotides, we found that inhibition of one of these genes, designated Disp1, results in a phenotype similar to that of the "you-type" mutants, previously implicated in signalling by Hedgehog proteins in the zebrafish embryo. Injection of disp1 mRNA into embryos homozygous for one such mutation, chameleon (con) results in rescue of the mutant phenotype. Radiation hybrid mapping localised disp1 to the same region of LG20 to which the con mutation was mapped by meiotic recombination analysis. Sequence analysis of disp1 cDNA derived from homozygous con mutant embryos revealed that both mutant alleles are associated with premature termination codons in the disp1 coding sequence. By analysing the expression of markers of specific cell types in the neural tube, pancreas and myotome of con mutant and Disp1 morphant embryos, we conclude that Disp1 activity is essential for the secretion of lipid-modified Hh proteins from midline structures.

Descriptors: zebrafish embryology, membrane, signal transduction , trans activators physiology, amino acid sequence, chromosome mapping, developmental gene expression regulation, Hedgehog proteins, molecular sequence data, mutation.

Nambiar, R.M. and P.D. Henion (2004). **Sequential antagonism of early and late Wnt-signaling by zebrafish colgate promotes dorsal and anterior fates.** *Developmental Biology* 267(1): 165-80. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The establishment of the vertebrate body plan involves patterning of the ectoderm, mesoderm, and endoderm along the dorsoventral and antero-posterior axes. Interactions among numerous signaling molecules from several multigene families, including Wnts, have been implicated in regulating these processes. Here we provide evidence that the zebrafish colgate(b382) (col) mutation results in increased Wnt signaling that leads to defects in dorsal and anterior development. col mutants display early defects in dorsoventral patterning manifested by a decrease in the expression of dorsal shield-specific markers and ectopic expression of ventrolaterally expressed genes during gastrulation. In addition to these early patterning defects, col mutants display a striking regional posteriorization within the neuroectoderm, resulting in a reduction in anterior fates and an expansion of posterior fates within the forebrain and midbrain-hindbrain regions. We are able to correlate these phenotypes to the overactivation of Wnt signaling in col mutants. The early dorsal and anterior patterning phenotypes of the col mutant embryos are selectively rescued by inactivation of Wnt8 function by morpholino translational interference. In contrast, the regionalized neuroectoderm posteriorioriorization phenotype is selectively rescued by morpholino-mediated inactivation of Wnt8b. These results suggest that col-mediated antagonism of early and late Wnt-signaling activity during gastrulation is normally required sequentially for both early dorsoventral patterning and the specification and patterning of regional fates within the anterior neuroectoderm.

Descriptors: zebrafish embryology, cell lineage, proto oncogene proteins metabolism, signal transduction physiology, proteins, immunohistochemistry, in situ hybridization, phenotype, Wnt proteins.

Nambiar, R.M., M.S. Ignatius, and P.D. Henion (2007). **Zebrafish colgate/hdac1 functions in the non-canonical Wnt pathway during axial extension and in Wnt-independent branchiomotor neuron migration.** *Mechanisms of Development* 124(9-10): 682-98. ISSN: 0925-4773.

NAL Call Number: QH607.A1C4

Abstract: Vertebrate gastrulation involves the coordinated movements of populations of cells. These movements include cellular rearrangements in which cells polarize along their medio-lateral axes leading to cell intercalations that result in elongation of the body axis. Molecular analysis of this process has implicated the non-canonical Wnt/Frizzled signaling pathway that is similar to the planar cell polarity pathway (PCP) in Drosophila. Here we describe a zebrafish mutant, colgate (col), which displays defects in the extension of the body axis and the migration of branchiomotor neurons. Activation of the non-canonical Wnt/PCP pathway in these mutant embryos by overexpressing DeltaNdishevelled, rho kinase2 and van gogh-like protein 2 (vangl2) rescues the extension defects suggesting that col acts as a positive regulator of the non-canonical Wnt/PCP pathway. Further, we show that col normally regulates the caudal migration of nVII facial hindbrain branchiomotor neurons and that the mutant phenotype can be rescued by misexpression of vangl2 independent of the Wnt/PCP pathway. We cloned the col locus and found that it encodes histone deacetylase1 (hdac1). Our previous results and studies by others have implicated hdac1 in repressing the canonical Wnt pathway. Here, we demonstrate novel roles for zebrafish hdac1 in activating non-canonical Wnt/PCP signaling underlying axial extension and in promoting Wnt-independent caudal migration of a subset of hindbrain branchiomotor neurons.

Descriptors: zebrafish embryos, axons physiology, body patterning physiology, cell movement physiology, histone deacetylases physiology, motor neurons physiology, signal transduction physiology, Wnt, axons enzymology, body patterning genetics, cell movement genetics, cell polarity genetics, cell polarity physiology, histone deacetylases genetics, mutation, rhombencephalon cytology, embryology and enzymology, signal transduction genetics, Wnt proteins genetics, proteins genetics.

Nechiporuk, A., T. Linbo, K.D. Poss, and D.W. Raible (2007). **Specification of epibranchial placodes in zebrafish.** *Development* 134(3): 611-23. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: In all vertebrates, the neurogenic placodes are transient ectodermal thickenings that give rise to sensory neurons of the cranial ganglia. Epibranchial (EB) placodes generate neurons of the distal facial, glossopharyngeal and vagal ganglia, which convey sensation from the viscera, including pharyngeal endoderm structures, to the CNS. Recent studies have implicated signals from pharyngeal endoderm in the initiation of neurogenesis from EB placodes; however, the signals underlying the formation of placodes are unknown. Here, we show that zebrafish embryos mutant for fgf3 and fgf8 do not express early EB placode markers, including foxi1 and pax2a. Mosaic analysis demonstrates that placodal cells must directly receive Fgf signals during a specific crucial period of development. Transplantation experiments and mutant analysis reveal that cephalic mesoderm is the source of Fgf signals. Finally, both Fgf3 and Fgf8 are sufficient to induce foxi1-positive placodal precursors in wild-type as well as Fgf3-plus Fgf8-depleted embryos. We propose a model in which mesoderm-derived Fgf3 and Fgf8 signals establish both the EB placodes and the development of the pharyngeal endoderm, the subsequent interaction of which promotes neurogenesis. The coordinated interplay between craniofacial tissues would thus assure proper spatial and temporal interactions in the shaping of the vertebrate head.

Descriptors: zebrafish , branchial region embryology, genetically modified, base sequence,

branchial region metabolism, fibroblast growth factor 3 genetics and metabolism, Fgf genetics and metabolism, forkhead transcription factors genetics and metabolism, developmental gene expression regulation, neurological models, mutation, oligodeoxyribonucleotides, antisense genetics, pax2 transcription factor genetics andmetabolism, signal transduction, metabolism.

Nechiporuk, A., T. Linbo, and D.W. Raible (2005). **Endoderm-derived Fgf3 is necessary and sufficient for inducing neurogenesis in the epibranchial placodes in zebrafish.** *Development* 132(16): 3717-30. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: In vertebrates, epibranchial placodes are transient ectodermal thickenings that contribute sensory neurons to the epibranchial ganglia. These ganglia innervate internal organs and transmit information on heart rate, blood pressure and visceral distension from the periphery to the central nervous system. Despite their importance, the molecular mechanisms that govern the induction and neurogenesis of the epibranchial placodes are only now being elucidated. In this study, we demonstrate that endoderm is required for neurogenesis of the zebrafish epibranchial placodes. Mosaic analyses confirm that endoderm is the source of the neurogenic signal. Using a morpholino knockdown approach, we find that fgf3 is required for the majority of placode cells to undergo neurogenesis. Tissue transplants demonstrate that fgf3 activity is specifically required in the endodermal pouches. Furthermore, ectopic fgf3 expression is sufficient for inducing phox2a-positive neurons in wild-type and endoderm-deficient embryos. Surprisingly, ectodermal foxi1 expression, a marker for the epibranchial placode precursors, is present in both endoderm-deficient embryos and fgf3 morphants, indicating that neither endoderm nor Fgf3 is required for initial placode induction. Based on these findings, we propose a model for epibranchial placode development in which Fgf3 is a major endodermal determinant required for epibranchial placode neurogenesis.

Descriptors: zebrafish embryology, embryonic structures physiology, endoderm metabolism, Fgf metabolism, morphogenesis, neurons physiology, proteins metabolism, body patterning, embryonic structures anatomy and histology, fibroblast growth factor 3, Fgf genetics, in situ hybridization, neurons cytology, antisense oligonucleotides genetics and metabolism, signal transduction physiology, metabolism.

Nechiporuk, A., K.D. Poss, S.L. Johnson, and M.T. Keating (2003). **Positional cloning of a temperature-sensitive mutant emmental reveals a role for sly1 during cell proliferation in zebrafish fin regeneration.** *Developmental Biology* 258(2): 291-306. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Here, we used classical genetics in zebrafish to identify temperature-sensitive mutants in caudal fin regeneration. Gross morphological, histological, and molecular analyses revealed that one of these strains, emmental (emm), failed to form a functional regeneration blastema. Inhibition of emm function by heat treatment during regenerative outgrowth rapidly blocked regeneration. This block was associated with reduced proliferation in the proximal blastema and expansion of the nonproliferative distal blastemal zone. Positional cloning revealed that the emm phenotype is caused by a mutation in the orthologue of yeast sly1, a gene product involved in protein trafficking. sly1 is upregulated in the newly formed blastema as well as during regenerative outgrowth. Thus, sly1 is essential for blastemal organization and proliferation during two stages of fin regeneration.

Descriptors: laminin physiology, regeneration, amino acid and base sequence, cell division, chromosome mapping, molecular cloning, complementary DNA genetics, extremities

growth and development, extremities physiology, gene expression, in situ hybridization, laminin genetics, molecular sequence data, mutation, amino acid sequence homology, nucleic acid sequence homology, temperature, proteins genetics.

Nechiporuk, T. and V. Vasioukhin (2006). **Planar cell polarity planes the inconveniences of cell division into a smooth morphogenetic process**. *Developmental Cell* 10(2): 153-154. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Cell divisions are necessary, but also very disruptive for morphogenesis. Dividing cells lose many intercellular contacts and polarized features. This breaks the magnificent topology of the developing embryo and, if left unrepaired, can lead to severe tissue disorganization. A recent study demonstrated that cells use the planar cell polarity pathway to reestablish polarity and reintegrate daughter cells into developing tissue. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, cell biology and development, cell division, morphogenesis, planar cell polarity pathway.

Negron, J.F. and R.A. Lockshin (2004). **Activation of apoptosis and caspase-3 in zebrafish early gastrulae.** *Developmental Dynamics* 231(1): 161-70. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Nonmammalian vertebrate embryos do not manifest apoptosis before gastrulation, and it has been suggested that their cells are inhibited from undergoing apoptosis. To study this interesting possibility, the zebrafish (Danio rerio) embryo is an excellent model. However, the appearance of apoptosis varies among species, and many components of cell death are not highly conserved. To undertake the larger investigation, we first need to document by several criteria that cell death in the zebrafish embryo is apoptotic. Exposure of gastrulating germ-ring stage embryos to cycloheximide or staurosporine elicits an arrest in development and cell death within 8 hr. Caspase-3 activity increases, followed by translocation of phosphatidylserine, loss of cell-cell adhesion, cleavage of poly (ADP-ribose) polymerase, terminal deoxynucleotidyl transferase-mediated dNTP-fluorescein nick end labeling (TUNEL)-positive nuclei, internucleosomal DNA fragmentation, chromatin condensation and margination, and blebbing of the nuclear membrane. Thus, by many criteria, cell death in zebrafish is apoptotic; many of the markers of apoptosis found in mammals are conserved in zebrafish; and post-midblastula transition embryos have the capacity to activate a caspase-dependent apoptotic response well before naturally occurring programmed cell death is seen.
Descriptors: zebrafish embryo, apoptosis physiology, caspases metabolism, cell adhesion physiology, phosphatidylserines metabolism, adenosine diphosphate ribose metabolism, apoptosis drug effects, caspase 3, cell adhesion drug effects, cell nucleus metabolism, cycloheximide pharmacology, cytology, metabolism, gastrula cytology and metabolism, sTaurosporine pharmacology.

Nelson, W.J. (2003). **Tube morphogenesis: closure, but many openings remain.** *Trends in Cell Biology* 13(12): 615-621. ISSN: 0962 8924.
NAL Call Number: QH573.T73
Descriptors: zebrafish, development, cell migration, cellular reorganization, dichotomous branching, directional cues, growth factor signaling, inductive signaling, protein trafficking, tube closure.

Ng, A.N., T.A. de Jong Curtain, D.J. Mawdsley, S.J. White, J. Shin, B. Appel, P.D. Dong, D.Y. Stainier, and J.K. Heath (2005). **Formation of the digestive system in zebrafish: III. Intestinal epithelium morphogenesis.** *Developmental Biology* 286(1): 114-35. ISSN: 0012 1606.
NAL Call Number: 442.8D49

Abstract: Recent analysis of a novel strain of transgenic zebrafish (gutGFP) has provided a detailed description of the early morphological events that occur during the development of the liver and pancreas. In this paper, we aim to complement these studies by providing an analysis of the morphological events that shape the zebrafish intestinal epithelium. One of our goals is to provide a framework for the future characterization of zebrafish mutant phenotypes in which intestinal epithelial morphogenesis has been disrupted. Our analysis encompasses the period between 26 and 126 h post-fertilization (hpf) and follows the growth, lumen formation and differentiation of a continuous layer of endoderm into a functional intestinal epithelium with three morphologically distinct segments: the intestinal bulb, mid-intestine and posterior intestine. Between 26 hpf and 76 hpf, the entire intestinal endoderm is a highly proliferative organ. To make a lumen, the zebrafish endoderm cells undergo apical membrane biogenesis, adopt a bilayer configuration and form small cavities that coalesce without cell death. Thereafter, the endoderm cells polarize and differentiate into distinct cell lineages. Enteroendocrine cells are distinguished first at 52 hpf in the caudal region of the intestine in a new stable transgenic line, Tg[nkx2.2a:mEGFP]. The differentiation of mucin-containing goblet cells is first evident at 100 hpf and is tightly restricted to a middle segment of the intestine, designated the mid-intestine, that is also demarcated by the presence of enterocytes with large supranuclear vacuoles. Meanwhile, striking expansion of the lumen in the rostral intestine forms the intestinal bulb. Here the epithelium elaborates folds and proliferating cells become progressively restricted to a basal compartment analogous to the crypts of Lieberkuhn in mammals. At 126 hpf, the posterior intestine remains an unfolded monolayer of simple columnar epithelium.

Descriptors: zebrafish, intestines embryology, genetically modified, base sequence, cell differentiation, cell polarity, cell proliferation, recombinant DNA genetics, endoderm cytology and metabolism, epithelial cells cytology, epithelial cells metabolism, epithelium embryology, epithelium metabolism, gene expression, green fluorescent intestines cytology and metabolism, morphogenesis, recombinant metabolism.

Nica, G., W. Herzog, C. Sonntag, M. Nowak, H. Schwarz, A.G. Zapata, and M. Hammerschmidt (2006). **Eya1 is required for lineage-specific differentiation, but not for cell survival in the zebrafish adenohypophysis.** *Developmental Biology* 292(1): 189-204. ISSN: 0012 1606.
NAL Call Number: 442.8D49

Abstract: The homeodomain transcription factor Six1 and its modulator, the protein phosphatase Eya1, cooperate to promote cell differentiation and survival during mouse organ development. Here, we studied the effects caused by loss of eya1 and six1 function on pituitary development in zebrafish. eya1 and six1 are co-expressed in all adenohypophyseal cells. Nevertheless, eya1 (aal, dog) mutants show lineage-specific defects, defining corticotropes, melanotropes, and gonadotropes as an Eya1-dependent lineage, which is complementary to the Pit1 lineage. Furthermore, eya1 is required for maintenance of pit1 expression, leading to subsequent loss of cognate hormone gene expression in thyrotropes and somatotropes of mutant embryos, whereas prolactin expression in lactotropes persists. In contrast to other organs, adenohypophyseal cells of eya1 mutants do not become apoptotic, and the adenohypophysis remains at rather normal size. Also, cells do not trans-differentiate, as in the case of pit1 mutants, but display morphological features characteristic for nonsecretory cells. Some of the adenohypophyseal defects of eya1 mutants are moderately enhanced in combination

with antisense-mediated loss of Six1 function, which per se does not affect pituitary cell differentiation. In conclusion, this is the first report of an essential role of Eya1 during pituitary development in vertebrates. Eya1 is required for lineage-specific differentiation of adenohypophyseal cells, but not for their survival, thereby uncoupling the differentiation-promoting and anti-apoptotic effects of Eya proteins seen in other tissues.
Descriptors: zebrafish embryos, cell differentiation physiology, cell lineage physiology, intracellular signaling peptides and, nuclear , anterior pituitary gland cytology and embryology, protein tyrosine phosphatase physiology, amino acid sequence, apoptosis genetics and physiology, cell differentiation genetics, cell lineage genetics, cell survival genetics, cell survival physiology, developmental gene expression regulation physiology, growth hormone biosynthesis and genetics, homeodomain proteins biosynthesis, genetics and physiology, intracellular signaling peptides and molecular sequence data, mutation, nerve tissue proteins biosynthesis and genetics, nuclear pro opiomelanocortin biosynthesis and genetics, protein tyrosine phosphatase genetics, thyrotropin biosynthesis and genetics, transcription factor pit 1 biosynthesis and genetics, transcription factors biosynthesis and genetics, proteins biosynthesis and genetics.

Niikura, Y., Y. Tabata, A. Tajima, I. Inoue, K. Arai, and S. Watanabe (2006). **Zebrafish Numb homologue: phylogenetic evolution and involvement in regulation of left-right asymmetry.** *Mechanisms of Development* 123(5): 407-14. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: Numb and its homologue, Numb-like (Numbl), play important roles in mammalian development, but their role in embryonic development of lower vertebrates remains unknown. We cloned a zebrafish numb homologue (znumb) by searching database. znumb shows approximately 60% identity with mammalian Numb orthologs. Interstingly, znumb lacks two specific sequence motifs unique to Numbl orthologs. However, chromosomal localization of znumb gene revealed colinearity with genes located around mouse and human Numbl genes. Furthermore, multi-species comparisons of conserved phosphotyrosine-binding (PTB) domain sequences in Numb and Numbl proteins suggest that znumb is more closely related to Numbl than Numb. znumb mRNA was expressed in a wide variety of zebrafish adult tissues. Overexpression of znumb in embryos resulted in an absence, or reversal, of the normal leftward shift of the developing heart tube. Furthermore, no or bi-lateral transcripts of lefty2 were observed in znumb-expressing embryos, suggesting that the Notch signaling was essential for left-right field formation and maintenance in zebrafish, and that znumb perturbed this process through down-regulation of endogenous Notch signaling.
Descriptors: zebrafish, body patterning genetics, *Drosophila* proteins metabolism, juvenile hormones metabolism, membrane proteins metabolism, phylogeny , proteins metabolism, molecular cloning, *Drosophila* proteins genetics molecular evolution, developmental gene expression regulation, juvenile hormones genetics, membrane tertiary protein structure, Notch receptors genetics, Notch receptors metabolism, amino acid sequence homology, signal transduction.

Nikaido, M., K. Doi, T. Shimizu, M. Hibi, Y. Kikuchi, and K. Yamasu (2007). **Initial specification of the epibranchial placode in zebrafish embryos depends on the fibroblast growth factor signal.** *Developmental Dynamics* 236(2): 564-71. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: In vertebrates, cranial sensory ganglia are mainly derived from ectodermal placodes, which are focal thickenings at characteristic positions in the embryonic head. Here, we provide the first description of the early development of the epibranchial placode in zebrafish

embryos using sox3 as a molecular marker. By the one-somite stage, we saw a pair of single sox3-expressing domains appear lateral to the future hindbrain. The sox3 domain, which is referred to here as the early lateral placode, is segregated during the early phase of segmentation to form a pax2a-positive medial area and a pax2a-negative lateral area. The medial area subsequently developed to form the otic placode, while the lateral area was further segregated along the anteroposterior axis, giving rise to four sox3-positive subdomains by 26 hr postfertilization. Given their spatial relationship with the expression of the markers for the epibranchial ganglion, as well as their positions and temporal changes, we propose that these four domains correspond to the facial, glossopharyngeal, vagal, and posterior lateral line placodes in an anterior-to-posterior order. The expression of sox3 in the early lateral placode was absent in mutants lacking functional fgf8, while implantation of fibroblast growth factor (FGF) beads restored the sox3 expression. Using SU5402, which inhibits the FGF signal, we were able to demonstrate that formation of both the early lateral domains and later epibranchial placodes depends on the FGF signal operating at the beginning of somitogenesis. Together, these data provide evidence for the essential role of FGF signals in the development of the epibranchial placodes.

Descriptors: zebrafish embryos, body patterning physiology, cell differentiation physiology, ectoderm physiology, fibroblast growth factor 8 metabolism, sensory ganglia embryology, signal transduction physiology, cell differentiation genetics, DNA binding proteins genetics, proteins metabolism, fibroblast growth factor 8 antagonists and inhibitors and physiology, high mobility group proteins metabolism, in situ hybridization, pyrroles pharmacology, transcription factors metabolism.

Nimura, T., N. Sueyoshi, A. Ishida, Y. Yoshimura, M. Ito, H. Tokumitsu, Y. Shigeri, N. Nozaki, and I. Kameshita (2007). **Knockdown of nuclear Ca2+/calmodulin-dependent protein kinase phosphatase causes developmental abnormalities in zebrafish.** *Archives of Biochemistry and Biophysics* 457(2): 205-16. ISSN: 0003 9861.

NAL Call Number: 381 Ar2

Abstract: Nuclear Ca2+/calmodulin-dependent protein kinase phosphatase (CaMKP-N) is an enzyme that dephosphorylates and concomitantly downregulates multifunctional Ca2+/calmodulin-dependent protein kinases (CaMKs) in vitro. However, the functional roles of this enzyme in vivo are not well understood. To investigate the biological significance of CaMKP-N during zebrafish embryogenesis, we cloned and characterized zebrafish CaMKP-N (zCaMKP-N). Based on the nucleotide sequences in the zebrafish whole genome shotgun database, we isolated a cDNA clone for zCaMKP-N, which encoded a protein of 633 amino acid residues. Transiently expressed full-length zCaMKP-N in mouse neuroblastoma, Neuro2a cells, was found to be localized in the nucleus. In contrast, the C-terminal truncated mutant lacking RKKRRLDVLPLRR (residues 575-587) had cytoplasmic staining, suggesting that the nuclear localization signal of zCaMKP-N exists in the C-terminal region. Ionomycin treatment of CaMKIV-transfected Neuro2a cells resulted in a marked increase in the phosphorylated form of CaMKIV. However, cotransfection with zCaMKP-N significantly decreased phospho-CaMKIV in ionomycin-stimulated cells. Whole mount in situ hybridization analysis of zebrafish embryos showed that zCaMKP-N is exclusively expressed in the head and neural tube regions. Gene knockdown of zCaMKP-N using morpholino-based antisense oligonucleotides induced significant morphological abnormalities in zebrafish embryos. A number of apoptotic cells were observed in brain and spinal cord of the abnormal embryos. These results suggest that zCaMKP-N plays a crucial role in the early development of zebrafish.

Descriptors: zebrafish embryos, Ca2+ calmodulin dependent protein kinase metabolism,

phosphoprotein phosphatase metabolism, proteins metabolism, amino acid sequence, apoptosis, base sequence, tumor cell line, cell nucleus metabolism, ionomycin pharmacology, ionophores pharmacology, mice, molecular sequence data, mutation, nuclear localization signals, phosphoprotein phosphatase genetics, phosphorylation, rats, zebrafish abnormalities.

Ninkovic, J., A. Tallafuss, C. Leucht, J. Topczewski, B. Tannhauser, L. Solnica Krezel, and L. Bally Cuif (2005). **Inhibition of neurogenesis at the zebrafish midbrain-hindbrain boundary by the combined and dose-dependent activity of a new hairy/E(spl) gene pair.** *Development* 132(1): 75-88. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The intervening zone (IZ) is a pool of progenitor cells located at the midbrain-hindbrain boundary (MHB) and important for MHB maintenance, midbrain-hindbrain growth and the generation of midbrain-hindbrain neurons. Recently, we implicated the Hairy/E(spl) transcription factor Her5 in the formation of the medial (most basal) part of the IZ (MIZ) in zebrafish; the molecular bases for lateral IZ (LIZ) formation, however, remain unknown. We now demonstrate that her5 is physically linked to a new family member, him, displaying an identical MHB expression pattern. Using single and double knockdowns of him and her5, as well as a him+her5 deletion mutant background (b404), we demonstrate that Him and Her5 are equally necessary for MIZ formation, and that they act redundantly in LIZ formation in vivo. We show that these processes do not involve cross-regulation between Him and Her5 expression or activities, although Him and Her5 can heterodimerize with high affinity. Increasing the function of one factor when the other is depleted further shows that Him and Her5 are functionally interchangeable. Together, our results demonstrate that patterning and neurogenesis are integrated by the her5-him gene pair to maintain a progenitor pool at the embryonic MHB. We propose a molecular mechanism for this process where the global 'Him+Her5' activity inhibits ngn1 expression in a dose-dependent manner and through different sensitivity thresholds along the medio-lateral axis of the neural plate.
Descriptors: zebrafish embryos, gene expression regulation, mesencephalon developmental embryology, nerve tissue, neurons pathology, rhombencephalon embryology, transcription factors physiology, amino acid sequence, basic helix loop helix transcription factors, Western blotting, body patterning, molecular cloning, DNA primers chemistry, immunohistochemistry, immunoprecipitation, in situ hybridization, molecular sequence data, neurons metabolism, neurons physiology, phylogeny, protein binding, RNA metabolism, messenger RNA metabolism, reverse transcriptase PCR, transcription factors biosynthesis, transgenes, two hybrid system techniques, zebrafish, proteins biosynthesis.

Ninomiya, H., R.P. Elinson, and R. Winklbauer (2004). **Antero-posterior tissue polarity links mesoderm convergent extension to axial patterning**. *Nature* 430(6997): 364-367. ISSN: 0028-0836.
NAL Call Number: 472 N21
Descriptors: zebrafish, embryonic development, genes, morphogenesis, *Danio rerio*, *Xenopus*, Antero-posterior tissue polarity, clawed frogs, convergent extension, planar cell polarity signalling.

Nissen, R.M., A. Amsterdam, and N. Hopkins (2006). **A zebrafish screen for craniofacial mutants identifies wdr68 as a highly conserved gene required for endothelin-1 expression.** *BioMed Central Developmental Biology* 6: 28. ISSN: 1471 213X.
NAL Call Number: QH491.B63
Abstract: BACKGROUND: Craniofacial birth defects result from defects in cranial neural

crest (NC) patterning and morphogenesis. The vertebrate craniofacial skeleton is derived from cranial NC cells and the patterning of these cells occurs within the pharyngeal arches. Substantial efforts have led to the identification of several genes required for craniofacial skeletal development such as the endothelin-1 (edn1) signaling pathway that is required for lower jaw formation. However, many essential genes required for craniofacial development remain to be identified. RESULTS: Through screening a collection of insertional zebrafish mutants containing approximately 25% of the genes essential for embryonic development, we present the identification of 15 essential genes that are required for craniofacial development. We identified 3 genes required for hyomandibular development. We also identified zebrafish models for Campomelic Dysplasia and Ehlers-Danlos syndrome. To further demonstrate the utility of this method, we include a characterization of the wdr68 gene. We show that wdr68 acts upstream of the edn1 pathway and is also required for formation of the upper jaw equivalent, the palatoquadrate. We also present evidence that the level of wdr68 activity required for edn1 pathway function differs between the 1st and 2nd arches. Wdr68 interacts with two minibrain-related kinases, Dyrk1a and Dyrk1b, required for embryonic growth and myotube differentiation, respectively. We show that a GFP-Wdr68 fusion protein localizes to the nucleus with Dyrk1a in contrast to an engineered loss of function mutation Wdr68-T284F that no longer accumulated in the cell nucleus and failed to rescue wdr68 mutant animals. Wdr68 homologs appear to exist in all eukaryotic genomes. Notably, we found that the Drosophila wdr68 homolog CG14614 could substitute for the vertebrate wdr68 gene even though insects lack the NC cell lineage. CONCLUSION: This work represents a systematic identification of approximately 25% of the essential genes required for craniofacial development. The identification of zebrafish models for two human disease syndromes indicates that homologs to the other genes are likely to also be relevant for human craniofacial development. The initial characterization of wdr68 suggests an important role in craniofacial development for the highly conserved Wdr68-Dyrk1 protein complexes.
Descriptors: zebrafish mutants, craniofacial abnormalities genetics , endothelin 1 genetics, mutation, nuclear, body patterning, conserved sequence, gene expression, neural crest, nuclear proteins genetics.

Nissen, R.M., J. Yan, A. Amsterdam, N. Hopkins, and S.M. Burgess (2003). **Zebrafish foxi one modulates cellular responses to Fgf signaling required for the integrity of ear and jaw patterning.** *Development* 130(11): 2543-54. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: We identified four insertional alleles of foxi one (foo), an embryonic lethal mutation in zebrafish that displays defects in both otic placode and the jaw. In foo/foo embryos the otic placode is split into two smaller placodes and mutant embryos show a dorsoventral (DV) cartilage defect manifested as a reduced hyomandibular and reduced third and fourth branchial arches. We identified foxi one (foo), the zebrafish ortholog of Foxi1 (FREAC6, FKHL10, HFH-3, Fkh10) and a member of the forkhead domain transcriptional regulator family, as the gene mutated in foo/foo embryos. foo is expressed in otic placode precursor cells, and foo/foo embryos lack placodal pax8 expression and have disorganized otic expression of pax2.1 and dlx3. Third stream neural crest cell migration, detected by dlx2 and krox20 expression, is aberrant in that it invades the otic placode territory. foo is expressed in pharyngeal pouch endoderm and is required for pouch expression of pax8 and proper patterning of other markers in the pouch such as nkx2.3. In foo/foo embryos, we observed a failure to maintain fgf3 expression in the pouches, followed by apoptosis of neural crest cells in adjacent arches. We conclude that foo expression is essential for pax8 expression probably downstream of Fgf signaling in a conserved pathway jointly required for integrity of pat-

terning in the otic placode and pharyngeal pouches. We propose that correct placement of survival/proliferation cues is essential for shaping the pharyngeal cartilages and that evolutionary links between jaw and ear formation can be traced to Fgf-Foxi1-Pax8 pathways.
Descriptors: zebrafish embryos, DNA binding proteins genetics, Fgf metabolism, nuclear proteins, transcription factors genetics, alleles, base sequence, body patterning genetics, branchial region embryology, cell movement, complementary DNA genetics, DNA binding proteins genetics, proteins metabolism, ear abnormalities, ear embryology, evolution, fibroblast growth factor 3, forkhead transcription factors, developmental gene expression regulation, jaw embryology, abnormalities, embryology, abnormalities and genetics, mutation, neural crest cytology, paired box transcription factors, proto oncogene proteins metabolism, signal transduction, trans activators genetics and metabolism, transcription factors metabolism.

Nixon, S.J., A. Carter, J. Wegner, C. Ferguson, M. Floetenmeyer, J. Riches, B. Key, M. Westerfield, and R.G. Parton (2007). **Caveolin-1 is required for lateral line neuromast and notochord development**. *Journal of Cell Science* 120(13): 2151-2161. ISSN: 0021-9533.
NAL Call Number: QH301.J6
Descriptors: zebrafish embryos, boundaries, caveolae, caveolin, developmental stages, embryogenesis, embryonic development, embryos, freshwater fish, heart, lateral line, notochord, sense organs, signal transduction, tomography, ultrastructure, vesicles, caveolin-1, neuromasts, *Danio rerio*.

Njiwa, J.K., P. Mueller, and R. Klein (2004). **Variations of sperm release in three batches of zebrafish.** *Journal of Fish Biology* 64(2): 475-482. ISSN: 0022 1112.
NAL Call Number: QL614.J68
Abstract: By collecting and counting the number of sperm released during separate matings in three batches of zebrafish *Danio rerio*, aged 3-4, 4-5 and 5-6 months, males were observed to release sperm before the female started laying their eggs. After the female left the nest, the number and motility of sperm and life span of sperm of younger fish were higher than those of older fish in water samples collected under the nest and at the surface of the tank. Sperm were released in the form of sperm trails laid on the nest surface, subsequently active spermatozoa left the trails and moved in the water for several minutes. Sperm trails consisted of bands of viscous material in which the sperm were embedded. In most cases eggs were not laid directly over the sperm trail, suggesting that sperm may contact the eggs after the latter are released into the water. In all the three tested groups there was no significant difference ($P > 0.05$) between the number of sperm collected on some portions of the acetate sheets which lined the nest ceiling. This result demonstrated that the greater activity of younger fish accelerated the sperm dispersal in water. Male sperm duct glands, seminal vesicles, known to secrete mucosubstances are probably involved in the production of sperm trails. The possible influence of insemination on the mating style of zebrafish is discussed.
Descriptors: zebrafish, *Danio rerio*, age , sperm dispersal and insemination success relationship, fertilization, reproductive behavior, egg and sperm synchronous spawning evaluation, breeding site, spawning site, evaluation of substrate or water column.

Nogare, D.E., A. Arguello, S. Sazer, and M.E. Lane (2007). **Zebrafish cdc25a is expressed during early development and limiting for post-blastoderm cell cycle progression.** *Developmental Dynamics* 236(12): 3427-35. ISSN: 1058 8388.
NAL Call Number: QL801.A4

Descriptors: zebrafish, Cdc25a, phosphatases, spatiotemporal dynamics, *Schizosaccharomyces pombe*, post-blastoderm cells, zebrafish.

Nogare, D.E.D. and M.E. Lane (2004). **Cloning and characterization of CDC25 homologues from the zebrafish, *danio rerio*.** *Developmental Biology* 271(2): 567. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, biochemistry and molecular biophysics, cloning, genetic techniques, cell cycle, spatiotemporal regulation.

Nojima, H., T. Shimizu, C.H. Kim, T. Yabe, Y.K. Bae, O. Muraoka, T. Hirata, A. Chitnis, T. Hirano, and M. Hibi (2004). **Genetic evidence for involvement of maternally derived Wnt canonical signaling in dorsal determination in zebrafish.** *Mechanisms of Development* 121(4): 371-86. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: In zebrafish, the program for dorsal specification begins soon after fertilization. Dorsal determinants are localized initially to the vegetal pole, then transported to the blastoderm, where they are thought to activate the canonical Wnt pathway, which induces the expression of dorsal-specific genes. We identified a novel maternal-effect recessive mutation, tokkaebi (tkk), that affects formation of the dorsal axis. Severely ventralized phenotypes, including a lack of dorso-anterior structures, were seen in 5-100% of the embryos obtained from tkk homozygous transmitting females. tkk embryos displayed defects in the nuclear accumulation of beta-catenin on the dorsal side, and reduced or absent expression of dorsal-specific genes. Mesoderm and endoderm formation outside the dorsal axis was not significantly affected. Injection of RNAs for activated beta-catenin, dominant-negative forms of Axin1 and GSK3beta, and wild-type Dvl3, into the tkk embryos suppressed the ventralized phenotypes and/or dorsalized the embryos, and restored or induced an ectopic and expanded expression of bozozok/dharma and goosecoid. However, dorsalization by wnt RNAs was affected in the tkk embryos. Inhibition of cytoplasmic calcium release elicited an ectopic and expanded expression of chordin in the wild-type, but did not restore chordin expression efficiently in the tkk embryos. These data indicate that the tkk gene product functions upstream of or parallel to the beta-catenin-degradation machinery to control the stability of beta-catenin. The tkk locus was mapped to chromosome 16. These data provide genetic evidence that the maternally derived canonical Wnt pathway upstream of beta-catenin is involved in dorsal axis formation in zebrafish.
Descriptors: zebrafish, body patterning genetics, proto oncogene signal transduction genetics, body patterning physiology, calcium signaling physiology, cell nucleus metabolism, chromosome mapping, genetic crosses, cytoskeletal proteins metabolism, ligands, organizers, embryonic physiology, proto oncogene proteins metabolism, signal transduction physiology, trans activators metabolism, Wnt proteins, metabolism, proteins, beta catenin.

Nolte, C., M. Rastegar, A. Amores, M. Bouchard, D. Grote, R. Maas, E.N. Kovacs, J. Postlethwait, I. Rambaldi, S. Rowan, Y.L. Yan, F. Zhang, and M. Featherstone (2006). **Stereo specificity and PAX6 function direct Hoxd4 neural enhancer activity along the antero-posterior axis**. *Developmental Biology* 299(2): 582-593. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The antero-posterior (AP) and dorso-ventral (DV) patterning of the neural tube is controlled in part by HOX and PAX transcription factors, respectively. We have reported on a neural enhancer of Hoxd4 that directs expression in the CNS with the correct anterior border in the hindbrain. Comparison to the orthologous enhancer of zebrafish revealed seven

conserved footprints including an obligatory retinoic acid response element (RARE), and adjacent sites D, E and F. Whereas enhancer function in the embryonic CNS is destroyed by separation of the RARE from sites DE-F by a half turn of DNA, it is rescued by one full turn, suggesting stereospecific constraints between DNA-bound retinoid receptors and the factor(s) recognizing sites D-E-F. Alterations in the DV trajectory of the Hoxd4 anterior expression border following mutation of site D or E implicated transcriptional regulators active across the DV axis. We show that PAX6 specifically binds sites D and E in vitro, and use chromatin immunoprecipitation to demonstrate recruitment of PAX6 to the Hoxd4 neural enhancer in mouse embryos. Hoxd4 expression throughout the CNS is reduced in Pax6 mutant Sey(Neu) animals on embryonic day 8. Additionally, stage-matched zebrafish embryos having decreased pax6a and/or pax6b activity display malformed rhombomere boundaries and an anteriorized hoxd4a expression border. These results reveal an evolutionarily conserved role for Pax6 in AP-restricted expression of vertebrate Hoxd4 orthologs. (c) 2006 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, development, molecular genetics, nervous system, neural coordination, stereospecificity, dorso ventral patterning, antero posterior axis patterning, neural enhancer.

North, T.E. and L.I. Zon (2003). **Modeling human hematopoietic and cardiovascular diseases in zebrafish.** *Developmental Dynamics* 228(3): 568-83. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish mutants, cardiovascular diseases genetics, hematologic diseases genetics, anemia genetics, disease models, gene expression regulation, heart diseases genetics, mutation, vascular diseases genetics.
Notes: A literature review.

Norton, W.H., J. Ledin, H. Grandel, and C.J. Neumann (2005). **HSPG synthesis by zebrafish Ext2 and Extl3 is required for Fgf10 signalling during limb development.** *Development* 132(22): 4963-73. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Heparan sulphate proteoglycans (HSPGs) are known to be crucial for signalling by the secreted Wnt, Hedgehog, Bmp and Fgf proteins during invertebrate development. However, relatively little is known about their effect on developmental signalling in vertebrates. Here, we report the analysis of daedalus, a novel zebrafish pectoral fin mutant. Positional cloning identified fgf10 as the gene disrupted in daedalus. We find that fgf10 mutants strongly resemble zebrafish ext2 and extl3 mutants, which encode glycosyltransferases required for heparan sulphate biosynthesis. This suggests that HSPGs are crucial for Fgf10 signalling during limb development. Consistent with this proposal, we observe a strong genetic interaction between fgf10 and extl3 mutants. Furthermore, application of Fgf10 protein can rescue target gene activation in fgf10, but not in ext2 or extl3 mutants. By contrast, application of Fgf4 protein can activate target genes in both ext2 and extl3 mutants, indicating that ext2 and extl3 are differentially required for Fgf10, but not Fgf4, signalling during limb development. This reveals an unexpected specificity of HSPGs in regulating distinct vertebrate Fgfs.
Descriptors: zebrafish embryos, extremities embryology, fibroblast growth factor 10 physiology, heparan sulfate proteoglycans biosynthesis, N acetylglucosaminyltransferases physiology , signal transduction physiology, fibroblast growth factor 10 deficiency and genetics, mutation, phenotype, signal transduction genetics, proteins genetics and physiology.

Norton, W.H., M. Mangoli, Z. Lele, H.M. Pogoda, B. Diamond, S. Mercurio, C. Russell, H. Teraoka, H.L. Stickney, G.J. Rauch, C.P. Heisenberg, C. Houart, T.F. Schilling, H.G. Frohnhoefer, S. Rastegar, C.J. Neumann, R.M. Gardiner, U. Straehle, R. Geisler, M. Rees, W.S. Talbot, and S.W. Wilson (2005). **Monorail/Foxa2 regulates floorplate differentiation and specification of oligodendrocytes, serotonergic raphe neurones and cranial motoneurones**. *Development* 132(4): 645-658. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: In this study, we elucidate the roles of the winged-helix transcription factor Foxa2 in ventral CNS development in zebrafish. Through cloning of monorail (mol), which we find encodes the transcription factor Foxa2, and phenotypic analysis of mol-/- embryos, we show that floorplate is induced in the absence of Foxa2 function but fails to further differentiate. In mol-/- mutants, expression of Foxa and Hh family genes is not maintained in floorplate cells and lateral expansion of the floorplate fails to occur. Our results suggest that this is due to defects both in the regulation of Hh activity in medial floorplate cells as well as cell-autonomous requirements for Foxa2 in the prospective laterally positioned floorplate cells themselves. Foxa2 is also required for induction and/or patterning of several distinct cell types in the ventral CNS. Serotonergic neurones of the raphe nucleus and the trochlear motor nucleus are absent in mol-/- embryos, and oculomotor and facial motoneurones ectopically occupy ventral CNS midline positions in the midbrain and hindbrain. There is also a severe reduction of prospective oligodendrocytes in the midbrain and hindbrain. Finally, in the absence of Foxa2, at least two likely Hh pathway target genes are ectopically expressed in more dorsal regions of the midbrain and hindbrain ventricular neuroepithelium, raising the possibility that Foxa2 activity may normally be required to limit the range of action of secreted Hh proteins. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryo mutant, Foxa 2, midbrain, hindbrain, molecular genetics, nervous system, neural coordination, gene cloning, genetic techniques, phenotypic analysis, ventricular neuroepithelium.

Novak, A.E., A.D. Taylor, R.H. Pineda, E.L. Lasda, M.A. Wright, and A.B. Ribera (2006). **Embryonic and larval expression of zebrafish voltage-gated sodium channel alpha-subunit genes.** *Developmental Dynamics* 235(7): 1962-73. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, sodium channels metabolism, proteins metabolism, amino acid sequence, metabolism, developmental gene expression regulation, ion channel gating, larva metabolism, molecular sequence data , myocardium metabolism, protein subunits genetics, protein subunits metabolism, sodium channels genetics.

Nyholm, M.K., S.F. Wu, R.I. Dorsky, and Y. Grinblat (2007). **The zebrafish zic2a-zic5 gene pair acts downstream of canonical Wnt signaling to control cell proliferation in the developing tectum.** *Development* 134(4): 735-46. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Wnt growth factors acting through the canonical intracellular signaling cascade play fundamental roles during vertebrate brain development. In particular, canonical Wnt signaling is crucial for normal development of the dorsal midbrain, the future optic tectum. Wnts act both as patterning signals and as regulators of cell growth. In the developing tectum, Wnt signaling is mitogenic; however, the mechanism of Wnt function is not known. As a step towards better understanding this mechanism, we have identified two new Wnt targets, the closely linked zic2a and zic5 genes. Using a combination of in vivo assays, we show that zic2a and zic5 transcription is activated by Tcf/Lef transcription factors in the

dorsal midbrain. Zic2a and Zic5, in turn, have essential, cooperative roles in promoting cell proliferation in the tectum, but lack obvious patterning functions. Collectively these findings suggest that Wnts control midbrain proliferation, at least in part, through regulation of two novel target genes, the zic2a-zic5 gene pair.

Descriptors: zebrafish, corpora quadrigemina growth and development, DNA binding proteins genetics, Wnt, body patterning, cell proliferation, corpora quadrigemina cytology and embryology, DNA binding proteins genetics, mesencephalon cytology and embryology and growth and development, signal transduction, trans activation genetics.

O'Brien, E.K., C. d'Alencon, G. Bonde, W. Li, J. Schoenebeck, M.L. Allende, B.D. Gelb, D. Yelon , J.S. Eisen, and R.A. Cornell (2004). **Transcription factor Ap-2alpha is necessary for development of embryonic melanophores, autonomic neurons and pharyngeal skeleton in zebrafish.** *Developmental Biology* 265(1): 246-61. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The genes that control development of embryonic melanocytes are poorly defined. Although transcription factor Ap-2alpha is expressed in neural crest (NC) cells, its role in development of embryonic melanocytes and other neural crest derivatives is unclear because mouse Ap-2alpha mutants die before melanogenesis. We show that zebrafish embryos injected with morpholino antisense oligonucleotides complementary to ap-2alpha (ap-2alpha MO) complete early morphogenesis normally and have neural crest cells. Expression of c-kit, which encodes the receptor for the Steel ligand, is reduced in these embryos, and, similar to zebrafish c-kit mutant embryos, embryonic melanophores are reduced in number and migration. The effects of ap-2alpha MO injected into heterozygous and homozygous c-kit mutants support the notion that Ap-2alpha works through C-kit and additional target genes to mediate melanophore cell number and migration. In contrast to c-kit mutant embryos, in ap-2alpha MO-injected embryos, melanophores are small and under-pigmented, and unexpectedly, analysis of mosaic embryos suggests Ap-2alpha regulates melanophore differentiation through cell non-autonomous targets. In addition to melanophore phenotypes, we document reduction of other neural crest derivatives in ap-2alpha MO-injected embryos, including jaw cartilage, enteric neurons, and sympathetic neurons. These results reveal that Ap-2alpha regulates multiple steps of melanophore development, and is required for development of other neuronal and non-neuronal neural crest derivatives.

Descriptors: zebrafish embryos, autonomic pathways embryology , branchial region embryology, DNA binding, developmental gene expression regulation, melanophores physiology, transcription factors physiology, amino acid sequence, antisense elements genetics, gene expression profiling, molecular sequence data, neural crest embryology, proto oncogene proteins C kit, reverse transcriptase PCR, sequence alignment, transcription factor ap 2, proteins.

O'Hara, F.P., E. Beck, L.K. Barr, L.L. Wong, D.S. Kessler, and R.D. Riddle (2005). **Zebrafish Lmx1b.1 and Lmx1b.2 are required for maintenance of the isthmic organizer.** *Development* 132(14): 3163-73. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: The mesencephalic and metencephalic region (MMR) of the vertebrate central nervous system develops in response to signals produced by the isthmic organizer (IsO). We have previously reported that the LIM homeobox transcription factor Lmx1b is expressed within the chick IsO, where it is sufficient to maintain expression of the secreted factor wnt1. In this paper, we show that zebrafish express two Lmx1b orthologs, lmx1b.1 and lmx1b.2, in the rostral IsO, and demonstrate that these genes are necessary for key aspects of MMR

development. Simultaneous knockdown of Lmx1b.1 and Lmx1b.2 using morpholino antisense oligos results in a loss of wnt1, wnt3a, wnt10b, pax8 and fgf8 expression at the IsO, leading ultimately to programmed cell death and the loss of the isthmic constriction and cerebellum. Single morpholino knockdown of either Lmx1b.1 or Lmx1b.2 has no discernible effect on MMR development. Maintenance of lmx1b.1 and lmx1b.2 expression at the isthmus requires the function of no isthmus/pax2.1, as well as Fgf signaling. Transient misexpression of Lmx1b.1 or Lmx1b.2 during early MMR development induces ectopic wnt1 and fgf8 expression in the MMR, as well as throughout much of the embryo. We propose that Lmx1b.1- and Lmx1b.2-mediated regulation of wnt1, wnt3a, wnt10b, pax8 and fgf8 maintains cell survival in the isthmocerebellar region.

Descriptors: zebrafish embryos, homeodomain, mesencephalon embryology, metencephalon embryology, transcription factors physiology, amino acid sequence, DNA binding proteins genetics, proteins metabolism, fibroblast growth factor 8, Fgf genetics and metabolism, developmental gene expression regulation physiology, homeodomain intercellular signaling peptides and proteins biosynthesis, intercellular signaling peptides and molecular sequence data, pax2 transcription factor, protein isoforms genetics, protein isoforms physiology, transcription factors genetics and metabolism, Wnt proteins, Wnt1 protein.

Oates, A.C., L.A. Rohde, and R.K. Ho (2005). **Generation of segment polarity in the paraxial mesoderm of the zebrafish through a T-box-dependent inductive event.** *Developmental Biology* 283(1): 204-14. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The first morphological sign of vertebrate postcranial body segmentation is the sequential production from posterior paraxial mesoderm of blocks of cells termed somites. Each of these embryonic structures is polarized along the anterior/posterior axis, a subdivision first distinguished by marker gene expression restricted to rostral or caudal territories of forming somites. To better understand the generation of segment polarity in vertebrates, we have studied the zebrafish mutant fused somites (fss), because its paraxial mesoderm lacks segment polarity. Previously examined markers of caudal half-segment identity are widely expressed, whereas markers of rostral identity are either missing or dramatically down-regulated, suggesting that the paraxial mesoderm of the fss mutant embryo is profoundly caudalized. These findings gave rise to a model for the formation of segment polarity in the zebrafish in which caudal is the default identity for paraxial mesoderm, upon which is patterned rostral identity in an fss-dependent manner. In contrast to this scheme, the caudal marker gene ephrinA1 was recently shown to be down-regulated in fss embryos. We now show that notch5, another caudal identity marker and a component of the Delta/Notch signaling system, is not expressed in the paraxial mesoderm of early segmentation stage fss embryos. We use cell transplantation to create genetic mosaics between fss and wild-type embryos in order to assay the requirement for fss function in notch5 expression. In contrast to the expression of rostral markers, which have a cell-autonomous requirement for fss, expression of notch5 is induced in fss cells at short range by nearby wild-type cells, indicating a cell-non-autonomous requirement for fss function in this process. These new data suggest that segment polarity is created in a three-step process in which cells that have assumed a rostral identity must subsequently communicate with their partially caudalized neighbors in order to induce the fully caudalized state.

Descriptors: zebrafish, body patterning, cell polarity, mesoderm cytology, morphogenesis, developmental gene expression regulation, genetic markers, in situ hybridization, mesoderm physiology, T Box, genetic mosaics.

Oates, A.C., C. Mueller, and R.K. Ho (2005). **Cooperative function of deltac and her7 in anterior segment formation**. *Developmental Biology* 280(1): 133-149. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Segmentation of paraxial mesoderm in vertebrates is regulated by a genetic oscillator that manifests as a series of wavelike or cyclic gene expression domains in the embryo. In zebrafish, this oscillator involves members of the Delta/Notch intercellular signaling pathway, and its down-stream targets, the Her family of transcriptional repressors. Loss of function of any one of the genes of this system, such as her7, gives rise to segmentation defects in the posterior trunk and tail, concomitant with a disruption of cyclic expression domains, indicating that the oscillator is required for posterior segmentation. Control of segmentation in the anterior trunk, and its relationship to that of the posterior is, however, not yet well understood.A combined loss of the cyclic Her genes her1 and her7 disrupts segmentation of both anterior and posterior paraxial mesoderm, indicating that her genes function redundantly in anterior segmentation. To test whether this anterior redundancy is specific to the her gene family, or alternatively is a more global feature of the segmentation oscillator, we looked at anterior segmentation after morpholino knock clown of the cyclic cell-surface Notch ligand deltaC (dlc), either alone or in combination with her7, or other Delta/Notch pathway genes. We find that dlc is required for coherence of wavelike expression domains of cyclic genes her1 and her7 and maintenance of their expression levels, as well as for cyclic transcription of dlc itself, confirming that We is a component of the segmentation oscillator. Dose dependent, posteriorly-restricted segmentation defects were seen in the dlc knock down, and in combination with the deltaD or notch 1a mutants. However, combined reduction of function of dlc and her7 results in defective segmentation of both anterior and posterior paraxial mesoderm, and a failure of cyclic expression domains to initiate, similar to loss of both her genes. Thus, anterior segmentation requires the functions of both her and delta family members in a parallel manner, suggesting that the segmentation oscillator operates in paraxial mesoderm along the entire vertebrate axis. (c) 2005 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, molecular genetics, biochemistry and molecular biophysics, genetic oscillator, anterior segmentation occillator, paraxial mesoderm.

Ober, E.A., H.A. Field, and D.Y. Stainier (2003). **From endoderm formation to liver and pancreas development in zebrafish.** *Mechanisms of Development* 120(1): 5-18. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: Recent studies in zebrafish have contributed to our understanding of early endoderm formation in vertebrates. Specifically, they have illustrated the importance of Nodal signaling as well as three transcription factors, Faust/Gata5, Bonnie and Clyde, and Casanova, in this process. Ongoing genetic and embryological studies in zebrafish are also contributing to our understanding of later aspects of endoderm development, including the formation of the gut and its associated organs, the liver and pancreas. The generation of transgenic lines expressing GFP in these organs promises to be particularly helpful in such studies.
Descriptors: zebrafish embryos, endoderm, liver embryology, pancreas embryology, embryonic induction, gastrula, mesoderm, morphogenesis.

Ochi, H., B.J. Pearson, P.T. Chuang, M. Hammerschmidt, and M. Westerfield (2006). **Hhip regulates zebrafish muscle development by both sequestering Hedgehog and modulating localization of Smoothened.** *Developmental Biology* 297(1): 127-40. ISSN: 0012 1606.
NAL Call Number: 442.8D49

Abstract: Sharp borders between cells with different developmental fates are important for patterning of invertebrates, but are not well understood in vertebrates. Zebrafish slow muscle cells develop from adaxial cells, a one-cell-diameter-thick pseudo-epithelium immediately adjacent to the notochord. Hedgehog (Hh) signals from notochord specify adaxial cells to form slow muscle cells. Cells next to adaxial cells form fast muscle. This suggests that Hh signaling is locally regulated to produce a sharp border that separates slow and fast muscle precursors. To understand how Hh activity is locally regulated, we characterized the dynamic roles of Hhip, a protein that binds Hedgehog at the cell surface. Hhip is strongly expressed by adaxial cells and, together with Patched, the Hedgehog receptor, limits transduction of the Hedgehog signaling by Smoothened to adaxial cells. Hhip protein lacking its membrane associated domain still suppresses Hh activity but no longer acts synergistically with Patched. Hhip and Smoothened colocalize at the cell surface and, in response to Hedgehog, internalize together. Knockdown of Hhip blocks Smoothened internalization while increasing Hedgehog signaling and slow muscle formation. These data support a model in which Hhip regulates muscle development both by sequestering Hedgehog and by modulating localization of Smoothened.

Descriptors: zebrafish embryos, membrane proteins metabolism, muscle development, G protein Coupled receptors metabolism, trans activators metabolism, proteins metabolism, cell membrane metabolism, *Drosophila* proteins genetics and metabolism, developmental gene expression regulation, Hedgehog proteins, membrane muscles cytology and embryology, tertiary protein structure, cell surface receptors genetics and metabolism, G protein Coupled receptors genetics, amino acid sequence homology, trans activators genetics, proteins genetics.

Ochi, H. and M. Westerfield (2007). **Signaling networks that regulate muscle development: lessons from zebrafish.** *Development Growth and Differentiation* 49(1): 1-11. ISSN: 0012 1592.

NAL Call Number: QL951.E4

Abstract: Locomotion mediated by skeletal muscle provides a basis for the behavioral repertoire of most animals. Embryological and genetic studies of mouse, bird, fish and frog embryos are providing insights into the functions of the myogenic regulatory factors (MRFs) and the signaling molecules that regulate activity of MRFs. Nevertheless, our understanding of muscle development remains somewhat limited. Fundamental goals are to elucidate how mesodermal cells are induced during gastrulation to form muscle precursor cells and how muscle precursor cells acquire specific cell fates, such as slow and fast muscle cells. In this review, we focus on studies of zebrafish muscle development that have advanced our understanding of the molecular genetics of muscle cell induction and specification.

Descriptors: zebrafish, mesoderm physiology, skeletal muscle embryology, myogenic regulatory factors metabolism, signal transduction physiology, stem cells physiology, gastrula cytology, gastrula physiology, mesoderm cytology, muscle development, myogenic regulatory factors genetics, stem cells cytology, .

Notes: A literature review.

Okuda, Y., H. Yoda, M. Uchikawa, M. Furutani Seiki, H. Takeda, H. Kondoh, and Y. Kamachi (2006). **Comparative genomic and expression analysis of group B1 sox genes in zebrafish indicates their diversification during vertebrate evolution.** *Developmental Dynamics* 235(3): 811-25. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: Group B1 Sox genes encode HMG domain transcription factors that play major

roles in neural development. We have identified six zebrafish B1 sox genes, which include pan-vertebrate sox1a/b, sox2, and sox3, and also fish-specific sox19a/b. SOX19A/B proteins show a transcriptional activation potential that is similar to other B1 SOX proteins. The expression of sox19a and sox3 begins at approximately the 1,000-cell stage during embryogenesis and becomes confined to the future ectoderm by the shield stage. This is reminiscent of the epiblastic expression of Sox2 and/or Sox3 in amniotes. As development progresses, these six B1 sox genes display unique expression patterns that overlap distinctly from one region to another. sox19a expression is widespread in the early neuroectoderm, resembling pan-neural Sox2 expression in amniotes, whereas zebrafish sox2 shows anterior-restricted expression. Comparative genomics suggests that sox19a/b and mammalian Sox15 (group G) have an orthologous relationship and that the B1/G Sox genes arose from a common ancestral gene through two rounds of genome duplication. It seems likely, therefore, that each B1/G Sox gene has gained a distinct expression profile and function during vertebrate evolution.
Descriptors: zebrafish, molecular evolution, gene expression, high mobility group genomics.

Olechowicz, S.L., J.N. Miller, and J.T.Jr. Warren (2006). **Analysis of the in vivo function of the zebrafish Fragile X gene family**. *Developmental Biology* 295(1): 351. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, behavior, development, molecular genetics, fragile X syndrome, congenital disease, behavioral and mental disorders.

Omata, Y., Y. Nojima, S. Nakayama, H. Okamoto, H. Nakamura, and J. Funahashi (2007). **Role of Bone morphogenetic protein 4 in zebrafish semicircular canal development.** *Development Growth and Differentiation* 49(9): 711-9. ISSN: 0012 1592.
NAL Call Number: QL951.E4
Abstract: Bone morphogenetic proteins (BMPs) are known to play roles in inner ear development of higher vertebrates. In zebrafish, there are several reports showing that members of the BMP family are expressed in the otic vesicle. We have isolated a novel zebrafish mutant gallery, which affects the development of the semicircular canal. Gallery merely forms the lateral and the immature anterior protrusion, and does not form posterior and ventral protrusions. We found that the expression of bmp2b and bmp4, both expressed in the normal optic vesicle at the protrusion stage, are extremely upregulated in the otic vesicle of gallery. To elucidate the role of BMPs in the development of the inner ear of zebrafish, we have applied excess BMP to the wild-type otic vesicle. The formation of protrusions was severely affected, and in some cases, they were completely lost in BMP4-treated embryos. Furthermore, the protrusions in gallery treated with Noggin were partially rescued. These data indicate that BMP4 plays an important role in the development of protrusions to form semicircular canals.
Descriptors: zebrafish, bone, morphogenesis, protein 4, zebrafish, semicircular canal, development.

Onichtchouk, D., K. Aduroja, H.G. Belting, L. Gnugge, and W. Driever (2003). **Transgene driving GFP expression from the promoter of the zona pellucida gene zpc is expressed in oocytes and provides an early marker for gonad differentiation in zebrafish.** *Developmental Dynamics* 228(3): 393-404. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: transgenic zebrafish, oocyte development, cell differentiation genetics, luminescent membrane glycooocytes physiology, promoter regions genetics, zona pellucida

physiology, amino acid sequence, genetically modified, basic helix loop helix transcription factors, consensus sequence, DNA binding proteins genetics, proteins chemistry and genetics, green fluorescent proteins, luminescent proteins analysis, molecular sequence data, recombinant proteins analysis, sequence alignment and homology, sex differentiation genetics, transcription factors chemistry and genetics.

Osborne, N., N.S. Glickman, J. Alexander, D. Yelon, and D.Y. Stainier (2005). **A novel component in signaling by a sphingosine 1-phosphate receptor.** *Developmental Biology* 283(2): 673-674. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, cardiovascular system, transport and circulation, phenotype, morphogenesis, gastrulation.

Ott, E.B., P.A. Sakalis, I.J. Marques, and C.P. Bagowski (2007). **Characterization of the enigma family in zebrafish.** *Developmental Dynamics* 236(11): 3144-54. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, enigma subfamily, proteins, enigma homologue, cypher, ZASP, PDZ, LIM, protein family, form specific expression patterns.

Padhi, B.K., L. Joly, P. Tellis, A. Smith, P. Nanjappa, M. Chevrette, M. Ekker, and M.A. Akimenko (2004). **Screen for genes differentially expressed during regeneration of the zebrafish caudal fin.** *Developmental Dynamics* 231(3): 527-41. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, extremities embryology, developmental gene expression regulation, genetic screening, regeneration genetics, amputation, base sequence, complementary DNA chemistry, complementary DNA genetics, isolation and purification expressed sequence tags, extremities physiology, in situ hybridization, keratins genetics, molecular sequence data, nucleic acid hybridization methods, physical chromosome mapping, PCR, reverse transcriptase PCR, up regulation, wound healing physiology.

Padhi, B.K., M. Ekker, and M.A. Akimenko (2004). **Characterization of two novel genes specifically involved in fin development in zebrafish**. *Journal of Morphology* 260(3): 319. ISSN: 0362 2525.
NAL Call Number: 444.8J826
Descriptors: zebrafish fin development, integumentary system, chemical coordination and homeostasis, gene expression.

Palaisa, K.A. and M. Granato (2007). **Analysis of zebrafish sidetracked mutants reveals a novel role for Plexin A3 in intraspinal motor axon guidance.** *Development* 134(18): 3251-7. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: One of the earliest guidance decisions for spinal cord motoneurons occurs when pools of motoneurons orient their growth cones towards a common, segmental exit point. In contrast to later events, remarkably little is known about the molecular mechanisms underlying intraspinal motor axon guidance. In zebrafish sidetracked (set) mutants, motor axons exit from the spinal cord at ectopic positions. By single-cell labeling and time-lapse analysis we show that motoneurons with cell bodies adjacent to the segmental exit point properly exit from the spinal cord, whereas those farther away display pathfinding errors. Misguided growth cones either orient away from the endogenous exit point, extend towards the endog-

enous exit point but bypass it or exit at non-segmental, ectopic locations. Furthermore, we show that sidetracked acts cell autonomously in motoneurons. Positional cloning reveals that sidetracked encodes Plexin A3, a semaphorin guidance receptor for repulsive guidance. Finally, we show that sidetracked (plexin A3) plays an additional role in motor axonal morphogenesis. Together, our data genetically identify the first guidance receptor required for intraspinal migration of pioneering motor axons and implicate the well-described semaphorin/plexin signaling pathway in this poorly understood process. We propose that axonal repulsion via Plexin A3 is a major driving force for intraspinal motor growth cone guidance.
Descriptors: zebrafish, axons physiology, morphogenesis genetics, motor neurons physiology, cell surface receptors physiology, spinal cord embryology, growth cones physiology, motor neurons cytology, mutation, cell surface receptors genetics, semaphorins physiology, spinal cord cytology, proteins genetics.

Panizzi, J.R., J.R. Jessen, and L. SolnicaKrezel (2005). **New functions for a vertebrate rho guanine nucleotide exchange factor in cilia-dependent processes.** *Developmental Biology* 283(2): 691. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, nervous system, neural coordination, development, biochemistry and molecular biophysics, somitogenesis.

Panzer, J.A., S.M. Gibbs, R. Dosch, D. Wagner, M.C. Mullins, M. Granato, and R.J. Balice Gordon (2005). **Neuromuscular synaptogenesis in wild-type and mutant zebrafish.** *Developmental Biology* 285(2): 340-57. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Genetic screens for synaptogenesis mutants have been performed in many organisms, but few if any have simultaneously screened for defects in pre- and postsynaptic specializations. Here, we report the results of a small-scale genetic screen, the first in vertebrates, for defects in synaptogenesis. Using zebrafish as a model system, we identified seven mutants that affect different aspects of neuromuscular synapse formation. Many of these mutant phenotypes have not been previously reported in zebrafish and are distinct from those described in other organisms. Characterization of mutant and wild-type zebrafish, from the time that motor axons first arrive at target muscles through adulthood, has provided the new information about the cellular events that occur during neuromuscular synaptogenesis. These include insights into the formation and dispersal of prepatterned AChR clusters, the relationship between motor axon elongation and synapse size, and the development of precise appositions between presynaptic clusters of synaptic vesicles in nerve terminals and postsynaptic receptor clusters. In addition, we show that the mechanisms underlying synapse formation within the myotomal muscle itself are largely independent of those that underlie synapse formation at myotendinous junctions and that the outgrowth of secondary motor axons requires at least one cue not necessary for the outgrowth of primary motor axons, while other cues are required for both. One-third of the mutants identified in this screen did not have impaired motility, suggesting that many genes involved in neuromuscular synaptogenesis were missed in large scale motility-based screens. Identification of the underlying genetic defects in these mutants will extend our understanding of the cellular and molecular mechanisms that underlie the formation and function of neuromuscular and other synapses.
Descriptors: zebrafish synaptogenesis mutants, morphogenesis genetics, neuromuscular junction embryology, phenotype, synapses genetics, axons physiology, genetic screening, in situ hybridization, in situ nick end labeling, video microscopy, movement physiology, mutation genetics, synapses physiology.

Parichy, D.M. and J.M. Turner (2003). **Temporal and cellular requirements for Fms signaling during zebrafish adult pigment pattern development.** *Development* 130(5): 817-33. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Ectothermic vertebrates exhibit a diverse array of adult pigment patterns. A common element of these patterns is alternating dark and light stripes each comprising different classes of neural crest-derived pigment cells. In the zebrafish, *Danio rerio*, alternating horizontal stripes of black melanophores and yellow xanthophores are a prominent feature of the adult pigment pattern. In fms mutant zebrafish, however, xanthophores fail to develop and melanophore stripes are severely disrupted. fms encodes a type III receptor tyrosine kinase expressed by xanthophores and their precursors and is the closest known homologue of kit, which has long been studied for roles in pigment pattern development in amniotes. In this study we assess the cellular and temporal requirements for Fms activity in promoting adult pigment pattern development. By transplanting cells between fms mutants and either wild-type or nacre mutant zebrafish, we show that fms acts autonomously to the xanthophore lineage in promoting the striped arrangement of adult melanophores. To identify critical periods for fms activity, we isolated temperature sensitive alleles of fms and performed reciprocal temperature shift experiments at a range of stages from embryo to adult. These analyses demonstrate that Fms is essential for maintaining cells of the xanthophore lineage as well as maintaining the organization of melanophore stripes throughout development. Finally, we show that restoring Fms activity even at late larval stages allows essentially complete recovery of xanthophores and the development of a normal melanophore stripe pattern. Our findings suggest that fms is not required for establishing a population of precursor cells during embryogenesis but is required for recruiting pigment cell precursors to xanthophore fates, with concomitant effects on melanophore organization.

Descriptors: zebrafish embryos, chromatophores metabolism, pigmentation physiology, receptor, macrophage colony stimulating factor metabolism, signal transduction physiology, growth and development, alleles, body patterning, cell death physiology, cell transplantation, chimera physiology, DNA binding proteins genetics, proteins genetics and metabolism, anatomy and histology, physiology, developmental gene expression regulation, genotype, in situ nick end labeling, microphthalmia associated transcription factor, morphogenesis, mutation, phenotype, pigmentation genetics, receptor, macrophage colony stimulating factor genetics, temperature, transcription factors genetics and metabolism.

Parichy, D.M. and J.M. Turner (2003). **Zebrafish puma mutant decouples pigment pattern and somatic metamorphosis.** *Developmental Biology* 256(2): 242-57. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish, puma mutant embryos, melanophores physiology, biological metamorphosis genetics, mutation, skin pigmentation genetics, cell differentiation genetics, embryo cytology and physiology, melanophores cytology, temperature.

Parichy, D.M., J.M. Turner, and N.B. Parker (2003). **Essential role for puma in development of postembryonic neural crest-derived cell lineages in zebrafish.** *Developmental Biology* 256(2): 221-41. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish embryos, cell lineage, neural crest embryology, immunohistochemistry, in situ hybridization, melanophores metabolism, skin pigmentation genetics, temperature shift experiments.

Parinov, S., I. Kondrichin, V. Korzh, and A. Emelyanov (2004). **Tol2 transposon-mediated enhancer trap to identify developmentally regulated zebrafish genes in vivo.** *Developmental Dynamics* 231(2): 449-59. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, DNA transposable elements, enhancer elements genetics, developmental gene expression regulation, genetically modified, base sequence, reporter genes, green fluorescent proteins genetics and metabolism, in situ hybridization, molecular sequence data, promoter regions genetics.

Park, H.C., J. Shin, R.K. Roberts, and B. Appel (2007). **An olig2 reporter gene marks oligodendrocyte precursors in the postembryonic spinal cord of zebrafish.** *Developmental Dynamics* 236(12): 3402-7. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish larvae and adults, olig2 reporter gene, oligodendrocyte, forebrain, spinal cord radial glia.

Park, K.W., L.D. Urness, M.M. Senchuk, C.J. Colvin, J.D. Wythe, C.B. Chien, and D.Y. Li (2005). **Identification of new netrin family members in zebrafish: developmental expression of netrin 2 and netrin 4.** *Developmental Dynamics* 234(3): 726-31. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, developmental gene expression regulation, nerve growth factors genetics and metabolism, amino acid sequence, embryology, metabolism, molecular sequence data, nerve growth factors chemistry, nerve growth factors classification, phylogeny , messenger RNA genetics, sequence alignment, amino acid sequence homology, proteins classification.

Pasini, A., Y.J. Jiang, and D.G. Wilkinson (2004). **Two zebrafish Notch-dependent hairy/enhancer-of-split-related genes, her6 and her4, are required to maintain the coordination of cyclic gene expression in the presomitic mesoderm.** *Development* 131(7): 1529-41. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Alterations of the Delta/Notch signalling pathway cause multiple morphogenetic abnormalities in somitogenesis, including defects in intersomitic boundary formation and failure in maintenance of somite regularity. Notch signalling has been implicated in establishing the anteroposterior polarity within maturing somites and in regulating the activity of a molecular segmentation clock operating in the presomitic mesoderm. The pleiotropy of Notch signalling obscures the roles of this pathway in different steps of somitogenesis. One possibility is that distinct Notch effectors mediate different aspects of Notch signalling. In this study, we focus on two zebrafish Notch-dependent hairy/Enhancer-of-split-related transcription factors, Her6 and Her4, which are expressed at the transition zone between presomitic mesoderm and the segmented somites. The results of overexpression/gain-of-function and of morpholino-mediated loss-of-function experiments show that Her6 and Her4 are Notch signalling effectors that feedback on the clock and take part in the maintenance of cyclic gene expression coordination among adjacent cells in the presomitic mesoderm.
Descriptors: zebrafish embryo, body patterning, DNA binding proteins genetics, proteins metabolism, developmental gene expression regulation, membrane proteins metabolism, mesoderm physiology, trans activators metabolism, transcription factors metabolism, proteins metabolism, basic helix loop helix transcription factors, biological clocks physiology, DNA binding proteins genetics, reporter genes, helix loop helix motifs, homeodomain proteins

genetics and metabolism, in situ hybridization, membrane mesoderm cytology, morphogenesis, antisense oligonucleotides genetics, antisense oligonucleotides metabolism, Notch receptors, signal transduction physiology, somites physiology, transcription factors genetics, Delta, Notch signalling pathway.

Patricia Hernandez, L., N.C. Bird, and K.L. Staab (2007). **Using zebrafish to investigate cypriniform evolutionary novelties: functional development and evolutionary diversification of the kinethmoid.** *Journal of Experimental Zoology. Part B. Molecular and Developmental Evolution* 308(5): 625-41. ISSN: 1552-5007.
NAL Call Number: QL1.J87
Descriptors: zebrafish, evolutionary innovations, cypriniformes anatomy, histology growth and development, evolution, comparative anatomy methods, biodiversity, cypriniformes classification, embryonic development, facial bones anatomy and histology, growth and development, feeding behavior, ligaments, animal models, zebrafish classification.

Pauls, S., E. Zecchin, N. Tiso, M. Bortolussi, and F. Argenton (2007). **Function and regulation of zebrafish nkx2.2a during development of pancreatic islet and ducts.** *Developmental Biology* 304(2): 875-90. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: In the mouse Nkx2.2 is expressed in the entire pancreatic anlage. Nevertheless, absence of Nkx2.2 only perturbs the development of endocrine cell types, notably beta-cells which are completely absent. In order to test the possibility that Nkx2.2 might fulfil additional functions during pancreas development we analysed its zebrafish homologue nkx2.2a using gene targeting and GFP-transgenic fish lines. Our results suggest similar roles for nkx2.2a and Nkx2.2 during the development of the endocrine pancreas. Morpholino-based knock-down of nkx2.2a leads to a reduction of alpha- and beta-cell number and an increase of ghrelin-producing cells but, as in mice, does not affect delta-cells. Moreover, like in the mouse, two spatially distinct promoters regulate expression of nkx2.2a in precursors and differentiated islet cells. In addition we found that in zebrafish nkx2.2a is also expressed in the anterior pancreatic bud and, later, in the differentiated pancreatic ducts. A nkx2.2a-transgenic line in which pancreatic GFP expression is restricted to the pancreatic ducts revealed that single GFP-positive cells leave the anterior pancreatic bud and move towards the islet where they form intercellular connections between each other. Subsequently, these cells generate the branched network of the larval pancreatic ducts. Morpholinos that block nkx2.2a function also lead to the absence of the pancreatic ducts. We observed the same phenotype in ptf1a-morphants that are additionally characterized by a reduced number of nkx2.2a-positive duct precursors. Whereas important details of the molecular program leading to the differentiation of endocrine cell types are conserved between mammals and zebrafish, our results reveal a new function for nkx2.2a in the development of the pancreatic ducts.
Descriptors: zebrafish embryos, homeodomain proteins metabolism, Islets of Langerhans metabolism, pancreatic ducts metabolism, transcription factors metabolism, base sequence, developmental gene expression regulation, Islets of Langerhans cytology and embryology, molecular sequence data, pancreatic ducts cytology and embryology, promoter regions genetics.

Paulus, J.D. and M.C. Halloran (2006). **Zebrafish bashful/laminin-alpha 1 mutants exhibit multiple axon guidance defects.** *Developmental Dynamics* 235(1): 213-24. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, axons physiology, laminin genetics, mutation, alleles, cell

adhesion physiology, cell movement genetics, cell nucleus metabolism, laminin metabolism, neurons cytology, rhombencephalon cytology and embryology, spinal cord cytology and embryology, zebrafish abnormalities.

Payne, F.T.L. and P.C. Yelick (2003). **Alk8 is required for neural crest cell formation and development of pharyngeal arch cartilages.** *Developmental Dynamics* 228(4): 638-696. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: The type I TGFbeta family member receptor alk8 acts in bone morphogenetic protein (BMP) signaling pathways to establish dorsoventral patterning in the early zebrafish embryo. Here, we present evidence that alk8 is required for neural crest cell (NCC) formation and that alk8 signaling gradients direct the proper patterning of premigratory NCCs. We extend our previous functional studies of alk8 to demonstrate that ectopic expression of constitutively active and dominant negative Alk8, consistently results in more medially or laterally positioned premigratory NCCs, respectively. We also demonstrate that patterning defects in premigratory NCCs, induced by alk8 misexpression, correlate with subsequent defects in NCC-derived pharyngeal arch cartilages. Furthermore, an anteroposterior effect is revealed, where overexpression of Alk8 more severely affects anterior arch cartilages and decreased Alk8 activity more severely affects posterior arch cartilage formation. Ectopic expression studies of alk8 are supported by analyses of zygotic and maternal-zygotic laf/alk8 mutants and of several BMP pathway mutants. Pharyngeal mesodermal and endodermal defects in laf/alk8 mutants suggest additional roles for alk8 in patterning of these tissues. Our results provide insight into alk8-mediated BMP signaling gradients and the establishment of premigratory NCC mediolateral positioning, and extend the model for BMP patterning of the neural crest to include that of NCC-derived pharyngeal arch cartilages. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryology, dental and oral system, ingestion and assimilation, development, nervous system, neural coordination, anteroposterior effect, cellular signaling, dorsoventral patterning, ectopic expression studies, embryonic patterning, functional studies, gene mutations, patterning defects, signaling pathways, tissue patterning, zebrafish.

Pei, W., H. Noushmehr, J. Costa, M.V. Ouspenskaia, A.G. Elkahloun, and B. Feldman (2007). **An early requirement for maternal FoxH1 during zebrafish gastrulation.** *Developmental Biology* 310(1): 10-22. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The Forkhead Box H1 (FoxH1) protein is a co-transcription factor recruited by phosphorylated Smad2 downstream of several TGFbetas, including Nodal-related proteins. We have reassessed the function of zebrafish FoxH1 using antisense morpholino oligonucleotides (MOs). MOs targeting translation of foxH1 disrupt embryonic epiboly movements during gastrulation and cause death on the first day of development. The FoxH1 morphant phenotype is much more severe than that of zebrafish carrying foxh1/schmalspur (sur) DNA-binding domain mutations, FoxH1 splice-blocking morphants or other Nodal pathway mutants, and it cannot be altered by concomitant perturbations in Nodal signaling. Apart from disrupting epiboly, FoxH1 MO treatment disrupts convergence and internalization movements. Late gastrula-stage FoxH1 morphants exhibit delayed mesoderm and endoderm marker gene expression and failed patterning of the central nervous system. Probing FoxH1 morphant RNA by microarray, we identified a cohort of five keratin genes--cyt1, cyt2, krt4, krt8 and krt18--that are normally transcribed in the embryo's enveloping layer (EVL) and which have significantly reduced expression in FoxH1-depleted embryos. Simultaneously dis-

rupting these keratins with a mixture of MOs reproduces the FoxH1 morphant phenotype. Our studies thus point to an essential role for maternal FoxH1 and downstream keratins during gastrulation that is epistatic to Nodal signaling.
Descriptors: zebrafish embryos, forkhead transcription factors physiology, gastrulation physiology, developmental gene expression regulation, keratins genetics, signal transduction, physiology, embryonic induction, antisense oligonucleotides, Smad2 protein physiology, transforming growth factor beta physiology.

Pei, W., P.H. Williams, M.D. Clark, D.L. Stemple, and B. Feldman (2007). **Environmental and genetic modifiers of squint penetrance during zebrafish embryogenesis.** *Developmental Biology* 308(2): 368-78. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The Nodal-related subgroup of the TGFbeta superfamily of secreted cytokines regulates the specification of the mesodermal and endodermal germ layers during gastrulation. Two Nodal-related proteins - Squint (Sqt) and Cyclops (Cyc) - are expressed during germ-layer specification in zebrafish. Genetic sqt mutant phenotypes have defined a variable requirement for zygotic Sqt, but not for maternal Sqt, in midline mesendoderm development. However a comparison of phenotypes arising from oocytes or zygotes injected with Sqt antisense morpholinos has suggested a novel requirement for maternal Sqt in dorsal specification. In this study we examined maternal-zygotic mutants for each of two sqt alleles and we also compared phenotypes of closely related zygotic and maternal-zygotic sqt mutants. Each of these approaches indicated there is no general requirement for maternal Sqt. To better understand the dispensability of maternal and zygotic Sqt, we sought out developmental contexts that more rigorously demand intact Sqt signalling. We found that sqt penetrance is influenced by genetic modifiers, by environmental temperature, by levels of residual Activin-like activity and by Heat-Shock Protein 90 (HSP90) activity. Therefore, Sqt may confer an evolutionary advantage by protecting early-stage embryos against detrimental interacting alleles and environmental challenges.
Descriptors: zebrafish embryology, activins metabolism, base sequence, DNA genetics, environment, HSP90 heat shock proteins antagonists and inhibitors, HSP90 heat shock mutation, penetrance, phenotype, messenger RNA genetics and metabolism, signal transduction, transforming growth factor beta metabolism, proteins deficiency, zygote metabolism.

Pelegri, F. (2003). **Maternal factors in zebrafish development.** *Developmental Dynamics* 228(3): 535-54. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, blastula physiology, physiology, morphogenesis physiology, fishes genetics, gastrula physiology, developmental gene expression regulation, animal models, oocytes cytology and physiology, oogenesis.

Pelegri, F., M.P. Dekens, S. Schulte Merker, H.M. Maischein, C. Weiler, and C. Nusslein Volhard (2004). **Identification of recessive maternal-effect mutations in the zebrafish using a gynogenesis-based method.** *Developmental Dynamics* 231(2): 324-35. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryo, anatomy and histology, drug effects, physiology, recessive genes, morphogenesis, mutation, body patterning, mutagens pharmacology, phenotype, physiology.

Penberthy, W.T., C. Zhao, Y. Zhang, J.R. Jessen, Z. Yang, O. Bricaud, A. Collazo, A. Meng , and S. Lin (2004). **Pur alpha and Sp8 as opposing regulators of neural gata2 expression**. *Developmental Biology* 275(1): 225-234. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, cell differentiation, cell fate, developmental genetics, embryogenesis, embryonic development, freshwater fish, GATA-2 protein, gene expression, morphogenesis, nervous system, ontogeny, pattern formation, transcription factors, *Danio rerio*, pur alpha protein, Sp8 protein.

Peng, G. and M. Westerfield (2006). **Lhx5 promotes forebrain development and activates transcription of secreted wnt antagonists**. *Development* 133(16): 3191-3200. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: In vertebrate embryos, induction and patterning of the forebrain require the local inhibition of caudalizing signals, such as Wnts, emanating from the mesendoderm and caudal brain. Here, we report that Lhx5, expressed in the rostral neuroectoderm, regulates the local inhibition of Wnts. Activation of Lhx5 expands forebrain structures, whereas inhibition of Lhx5 function compromises forebrain development in zebrafish embryos. Lhx5 can rescue forebrain deficiencies caused by excess Wnt activity, and inhibition of Lhx5 function results in ectopic activation of Wnt signaling. Lhx5 regulates the expression of two secreted Frizzled-related Wnt antagonists, Sfrp1a and Sfrp5. These Sfrps can reduce the ectopic activation of Wnt signaling and rescue the forebrain deficiencies caused by inhibition of Lhx5 function. Our results demonstrate that Lhx5 is a required factor that promotes forebrain development and inhibits Wnt signaling by activating the transcription of secreted Wnt antagonists. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, biochemistry and molecular biophysics, forebrain development, nervous system: neural coordination, Lhx5, Wnt inhibition.

Perkins, B.D., C.S. Nicholas, L.M. Baye, B.A. Link, and J.E. Dowling (2005). **Dazed gene is necessary for late cell type development and retinal cell maintenance in the zebrafish retina.** *Developmental Dynamics* 233(2): 680-94. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, cell differentiation, eye proteins metabolism, retina cytology and embryology, metabolism, aging physiology, genetically modified, blindness genetics, pathology and physiopathology, cell death, cell proliferation, cell survival, cytology, embryology, metabolism, eye developmental gene expression regulation, heterozygote, light, mutation genetics, retina metabolism, retina radiation effects, retinal rodscytology, time factors, proteins genetics.

Perner, B., C. Englert, and F. Bollig (2007). **The Wilms tumor genes Wt1a and Wt1b control different steps during formation of the zebrafish pronephros.** *Developmental Biology* 309(1): 87-96. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The Wilms tumor protein WT1 is an essential factor for kidney development. In humans, mutations in WT1 lead to Wilms tumor, a pediatric kidney cancer as well as to developmental anomalies concerning the urogenital tract. Inactivation of Wt1 in mice causes multiple organ defects most notably agenesis of the kidneys. In zebrafish, two paralogous wt1 genes exist, wt1a and wt1b. The wt1 genes are expressed in a similar and overlapping but not identical pattern. Here, we have examined the role of both wt1 genes in early kidney development employing a transgenic line with pronephros specific GFP expression and morpholino

knockdown experiments. Inactivation of wt1a led to failure of glomerular differentiation and morphogenesis resulting in a rapidly expanding general body edema. In contrast, knockdown of wt1b was compatible with early glomerular development. After 48 h, however, wt1b morphant embryos developed cysts in the region of the glomeruli and tubules and subsequent pericardial edema at 4 days post-fertilization. Thus, our data suggest different functions for wt1a and wt1b in zebrafish nephrogenesis. While wt1a has a more fundamental and early role in pronephros development and is essential for the formation of glomerular structures, wt1b functions at later stages of nephrogenesis.
Descriptors: zebrafish embryo, nephrons embryology, Wt1 proteins metabolism, genetically modified, body patterning physiology, metabolism, developmental gene expression regulation, antisense oligonucleotides pharmacology, Wt1 proteins genetics.

Pham, V.a.n.N., N.D. Lawson, J.W. Mugford, L. Dye, D. Castranova, B. Lo, and B.M. Weinstein (2007). **Combinatorial function of ETS transcription factors in the developing vasculature**. *Developmental Biology* 303(2): 772-783. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, angiogenesis, differentiation, ETS protein, endothelial cells, Ets-1 protein, freshwater fish, gene expression, hemopoiesis, phylogenetics, transcription, transcription factors, vascular system, *Danio rerio.*

Phillips, B.T., H.J. Kwon, C. Melton, P. Houghtaling, A. Fritz, and B.B. Riley (2006). **Zebrafish msxB, msxC and msxE function together to refine the neural-nonneural border and regulate cranial placodes and neural crest development.** *Developmental Biology* 294(2): 376-90. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The zebrafish muscle segment homeobox genes msxB, msxC and msxE are expressed in partially overlapping domains in the neural crest and preplacodal ectoderm. We examined the roles of these msx genes in early development. Disrupting individual msx genes causes modest variable defects, whereas disrupting all three produces a reproducible severe phenotype, suggesting functional redundancy. Neural crest differentiation is blocked at an early stage. Preplacodal development begins normally, but placodes arising from the msx expression domain later show elevated apoptosis and are reduced in size. Cell proliferation is normal in these tissues. Unexpectedly, Msx-deficient embryos become ventralized by late gastrulation whereas misexpression of msxB dorsalizes the embryo. These effects appear to involve Distal-less (Dlx) protein activity, as loss of dlx3b and dlx4b suppresses ventralization in Msx-depleted embryos. At the same time, Msx-depletion restores normal preplacodal gene expression to dlx3b-dlx4b mutants. These data suggest that mutual antagonism between Msx and Dlx proteins achieves a balance of function required for normal preplacodal differentiation and placement of the neural-nonneural border.
Descriptors: zebrafish embryos, embryonic structures physiology, homeodomain proteins metabolism, neural crest embryology, neurons physiology, transcription factors metabolism, proteins metabolism, cell proliferation and survival, anatomy and histology, physiology, embryonic structures anatomy and histology, developmental gene expression regulation, homeodomain in situ hybridization, neural crest cytology, neurons cytology, antisense oligonucleotides genetics , antisense oligonucleotides metabolism, phenotype, transcription factors genetics.

Phillips, B.T., E.M. Storch, A.C. Lekven, and B.B. Riley (2004). **A direct role for Fgf but not Wnt in otic placode induction.** *Development* 131(4): 923-931. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Induction of the otic placode, which gives rise to all tissues comprising the inner ear, is a fundamental aspect of vertebrate development. A number of studies indicate that fibroblast growth factor (Fgf), especially Fgf3, is necessary and sufficient for otic induction. However, an alternative model proposes that Fgf must cooperate with Wnt8 to induce otic differentiation. Using a genetic approach in zebrafish, we tested the roles of Fgf3, Fgf8 and Wnt8. We demonstrate that localized misexpression of either Fgf3 or Fgf8 is sufficient to induce ectopic otic placodes and vesicles, even in embryos lacking Wnt8. Wnt8 is expressed in the hindbrain around the time of otic induction, but loss of Wnt8 merely delays expression of preotic markers and otic vesicles form eventually. The delay in otic induction correlates closely with delayed expression of fgf3 and fgf8 in the hindbrain. Localized misexpression of Wnt8 is insufficient to induce ectopic otic tissue. By contrast, global misexpression of Wnt8 causes development of supernumerary placodes/vesicles, but this reflects posteriorization of the neural plate and consequent expansion of the hindbrain expression domains of Fgf3 and Fgf8. Embryos that misexpress Wnt8 globally but are depleted for Fgf3 and Fgf8 produce no otic tissue. Finally, cells in the preotic ectoderm express Fgf (but not Wnt) reporter genes. Thus, preotic cells respond directly to Fgf but not Wnt8. We propose that Wnt8 serves to regulate timely expression of Fgf3 and Fgf8 in the hindbrain, and that Fgf from the hindbrain then acts directly on preplacodal cells to induce otic differentiation. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryology, development, endocrine system: chemical coordination and homeostasis, molecular genetics, nervous system, neural coordination, sense organs, sensory reception, development, otic placade, hindbrain development.

Pierce, L.X., D. Harrison, and J.O. Liang (2007). **The time reaper 5-channel automatic liquid dispenser: a new tool for studying zebrafish development.** *Zebrafish* 4(3): 169-77. ISSN: 1545-8547.
Abstract: Patterning of zebrafish and other vertebrate embryos proceeds according to consistent, predictable developmental time courses. Because zebrafish spawn primarily during the first few hours after dawn, many important developmental stages typically occur during the middle of the night. As an automatic, accurate way to fix embryos at these inconvenient times, we have developed the Time Reaper 5-Channel Automatic Liquid Dispenser (TimeR). The TimeR delivers up to 50 mL of liquid to embryos in a Petri dish at preset times. We have used the TimeR to deliver paraformaldehyde and fix zebrafish embryos at different stages of development. We find that the pattern of expression for a number of genes is indistinguishable between embryos fixed manually and with the TimeR. The TimeR is also suitable for fixing embryos for whole-mount immunostaining, but care needs to be taken to find conditions that preserve the antibody's epitope. The TimeR is inexpensive to make, and can be constructed using tools present in most machine shops. In addition to fixing embryos, the TimeR will be useful for any experiment that requires automatic delivery of milliliter amounts of liquid.
Descriptors: zebrafish, zebrafish, development, liquid dispenser, time reaper, Timer.

Pietsch, J., J.M. Delalande, B. Jakaitis, J.D. Stensby, S. Dohle, W.S. Talbot, D.W. Raible, and I.T. Shepherd (2006). **Lessen encodes a zebrafish trap100 required for enteric nervous system development.** *Development* 133(3): 395-406. ISSN: 0950 1991.
NAL Call Number: QL951.D38

Abstract: The zebrafish enteric nervous system (ENS), like those of all other vertebrate species, is principally derived from the vagal neural crest. The developmental controls that govern the specification and patterning of the ENS are not well understood. To identify genes required for the formation of the vertebrate ENS, we preformed a genetic screen in zebrafish. We isolated the lessen (lsn) mutation that has a significant reduction in the number of ENS neurons as well as defects in other cranial neural crest derived structures. We show that the lsn gene encodes a zebrafish orthologue of Trap100, one of the subunits of the TRAP/mediator transcriptional regulation complex. A point mutation in trap100 causes a premature stop codon that truncates the protein, causing a loss of function. Antisense-mediated knockdown of trap100 causes an identical phenotype to lsn. During development trap100 is expressed in a dynamic tissue-specific expression pattern consistent with its function in ENS and jaw cartilage development. Analysis of neural crest markers revealed that the initial specification and migration of the neural crest is unaffected in lsn mutants. Phosphohistone H3 immunocytochemistry revealed that there is a significant reduction in proliferation of ENS precursors in lsn mutants. Using cell transplantation studies, we demonstrate that lsn/trap100 acts cell autonomously in the pharyngeal mesendoderm and influences the development of neural crest derived cartilages secondarily. Furthermore, we show that endoderm is essential for ENS development. These studies demonstrate that lsn/trap100 is not required for initial steps of cranial neural crest development and migration, but is essential for later proliferation of ENS precursors in the intestine.
Descriptors: zebrafish, body patterning, enteric nervous system embryology, transcription factors genetics and, cell movement, molecular cloning, endoderm physiology, enteric nervous system physiology, facial bones anatomy, histology and embryology, developmental gene expression regulation, in situ hybridization, mutation, neural crest cytology and physiology, antisense oligonucleotides genetics, antisense oligonucleotides metabolism, phenotype, skull anatomy and histology and embryology, thymus gland anatomy, histology and embryology.

Pillai, R., L.E. Coverdale, G. Dubey, and C.C. Martin (2004). **Histone deacetylase 1 (HDAC-1) required for the normal formation of craniofacial cartilage and pectoral fins of the zebrafish.** *Developmental Dynamics* 231(3): 647-54. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, cartilage embryology, extremities embryology, histone deacetylases physiology, skull embryology, alcian blue metabolism, embryo drug effects embryonic development, developmental gene expression regulation, histone deacetylases metabolism, homozygote, in situ hybridization, insertional mutagenesis, antisense oligonucleotides pharmacology, time factors.

Pillai, R., L.E. Coverdale, G. Dubey, and C.C. Martin (2004). **Histone deacetylase 1 (hdac-1) essential for craniofacial cartilage and pectoral fin development in zebrafish, *danio rerio*.** *Developmental Biology* 271(2): 564. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, development, skeletal system, craniofacial cartilage and pectoral fin, movement and support, molecular genetics, enzymology, histone deacetylase 1.

Pineda, R.H., K.R. Svoboda, M.A. Wright, A.D. Taylor, A.E. Novak, J.T. Gamse, J.S. Eisen, and A.B. Ribera (2006). **Knockdown of Nav1.6a Na+ channels affects zebrafish motoneuron development.** *Development* 133(19): 3827-36. ISSN: 0950 1991.
NAL Call Number: QL951.D38

Abstract: In addition to rapid signaling, electrical activity provides important cues to developing neurons. Electrical activity relies on the function of several different types of voltage-gated ion channels. Whereas voltage-gated Ca2+ channel activity regulates several aspects of neuronal differentiation, much less is known about developmental roles of voltage-gated Na+ channels, essential mediators of electrical signaling. Here, we focus on the zebrafish Na+ channel isotype, Nav1.6a, which is encoded by the scn8a gene. A restricted set of spinal neurons, including dorsal sensory Rohon-Beard cells, two motoneuron subtypes with different axonal trajectories, express scn8a during embryonic development. CaP, an early born primary motoneuron subtype with ventrally projecting axons expresses scn8a, as does a class of secondary motoneurons with axons that project dorsally. To test for developmental roles of scn8a, we knocked down Nav1.6a protein using antisense morpholinos. Na+ channel protein and current amplitudes were reduced in neurons that express scn8a. Furthermore, Nav1.6a knockdown altered axonal morphologies of some but not all motoneurons. Dorsally projecting secondary motoneurons express scn8a and displayed delayed axonal outgrowth. By contrast, CaP axons developed normally, despite expression of the gene. Surprisingly, ventrally projecting secondary motoneurons, a population in which scn8a was not detected, displayed aberrant axonal morphologies. Mosaic analysis indicated that effects on ventrally projecting secondary motoneurons were non cell-autonomous. Thus, voltage-gated Na+ channels play cell-autonomous and non cell-autonomous roles during neuronal development.
Descriptors: zebrafish embryos, axons ultrastructure, motor neurons cytology, spinal cord embryology, proteins antagonists and inhibitors, axons chemistry and metabolism, cell survival, drug effects, metabolism , embryonic development drug effects and genetics, motor neurons chemistry and metabolism, antisense oligonucleotides pharmacology, messenger RNA analysis and metabolism, sodium channels genetics and metabolism, spinal cord cytology and metabolism, proteins genetics and metabolism.

Piotrowski, T., D.G. Ahn, T.F. Schilling, S. Nair, I. Ruvinsky, R. Geisler, G.J. Rauch, P. Haffter, L.I. Zon, Y. Zhou, H. Foott, I.B. Dawid, and R.K. Ho (2003). **The zebrafish van gogh mutation disrupts tbx1, which is involved in the DiGeorge deletion syndrome in humans.** *Development* 130(20): 5043-52. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The van gogh (vgo) mutant in zebrafish is characterized by defects in the ear, pharyngeal arches and associated structures such as the thymus. We show that vgo is caused by a mutation in tbx1, a member of the large family of T-box genes. tbx1 has been recently suggested to be a major contributor to the cardiovascular defects in DiGeorge deletion syndrome (DGS) in humans, a syndrome in which several neural crest derivatives are affected in the pharyngeal arches. Using cell transplantation studies, we demonstrate that vgo/tbx1 acts cell autonomously in the pharyngeal mesendoderm and influences the development of neural crest-derived cartilages secondarily. Furthermore, we provide evidence for regulatory interactions between vgo/tbx1 and edn1 and hand2, genes that are implicated in the control of pharyngeal arch development and in the etiology of DGS.
Descriptors: zebrafish, DiGeorge syndrome genetics, T-box domain proteins genetics, metabolism, amino acid sequence, branchial region metabolism, ear embryology, endoderm metabolism, mesoderm metabolism, molecular sequence data, mutation, sequence deletion, T-box domain proteins metabolism.

Pisharath, H., J.M. Rhee, M.A. Swanson, S.D. Leach, and M.J. Parsons (2007). **Targeted ablation of beta cells in the embryonic zebrafish pancreas using *E. coli* nitroreductase.** *Mechanisms of Development* 124(3): 218-29. ISSN: 0925-4773.

NAL Call Number: QH607.A1C4

Abstract: In order to generate a zebrafish model of beta cell regeneration, we have expressed an *Escherichia coli* gene called nfsB in the beta cells of embryonic zebrafish. This bacterial gene encodes a nitroreductase (NTR) enzyme, which can convert prodrugs such as metronidazole (Met) to cytotoxins. By fusing nfsB to mCherry, we can simultaneously render beta cells susceptible to prodrug and visualize Met dependent cell ablation. We show that the neighboring alpha and delta cells are unaffected by prodrug treatment and that ablation is beta cell specific. Following drug removal and 36h of recovery, beta cells regenerate. Using ptf1a morphants, it is clear that this beta cell recovery occurs independently of the presence of the exocrine pancreas. Also, by using photoconvertible Kaede to cell lineage trace and BrdU incorporation to label proliferation, we investigate mechanisms for beta regeneration. Therefore, we have developed a unique resource for the study of beta cell regeneration in a living vertebrate organism, which will provide the opportunity to conduct large-scale screens for pharmacological and genetic modifiers of beta cell regeneration.

Descriptors: zebrafish embryos, apoptosis genetics, *Escherichia coli* enzymology and, insulin secreting cells metabolism, nitroreductases physiology, pancreas enzymology, diabetes mellitus, disease models, insulin secreting cells pathology, pancreas cytology, embryology and pathology, regeneration genetics.

Plaster, N., C. Sonntag, T.F. Schilling, and M. Hammerschmidt (2007). **REREa/Atrophin-2 interacts with histone deacetylase and Fgf8 signaling to regulate multiple processes of zebrafish development.** *Developmental Dynamics* 236(7): 1891-904. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryos, Fgf physiology, histone deacetylases physiology, intracellular signaling peptides and, signal transduction physiology, down regulation physiology.

Pogoda, H.M. and M. Hammerschmidt (2007). **Molecular genetics of pituitary development in zebrafish.** *Seminars in Cell and Developmental Biology* 18(4): 543-58. ISSN: 1084-9521.

NAL Call Number: QH573

Abstract: The pituitary gland of vertebrates consists of two major parts, the neurohypophysis (NH) and the adenohypophysis (AH). As a central part of the hypothalamo-hypophyseal system (HHS), it constitutes a functional link between the nervous and the endocrine system to regulate basic body functions, such as growth, metabolism and reproduction. The development of the AH has been intensively studied in mouse, serving as a model for organogenesis and differential cell specification. However, given that the AH is a relatively recent evolutionary advance of the chordate phylum, it is also interesting to understand its development in lower chordate systems. In recent years, the zebrafish has emerged as a powerful lower vertebrate system for developmental studies, being amenable for large-scale genetic approaches, embryological manipulations, and in vivo imaging. Here, we present an overview of current knowledge of the mechanisms and genetic control of pituitary formation during zebrafish development. First, we describe the components of the zebrafish HHS, and the different pituitary cell types and hormones, followed by a description of the different steps of normal pituitary development. The central part of the review deals with the genes found to be essential for zebrafish AH development, accompanied by a description of the corresponding mutant phenotypes. Finally, we discuss future directions, with particular focus on evolutionary aspects, and some novel functional aspects with growing medical and social relevance.

Descriptors: zebrafish embryos, pituitary gland embryology, basic helix loop helix transcription factors metabolism, body patterning, molecular evolution, fibroblast growth factor 3 metabolism, homeodomain proteins metabolism, intracellular signaling peptides and proteins metabolism, nuclear proteins metabolism, pituitary gland growth and development and metabolism, anterior pituitary gland embryology, growth, development and metabolism, pituitary gland, posterior embryology, growth, development and metabolism, protein tyrosine phosphatases metabolism, transcription factor pit 1 metabolism, zebrafish growth, development and metabolism.

Pogoda, H.M., N. Sternheim, D.A. Lyons, B. Diamond, T.A. Hawkins, I.G. Woods, D.H. Bhatt, C. Franzini Armstrong, C. Dominguez, N. Arana, J. Jacobs, R. Nix, J.R. Fetcho, and W.S. Talbot (2006). **A genetic screen identifies genes essential for development of myelinated axons in zebrafish.** *Developmental Biology* 298(1): 118-31. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The myelin sheath insulates axons in the vertebrate nervous system, allowing rapid propagation of action potentials via saltatory conduction. Specialized glial cells, termed Schwann cells in the PNS and oligodendrocytes in the CNS, wrap axons to form myelin, a compacted, multilayered sheath comprising specific proteins and lipids. Disruption of myelinated axons causes human diseases, including multiple sclerosis and Charcot-Marie-Tooth peripheral neuropathies. Despite the progress in identifying human disease genes and other mutations disrupting glial development and myelination, many important unanswered questions remain about the mechanisms that coordinate the development of myelinated axons. To address these questions, we began a genetic dissection of myelination in zebrafish. Here we report a genetic screen that identified 13 mutations, which define 10 genes, disrupting the development of myelinated axons. We present the initial characterization of seven of these mutations, defining six different genes, along with additional characterization of mutations that we have described previously. The different mutations affect the PNS, the CNS, or both, and phenotypic analyses indicate that the genes affect a wide range of steps in glial development, from fate specification through terminal differentiation. The analysis of these mutations will advance our understanding of myelination, and the mutants will serve as models of human diseases of myelin.
Descriptors: zebrafish, axons metabolism, mutation, disruption of development, myelin sheath metabolism, nerve fibers, myelinated metabolism, body patterning, central nervous system metabolism developmental gene expression regulation, peripheral nervous system metabolism, phenotype.

Pogoda, H.M., S. von der Hardt, W. Herzog, C. Kramer, H. Schwarz, and M. Hammerschmidt (2006). **The proneural gene ascl1a is required for endocrine differentiation and cell survival in the zebrafish adenohypophysis.** *Development* 133(6): 1079-89. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Mammalian basic helix-loop-helix proteins of the achaete-scute family are proneural factors that, in addition to the central nervous system, are required for the differentiation of peripheral neurons and sensory cells, derivatives of the neural crest and placodal ectoderm. Here, in identifying the molecular nature of the pia mutation, we investigate the role of the zebrafish achaete-scute homologue ascl1a during development of the adenohypophysis, an endocrine derivative of the placodal ectoderm. Similar to mutants deficient in Fgf3 signaling from the adjacent ventral diencepahalon, pia mutants display failure of endocrine differentiation of all adenohypophyseal cell types. Shortly after the failed first phase of cell differentiation, the adenohypophysis of pia mutants displays a transient phase of cell death,

which affects most, but not all adenohypophyseal cells. Surviving cells form a smaller pituitary rudiment, lack expression of specific adenohypophyseal marker genes (pit1, neurod), while expressing others (lim3, pitx3), and display an ultrastructure reminiscent of precursor cells. During normal development, ascl1a is expressed in the adenohypophysis and the adjacent diencephalon, the source of Fgf3 signals. However, chimera analyses show that ascl1a is required cell-autonomously in adenohypophyseal cells themselves. In fgf3 mutants, adenohypophyseal expression of ascl1a is absent, while implantation of Fgf3-soaked beads into pia mutants enhances ascl1a, but fails to rescue pit1 expression. Together, this suggests that Ascl1a might act downstream of diencephalic Fgf3 signaling to mediate some of the effects of Fgf3 on the developing adenohypophysis.
Descriptors: zebrafish embryos, basic helix loop helix transcription factors metabolism, endocrine system cytology and metabolism, anterior pituitary gland embryology and metabolism, proteins metabolism, genetically modified, basic helix loop helix transcription factors genetics, cell differentiation, cell survival, diencephalon embryology and metabolism, endocrine system embryology, fibroblast growth factor 3 metabolism, developmental gene expression regulation, electron microscopy, mutation genetics, anterior pituitary gland cytology, proteins genetics.

Poliakov, A., A. Pasini, and D.G. Wilkinson (2004). **Roles of fgf-receptor signaling in modulation of ephrinb/ephb-receptor system.** *Developmental Biology* 271(2): 602. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, FGF-receptor, development, biochemistry, cell culture, culturing techniques.

Pollard, S.M., M.J. Parsons, M. Kamei, R.N.W. Kettleborough, K.A. Thomas, V.N. Pham, M.K. Bae, A. Scott, B.M. Weinstein, and D.L. Stemple (2006). **Essential and overlapping roles for laminin alpha chains in notochord and blood vessel formation**. *Developmental Biology* 289(1): 64-76. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Laminins are mal. or constituents of basement membranes and have wide ranging functions during development and in the adult. They are a family of heterotrimeric molecules created through association of an alpha, beta and gamma chain. We previously reported that two zebrafish loci, grumpy (gup) and sleepy (sly), encode laminin beta 1 and gamma 1, which are important both for notochord differentiation and for proper intersegmental blood vessel (ISV) formation. In this study we show that bashful (bal) encodes laminin alpha 1 (lama 1). Although the strongest allele, bal(m190), is fully penetrant, when compared to gup or sly mutant embryos, bal mutants are not as severely affected, as only anterior notochord fails to differentiate and ISVs are unaffected. This suggests that other a chains, and hence other isoforms, act redundantly to laminin 1 in posterior notochord and ISV development. We identified cDNA sequences for lama2, lama4 and lama5 and disrupted the expression of each alone or in mutant embryos also lacking laminin alpha 1. When expression of laminin alpha 4 and laminin alpha 1 are simultaneously disrupted, notochord differentiation and ISVs are as severely affected as sly or gup mutants. Moreover, live imaging of transgenic embryos expressing enhanced green fluorescent protein in forming ISVs reveals that the vascular defects in these embryos are due to an inability of ISV sprouts to migrate correctly along the intersegmental, normally laminin-rich regions. (c) 2005 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish transgenic embryos , grumpy and sleepy loci, laminin alpha 1 (lama

1), development, molecular genetics, cardiovascular system, transport and circulation, blood vessel development.

Poss, K.D., M.T. Keating, and A. Nechiporuk (2003). **Tales of regeneration in zebrafish.** *Developmental Dynamics* 226(2): 202-10. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, regeneration physiology, wound healing physiology.

Poulain, M., M. Furthauer, B. Thisse, C. Thisse, and T. Lepage (2006). **Zebrafish endoderm formation is regulated by combinatorial Nodal, FGF and BMP signalling.** *Development* 133(11): 2189-200. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: In the zebrafish embryo, the mesoderm and endoderm originate from common precursors and segregate during gastrulation by mechanisms that are largely unknown. Understanding how the signalling pathways that regulate endoderm and mesoderm formation interact is crucial to understanding how the germ layers are established. Here, we have analysed how the FGF and BMP pathways interact with Nodal signalling during the process of endoderm formation. We found that activation of the FGF/ERK pathway disrupts endoderm formation in the embryo and antagonizes the ability of an activated form of Tar/Acvr1b to induce endoderm at the animal pole. By contrast, inhibition of FGF signalling increases the number of endodermal precursors and potentiates the ability of Tar*/Acvr1b to induce endoderm at the animal pole. Using a pharmacological inhibitor of the FGF receptor, we show that reducing FGF signalling partially rescues the deficit of endoderm precursors in bon mutant embryos. Furthermore, we found that overexpression of BMPs compromises endoderm formation, suggesting that formation of endoderm precursors is negatively regulated by BMPs on the ventral side. We show that simultaneous inhibition of the FGF/Ras and BMP pathways results in a dramatic increase in the number of endoderm precursors. Taken together, these data strongly suggest that BMP and FGF-ERK pathways cooperate to restrict the number of endodermal progenitors induced in response to Nodal signalling. Finally, we investigated the molecular basis for the FGF-MAPK-dependent repression of endoderm formation. We found that FGF/ERK signalling causes phosphorylation of Casanova/Sox32, an important regulator of endoderm determination, and provide evidence that this phosphorylation attenuates its ability to induce sox17. These results identify a molecular mechanism whereby FGF attenuates Nodal-induced endodermal transcription factors and highlight a potential mechanism whereby mesoderm and endoderm fates could segregate from each other.
Descriptors: zebrafish embryos, bone morphogenetic proteins metabolism, endoderm metabolism, Fgf metabolism, signal transduction, transforming growth factor beta metabolism, Type I activin receptors metabolism, amino acid sequence, conserved sequence, DNA binding proteins genetics, proteins chemistry, DNA binding proteins genetics, proteins metabolism, embryology, metabolism, extracellular signal regulated MAP kinases metabolism, Fgf genetics, developmental gene expression regulation, high mobility group proteins chemistry and metabolism, homeodomain proteins genetics and metabolism, mitogen activated protein kinases metabolism, molecular sequence data, mutation genetics, phenotype, phosphorylation, sequence alignment, transcription factors chemistry, transcription factors geneticsand metabolism, transforming growth factor beta genetics, proteins chemistry.

Prabhudesai, S.N., D.A. Cameron, and D.L. Stenkamp (2005). **Targeted effects of retinoic acid signaling upon photoreceptor development in zebrafish.** *Developmental Biology* 287(1): 157-67. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Retinoic acid (RA) is a signaling molecule important for photoreceptor development in vertebrates. The purpose of this study was to examine the mechanisms of the effects of RA upon developing rod and cone photoreceptors in the embryonic zebrafish. Exposure to exogenous RA increased the number of photoreceptors expressing rod opsin and red cone opsin, and decreased the number of photoreceptors expressing the blue and UV cone opsins, suggesting targeted effects of RA on photoreceptor development. RA exposure also increased opsin expression in individual rods and red cones, but decreased opsin expression in individual blue and UV cones, as indicated by differences in the strength of opsin hybridization in identified photoreceptors. RA exposure did not, however, significantly alter quantitative measures of photoreceptor pattern in a manner expected for changes in photoreceptor fate. These observations collectively indicate that RA treatment does not affect photoreceptor fate, but rather differentially influences opsin transcription in determined photoreceptors. An enzyme involved in RA synthesis, RALDH2, was immunocytochemically localized to retinal progenitor cells and the retinal pigmented epithelium (RPE), suggesting the presence of RA in the vicinity of developing photoreceptors. However, expression of an RA response element-driven transgene was restricted to the RPE, retinal progenitors, and a small population of neurons in ventral retina, suggesting that the endogenous RA signaling system is spatially limited within the eye.

Descriptors: zebrafish embryos, cell differentiation physiology, vertebrate photoreceptors metabolism, signal transduction physiology, tretinoin physiology, aldehyde oxidoreductases biosynthesis and genetics, genetically modified, retina cones cytology and metabolism, gene expression physiology, reporter genes, opsin biosynthesis, opsin genetics, vertebrate photoreceptors cytology, retinal rodscytology, retinal rods metabolism.

Pujic, Z. and J. Malicki (2004). **Retinal pattern and the genetic basis of its formation in zebrafish.** *Seminars in Cell and Developmental Biology* 15(1): 105-14. ISSN: 1084-9521.

NAL Call Number: QH573

Abstract: The vertebrate nervous system contains an immense diversity of distinct cellular components that are organized into precise spatial patterns. The importance of accurate neuronal architecture is particularly obvious in the retina, where it is necessary for the formation of visual images. The retina is structured in a distinct layered pattern that is remarkably conserved in evolution, including phyla as diverse as primates and teleost fish. Genetic analysis in zebrafish reveals mechanisms that are essential for the formation of this architecture.

Descriptors: zebrafishembryos, retina embryology, cell differentiation genetics and physiology, central nervous system cytology, embryology, metabolism, embryology, metabolism, eye anatomy and histology, eye cytology and embryology, developmental gene expression regulation, Hedgehog proteins, isoenzymes, mutation, neurons cytology and physiology, protein kinase C physiology, retina anatomy and histology, retina cytology, trans activators physiology.

Pyati, U.J., M.S. Cooper, A.J. Davidson, A. Nechiporuk, and D. Kimelman (2006). **Sustained Bmp signaling is essential for cloaca development in zebrafish.** *Development* 133(11): 2275-84. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Bone morphogenetic protein (Bmp) signaling has long been known to be impor-

tant for the early development of the ventral mesoderm, including blood, vasculature and kidney cells. Although Bmp genes are continually expressed in the ventral cells throughout gastrulation and somitogenesis, previous studies in zebrafish have not addressed how the role of Bmp signaling changes over time to regulate ventral mesoderm development. Here, we describe the use of a transgenic inducible dominant-negative Bmp receptor line to examine the temporal roles of Bmp signaling in ventral mesoderm patterning. Surprisingly, we find that Bmp signaling from the mid-gastrula stage through early somitogenesis is important for excluding blood and vascular precursors from the extreme ventral mesoderm, and we show that this domain is normally required for development of the cloaca (the common gut and urogenital opening). Using a novel assay for cloacal function, we find that larvae with reduced mid-gastrula Bmp signaling cannot properly excrete waste. We show that the cloacal defects result from alterations in the morphogenesis of the cloaca and from changes in the expression of genes marking the excretory system. Finally, we show that HrT, a T-box transcription factor, is a Bmp-regulated gene that has an essential function in cloacal development. We conclude that sustained Bmp signaling plays an important role in specification of the zebrafish cloaca by maintaining the fate of extreme ventral cells during the course of gastrulation and early somitogenesis. Furthermore, our data suggest that alterations in Bmp signaling are one possible cause of anorectal malformations during human embryogenesis.
Descriptors: zebrafish, bone morphogenetic proteins metabolism, cloaca embryology and metabolism, signal transduction, metabolism, genetically modified, bone morphogenetic cell lineage, cloaca blood supply and cytology, cytology, embryology, metabolism, developmental gene expression regulation, mesoderm cytology, mesoderm metabolism, stem cells metabolism, time factors, transcription factors metabolism.

Pyati, U.J., A.E. Webb, and D. Kimelman (2005). **Transgenic zebrafish reveal stage-specific roles for Bmp signaling in ventral and posterior mesoderm development.** *Development* 132(10): 2333-43. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Bone morphogenetic protein (Bmp) signaling is crucial for the formation and patterning of zebrafish ventral and posterior mesoderm. Mutants defective in the Bmp pathway have expanded trunk muscle, abnormal tails and severely impaired development of ventral mesodermal derivatives such as vasculature, blood and pronephros. As Bmps continue to be expressed in the ventral and posterior mesoderm after gastrulation, it is likely that Bmp signaling continues to play an important developmental role during outgrowth of the posterior body. However, because Bmp signaling plays an essential role during the gastrula stages, it has not been possible with mutants or standard disruption techniques to determine the later functions of the Bmp pathway. To study the role of Bmp signaling in the ventral and posterior mesoderm during trunk and tail outgrowth, we generated a transgenic zebrafish line containing a heatshock-inducible dominant-negative Bmp receptor-GFP fusion. Our data show that Bmps are important for tail organizer formation and for patterning the ventral mesoderm during early gastrulation. However, from mid-gastrulation to the early somitogenesis stages, Bmp signaling is important for ventral tail fin development and for preventing secondary tail formation. We conclude that the role of Bmp signaling in the ventral and posterior mesoderm changes as gastrulation proceeds.
Descriptors: zebrafish embryos, body patterning physiology, developmental gene expression regulation, mesoderm physiology, protein serine threonine kinases metabolism, growth factor receptors metabolism, signal transduction physiology, genetically modified, bone morphogenetic protein receptors, Type I, DNA primers, green fluorescent proteins metabolism, heat,

confocal microscopy, protein serine threonine kinases physiology, growth factor receptors physiology, recombinant fusion proteins metabolism, tail embryology.

Pyron, M. (2003). **Female preferences and male male interactions in zebrafish (*Danio rerio*).** *Canadian Journal of Zoology/Revue Canadienne De Zoologie* 81(1): 122-125. ISSN: 0008-4301.
Descriptors: zebrafish, body size, mate selection, reproductive behavior, sexual selection, *Danio rerio*.

Qian, F., F. Zhen, C. Ong, S.W. Jin, H. Meng Soo, D.Y. Stainier, S. Lin, J. Peng, and Z. Wen (2005). **Microarray analysis of zebrafish cloche mutant using amplified cDNA and identification of potential downstream target genes.** *Developmental Dynamics* 233(3): 1163-72. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Zebrafish is an excellent model organism for studying vertebrate development and human disease. With the availability of increased numbers of zebrafish mutants and microarray chips, gene expression profiling has become a powerful tool for identification of downstream target genes perturbed by a specific mutation. One of the obstacles often encountered, however, is to isolate large numbers of zebrafish mutant embryos that are indistinguishable in morphology from the wild-type siblings for microarray analysis. Here, we report a method using amplified cDNA derived from five embryos for gene expression profiling of the 18-somite zebrafish cloche (clo) mutant, in which development of hematopoietic and endothelial lineages is severely impaired. In total, 31 differentially expressed target genes are identified, of which 13 have not been reported previously. We further determine that of these 13 new targets, 8 genes, including coproporphyrinogen oxidase (cpo), carbonic anhydrase (cahz), claudin g (cldn g), zinc-finger-like gene 2 (znfl2), neutrophil cytosol factor 1 (ncf1), matrix metalloproteinase 13 (mmp13), dual specificity phosphatase 5 (dusp5), and a novel gene referred as zebrafish vessel-specific gene 1 (zvsg1) are predominantly expressed in hematopoietic and endothelial cells. Comparative analysis demonstrates that this method is comparable and complementary to that of the conventional approach using unamplified sample. Our study provides valuable information for studying hematopoiesis and vessel formation. The method described here offers a powerful tool for gene expression profiling of zebrafish mutants in general.
Descriptors: zebrafish embryos, complementary DNA genetics, mutation genetics, nucleic acid amplification techniques, oligonucleotide array sequence analysis, proteins genetics and metabolism, down regulation, embryology , metabolism, gene expression profiling and regulation, genotype, homozygote, messenger RNA genetics and metabolism, somites metabolism.

Qiu, X., H. Xu, C. Haddon, J. Lewis, and Y.J. Jiang (2004). **Sequence and embryonic expression of three zebrafish fringe genes: lunatic fringe, radical fringe, and manic fringe.** *Developmental Dynamics* 231(3): 621-30. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish fringe genes, developmental gene expression regulation, glycosyltransferases metabolism, n acetylglucosaminyltransferases metabolism, protein sequence analysis, proteins metabolism, amino acid motifs, amino acid sequence, avian proteins, central nervous system metabolism, molecular cloning, conserved sequence, cysteine chemistry, *Drosophila* proteins embryonic development, gene library, glycosyltransferases chemistry and genetics, in situ hybridization, molecular sequence data, n acetylglucosaminyltransferases

chemistry and genetics, phylogeny, reverse transcriptase PCR, amino acid sequence homology, signal transduction, somites metabolism, tissue distribution genetics, proteins chemistry and genetics.

Quigley, I.K., J.M. Turner, R.J. Nuckels, J.L. Manuel, E.H. Budi, E.L. Macdonald, and D.M. Parichy (2004). **Pigment pattern evolution by differential deployment of neural crest and post-embryonic melanophore lineages in danio fishes**. *Development* 131(24): 6053-6069. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Latent precursors or stem cells of neural crest origin are present in a variety of post-embryonic tissues. Although these cells are of biomedical interest for roles in human health and disease, their potential evolutionary significance has been underappreciated. As a first step towards elucidating the contributions of such cells to the evolution of vertebrate form, we investigated the relative roles of neural crest cells and post-embryonic latent precursors during the evolutionary diversification of adult pigment patterns in Danio fishes. These pigment patterns result from the numbers and arrangements of embryonic melanophores that are derived from embryonic neural crest cells, as well as from post-embryonic metamorphic melanophores that are derived from latent precursors of presumptive neural crest origin. In the zebrafish *D. rerio*, a pattern of melanophore stripes arises during the larval-to-adult transformation by the recruitment of metamorphic melanophores from latent precursors. Using a comparative approach in the context of new phylogenetic data, we show that adult pigment patterns in five additional species also arise from metamorphic melanophores, identifying this as an ancestral mode of adult pigment pattern development. By contrast, superficially similar adult stripes of *D. nigrofasciatus* (a sister species to *D. rerio*) arise by the reorganization of melanophores that differentiated at embryonic stages, with a diminished contribution from metamorphic melanophores. Genetic mosaic and molecular marker analyses reveal evolutionary changes that are extrinsic to *D. nigrofasciatus* melanophore lineages, including a dramatic reduction of metamorphic melanophore precursors. Finally, interspecific complementation tests identify a candidate genetic pathway for contributing to the evolutionary reduction in metamorphic melanophores and the increased contribution of early larval melanophores to *D. nigrofasciatus* adult pigment pattern development. These results demonstrate an important role for latent precursors in the diversification of pigment patterns across danios. More generally, differences in the deployment of post-embryonic neural crest-derived stem cells or their specified progeny may contribute substantially to the evolutionary diversification of adult form in vertebrates, particularly in species that undergo a metamorphosis. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, evolution and adaptation, marine ecology, environmental sciences, pigment pattern evolution, genetic marker analysis, genetic techniques , genetic mosaic analysis, embryology, pigment pattern.

Raeker, M.O., F. Su, S.B. Geisler, A.B. Borisov, A. Kontrogianni Konstantopoulos, S.E. Lyons, and M.W. Russell (2006). **Obscurin is required for the lateral alignment of striated myofibrils in zebrafish.** *Developmental Dynamics* 235(8): 2018-29. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: Obscurin/obscurin-MLCK is a giant sarcomere-associated protein with multiple isoforms whose interactions with titin and small ankyrin-1 suggest that it has an important role in myofibril assembly, structural support, and the sarcomeric alignment of the sarcoplasmic reticulum. In this study, we characterized the zebrafish orthologue of obscurin and examined its role in striated myofibril assembly. Zebrafish obscurin was expressed in the

somites and central nervous system by 24 hours post-fertilization (hpf) and in the heart by 48 hpf. Depletion of obscurin using two independent morpholino antisense oligonucleotides resulted in diminished numbers and marked disarray of skeletal myofibrils, impaired lateral alignment of adjacent myofibrils, disorganization of the sarcoplasmic reticulum, somite segmentation defects, and abnormalities of cardiac structure and function. This is the first demonstration that obscurin is required for vertebrate cardiac and skeletal muscle development. The diminished capacity to generate and organize new myofibrils in response to obscurin depletion suggests that it may have a vital role in the causation of or adaptation to cardiac and skeletal myopathies.

Descriptors: zebrafish, guanine nucleotide exchange factors metabolism, muscle proteins metabolism, myofibrils metabolism, amino acid sequence, body patterning, conserved sequence, developmental gene expression regulation, guanine nucleotide exchange factors chemistry and genetics, heart embryology, electron transmission microscopy, molecular sequence data, muscle proteins chemistry and genetics, myocardium metabolism, myofibrils ultrastructure, myosin light chain kinase metabolism, phylogeny, sequence alignment, somites metabolism, somites ultrastructure, proteins chemistry.

Ragland, J.W. and D.W. Raible (2004). **Signals derived from the underlying mesoderm are dispensable for zebrafish neural crest induction.** *Developmental Biology* 276(1): 16-30. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Signals from the non-neural ectoderm, the neural ectoderm, and the underlying mesoderm have all been implicated in the induction of neural crest. Bone morphogenetic protein (BMP) signaling in particular has an important role in this process; however, it is unclear whether this activity of BMP is due to its effects on patterning the underlying mesoderm, to its ability to establish a competent neural plate boundary zone, or to the direct specification of neural crest at intermediate levels of activity within a BMP gradient. We show neural crest induction occurs in zebrafish in the absence of involuted mesoderm, indicating that this tissue and signals derived from it are dispensable for the formation of neural crest. Dorsal-involuted mesoderm is a major source of secreted BMP antagonists, and the activity of BMP signaling is thought to depend on the presence of the opposing activity of these antagonists. We find that the three BMP antagonists known to be expressed during gastrulation in zebrafish, noggin1, follistatin, and chordin, are dispensable for neural crest induction. These results suggest that mechanisms for restricting the spatio-temporal pattern of BMP expression may compensate for the loss of secreted BMP antagonist activity in establishing dorso-ventral patterning, neural induction, and the neural crest.

Descriptors: zebrafish embryos, embryonic induction, mesoderm metabolism, neural crest metabolism, proteins, body patterning, bone morphogenetic proteins antagonists and inhibitors embryonic development, follistatin, glycoproteins, intercellular signaling peptides and proteins, neural crest embryology, signal transduction.

Notes: Comments: Erratum In: Dev Biol. 2005 Mar 1;279(1):268-9

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Ramasamy, S., H. Wang, H.N. Quach, and K. Sampath (2006). **Zebrafish Staufen1 and Staufen2 are required for the survival and migration of primordial germ cells.** *Developmental Biology* 292(2): 393-406. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: In sexually reproducing organisms, primordial germ cells (PGCs) give rise to the cells of the germ line, the gametes. In many animals, PGCs are set apart from somatic cells

early during embryogenesis. Work in Drosophila, C. elegans, Xenopus, and zebrafish has shown that maternally provided localized cytoplasmic determinants specify the germ line in these organisms (Raz, E., 2003. Primordial germ-cell development: the zebrafish perspective. Nat. Rev., Genet. 4, 690--700; Santos, A.C., Lehmann, R., 2004. Germ cell specification and migration in Drosophila and beyond. Curr. Biol. 14, R578-R589). The Drosophila RNA-binding protein, Staufen is required for germ cell formation, and mutations in stau result in a maternal effect grandchild-less phenotype (Schupbach,T., Weischaus, E., 1989. Female sterile mutations on the second chromosome of Drosophila melanogaster:1. Maternal effect mutations. Genetics 121, 101-17). Here we describe the functions of two zebrafish Staufen-related proteins, Stau1 and Stau2. When Stau1 or Stau2 functions are compromised in embryos by injecting antisense morpholino modified oligonucleotides or dominant-negative Stau peptides, germ layer patterning is not affected. However, expression of the PGC marker vasa is not maintained. Furthermore, expression of a green fluorescent protein (GFP):nanos 3'UTR fusion protein in germ cells shows that PGC migration is aberrant, and the mis-migrating PGCs do not survive in Stau-compromised embryos. Stau2 is also required for survival of neurons in the central nervous system (CNS). These phenotypes are rescued by co-injection of Drosophila stau mRNA. Thus, staufen has an evolutionarily conserved function in germ cells. In addition, we have identified a function for Stau proteins in PGC migration.

Descriptors: zebrafish embryos, cell movement, cell survival , germ cells cytology and physiology, RNA binding, amino acid sequence, apoptosis, conserved sequence glutathione transferase metabolism, immunohistochemistry, in situ hybridization, video microscopy, molecular sequence data, antisense oligonucleotides pharmacology, tertiary protein structure, RNA metabolism, RNA binding proteins chemistry and genetics, radiation hybrid mapping, recombinant fusion proteins chemistry and metabolism, reverse transcriptase PCR, amino acid sequence homology, proteins chemistry.

Ramasamy, S., W. Hui, and K. Sampath (2005). **Zebrafish staufen in germ cell specification.** *Developmental Biology* 283(2): 648. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish, molecular genetics, phenotype, cell death, embryogenesis, cell specification, germ line specification.

Ramel, M.C., G.R. Buckles, K.D. Baker, and A.C. Lekven (2005). **WNT8 and BMP2B co-regulate non-axial mesoderm patterning during zebrafish gastrulation.** *Developmental Biology* 287(2): 237-48. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: During vertebrate mesoderm formation, fates are established according to position in the dorsoventral (D/V) axis of the embryo. Initially, maternal signaling divides nascent mesoderm into axial (dorsal) and non-axial (ventral) domains. Although the subsequent subdivision of non-axial mesoderm into multiple D/V fate domains is known to involve zygotic Wnt8 and BMP signaling as well as the Vent/Vox/Ved family of transcriptional repressors, how levels of signaling activity are translated into differential regulation of fates is not well understood. To address this question, we have analyzed zebrafish embryos lacking Wnt8 and BMP2b. Zebrafish wnt8; swr (bmp2b) double mutants display a progressive loss of non-axial mesoderm and a concomitant expansion of axial mesoderm during gastrulation. Mesoderm induction and specification of the axial domain occur normally in wnt8; swr mutants, but dorsal mesoderm genes eventually come to be expressed throughout the mesoderm, suggesting that the establishment of non-axial mesoderm identity requires

continual repression of dorsal mesoderm factors, including repressors of ventral genes. Loss-of-function for Vent, Vox, and Ved phenocopies the wnt8; swr mutant phenotype, consistent with Wnt8 and BMP2b maintaining non-axial mesoderm identity during gastrulation through the regulation of these three transcriptional repressors. We postulate that timely differentiation of the mesoderm requires the maintenance of non-axial mesoderm identity by Wnt8 and BMP2b at the onset of gastrulation followed by subdivision of the non-axial mesoderm into different functional domains during gastrulation.
Descriptors: zebrafish, bone morphogenetic proteins metabolism, cytoskeletal proteins metabolism, gastrula metabolism, mesoderm metabolism, Wnt proteins metabolism, body patterning, bone morphogenetic cytoskeletal embryonic induction, gastrula cytology, developmental gene expression regulation, mesoderm cytology, mutation, phenotype, Wnt proteins genetics.

Ramel, M.C. and A.C. Lekven (2004). **Repression of the vertebrate organizer by wnt8 is mediated by vent and vox**. *Development* 131(16): 3991-4000. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Dorsoventral (DV) patterning of vertebrate embryos requires the concerted action of the Bone Morphogenetic Protein (BMP) and Wnt signaling pathways. In contrast to our understanding of the role of BMP in establishing ventral fates, our understanding of the role of Wnts in ventralizing embryos is less complete. Wnt8 is required for ventral patterning in both Xenopus and zebrafish; however, its mechanism of action remains unclear. We have used the zebrafish to address the requirement for Wnt8 in restricting the size of the dorsal organizer. Epistasis experiments suggest that Wnt8 achieves this restriction by regulating the early expression of the transcriptional repressors Vent and Vox. Our data show that vent and vox are direct transcriptional targets of Wnt8/beta-catenin. Additionally, we show that Wnt8 and Bmp2b co-regulate vent and vox in a dynamic fashion. Thus, whereas both Wnt8 and zygotic BMP are ventralizing agents that regulate common target genes, their temporally different modes of action are necessary to pattern the embryo harmoniously along its DV axis.
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Descriptors: zebrafish, development, reproductive system , in situ hybridization, genetic techniques, vent and vox, Wnt8, zygotic BMP.

Ramos-Mejia, V., D. Escalante-Alcalde, T. Kunath , L. Ramirez, M. Gertsenstein, A. Nagy, and H. Lomeli (2005). **Phenotypic analyses of mouse embryos with ubiquitous expression of oct4: effects on mid-hindbrain patterning and gene expression**. *Developmental Dynamics* 232(1): 180-190. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Oct4 is a transcription factor that has been associated with pluripotency and fate determination in the initial cell lineages of mammals. On the other hand, Pou2, the ortholog of Oct4 in zebrafish, serves additional later functions during brain development acting as a differentiation switch. In mice, Oct4 is expressed throughout the neural plate of embryos until embryonic day (E) 8.0. In this study, we produced transgenic mouse embryos that ubiquitously express Oct4 and analyzed the consequences during development. We show that, at E8.0, a higher dosage of Oct4 in the neuroectoderm is sufficient to transiently alter mid-hindbrain patterning and produced a strong up-regulation of Pax2, indicating that Oct4 can regulate this gene in vivo. After E9.5, ectopic Oct4 in this region produced cell death and affected the development of the forebrain, suggesting that, at these later stages, Oct4 down-regulation is necessary for normal development to proceed. The phenotype of the transgenic embryos was also accompanied with an increase of Fgf8 expression in

several of its endogenous domains, suggesting the possibility that Oct4 can participate in the regulation of expression of this ligand. Our observations support the hypothesis that Oct4, like zebrafish Pou2, has a conserved function during early brain patterning in mouse. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, development, molecular genetics, nervous system, neural coordination, cell death, fate determination, pluripotency.

Rand, K., M. Itoh, G. Palardy, M. Matsuda, S.-Y. Yeo, M. Goswami, and M. Chitnis (2007). **Cis-inhibition of Notch signaling in para-boundary cells in the zebrafish hindbrain**. *Developmental Biology* 306(1): 301. ISSN: 0012-1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish, cytology, *Danio rerio.*

Rasooly, R.S., D. Henken, N. Freeman, L. Tompkins, D. Badman, J. Briggs, and A.T. Hewitt (2003). **Genetic and genomic tools for zebrafish research: the NIH zebrafish initiative.** *Developmental Dynamics* 228(3): 490-6. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: The National Institutes of Health (NIH) has been a leading advocate of the zebrafish as a model organism for the study of vertebrate development, physiology, and disease. Genomic tools, developed with the support of NIH funding, have made zebrafish even more attractive as a genetic system and have stimulated research using this model. The NIH continues to provide support for new and existing community resources, such as the Zebrafish International Resource Center and the Zebrafish Information Network, research tool development and mutant screens, and a wide array of investigator-initiated studies that are using zebrafish to elucidate many aspects of vertebrate biology.

Descriptors: zebrafish, genome, The National Institutes of Health U.S., research methods, brain physiology, genomics, research organization and administration, research trends, research support, model of vertebrate development, United States.

Rau, M.J., S. Fischer, and C.J. Neumann (2006). **Zebrafish Trap230/Med12 is required as a coactivator for Sox9-dependent neural crest, cartilage and ear development.** *Developmental Biology* 296(1): 83-93. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The vertebrate Sox9 transcription factor directs the development of neural crest, otic placodes, cartilage and bone. In zebrafish, there are two Sox9 orthologs, Sox9a and Sox9b, which together perform the functions of the single-copy tetrapod Sox9. In a large-scale genetic screen, we have identified a novel zebrafish mutant that strongly resembles the Sox9a/Sox9b double mutant phenotype. We show that this mutation disrupts the zebrafish Trap230/Med12 ortholog, a member of the Mediator complex. Mediator is a coactivator complex transducing the interaction of DNA-binding transcription factors with RNA polymerase II, and our results reveal a critical function of the Trap230 subunit as a coactivator for Sox9.

Descriptors: zebrafish embryos, cartilage embryology, ear embryology, hmgb, high mobility group, intracellular signaling peptides and and genetics, neural crest embryology, receptors, thyroid hormone physiology, transcription factors physiology, phenotype, protein subunits genetics, protein subunits physiology, receptors, thyroid hormone genetics, trans activators genetics andphysiology, transcription factors genetics.

Rawls, J.F. and S.L. Johnson (2003). **Temporal and molecular separation of the kit receptor tyrosine kinase's roles in zebrafish melanocyte migration and survival.** *Developmental Biology* 262(1): 152-61. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The Kit receptor tyrosine kinase is required by vertebrate melanocytes for their migration and survival. The relationship between these developmental roles of Kit, however, remains poorly understood. Here, we use two genetic approaches to demonstrate that Kit's roles in the migration and survival of embryonic melanocytes in the zebrafish (*Danio rerio)* are temporally and functionally independent. We use a temperature-sensitive kit mutation to show that kit promotes melanocyte migration and survival during distinct stages of development. These experiments additionally reveal that melanocyte migration is neither necessary nor sufficient for subsequent survival. We also identify kit alleles that molecularly separate kits roles in migration and survival. These results suggest that the melanocyte changes its response to Kit receptor signaling and function during development, first to promote migration, then to promote survival through distinct Kit-dependent mechanisms.

Descriptors: zebrafish embryos, melanocytes physiology, oncogene, alleles, melanocyte, cell movement and survival, oncogene proto oncogene proteins C kit.

Rawls, J.F., B.S. Samuel, and J.I. Gordon (2004). **Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota.** *Proceedings of the National Academy of Sciences of the United States of America* 101(13): 4596-601. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: Animals have developed the means for supporting complex and dynamic consortia of microorganisms during their life cycle. A transcendent view of vertebrate biology therefore requires an understanding of the contributions of these indigenous microbial communities to host development and adult physiology. These contributions are most obvious in the gut, where studies of gnotobiotic mice have disclosed that the microbiota affects a wide range of biological processes, including nutrient processing and absorption, development of the mucosal immune system, angiogenesis, and epithelial renewal. The zebrafish *(Danio rerio*) provides an opportunity to investigate the molecular mechanisms underlying these interactions through genetic and chemical screens that take advantage of its transparency during larval and juvenile stages. Therefore, we developed methods for producing and rearing germ-free zebrafish through late juvenile stages. DNA microarray comparisons of gene expression in the digestive tracts of 6 days post fertilization germ-free, conventionalized, and conventionally raised zebrafish revealed 212 genes regulated by the microbiota, and 59 responses that are conserved in the mouse intestine, including those involved in stimulation of epithelial proliferation, promotion of nutrient metabolism, and innate immune responses. The microbial ecology of the digestive tracts of conventionally raised and conventionalized zebrafish was characterized by sequencing libraries of bacterial 16S rDNA amplicons. Colonization of germ-free zebrafish with individual members of its microbiota revealed the bacterial species specificity of selected host responses. Together, these studies establish gnotobiotic zebrafish as a useful model for dissecting the molecular foundations of host-microbial interactions in the vertebrate digestive tract.

Descriptors: zebrafish embryos to late juvenile stages, digestive system microbiology, evolution, intestines microbiology, zebrafish microbiology, air sacs microbiology, epithelial cells cytology, epithelial cells immunology, fisheries, germ free life, intestinal mucosa microbiology, mammals microbiology, molecular sequence data, molecular foundations of host-microbial interactions, genetic transcription.

Raymond, P.A. and L.K. Barthel (2004). **A moving wave patterns the cone photoreceptor mosaic array in the zebrafish retina.** *International Journal of Developmental Biology, The* 48(8-9): 935-45. ISSN: 0214-6282.

Abstract: In this paper, we describe the embryonic origin and patterning of the planar mosaic array of cone photoreceptor spectral subtypes in the zebrafish retina. A discussion of possible molecular mechanisms that might generate the cone mosaic array considers but discards a model that accounts for formation of neuronal mosaics in the inner retina and discusses limitations of mathematical simulations that reproduce the zebrafish cone mosaic pattern. The formation and organization of photoreceptors in the ommatidia of the compound eye of *Drosophila* is compared with similar features in the developing zebrafish cone mosaic, and a model is proposed that invokes spatiotemporally coordinated cell-cell interactions among cone progenitors to determine the identity and positioning of cone spectral subtypes.

Descriptors: zebrafish embryos, retina cones embryology, developmental gene expression regulation, vertebrate photoreceptors physiology, retina embryology, body patterning, cell differentiation, cell lineage, *Drosophila* embryology, embryonic development, in situ hybridization, neurons metabolism, phylogeny, retina metabolism, retinal pigments, time factors.

Raymond, P.A., L.K. Barthel, R.L. Bernardos, and J.J. Perkowski (2006). **Molecular characterization of retinal stem cells and their niches in adult zebrafish.** *BioMed Central Developmental Biology* 6: 36. ISSN: 1471 213X.

NAL Call Number: QH491.B63

Abstract: BACKGROUND: The persistence in adult teleost fish of retinal stem cells that exhibit all of the features of true 'adult stem cells'--self-renewal, multipotency, and the capacity to respond to injury by mitotic activation with the ability to regenerate differentiated tissues--has been known for several decades. However, the specialized cellular and molecular characteristics of these adult retinal stem cells and the microenvironmental niches that support their maintenance in the differentiated retina and regulate their activity during growth and regeneration have not yet been elucidated. RESULTS: Our data show that the zebrafish retina has two kinds of specialized niches that sustain retinal stem cells: 1) a neuroepithelial germinal zone at the interface between neural retina and ciliary epithelium, called the ciliary marginal zone (CMZ), a continuous annulus around the retinal circumference, and 2) the microenvironment around some Muller glia in the differentiated retina. In the uninjured retina, scattered Muller glia (more frequently those in peripheral retina) are associated with clusters of proliferating retinal progenitors that are restricted to the rod photoreceptor lineage, but following injury, the Muller-associated retinal progenitors can function as multipotent retinal stem cells to regenerate other types of retinal neurons. The CMZ has several features in common with the neurogenic niches in the adult mammalian brain, including access to the apical epithelial surface and a close association with blood vessels. Muller glia in the teleost retina have a complex response to local injury that includes some features of reactive gliosis (up-regulation of glial fibrillary acidic protein, GFAP, and re-entry into the cell cycle) together with dedifferentiation and re-acquisition of phenotypic and molecular characteristics of multipotent retinal progenitors in the CMZ (diffuse distribution of N-cadherin, activation of Notch-Delta signaling, and expression of rx1, vsx2/Chx10, and pax6a) along with characteristics associated with radial glia (expression of brain lipid binding protein, BLBP). We also describe a novel specific marker for Muller glia, apoE. CONCLUSION: The stem cell niches that support multi-lineage retinal progenitors in the intact, growing and regenerating teleost retina have properties characteristic of neuroepithelia and neurogenic radial glia. The regenerative capacity of the adult zebrafish retina with its ability

to replace lost retinal neurons provides an opportunity to discover the molecular regulators that lead to functional repair of damaged neural tissue.
Descriptors: zebrafish, aging physiology, Muller glia, apoE, retina cytology, stem cells cytology, stem cells metabolism, cell differentiation and proliferation, developmental gene expression regulation, in situ hybridization, growth and development.

Raz, E. (2004). **Cellular and molecular mechanisms governing early primordial germ cell migration in zebrafish**. *Molecular Biology of the Cell* 15(Suppl. S): 2A. ISSN: 1059 1524.
NAL Call Number: QH604.C452
Descriptors: zebrafish embryos, development, cell biology, time lapse microscopy, imaging and microscopy techniques, cluster migration.

Reichman-Fried, M., S. Minina, and E. Raz (2004). **Autonomous modes of behavior in primordial germ cell migration**. *Developmental Cell* 6(4): 589-596 . ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Zebrafish primordial germ cells (PGCs) are guided toward their targets by the chemokine SDF-1a. PGCs were followed during three phases of their migration: when migrating as individual cells, while remaining in a clustered configuration, and when moving as a cell cluster within the embryo. We found that individually migrating PGCs alternate between migratory and pausing modes. Pausing intervals are characterized by loss of cell polarity and correlate with subsequent changes in the direction of migration. These properties constitute an intrinsic behavior of PGCs, enabling erasure of prior polarity and re-sampling of the environment. Following migration arrest at a site of high SDF-1a levels, PGCs resume migration as a cluster. The seemingly coordinated cluster migration is a result of single-cell movement in response to local variations in SDF-1a distribution. Together, these behavioral modes allow the cells to arrive at specific destinations with high fidelity and remain at their target site. Copyright©Thomson Reuters 2009
Descriptors: zebrafish primordial germ cells, cell biology, development, cell migration, cell polarity, cellular behavior, migration arrest.

Reijntjes, S., A. Rodaway, and M. Maden (2007). **The retinoic acid metabolising gene, CYP26B1, patterns the cartilaginous cranial neural crest in zebrafish**. *International Journal of Developmental Biology* 51(5): 351-360. ISSN: 0214-6282.
Descriptors: zebrafish embryos, antisense oligonucleotides, cartilage, cell migration, embryonic development, embryos, enzymes, genes, head, hindbrain, Jaw, metabolism, neural crest, neurons, oligonucleotides, pattern formation, retinoic acid, skull, Vagus nerve, *Danio rerio*.

Reim, G. and M. Brand (2006). **Maternal control of vertebrate dorsoventral axis formation and epiboly by the pou domain protein spg/pou2/oct4**. *Development* 133(14): 2757-2770. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Dorsoventral (DV) axis formation of the vertebrate embryo is controlled by the maternal genome and is subsequently refined zygotically. In the zygote, repression of ventralizing Bmp activity on the dorsal side through chordin and noggin is crucial for establishment of a dorsally located organizer. This interplay generates a zygotic Bmp activity gradient that defines distinct positional values along the DV axis. The maternal processes that control expression of the zygotic genes implicated in DV patterning are largely unknown. spiel-ohne-grenzen (spg/pou2) is a maternally and zygotically expressed zebrafish gene that encodes the POU domain transcription factor Pou2, an ortholog of mammalian Oct4/Pou5f1. We

show that embryos that are genetically depleted of both maternal and zygotic pou2 function (MZspg) exhibit extreme DV patterning defects and, independently, a blastoderm-specific arrest of epiboly. Dorsal tissues expand to the ventral side at the expense of ventrolateral tissue in MZspg embryos. Dorsally expressed Bmp-antagonists, such as Chd and Nog1, and Gsc are ectopically activated at ventral levels in MZspg. Lack of ventral specification is apparent very early, suggesting that maternal processes are affected in MZspg. Indeed, maternal pou2 function is necessary to initiate zygotic expression of ventrally expressed genes such as bmp2b and bmp4, and for proper activation of bmp7, vox, vent and eve1. A constitutively active Alk8-TGF beta-receptor can ectopically induce bmp2b and bmp4 and rescues the dorsalization of MZspg. This indicates that pou2 acts upstream of Alk8, a maternally provided receptor implicated in the activation of zygotic bmp2b and bmp4 transcription. Consistent with this possibility, Bmp gene misexpression can rescue MZspg embryos, indicating that TGF beta-mediated signal transduction itself is intact in absence of Pou2. Inhibition of Fgf signaling, another pathway with early dorsalizing activity, can also restore and even ventralize MZspg embryos. The requirement for pou2 to initiate bmp2b expression can therefore be bypassed by releasing the repressive function of Fgf signaling upon bmp2b transcription. In transplantation experiments, we find that dorsalized cells from prospective ventrolateral regions of MZspg embryos are non cell-autonomously respecified to a ventral fate within wild-type host embryos. Analysis of pou2 mRNA injected MZspg embryos shows that pou2 is required on the ventral side of cleavage stage embryos. Based on the maternal requirement for pou2 in ventral specification, we propose that ventral specification employs an active, pou2-dependent maternal induction step, rather than a default ventralizing program. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, development, molecular genetics, maternal control, epiboly, dorsoventral axis formation.

Reim, G., T. Mizoguchi, D.Y. Stainier, Y. Kikuchi, and M. Brand (2004). **The pou domain protein spg (pou2/oct4) is essential for endoderm formation in cooperation with the hmg domain protein casanova**. *Developmental Cell* 6(1): 91-101. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: The gastrulating vertebrate embryo develops three germlayers: ectoderm, mesoderm, and endoderm. Zebrafish endoderm differentiation starts with the activation of sox17 by casanova (cas). We report that spg (pou2/Oct4) is essential for endoderm formation. Embryos devoid of maternal and zygotic spg function (MZspg) lack endodermal precursors. Cell transplantations show that spg acts in early endodermal precursors, and cas mRNA-injection into MZspg embryos does not restore endoderm development. spg and cas together are both necessary and sufficient to activate endoderm development, and stimulate expression of a sox17 promoter-luciferase reporter. Endoderm and mesoderm derive from a common origin, mesendoderm. We propose that Spg and Cas commit mesendodermal precursors to an endodermal fate. The joint control of endoderm formation by spg and cas suggests that the endodermal germlayer may be a tissue unit with distinct genetic control, thus adding genetic support to the germlayer concept in metazoan development. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, development, reproductive system, reproduction, zebrafish endoderm differentiation.

Rentzsch, F., J. Zhang, C. Kramer, W. Sebald, and M. Hammerschmidt (2006). **Crossveinless 2 is an essential positive feedback regulator of Bmp signaling during zebrafish gastrulation.** *Development* 133(5): 801-11. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Signaling by bone morphogenetic proteins (Bmps) plays a pivotal role in developmental and pathological processes, and is regulated by a complex interplay with secreted Bmp binding factors, including Crossveinless 2 (Cvl2). Although structurally related to the Bmp antagonist Chordin, Crossveinless 2 has been described to be both a Bmp agonist and antagonist. Here, we present the first loss-of-function study of a vertebrate cvl2 homologue, showing that zebrafish cvl2 is required in a positive feedback loop to promote Bmp signaling during embryonic dorsoventral patterning. In vivo, Cvl2 protein undergoes proteolytic cleavage and this cleavage converts Cvl2 from an anti- to a pro-Bmp factor. Embryonic epistasis analyses and protein interaction assays indicate that the pro-Bmp function of Cvl2 is partly accomplished by competing with Chordin for binding to Bmps. Studies in cell culture and embryos further suggest that the anti-Bmp effect of uncleaved Cvl2 is due to its association with the extracellular matrix, which is not found for cleaved Cvl2. Our data identify Cvl2 as an essential pro-Bmp factor during zebrafish embryogenesis, emphasizing the functional diversity of Bmp binding CR-domain proteins. Differential proteolytic processing as a mode of regulation might account for anti-Bmp effects in other contexts.

Descriptors: zebrafish embryos, body patterning genetics, bone morphogenetic proteins metabolism, GTPase activatinG proteins metabolism, up regulation genetics, proteins metabolism, bone morphogenetic metabolism, epistasis, genetic, GTPase activatinG gastrula cytology and metabolism, glycoglycoproteins metabolism, intercellular signaling peptides and proteins genetics and metabolism, mutation, signal transduction.

Rentzsch, F., J. Bakkers, C. Kramer, and M. Hammerschmidt (2004). **Fgf signaling induces posterior neuroectoderm independently of bmp signaling inhibition**. *Developmental Dynamics* 231(4): 750-757. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: Whereas according to the neural default model, neural specification is induced by extracellular inhibitors of bone morphogenetic proteins (Bmps), the role of fibroblast growth factors (Fgfs) during neural induction is heavily debated. Here, we show that, in zebrafish embryos, Bmps and Fgfs play differential roles during the induction and patterning of the anterior vs. the posterior neuroectoderm. Induction of anterior neuroectoderm, giving rise to fore- and midbrain, is accomplished by Bmp inhibition, with Fgfs playing a moderate posteriorizing/patterning role, possibly by blocking Bmp signaling at the level of Smad proteins. In contrast, in the posterior-most neuroectoderm, which is located in marginal regions of the early gastrula embryo to give rise to spinal cord and hindbrain, Fgfs play a neural-inducing rather than a neural-patterning role. This Fgf-dependent posterior neural induction takes place during late blastula and early gastrula stages, after mesoderm has been induced and cannot be blocked by Bmps or the Bmp target gene and downstream effector DELTANp63alpha, indicating that here, Fgfs act independently of Bmp signaling inhibition.
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Descriptors: zebrafish embryos, development, molecular genetics, nervous system, neural coordination, neural induction, gastrula, spinal cord and hindbrain.

Reugels, A.M., B. Boggetti, N. Scheer, and J.A. Campos Ortega (2006). **Asymmetric localization of Numb:EGFP in dividing neuroepithelial cells during neurulation in *Danio rerio.*** *Developmental Dynamics* 235(4): 934-48. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish, cell polarity, *Drosophila* proteins metabolism, green fluorescent proteins metabolism, juvenile hormones metabolism, neuroepithelial cells physiology, amino

acid sequence, cell membrane metabolism, molecular cloning, conserved sequence, *Drosophila* proteins chemistry and genetics gene deletion, introns, juvenile hormones chemistry and genetics, membrane proteins, confocal microscopy, video microscopy, mitotic spindle apparatus physiology, molecular sequence data, insertional mutagenesis, neuroepithelial cells cytology, phylogeny, protein isoforms chemistry and genetics, tertiary protein structure, recombinant fusion proteins chemistry and metabolism, reverse transcriptase PCR, amino acid sequence homology.

Reyes, R., M. Haendel, D. Grant, E. Melancon, and J.S. Eisen (2004). **Slow degeneration of zebrafish Rohon-Beard neurons during programmed cell death.** *Developmental Dynamics* 229(1): 30-41. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, neurons cytology, genetically modified, apoptosis, DNA fragmentation, ganglia, spinal cytology, spinal ganglia embryology, green fluorescent proteins, luminescent nerve degeneration, recombinant proteins genetics.

Rhinn, M., K. Lun, A. Amores, Y.L. Yan, J.H. Postlethwait, and M. Brand (2003). **Cloning, expression and relationship of zebrafish gbx1 and gbx2 genes to Fgf signaling.** *Mechanisms of Development* 120(8): 919-36. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: The organizer at the midbrain-hindbrain boundary (MHB) forms at the interface between Otx2 and Gbx2 expressing cell populations, but how these gene expression domains are set up and integrated with the remaining machinery controlling MHB development is unclear. Here we report the isolation, mapping, chromosomal synteny and spatiotemporal expression of gbx1 and gbx2 in zebrafish. We focus in particular on the expression of these genes during development of the midbrain-hindbrain territory. Our results suggest that these genes function in this area in a complex fashion, as evidenced by their highly dynamic expression patterns and relation to Fgf signaling. Analysis of gbx1 and gbx2 expression during formation of the MHB in mutant embryos for pax2.1, fgf8 and pou2 (noi, ace, spg), as well as Fgf-inhibition experiments, show that gbx1 acts upstream of these genes in MHB development. In contrast, gbx2 activation requires ace (fgf8) function, and in the hindbrain primordium, also spg (pou2). We propose that in zebrafish, gbx genes act repeatedly in MHB development, with gbx1 acting during the positioning period of the MHB at gastrula stages, and gbx2 functioning after initial formation of the MHB, from late gastrulation stages onwards. Transplantation studies furthermore reveal that at the gastrula stage, Fgf8 signals from the hindbrain primordium into the underlying mesendoderm. Apart from the general involvement of gbx genes in MHB development reported also in other vertebrates, these results emphasize that early MHB development can be divided into multiple steps with different genetic requirements with respect to gbx gene function and Fgf signaling. Moreover, our results provide an example for switching of a specific gene function of gbx1 versus gbx2 between orthologous genes in zebrafish and mammals.
Descriptors: zebrafish, Fgf metabolism, gene expression, homeodomain proteins metabolism, amino acid sequence, fibroblast growth factor 8, mesencephalon embryology and metabolism, rhombencephalon embryology and metabolism, sequence alignment, amino acid sequence homology.

Rhinn, M., K. Lun, M. Luz, M.A. Werner, and M. Brand (2005). **Positioning of the midbrain-hindbrain boundary organizer through global posteriorization of the neuroectoderm mediated by Wnt8 signaling**. *Development* 132(6): 1261-1272. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: The organizing center located at the midbrain-hindbrain boundary (MHB) patterns the midbrain and hindbrain primordia of the neural plate. Studies in several vertebrates showed that the interface between cells expressing Otx and Gbx transcription factors marks the location in the neural plate where the organizer forms, but it is unclear how this location is set up. Using mutant analyses and shield ablation experiments in zebrafish, we find that axial mesendoderm, as a candidate tissue, has only a minor role in positioning the MHB. Instead, the blastoderm margin of the gastrula embryo acts as a source of signal(s) involved in this process. We demonstrate that positioning of the MHB organizer is tightly linked to overall neuroectodermal posteriorization, and specifically depends on Wnt8 signaling emanating from lateral mesendodermal precursors. Wnt8 is required for the initial subdivision of the neuroectoderm, including onset of posterior gbx1 expression and establishment of the posterior border of otx2 expression. Cell transplantation experiments further show that Wnt8 signaling acts directly and non-cell-autonomously. Consistent with these findings, a GFP-Wnt8 fusion protein travels from donor cells through early neural plate tissue. Our findings argue that graded Wnt8 activity mediates overall neuroectodermal posteriorization and thus determines the location of the MHB organizer. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, nervous system, neural coordination, molecular genetics, reproductive system and mutant analysis, genetic techniques, midbrain hindbrain boundary, global posteriorization, isthmic organizer.

Rhodes, J., A. Hagen, K. Hsu, M. Deng, T.X. Liu, A.T. Look, and J.P. Kanki (2005). **Interplay of pu.1 and gata1 determines myelo-erythroid progenitor cell fate in zebrafish.** *Developmental Cell* 8(1): 97-108. ISSN: 1534 5807.

NAL Call Number: QH573.C42

Abstract: The zebrafish is a powerful model system for investigating embryonic vertebrate hematopoiesis, allowing for the critical in vivo analysis of cell lineage determination. In this study, we identify zebrafish myeloerythroid progenitor cells (MPCs) that are likely to represent the functional equivalent of mammalian common myeloid progenitors. Utilizing transgenic pu.1-GFP fish, real-time MPC differentiation was correlated with dynamic changes in cell motility, morphology, and gene expression. Unlike mammalian hematopoiesis, embryonic zebrafish myelopoiesis and erythropoiesis occur in anatomically separate locations. Gene knockdown experiments and transplantation assays demonstrated the reciprocal negative regulation of pu.1 and gata1 and their non-cell-autonomous regulation that determines myeloid versus erythroid MPC fate in the distinct blood-forming regions. Furthermore, forced expression of pu.1 in the bloodless mutant cloche resulted in myelopoietic rescue, providing intriguing evidence that this gene can function in the absence of some stem cell genes, such as scl, in governing myelopoiesis.

Descriptors: zebrafish embryo, DNA binding, erythroid progenitor cells physiology, myeloid progenitor cells physiology, proto oncogene, trans activators physiology, transcription factors physiology, genetically modified, cell differentiation physiology, cell movement physiology, DNA binding proteins genetics, embryonic induction, erythroid specific DNA binding proteins genetics, factors, flow cytometry methods, GATA1 transcription factor, developmental gene expression regulation physiology, genotype, green fluorescent proteins genetics and metabolism, hematopoiesis physiology, in situ hybridization methods, microinjections methods, proto oncogene messenger RNA biosynthesis, reverse transcriptase PCR methods, trans activators genetics, transcription factors genetics, transplantation methods.

Rich, A., S.A. Leddon, S.L. Hess, S.J. Gibbons, S. Miller, X. Xu, and G. Farrugai (2007). **Kit-like immunoreactivity in the zebrafish gastrointestinal tract reveals putative ICC.** *Developmental Dynamics* 236(3): 903-11 . ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Gastrointestinal (GI) motility results from the coordinated actions of enteric neurons, interstitial cells of Cajal (ICC), and smooth muscle cells. The GI tract of the zebrafish has a cellular anatomy that is essentially similar to humans. Although enteric nerves and smooth muscle cells have been described, it is unknown if ICC are present in the zebrafish. Immunohistochemistry and PCR were used determine expression for the zebrafish Kit orthologue in the zebrafish gastrointestinal tract. Cells displaying Kit-like immunoreactivity were identified in the muscular layers of the adult zebrafish gastrointestinal tract. Two layers of Kit-positive cells were identified, one with multipolar cells located between the longitudinal and circular smooth muscle layers and one with simple bipolar cells located deep in the circular muscle layer. Primers specifically designed to amplify mRNA coding for two zebrafish kit genes, kita and kitb, and two kit ligands, kitla and kitlb, amplified the expected transcript from total RNA isolated from zebrafish GI tissues. The Sparse mutant, a kita null mutant, showed reduced contraction frequency and increased size of the GI tract indicating a functional role for kita. These data establish the presence of a cellular network with Kit-like immunoreactivity in the myenteric plexus region of the zebrafish GI tract, adjacent to enteric neurons. Expression of kita and kitb, and the ligands kitla and kitlb, were verified in the adult GI tract. The anatomical arrangement of the Kit-positive cells strongly suggests that they are ICC.
Descriptors: zebrafish embryos, gastrointestinal tract metabolism, myenteric plexus metabolism, proto oncogene proteins C kit metabolism, gastrointestinal motility genetics and physiology, gastrointestinal tract cytology and embryology, immunohistochemistry, mutation, myenteric plexus cytology and embryology, proto oncogene proteins C kit genetics, reverse transcriptase PCR.

Ricker, D.D. and J.P. Thompson (2005). **Molecular laboratory activities for undergraduate developmental biology courses: examining the expression of tie-2 protein and mRNA during zebrafish (*danio rerio)* embryo development using Elisa and rt-pcr.** *Developmental Biology* 283(2): 591. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, methods and techniques, molecular genetics, Elisa, immunologic techniques, reverse transcriptase PCR, genetic techniques, molecular technique, gene expression, molecular laboratory activity, undergraduate developmental biology course.

Rida, P.C.G., N. Le Minh, and Y.J. Jiang (2004). **A Notch feeling of somite segmentation and beyond**. *Developmental Biology* 265(1): 2-22. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, biological clocks, embryonic development, gene expression, mathematical models, segmentation, transcription, *Danio rerio*, *Xenopus laevis*, African clawed frog, FGF8 gene, gene regulation, hairy gene, Notch gene, vertebrate segmentation, Wnt3a gene.

Riedel-Kruse, I.H., C. Muller, and A.C. Oates (2007). **Synchrony dynamics during initiation, failure, and rescue of the segmentation clock**. *Science* 317(5846): 1911-1915. ISSN: 0036-8075.
NAL Call Number: 470Sci2

Descriptors: zebrafish embryos, abnormalities, developmental stages, embryonic development, embryos, freshwater fish, Notch protein, oscillators, segmentation, signal transduction.

Rieger, S., R.P. Kulkarni, D. Darcy, S.E. Fraser, and R.W. Koster (2005). **Quantum dots are powerful multipurpose vital labeling agents in zebrafish embryos.** *Developmental Dynamics* 234(3): 670-81. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, embryology, quantum dots, staining and labeling methods, axons physiology, cell lineage, contrast media, blood supply and cytology, fluorescence, green fluorescent proteins analysis and genetics, immunohistochemistry, larva growth and development, fluorescence microscopy, staining and labeling instrumentation, streptavidin.

Riley, B.B., M.Y. Chiang, E.M. Storch, R. Heck, G.R. Buckles, and A.C. Lekven (2004). **Rhombomere boundaries are Wnt signaling centers that regulate metameric patterning in the zebrafish hindbrain.** *Developmental Dynamics* 231(2): 278-91. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, body patterning, embryonic structures physiology, intercellular signaling peptides and proteins metabolism, morphogenesis, rhombencephalon embryology, signal transduction physiology, DNA binding proteins genetics, proteins genetics and metabolism, embryonic structures anatomy and histology, hepatocyte nuclear factor 3 beta, in situ hybridization, intercellular signaling peptides and membrane proteins genetics and metabolism, mutation, nuclear proteins genetics and metabolism, Notch receptors, rhombencephalon anatomy, histology and physiology, transcription factors genetics and metabolism, Wnt proteins, Wnt1 protein, proteins metabolism.

Rizzi, B., M. Campana, C. Zanella, C. Melani, R. Cunderlik, Z. Kriva, P. Bourgine, K. Mikula, N. Peyrieras, and A. Sarti (2007). **3-d zebrafish embryo image filtering by nonlinear partial differential equations.** *Engineering in Medicine and Biology Magazine* 1: 6251-4. ISSN: 0739 5175.
Abstract: We discuss application of nonlinear PDE based methods to filtering of 3-D confocal images of embryogenesis. We focus on the mean curvature driven and the regularized Perona-Malik equations, where standard as well as newly suggested edge detectors are used. After presenting the related mathematical models, the practical results are given and discussed by visual inspection and quantitatively using the mean Hausdorff distance.
Descriptors: zebrafish embryo, Hausdorff distance, Perona-Malik equations, 3-d confocal image, nonlinear partial differential equations.

Robertson, G.N., C.A. McGee, T.C. Dumbarton, R.P. Croll, and F.M. Smith (2007). **Development of the swimbladder and its innervation in the zebrafish, *Danio rerio*.** *Journal of Morphology* 268(11): 967-85. ISSN: 0362-2525.
NAL Call Number: 444.8J826
Abstract: Many teleosts including zebrafish, *Danio rerio*, actively regulate buoyancy with a gas-filled swimbladder, the volume of which is controlled by autonomic reflexes acting on vascular, muscular, and secretory effectors. In this study, we investigated the morphological development of the zebrafish swimbladder together with its effectors and innervation. The swimbladder first formed as a single chamber, which inflated at 1-3 days posthatching (dph), 3.5-4 mm body length. Lateral nerves were already present as demonstrated by the antibody zn-12, and blood vessels had formed in parallel on the cranial aspect to supply blood to anastomotic capillary loops as demonstrated by Tie-2 antibody staining. Neuropeptide Y-(NPY-)

like immunoreactive (LIR) fibers appeared early in the single-chambered stage, and vasoactive intestinal polypeptide (VIP)-LIR fibers and cell bodies developed by 10 dph (5 mm). By 18 dph (6 mm), the anterior chamber formed by evagination from the cranial end of the original chamber; both chambers then enlarged with the ductus communicans forming a constriction between them. The parallel blood vessels developed into an arteriovenous rete on the cranial aspect of the posterior chamber and this region was innervated by zn-12-reactive fibers. Tyrosine hydroxylase- (TH-), NPY-, and VIP-LIR fibers also innervated this area and the lateral posterior chamber. Innervation of the early anterior chamber was also demonstrated by VIP-LIR fibers. By 25-30 dph (8-9 mm), a band of smooth muscle formed in the lateral wall of the posterior chamber. Although gas in the swimbladder increased buoyancy of young larvae just after first inflation, our results suggest that active control of the swimbladder may not occur until after the formation of the two chambers and subsequent development and maturation of vasculature, musculature and innervation of these structures at about 28-30 dph.
Descriptors: zebrafish, air sacs growth and development and innervation, adrenergic fibers ultrastructure, air sacs blood supply, air sacs ultrastructure, blood vessels growth and development, cholinergic fibers ultrastructure, life cycle stages physiology, muscle development physiology, muscles innervation.

Rodino Klapac, L.R. and C.E. Beattie (2004). **Zebrafish topped is required for ventral motor axon guidance.** *Developmental Biology* 273(2): 308-20. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Zebrafish primary motor axons extend along stereotyped pathways innervating distinct regions of the developing myotome. During development, these axons make stereotyped projections to ventral and dorsal myotome regions. Caudal primary motoneurons, CaPs, pioneer axon outgrowth along ventral myotomes; whereas, middle primary motoneurons, MiPs, extend axons along dorsal myotomes. Although the development and axon outgrowth of these motoneurons has been characterized, cues that determine whether axons will grow dorsally or ventrally have not been identified. The topped mutant was previously isolated in a genetic screen designed to uncover mutations that disrupt primary motor axon guidance. CaP axons in topped mutants fail to enter the ventral myotome at the proper time, stalling at the nascent horizontal myoseptum, which demarcates dorsal from ventral axial muscle. Later developing secondary motor nerves are also delayed in entering the ventral myotome whereas all other axons examined, including dorsally projecting MiP motor axons, are unaffected in topped mutants. Genetic mosaic analysis indicates that Topped function is non-cell autonomous for motoneurons, and when wild-type cells are transplanted into topped mutant embryos, ventromedial fast muscle are the only cell type able to rescue the CaP axon defect. These data suggest that Topped functions in the ventromedial fast muscle and is essential for motor axon outgrowth into the ventral myotome.
Descriptors: zebrafish embryos, efferent pathways embryology, motor neurons cytology and physiology, axons physiology, efferent pathways cytology, lethal genes, recessive genes, mosaicism, motor neurons physiology, fast twitch muscle fibers cytology and physiology, mutation.

Rohde, L.A., A.C. Oates, and R.K. Ho (2004). **A crucial interaction between embryonic red blood cell progenitors and paraxial mesoderm revealed in spadetail embryos**. *Developmental Cell* 7(2): 251-262. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Zebrafish embryonic red blood cells (RBCs) develop in trunk intermediate

mesoderm (IM), and early macrophages develop in the head, suggesting that local microenvironmental cues regulate differentiation of these two blood lineages. spadetail (spt) mutant embryos, which lack trunk paraxial mesoderm (PM) due to a cell-autonomous defect in tbx16, fail to produce embryonic RBCs but retain head macrophage development. In spt mutants, initial hematopoietic gene expression is absent in trunk IM, although endothelial and pronephric expression is retained, suggesting that early blood progenitor development is specifically disrupted. Using cell transplantation, we reveal that spt is required cell autonomously for early hematopoietic gene expression in trunk IM. Further, we uncover an interaction between embryonic trunk PM and blood progenitors that is essential for RBC development. Importantly, our data identify a hematopoietic microenvironment that allows embryonic RBC production in the zebrafish. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, blood and lymphatics, transport and circulation, cell biology, development, molecular genetics, zebrafish embryonic red blood cells, spadetail mutation.

Rojas-Munoz, A., R. Dahm, and C. Nuesslein-Volhard (2005). **Chokh/Rx3 specifies the retinal pigment epithelium fate independently of eye morphogenesis**. *Developmental Biology* 288(2): 348-362. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Despite the importance of the retinal pigment epithelium (RPE) for vision. the molecular processes involved in its specification are poorly understood. We identified two new mutant alleles for the zebrafish gene chokh (chk), which display a reduction or absence of the RPE. Unexpectedly, the neural retina (NR) in chk is specified and laminated, indicating that the regulatory network leading to NR development is largely independent of the RPE. Genetic mapping and molecular characterization revealed that chk encodes Rx3. Expression analyses show that otx2 and mitfb are not expressed in the prospective RPE of chk, indicating that the retinal homeobox gene rx3 acts upstream of the molecular network controlling RPE specification. Cellular transplantations demonstrate that rx3 function is autonomously required to specify the prospective RPE. Though rx2 is also absent in chk, neither rx2 nor rx1 is required for RPE development. Thus, our data provide the first indication that, in addition to controlling optic lobe evagination and proliferation, chk/rx3 also determines cellular late. (c) 2005 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, vision, development, sense organs, sensory reception, genetic mapping and techniques, gene expression analysis, cellular fate.

Rothschild, S.C., J.A. Lister, and R.M. Tombes (2007). **Differential expression of CaMK-II genes during early zebrafish embryogenesis.** *Developmental Dynamics* 236(1): 295-305. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: CaMK-II is a highly conserved Ca(2+)/calmodulin-dependent protein kinase expressed throughout the lifespan of all vertebrates. During early development, CaMK-II regulates cell cycle progression and "non-canonical" Wnt-dependent convergent extension. In the zebrafish, *Danio rerio*, CaMK-II activity rises within 2 hr after fertilization. At the time of somite formation, zygotic expression from six genes (camk2a1, camk2b1, camk2g1, camk2g2, camk2d1, camk2d2) results in a second phase of increased activity. Zebrafish CaMK-II genes are 92-95% identical to their human counterparts in the non-variable regions. During the first three days of development, alternative splicing yields at least 20 splice variants, many of which are unique. Whole-mount in situ hybridization reveals that

camk2g1 comprises the majority of maternal expression. All six genes are expressed strongly in ventral regions at the 18-somite stage. Later, camk2a1 is expressed in anterior somites, heart, and then forebrain. Camk2b1 is expressed in somites, mid- and forebrain, gut, retina, and pectoral fins. Camk2g1 appears strongly along the midline and then in brain, gut, and pectoral fins. Camk2g2 is expressed early in the midbrain and trunk and exhibits the earliest retinal expression. Camk2d1 is elevated early at somite boundaries, then epidermal tissue, while camk2d2 is expressed in discrete anterior locations, steadily increasing along either side of the dorsal midline and then throughout the brain, including the retina. These findings reveal a complex pattern of CaMK-II gene expression consistent with pleiotropic roles during development.

Descriptors: zebrafish embryos, Ca2+ calmodulin dependent protein kinase genetics, embryonic development genetics, amino acid sequence, Ca2+ calmodulin dependent protein kinase metabolism, enzymology, gene expression, in situ hybridization, molecular sequence data, protein isoforms genetics and metabolism, sequence alignment, zebrafish.

Roustaian, P. and M.K. Litvak (2007). **Effect of yolk redistribution at different developmental stages on survivorship of zebrafish embryos**. *Journal of Fish Biology* 70(5): 1642-1646. ISSN: 0022 1112.

NAL Call Number: QL614.J68

Abstract: Survival of zebrafish *Danio rerio* embryos subjected to yolk redistribution during early development (cleavage to segmentation) was found to be dependent on the stage of manipulation and the quantity of redistributed yolk. The findings point to the presence and importance of yolk intrinsic organization during early development. Copyright © 2008 CABI

Descriptors: zebrafish embryos, zebrafish developmental stages, egg yolk, embryonic development, embryos, morphogenesis, ontogeny, survival, *Danio rerio*.

Runko, A.P. and C.G. Sagerstrom (2003). **Nlz belongs to a family of zinc-finger-containing repressors and controls segmental gene expression in the zebrafish hindbrain.** *Developmental Biology* 262(2): 254-67. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The zebrafish nlz gene has a rostral expression limit at the presumptive rhombomere (r) 3/r4 boundary during gastrula stages, and its expression progressively expands rostrally to encompass both r3 and r2 by segmentation stages, suggesting a role for nlz in hindbrain development. We find that Nlz is a nuclear protein that associates with the corepressor Groucho, suggesting that Nlz acts to repress transcription. Consistent with a role as a repressor, misexpression of nlz causes a loss of gene expression in the rostral hindbrain, likely due to ectopic nlz acting prematurely in this domain, and this repression is accompanied by a partial expansion in the expression domains of r4-specific genes. To interfere with endogenous nlz function, we generated a form of nlz that lacks the Groucho binding site and demonstrate that this construct has a dominant negative effect. We find that interfering with endogenous Nlz function promotes the expansion of r5 and, to a lesser extent, r3 gene expression into r4, leading to a reduction in the size of r4. We conclude that Nlz is a transcriptional repressor that controls segmental gene expression in the hindbrain. Lastly, we identify additional nlz-related genes, suggesting that Nlz belongs to a family of zinc-finger proteins.

Descriptors: zebrafish embryology, developmental gene expression regulation physiology, repressor proteins metabolism, rhombencephalon embryology, basic helix loop helix transcription factors, binding sites, DNA binding proteins genetics, proteins metabolism, mice,

phylogeny, recombinant fusion proteins, rhombencephalon metabolism, zinc fingers physiology.

Rutenberg, J.B., A. Fischer, H. Jia, M. Gessler, T.P. Zhong, and M. Mercola (2006). **Developmental patterning of the cardiac atrioventricular canal by notch and hairy-related transcription factors**. *Development* 133(21): 4381-4390. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Mutations in Notch2, Jagged1 or homologs of the Hairy-related transcriptional repressor Hey2 cause congenital malformations involving the non-chamber atrioventricular canal (AVC) and inner curvature (IC) regions of the heart, but the underlying mechanisms have not been investigated. By manipulating signaling directly within the developing chick heart, we demonstrated that Notch2, Hey1 and Hey2 initiate a signaling cascade that delimits the non-chamber AVC and IC regions. Specifically, misactivation of Notch2 signaling, or misexpression of either Hey1 or Hey2, repressed Bmp2. Because Jagged (also known as Serrate in non-mammalian species) ligands were found to be present in prospective chamber myocardium, these data support the model that Notch2 and Hey proteins cause the progressive restriction of Bmp2 expression to within the developing AVC and IC, where it is essential for differentiation. Misactivation or inhibition of Notch2 specifically induced or inhibited Hey1, respectively, but these manipulations did not affect Hey2, implicating Hey1 as the direct mediator of Notch2. Bmp2 within the developing AVC and IC has been shown to induce Tbx2, and we found that Tbx2 misexpression inhibited the expression of both Hey1 and Hey2. Tbx2, therefore, is envisaged to constitute a feedback loop that sharpens the border with the developing AVC and IC by delimiting Hey gene expression to within prospective chamber regions. Analysis of the loss-of-function phenotype in mouse embryos homozygous for targeted disruption of Hey2 revealed an expanded AVC domain of Bmp2. Similarly, zebrafish gridlock (Hey2 homolog) mutant embryos showed ectopic expression of Bmp4, which normally marks AVC myocardium in this species. Thus, Hey pathway regulation of cardiac Bmp appears to be an evolutionarily conserved mechanism to delimit AVC and IC fate, and provides a potential mechanistic explanation for cardiac malformations caused by mutations in Serrate/Jagged1 and Notch signaling components. Copyright©Thomson Reuters 2009
Descriptors: zebrafish mutant embryos, atrioventricular canal, cardiovascular system, transport and circulation, developmental patterning, Notch2, JaggeD1, mice, chicken heart.

Ryu, S. and W. Driever (2006). **Minichromosome maintenance proteins as markers for proliferation zones during embryogenesis**. *Cell Cycle* 5(11): 1140-1142. ISSN: 1538 4101.
Abstract: Regulation of cell proliferation is of fundamental importance for growth and prevention of cancer. An obligatory step in the regulation of cell proliferation is the control of the initiation of DNA synthesis. The minichromosome maintenance (MCM) proteins are essential DNA replication factors crucial for initiating DNA synthesis once every cell cycle. Recent studies show that the level of MCM proteins is stringently regulated to correlate with cell proliferation and carcinogenesis. Here we discuss recent data, which highlights the usefulness of minichromosome maintenance (MCM) gene expression for detecting proliferating cells as well as zones containing proliferative/stem cells during embryogenesis in a whole organism. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryo, development, minichromosome maintenance proteins, DNA replication factors, embryogenesis.

Sabaliauskas, N.A., C.A. Foutz, J.R. Mest, L.R. Budgeon, A.T. Sidor, J.A. Gershenson, S.B. Joshi, and K.C. Cheng (2006). **High-throughput zebrafish histology.** *Methods* 39(3): 246-54. ISSN: 0882-4010.

NAL Call Number: QP519.7.M47

Abstract: The morphological effects of mutation and disease are often critical to our understanding of normal and abnormal function. The power and popularity of zebrafish as a forward and reverse genetic vertebrate model system, combined with its small size, have made it an ideal model in which to study the genetics of histologically scorable phenotypes. The presence of multiple tissue types in this organism's small larvae also makes it a potentially important model for toxicological analysis. Studying histological phenotypes is greatly enhanced by high-throughput methods of histology. Here, we describe details of high-throughput histology of the zebrafish using larval arrays, along with recent advances in mold design and discussion of work in progress that will lead to easier ways for people in the field to more rapidly score phenotypes in arrays. These detailed descriptions, together with the troubleshooting guide, should enable any laboratory with ties to a histology facility to perform high-throughput histology of zebrafish.

Descriptors: histological techniques instrumentation, tissue array analysis methods, larva, microtomy instrumentation, paraffin embedding instrumentation, phenotype, software, tissue fixation, zebrafish growth and development.

Sadler, K.C., A. Amsterdam, C. Soroka, J. Boyer, and N. Hopkins (2005). **A genetic screen in zebrafish identifies the mutants vps18, nf2 and foie gras as models of liver disease.** *Development* 132(15): 3561-72. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Hepatomegaly is a sign of many liver disorders. To identify zebrafish mutants to serve as models for hepatic pathologies, we screened for hepatomegaly at day 5 of embryogenesis in 297 zebrafish lines bearing mutations in genes that are essential for embryonic development. Seven mutants were identified, and three have phenotypes resembling different liver diseases. Mutation of the class C vacuolar protein sorting gene vps18 results in hepatomegaly associated with large, vesicle-filled hepatocytes, which we attribute to the failure of endosomal-lysosomal trafficking. Additionally, these mutants develop defects in the bile canaliculi and have marked biliary paucity, suggesting that vps18 also functions to traffic vesicles to the hepatocyte apical membrane and may play a role in the development of the intrahepatic biliary tree. Similar findings have been reported for individuals with arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome, which is due to mutation of another class C vps gene. A second mutant, resulting from disruption of the tumor suppressor gene nf2, develops extrahepatic choledochal cysts in the common bile duct, suggesting that this gene regulates division of biliary cells during development and that nf2 may play a role in the hyperplastic tendencies observed in biliary cells in individuals with choledochal cysts. The third mutant is in the novel gene foie gras, which develops large, lipid-filled hepatocytes, resembling those in individuals with fatty liver disease. These mutants illustrate the utility of zebrafish as a model for studying liver development and disease, and provide valuable tools for investigating the molecular pathogenesis of congenital biliary disorders and fatty liver disease.

Descriptors: zebrafish embryology, fatty liver genetics, hepatomegaly genetics, liver diseases genetics, mutation, neurofibromin 2 genetics, ubiquitin protein ligases genetics, base sequence, DNA primers, disease models, genetic screening, hepatocytes cytology, hepatocytes physiology, liver diseases embryology, morphogenesis, reverse transcriptase PCR.

Sagasti, A., M.R. Guido, D.W. Raible, and A.F. Schier (2005). **Repulsive interactions shape the morphologies and functional arrangement of zebrafish peripheral sensory arbors.** *Current Biology* 15(9): 804-14. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: BACKGROUND: Trigeminal sensory neurons detect thermal and mechanical stimuli in the skin through their elaborately arborized peripheral axons. We investigated the developmental mechanisms that determine the size and shape of individual trigeminal arbors in zebrafish and analyzed how these interactions affect the functional organization of the peripheral sensory system. RESULTS: Time-lapse imaging indicated that direct repulsion between growing axons restricts arbor territories. Removal of one trigeminal ganglion allowed axons of the contralateral ganglion to cross the midline, and removal of both resulted in the expansion of spinal cord sensory neuron arbors. Generation of embryos with single, isolated sensory neurons resulted in axon arbors that possessed a vast capacity for growth and expanded to encompass the entire head. Embryos in which arbors were allowed to aberrantly cross the midline were unable to respond in a spatially appropriate way to mechanical stimuli. CONCLUSIONS: Direct repulsive interactions between developing trigeminal and spinal cord sensory axon arbors determine sensory neuron organization and control the shapes and sizes of individual arbors. This spatial organization is crucial for sensing the location of objects in the environment. Thus, a combination of undirected growth and mutual repulsion results in the formation of a functionally organized system of peripheral sensory arbors.

Descriptors: zebrafish embryos, axons metabolism, afferent neurons cytology, trigeminal nerve embryology, trigeminal nerve metabolism, embryology, green fluorescent proteins, confocal microscopy, afferent neurons metabolism, trans trigeminal nerve anatomy and histology.

Sagerstrom, C.G., L.S. Gammill, R. Veale, and H. Sive (2005). **Specification of the enveloping layer and lack of autoneuralization in zebrafish embryonic explants.** *Developmental Dynamics* 232(1): 85-97. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: We have analyzed the roles of cell contact during determination of the outermost enveloping layer (EVL) and deeper neurectoderm in zebrafish embryos. Outer cells, but not deeper cells, are specified to express the EVL-specific marker, cyt1 by late blastula. EVL specification requires cell contact or close cell proximity, because cyt1 is not expressed after explant dissociation. The EVL may be homologous to the Xenopus epithelial layer, including the ventral larval epidermis. While Xenopus epidermal cytokeratin gene expression is activated by bone morphogenetic protein (BMP) signaling, zebrafish cyt1 is not responsive to BMPs. Zebrafish early gastrula ectodermal explants are specified to express the neural markers opl (zic1) and otx2, and this expression is prevented by BMP4. Dissociation of zebrafish explants prevents otx2 and opl expression, suggesting that neural specification in zebrafish requires cell contact or close cell proximity. This finding is in contrast to the case in Xenopus, where ectodermal dissociation leads to activation of neural gene expression, or autoneuralization. Our data suggest that distinct mechanisms direct development of homologous lineages in different vertebrates.

Descriptors: zebrafish embryos, embryonic development, developmental gene expression regulation, keratins biosynthesis, genetics and physiology, proteins biosynthesis and physiology, amino acid and base sequence, Northern blotting, bone morphogenetic proteins metabolism, cell communication, cell differentiation, cell lineage, molecular cloning, epithelium embryology, homeodomain proteins biosynthesis, in situ hybridization, molecular

sequence data, neurons cytology, oligonucleotides genetics, otx transcription factors, reverse transcriptase PCR, signal transduction, time factors, transcription factors biosynthesis, *Xenopus* proteins biosynthesis, proteins genetics.

Sagerstrom, C., S.K. Choe, and N. Hirsch (2005). **Genetic pathways regulating rhombomere 4/5 formation.** *Developmental Biology* 283(2): 660-661. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, development, molecular genetics, genetic pathway, rhombomere 4 , 5.

Saito, T., T. Fujimoto, S. Maegawa, K. Inoue, M. Tanaka, K. Arai, and E. Yamaha (2006). **Visualization of primordial germ cells in vivo using GFP-nos1 3'UTR mRNA**. *International Journal of Developmental Biology* 50(8): 691-700. ISSN: 0214 6282.
Abstract: In some teleost fish, primordial germ cells (PGCs) inherit specific maternal cytoplasmic factors such as vasa and nanos 1 (nos1) mRNA. It has been shown that the 3'untranslated regions (UTRs) of vasa and nos1 have critical roles for stabilization of these RNAs in zebrafish PGCs. In this study, to determine whether this role of the nos 1 3'UTR is conserved between teleost species, we injected artificially synthesized mRNA, combining green fluorescent protein (GFP) and the zebrafish nos 1 3'UTR (GFP-nos 1 3'UTR mRNA), into the fertilized eggs of various fish species. The 3'UTR of the Oryzias latipes vasa homologue (olvas) mRNA was assayed in the same manner. We demonstrate that the PGCs of seven teleost species could be visualized using GFP-nos 1 3'UTR mRNA. GFP-olvas 3'UTR mRNA did not identify PGCs in herring or loach embryos, but did enable visualization of the PGCs in medaka embryos. Our results indicate that the 3'UTR of the zebrafish nos1 mRNA can promote maintenance of RNAs in the PGCs of different fish species. Finally, we describe and compare the migration routes of PGCs in seven teleost species. Copyright © 2008 CABI
Descriptors: zebrafish, embryos, germ cells, maternal cytoplasmic factors, messenger RNA, *Danio rerio*, fishes, herrings, *Oryzias latipes*.

Sakaguchi, T., Y. Kikuchi, A. Kuroiwa, H. Takeda, and D.Y. Stainier (2006). **The yolk syncytial layer regulates myocardial migration by influencing extracellular matrix assembly in zebrafish.** *Development* 133(20): 4063-72. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The roles of extra-embryonic tissues in early vertebrate body patterning have been extensively studied, yet we know little about their function during later developmental events. Here, we analyze the function of the zebrafish extra-embryonic yolk syncytial layer (YSL) specific transcription factor, Mtx1, and find that it plays an essential role in myocardial migration. Downregulating the function of Mtx1 in the YSL leads to cardia bifida, a phenotype in which the myocardial cells fail to migrate to the midline. Mtx1 in the extra-embryonic YSL appears to regulate the embryonic expression of fibronectin, a gene previously implicated in myocardial migration. We further show dosage-sensitive genetic interactions between mtx1 and fibronectin. Based on these data, we propose that the extra-embryonic YSL regulates myocardial migration, at least in part by influencing fibronectin expression and subsequent assembly of the extracellular matrix in embryonic tissues.
Descriptors: zebrafish embryos, body patterning genetics, developmental gene expression regulation, heart embryology, membrane, cardiac myocytes physiology, body patterning drug effects, cell movement genetics, chemistry, drug effects, metabolism, endoderm cytology and physiology, fibronectins analysis and genetics, membrane proteins antagonists and inhibitors

and genetics, myocardium chemistry and cytology, cardiac myocytes chemistry and cytology, antisense oligonucleotides pharmacology, yolk sac chemistry and metabolism, immunology, proteins antagonists and inhibitors.

Sakai, J.A. and M.C. Halloran (2006). **Semaphorin 3d guides laterality of retinal ganglion cell projections in zebrafish.** *Development* 133(6): 1035-44. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The optic chiasm is an important choice point at which retinal ganglion cell (RGC) axons either cross the midline to innervate the contralateral brain or turn back to innervate the ipsilateral brain. Guidance cues that regulate this decision, particularly those directing the midline crossing of contralateral axons, are still not well understood. Here we show that Sema3d, a secreted semaphorin expressed at the midline, guides the crossing of RGC axons in zebrafish. Both Sema3d knockdown and ubiquitous overexpression induced aberrant ipsilateral projections, suggesting that Sema3d normally guides axons into the contralateral optic tract. Live imaging in vivo showed that RGC growth cones responded to ubiquitous Sema3d overexpression by pausing for extended periods and increasing their exploratory behavior at the midline, suggesting that Sema3d overexpression causes the midline environment to become less favorable for RGC axon extension. Interestingly, Sema3d overexpression did not affect growth cone behaviors before the midline, suggesting that RGC axons normally respond to Sema3d only upon reaching the midline. After Sema3d knockdown, growth cones grew across the midline but then paused or repeatedly retracted, impairing their ability to leave the midline region. Our results indicate that a proper balance of Sema3d is needed at the midline for the progression of RGC axons from the chiasm midline into the contralateral optic tract.
Descriptors: zebrafish embryos, functional laterality physiology, nerve growth factors metabolism, retinal ganglion cells metabolism, semaphorins metabolism, vision physiology, cell proliferation, antisense DNAgenetics, developmental gene expression regulation, growth cones metabolism, nerve growth factors genetics, retinal ganglion cells cytology, semaphorins genetics, time factors, proteins genetics.

Sanders, L.H. and K.E. Whitlock (2003). **Phenotype of the zebrafish masterblind (mbl) mutant is dependent on genetic background.** *Developmental Dynamics* 227(2): 291-300. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, cell death genetics, eye abnormalities genetics, homeodomain transcription factors genetics, jaw embryology, jaw physiology, mutation, olfactory pathways abnormalities physiology and phenotype, prosencephalon abnormalities and physiology.

Sarmah, B., A.J. Latimer, B. Appel, and S.R. Wente (2005). **Inositol polyphosphates regulate zebrafish left-right asymmetry.** *Developmental Cell* 9(1): 133-45. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Vertebrate body plans have a conserved left-right (LR) asymmetry manifested in the position and anatomy of the heart, visceral organs, and brain. Recent studies have suggested that LR asymmetry is established by asymmetric Ca2+ signaling resulting from cilia-driven flow of extracellular fluid across the node. We report here that inositol 1,3,4,5,6-pentakisphosphate 2-kinase (Ipk1), which generates inositol hexakisphosphate, is critical for normal LR axis determination in zebrafish. Zebrafish embryos express ipk1 symmetrically during gastrulation and early segmentation. ipk1 knockdown by antisense

morpholino oligonucleotide injection randomized LR-specific gene expression and organ placement, effects that were associated with reduced intracellular Ca2+ flux in cells surrounding the ciliated Kupffer's vesicle, a structure analogous to the mouse node. Our data suggest that the pathway for inositol hexakisphosphate production is a key regulator of asymmetric Ca(2+) flux during LR specification.
Descriptors: zebrafish embryos, body patterning, phosphotransferases alcohol group acceptor metabolism, zebrafish proteins metabolism and genetics, amino acid sequence , calcium signaling, metabolism, molecular sequence data, phosphotransferases alcohol group acceptor genetics, phytic acid biosynthesis, amino acid sequence homology.

Sarmah, B., V.P. Winfrey, G.E. Olson, B. Appel, and S.R. Wente (2007). **A role for the inositol kinase Ipk1 in ciliary beating and length maintenance**. *Proceedings of the National Academy of Sciences, USA* 104(50): 19843-19848 . ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, basal bodies, canals, centrosomes, cilia, electron microscopy, embryos, inositol, microtubules, vesicles, *Danio rerio*.

Sarrazin, A.F., E.J. Villablanca, V.A. Nunez, P.C. Sandoval, A. Ghysen, and M.L. Allende (2006). **Proneural gene requirement for hair cell differentiation in the zebrafish lateral line.** *Developmental Biology* 295(2): 534-45. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The lateral line system comprises an array of mechanosensory organs, the neuromasts, distributed over the body surface. Each neuromast consists of a patch of mechanosensory hair cells surrounded by support cells. We show that, in the zebrafish, two proneural genes are essential for differentiation of the hair cells, neuroD (nrd) and atonal homolog 1 (ath1). Gene knockdown experiments demonstrate that loss of function of either gene, but not of the related proneural gene neurogenin1 (ngn1), abrogate the appearance of hair cell markers. This is in contrast to other sensory systems, such as the neurons of the lateral line ganglion, where nrd is regulated by ngn1 and not by ath1. Overexpression of ath1 can induce nrd, and the phenotype produced by loss of ath1 function can be partially rescued by injection of nrd mRNA. This supports the conclusion that the activation of nrd probably requires ath1 in the hair cell lineage, whereas in sensory neurons nrd activation requires ngn1. We propose that the emergence of two atonal homologs, ath1 and ngn1, allowed the cellular segregation of mechanoreception and signal transmission that were originally performed by a single cell type as found in insects.
Descriptors: zebrafish, basic helix loop helix transcription factors genetics, hair cell differentiation genetics and cytology, mechanoreceptors, nerve tissue transcription factors genetics, cell lineage, gene expression regulation physiology, signal transduction genetics, atonal homologs, ath1 and ngn1.

Sassa, T., H. Aizawa, and H. Okamoto (2007). **Visualization of two distinct classes of neurons by gad2 and zic1 promoter/enhancer elements in the dorsal hindbrain of developing zebrafish reveals neuronal connectivity related to the auditory and lateral line systems.** *Developmental Dynamics* 236(3): 706-18 . ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: The dorsal hindbrain includes distinct classes of neurons for processing various sensory stimuli, but the developmental aspects of these neurons remain largely unknown. We identify here two distinct classes of neurons in the dorsal hindbrain of developing zebrafish: (1) neurons that express the inhibitory neuronal marker Gad1/2, and (2) neurons

that express the zn-5 antigen and Lhx2/9 and require the basic helix-loop-helix transcription factor Atoh1a for development. Neurons were traced to their axon terminals by expressing green fluorescent protein using the Gal4VP16-UAS (UAS, upstream activating sequences) system in combination with the promoter/enhancer regions of gad2 for the Gad1/2(+) neurons and zic1 for the zn-5(+)Lhx2/9(+) neurons. The Gad1/2(+) neurons projected to the contralateral hindbrain, while the zn-5(+)Lhx2/9(+) neurons projected to the contralateral midbrain torus semicircularis, suggesting a role in auditory and lateral line sensory processing. Comparison of these projections with those from the cochlear nuclei to the inferior colliculus in mammals suggests similarities across vertebrate species.

Descriptors: zebrafish embryos, glutamate decarboxylase genetics, lateral line system metabolism, neurons metabolism, rhombencephalon metabolism, transcription factors genetics, basic helix loop helix transcription factors genetics and metabolism, enhancer elements genetics, developmental gene expression regulation, glutamate decarboxylase metabolism, green fluorescent proteins genetics and metabolism, immunohistochemistry, in situ hybridization, lateral line system cytology, lateral line system embryology, fluorescence microscopy, neurons cytology, promoter regions genetics, recombinant fusion proteins genetics and metabolism, rhombencephalon cytology and embryology, transcription factors metabolism .

Sato, M. and H.J. Yost (2003). **Cardiac neural crest contributes to cardiomyogenesis in zebrafish.** *Developmental Biology* 257(1): 127-39. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: In birds and mammals, cardiac neural crest is essential for heart development and contributes to conotruncal cushion formation and outflow tract septation. The zebrafish prototypical heart lacks outflow tract septation, raising the question of whether cardiac neural crest exists in zebrafish. Here, results from three distinct lineage-labeling approaches identify zebrafish cardiac neural crest cells and indicate that these cells have the ability to generate MF20-positive muscle cells in the myocardium of the major chambers during development. Fate-mapping demonstrates that cardiac neural crest cells originate both from neural tube regions analogous to those found in birds, as well as from a novel region rostral to the otic vesicle. In contrast to other vertebrates, cardiac neural crest invades the myocardium in all segments of the heart, including outflow tract, atrium, atrioventricular junction, and ventricle in zebrafish. Three distinct groups of premigratory neural crest along the rostrocaudal axis have different propensities to contribute to different segments in the heart and are correspondingly marked by unique combinations of gene expression patterns. Zebrafish will serve as a model for understanding interactions between cardiac neural crest and cardiovascular development.

Descriptors: zebrafish, heart embryology, neural crest embryology, genetically modified, gene expression profiling, reporter genes, HSP70 heat shock proteins genetics and metabolism, lasers, neural crest transplantation.

Sato Maeda, M., H. Tawarayama, M. Obinata, J.Y. Kuwada, and W. Shoji (2006). **Sema3a1 guides spinal motor axons in a cell- and stage-specific manner in zebrafish.** *Development* 133(5): 937-47. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: In order for axons to reach their proper targets, both spatiotemporal regulation of guidance molecules and stepwise control of growth cone sensitivity to guidance molecules is required. Here, we show that, in zebrafish, Sema3a1, a secreted class 3 semaphorin, plays an essential role in guiding the caudal primary (CaP) motor axon that pioneers the initial region of the motor pathway. The expression pattern of Sema3a1 suggests that it delim-

its the pioneer CaP axons to the initial, common pathway via a repulsive action, but then CaP axons become insensitive to Sema3a1 beyond the common pathway. Indeed, nrp1a, which probably encodes a component of the Sema3a1 receptor, is specifically expressed by CaP during the early part of its outgrowth but not during later stages when extending into sema3a1-expressing muscle cells. To examine this hypothesis directly, expression of sema3a1 and/or nrp1a was manipulated in several ways. First, antisense knockdown of Sema3a1 induced CaP axons to branch excessively, stall and/or follow aberrant pathways. Furthermore, dynamic analysis showed they extended more lateral filopodia and often failed to pause at the horizontal myoseptal choice point. Second, antisense knockdown of Nrp1a and double knockdown of Nrp1a/Sema3a1 induced similar outgrowth defects in CaP. Third, CaP axons were inhibited by focally misexpressed sema3a1 along the initial common pathway but not along their pathway beyond the common pathway. Thus, as predicted, Sema3a1 is repulsive to CaP axons in the common region of the pathway, but not beyond the common pathway. Fourth, induced ubiquitous overexpression of sema3a1 caused the CaP axons but not the other primary motor axons to follow aberrant pathways. These results suggest that the repulsive response to Sema3a1 of the primary motor axons along the common pathway is both cell-type specific and dynamically regulated, perhaps via regulation of nrp1a.
Descriptors: zebrafish embryos, axons physiology, growth cones physiology, motor neurons cytology, semaphorins metabolism, spinal cord embryology, proteins metabolism, axons metabolism , growth cones metabolism, motor neurons metabolism, neuropilin 1 genetics and metabolism, semaphorins genetics, spinal cord cytology, spinal cord metabolism, metabolism.

Sato-Maeda, M., M. Obinata, and W. Shoji (2008). **Position fine-tuning of caudal primary motoneurons in the zebrafish spinal cord**. *Development* 135(2): 323-332. ISSN: 0950-1991.
NAL Call Number: QL951.D38
Descriptors: zebrafish, axonogenesis, cell body, cell migration, embryos, motor neurons, signal transduction, spinal cord, neuropilin, semaphorins, somites, *Danio rerio*.

Sawada, A., Y. Nishizaki, H. Sato, Y. Yada, R. Nakayama, S. Yamamoto, N. Nishioka, H. Kondoh, and H. Sasaki (2005). **Tead proteins activate the foxa2 enhancer in the node in cooperation with a second factor**. *Development* 132(21): 4719-4729. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The cell population and the activity of the organizer change during the course of development. We addressed the mechanism of mouse node development via an analysis of the node/notochord enhancer (NE) of Foxa2. We first identified the core element (CE) of the enhancer, which in multimeric form drives gene expression in the node. The CE was activated in Wnt/beta-catenin-treated P19 cells with a time lag, and this activation was dependent on two separate sequence motifs within the CE. These same motifs were also required for enhancer activity in transgenic embryos. We identified the Tead family of transcription factors as binding proteins for the 3' motif. Teads and their co-factor YAP65 activated the CE in P19 cells, and binding of Tead to CE was essential for enhancer activity. Inhibition of Tead activity by repressor-modified Tead compromised NE enhancer activation and notochord development in transgenic mouse embryos. Furthermore, manipulation of Tead activity in zebrafish embryos led to altered expression of foxa2 in the embryonic shield. These results suggest that Tead activates the Foxa2 enhancer core element in the mouse node in cooperation with a second factor that binds to the 5' element, and that a similar mechanism also operates in the zebrafish shield. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, mouse, shield, development, immune system, chemical coordination and homeostasis, cell biology, node development, Tead, Foxa2.

Sbrogna, J.L., M.J. Barresi, and R.O. Karlstrom (2003). **Multiple roles for Hedgehog signaling in zebrafish pituitary development.** *Developmental Biology* 254(1): 19-35. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The endocrine-secreting lobe of the pituitary gland, or adenohypophysis, forms from cells at the anterior margin of the neural plate through inductive interactions involving secreted morphogens of the Hedgehog (Hh), fibroblast growth factor (FGF), and bone morphogenetic protein (BMP) families. To better understand when and where Hh signaling influences pituitary development, we have analyzed the effects of blocking Hh signaling both pharmacologically (cyclopamine treatments) and genetically (zebrafish Hh pathway mutants). While current models state that Shh signaling from the oral ectoderm patterns the pituitary after placode induction, our data suggest that Shh plays a direct early role in both pituitary induction and patterning, and that early Hh signals comes from adjacent neural ectoderm. We report that Hh signaling is necessary between 10 and 15 h of development for induction of the zebrafish adenohypophysis, a time when shh is expressed only in neural tissue. We show that the Hh responsive genes ptc1 and nk2.2 are expressed in preplacodal cells at the anterior margin of the neural tube at this time, indicating that these cells are directly receiving Hh signals. Later (15-20 h) cyclopamine treatments disrupt anterior expression of nk2.2 and Prolactin, showing that early functional patterning requires Hh signals. Consistent with a direct role for Hh signaling in pituitary induction and patterning, overexpression of Shh results in expanded adenohypophyseal expression of lim3, expansion of nk2.2 into the posterior adenohypophysis, and an increase in Prolactin- and Somatolactin-secreting cells. We also use the zebrafish Hh pathway mutants to document the range of pituitary defects that occur when different elements of the Hh signaling pathway are mutated. These defects, ranging from a complete loss of the adenohypophysis (smu/smo and yot/gli2 mutants) to more subtle patterning defects (dtr/gli1 mutants), may correlate to human Hh signaling mutant phenotypes seen in Holoprosencephaly and other congenital disorders. Our results reveal multiple and distinct roles for Hh signaling in the formation of the vertebrate pituitary gland, and suggest that Hh signaling from neural ectoderm is necessary for induction and functional patterning of the vertebrate pituitary gland.
Descriptors: zebrafish embryos, anterior pituitary gland embryology, signal transduction, trans activators metabolism, gene expression, Hedgehog proteins, mutation, genetics.

Schaefer, J. and A. Ryan (2006). **Developmental plasticity in the thermal tolerance of zebrafish *danio rerio*.** *Journal of Fish Biology* 69(3): 722-734. ISSN: 0022 1112.
NAL Call Number: QL614.J68
Abstract: To evaluate developmental plasticity in thermal tolerance of zebrafish *Danio rerio*, common-stock zebrafish were reared from fertilization to adult in the five thermal regimes (two stable, two with constant diel cycles and one stochastic diel cycle) and their thermal tolerance at three acclimation temperatures compared. The energetic cost of developing in the five regimes was assessed by measuring body size over time. While acclimation accounted for most of the variability in thermal tolerance, there were also significant differences among fish reared in the different regimes, regardless of acclimation. Fish reared in more variable environments (as much as +/- 6 degrees C diel cycle) had a greater tolerance than those from non-variable environments at the same mean temperature. Fish from the more variable environments were also significantly smaller than those from non-variable environments. These results indicate that the thermal history of individual zebrafish induces irreversible changes

to the thermal tolerance of adults. (c) 2006 The Authors Journal compilation (c) 2006 The Fisheries Society of the British Isles. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, reproduction, aquaculture, fertilization , body size, developmental plasticity, energetic cost, thermal tolerance, acclimation temperature, rearing system, diel cycle.

Schafer, M., D. Kinzel, C. Neuner, M. Schartl, J.N. Volff, and C. Winkler (2005). **Hedgehog and retinoid signalling confines nkx2.2b expression to the lateral floor plate of the zebrafish trunk.** *Mechanisms of Development* 122(1): 43-56. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: The ventral neural tube of vertebrates consists of distinct neural progenitor domains positioned along the dorsoventral (DV) axis that develop different types of moto- and interneurons. Several signalling molecules, most notably Sonic Hedgehog (Shh), retinoic acid (RA) and fibroblast growth factor (FGF) have been implicated in the generation of these domains. Shh is secreted from the floor plate, the ventral most neural tube structure that consists of the medial (MFP) and the lateral floor plate (LFP). While the MFP is well characterized, organization and function of the LFP remains unclear. Here, we describe the novel homeobox gene nkx2.2b that is strongly expressed in the trunk LFP of zebrafish and thus represents a unique marker for the characterization of LFP formation and the identification of LFP deficient mutants. nkx2.2b and its paralog nkx2.2a (formerly known as nk2.2 and nkx2.2) arose by gene duplication in zebrafish. Both duplicates show significant differences in their expression patterns. For example, while prominent nkx2.2a expression has been described in the ventral brain [Barth, K.A., Wilson, S.W., 1995. Expression of zebrafish nk2.2 is influenced by sonic hedgehog/vertebrate hedgehog-1 and demarcates a zone of neuronal differentiation in the embryonic forebrain. Development 121, 1755-1768], hardly any expression can be found in the trunk LFP, which is in contrast to nkx2.2b. Overexpression, mutant and inhibitor analyses show that nkx2.2b expression in the LFP is up-regulated by Shh, but repressed by retinoids and ectopic islet-1 (isl1) expression. In contrast to previously described zebrafish trunk LFP markers, like e.g. tal2 or foxa2, nkx2.2b is exclusively expressed in the LFP. Thus, it represents a unique tool to analyse the mechanisms of ventral neural tube patterning in zebrafish.
Descriptors: zebrafish embryology, homeodomain trans activators metabolism, transcription factors genetics, tretinoin metabolism, amino acid sequence gene duplication, expression and expression profiling, developmental gene expression regulation, Hedgehog proteins, homeodomain proteins metabolism, molecular sequence data, phylogeny, radiation hybrid mapping, signal transduction, transcription factors metabolism.

Schafer, M., D. Kinzel, and C. Winkler (2007). **Discontinuous organization and specification of the lateral floor plate in zebrafish.** *Developmental Biology* 301(1): 117-29. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The floor plate is a signaling center in the ventral neural tube of vertebrates with important functions during neural patterning and axon guidance. It is composed of a centrally located medial floor plate (MFP) and a bilaterally positioned lateral floor plate (LFP). While the role of the MFP as source of signaling molecules like, e.g., Sonic Hedgehog (Shh) is well understood, the exact organization and function of the LFP are currently unclear. Based on expression analyses, the one cell wide LFP in zebrafish has been postulated to be a homogenous structure. We instead show that the zebrafish trunk LFP is discontinuously arranged. Single LFP cells alternate with p3 neuronal precursor cells, which develop V3

interneurons along the anteroposterior (AP) axis. Our mutant analyses indicate that both, formation of LFP and p3 cells require Delta-Notch signaling. Importantly, however, the two cell types are differentially regulated by Hedgehog (HH) and Nkx2.2 activities. This implicates a novel mechanism of neural tube patterning, in which distinct cell populations within one domain of the ventral neural tube are differently specified along the AP axis. We conclude that different levels of HH and Nkx2.2 activities are responsible for the alternating appearance of LFP and p3 neuronal progenitor cells in the zebrafish ventral neural tube.
Descriptors: zebrafish embryos, body patterning, base sequence, DNA primers, gene expression profiling, Hedgehog proteins metabolism, nervous system embryology, nervous system metabolism, Notch receptors genetics and metabolism, signal transduction.

Schatten, G. (2005). ***Current Topics in Developmental Biology*** , Vol. 71, ELSEVIER ACADEMIC PRESS INC: SAN DIEGO, CA 92101-4495 USA, 360 p. ISBN: 0121531716.
Abstract: This 360-page book is volume 71 in the series Current Topics in Developmental Biology and this volume, like other volumes in the series, provides a comprehensive survey of major issues at the forefront of modern developmental biology and regenerative medicine. This volume is structured into 9 individually-authored chapters. The text (i)s in English and each chapter is individually and extensively referenced. The first chapter focuses on the choroid plexus-CSF system from development to aging, and chapter 2 discusses zebrafish genetics and formation of embryonic vasculature. Topics covered in chapters 3-5 include, respectively: leaf senescence in terms of signals, execution and regulation; muscle stem cells and regenerative myogenesis; and gene regulation in spermatogenesis. Chapter 6 discusses modeling age-related diseases in Drosophila and the seventh chapter focuses on cell death and organ development in plants. Chapter 8 discusses the blood-testis barrier in terms of its biology, regulation and physiological role in spermatogenesis, and the ninth and final chapter deals with angiogenic factors in the pathogenesis of preeclampsia. The final chapter is followed by an index and a reference to the contents of previous volumes. The book also includes a list of the contributors and their respective institutions. This book will be of interest to developmental biologists, cell biologists, geneticists, molecular biologists, and plant and animal biologists. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics, cell biology, development, aging , preeclampsia , vascular disease, reproductive system disease, female, etiology, development, spermatogenesis , aging , cell death, gene regulation, leaf senescence, organ development, regenerative myogenesis, regulating signals, zebrafish.

Schibler, A. and J. Malicki (2007). **A screen for genetic defects of the zebrafish ear.** *Mechanisms of Development* 124(7-8): 592-604. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: To advance the understanding of genetic mechanisms involved in the patterning and the differentiation of the vertebrate auditory system, we screened for mutations affecting ear development in the zebrafish larva. Fifteen recessive mutant alleles have been isolated and analyzed. The phenotypes of these mutants involve abnormalities in ear morphology, otolith formation, or both processes in parallel. Among morphological defects, we found mutations affecting early patterning of the otic vesicle, the morphogenesis of semicircular canals, and the expansion of the ear lumen. The two most severe mutant phenotypes involve the absence of anterior and posterior cristae, as well as a severely misshapen morphology of the ear. In the category of otolith mutants, we found defects in otolith formation, growth, and shape. As it proved to be the case in past screening efforts of this type, these mutant lines represent an asset in the studies of molecular mechanisms that regulate vertebrate ear development.

Descriptors: zebrafish larva, recessive, mutant, alleles, otolith mutants, zebrafish larva, semicircular canals, ear morphology.

Schiffmann, Y. (2006). **Symmetry breaking and convergent extension in early chordate development**. *Progress in Biophysics and Molecular Biology* 92(2): 209-231. ISSN: 0079 6107.
NAL Call Number: 444 P944
Abstract: The initiation of axis, polarity, cell differentiation, and gastrulation in the very early chordate development is due to the breaking of radial symmetry. It is believed that this occurs by an external signal. We suggest instead spontaneous symmetry breaking through the agency of the Turing-Child field. Increased size or decreased diffusivity, both brought about by mitotic activity, cause the spontaneous loss of stability of the homogeneous state and the evolution of the metabolic pattern during development. The polar metabolic pattern is the cause of polar gene expression, polar morphogenesis (gastrulation), and polar mitotic activity. The Turing-Child theory explains not only the spontaneous formation of the invagination in gastrulation but also the coherent cell movement observed in convergence and extension during gastrulation and neurulation. The theory is demonstrated with respect to experimental observations on the early development of fish, amphibian, and the chick. The theory can explain a multitude of experimental details. For example, it explains the splayed polar progression of reduction in the fish blastoderm. Reduction starts on that side of the blastoderm margin, which will initiate invagination several hours later. It progresses toward the blastoderm center and somewhat laterally from this future "dorsal lip". This is precisely as predicted by a Turing-Child system in a circle. And for a fish like zebrafish with a blastoderm that is slightly oval, reduction is observed to progress along the long axis of the ellipse, which is what Turing-Child theory predicts. In general the shape and the chemical nature of the experimental patterns are the same as predicted by the Turing couple (CAMP, ATP). Embryological polarity and convergent extension are based on polar eigenfunction and saddle-shaped eigenfunction, respectively. (c) 2005 Elsevier Ltd. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, bioenergetics, cell differentiation, gastrulation, convergent extension, neurulation, cell movement, metabolic pattern, symmetry breaking, Turing-Child theory.

Schlombs, K., T. Wagner, and J. Scheel (2003). **Site-1 protease is required for cartilage development in zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 100(24): 14024-9. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: Gonzo (goz) is a zebrafish mutant with defects in cartilage formation. The goz phenotype comprises cartilage matrix defects and irregular chondrocyte morphology. Expression of endoderm, mesoderm, and cartilage marker genes is, however, normal, indicating a defect in chondrocyte morphogenesis. The mutated gene responsible for the goz phenotype, identified by positional cloning and confirmed by phosphomorpholino knockdown, encodes zebrafish site-1 protease (s1p). S1P has been shown to process and activate sterol regulatory element-binding proteins (SREBPs), which regulate expression of key enzymes of lipid biosynthesis or transport. This finding is consistent with the abnormal distribution of lipids in goz embryos. Knockdown of site-2 protease, which is also involved in activation of SREBPs, results in similar lipid and cartilage phenotypes as S1P knockdown. However, knockdown of SREBP cleavage-activating protein, which forms a complex with SREBP and is essential for S1P cleavage, results only in lipid phenotypes, whereas cartilage appears normal. This indicates that the cartilage phenoptypes of goz are caused independently of the lipid defects.

Descriptors: zebrafish, cartilage enzymology, growth and development, proprotein Convertases metabolism, serine endopeptidases metabolism, transcription factors, zebrafish growth and development and metabolism, base sequence, ccaat enhancer binding ccaat enhancer binding proteins metabolism, chondrocytes metabolism, chondrogenesis, complementary DNA genetics, DNA binding proteins genetics, proteins genetics and metabolism, endopeptidases genetics, endopeptidases metabolism, developmental gene expression regulation, gene targeting, in situ hybridization, lipid metabolism, molecular sequence data, mutation, phenotype, proprotein Convertases genetics, serine endopeptidases genetics, sterol regulatory element binding protein 1.

Schlueter, P.J., X. Sang, C. Duan, and A.W. Wood (2007). **Insulin-like growth factor receptor 1b is required for zebrafish primordial germ cell migration and survival.** *Developmental Biology* 305(1): 377-87. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Insulin-like growth factor (IGF) signaling is a critical regulator of somatic growth during fetal and adult development, primarily through its stimulatory effects on cell proliferation and survival. IGF signaling is also required for development of the reproductive system, although its precise role in this regard remains unclear. We have hypothesized that IGF signaling is required for embryonic germline development, which requires the specification and proliferation of primordial germ cells (PGCs) in an extragonadal location, followed by directed migration to the genital ridges. We tested this hypothesis using loss-of-function studies in the zebrafish embryo, which possesses two functional copies of the Type-1 IGF receptor gene (igf1ra, igf1rb). Knockdown of IGF1Rb by morpholino oligonucleotides (MO) results in mismigration and elimination of primordial germ cells (PGCs), resulting in fewer PGCs colonizing the genital ridges. In contrast, knockdown of IGF1Ra has no effect on PGC migration or number despite inducing widespread somatic cell apoptosis. Ablation of both receptors, using combined MO injections or overexpression of a dominant-negative IGF1R, yields embryos with a PGC-deficient phenotype similar to IGF1Rb knockdown. TUNEL analyses revealed that mismigrated PGCs in IGF1Rb-deficient embryos are eliminated by apoptosis; overexpression of an antiapoptotic gene (Bcl2l) rescues ectopic PGCs from apoptosis but fails to rescue migration defects. Lastly, we show that suppression of IGF signaling leads to quantitative changes in the expression of genes encoding CXCL-family chemokine ligands and receptors involved in PGC migration. Collectively, these data suggest a novel role for IGF signaling in early germline development, potentially via cross-talk with chemokine signaling pathways.
Descriptors: zebrafish , cell movement physiology, cell survival physiology, germ cells physiology, signal transduction physiology, somatomedins metabolism, proteins metabolism, analysis of variance, Western blotting, chemokines metabolism, DNA primers, in situ hybridization, in situ nick end labeling, fluorescence microscopy, oligonucleotides, reverse transcriptase PCR, somatomedins genetics.

Schlueter, P., G. Peng, Y. Li, M. Westerfield, and C. Duan (2006). **Insulin-like growth factor signaling regulates cell survival and cell cycle progression in developing zebrafish embryos**. *Developmental Biology* 295(1): 360. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, biochemistry and molecular biophysics, development, cell cycle and survival, cell apoptosis, vertebrate development.

Schmidt, K. and J.M. Starck (2005). **Developmental plasticity vs. Phylotypic constraints during early embryogenesis of zebra fish, *danio rerio***. *Integrative and Comparative Biology* 45(6): 1069. ISSN: 1540 7063.
NAL Call Number: QL1.I67
Descriptors: zebrafish embryo, development, embryogenesis, developmental plasticity, phylotypic constraints.

Schmidt, K. and J.M. Starck (2004). **Developmental variability during early embryonic development of zebra fish, *Danio rerio*.** *Journal of Experimental Zoology. Part B. Molecular and Developmental Evolution* 302(5): 446-57. ISSN: 1552-5007.
NAL Call Number: QL1.J87
Abstract: Early vertebrate embryos pass through a period of remarkable morphological similarity. Possible causes for such similarity of early embryos include modularity, developmental constraints, stabilizing selection, canalization, and exhausted genetic variability. Supposedly, each process creates different patterns of variation and covariation of embryonic traits. We study the patterns of variation of the embryonic phenotype to test ideas about possible evolutionary mechanisms shaping the early embryonic development. We use the zebra fish, Danio rerio, as a model organism and apply repeated measures of individual embryos to study temporal changes of phenotypic variability during development. In particular, we are looking at the embryonic development from 12 hours post fertilization until 27 hours post fertilization. During this time period, the development of individual embryos is documented at hourly intervals. We measured maximum diameter of the eye, length of embryo, number of somites, inclination of somites, and the yolk size (as a maternal effect). The coefficient of variation (CV) was used as a measure of variability that was independent of size. We used a principal component analysis for analysis of morphological integration. The experimental setup kept environment x genotype interactions constant. Nongenetic parental contributions had no significant effects on interindividual variability. Thus all observed phenotypic variation was based on additive genetic variance and error variance. The average CV declined from 14% to 7.7%. The decline of the CV was in particular expressed during 15-19 h post fertilization and occurred in association with multiple correlations among embryonic traits and a relatively high degree of morphological integration. We suggest that internal constraints determine the patterns of variability during early embryonic development of zebra fish.
Descriptors: zebrafish, evolution, morphogenesis physiology, phenotype, analysis of variance, biometry, body weights and measures, embryology, eye anatomy and histology, morphogenesis genetics, principal component analysis, somites cytology, time factors, variation genetics.

Schmidt, K.U. and J.M. Starck (2004). **Is early embryonic development of zebrafish, *danio rerio*, accessible to selection? A quantitative genetic approach to study phenotypic variation during early ontogeny**. *Journal of Morphology* 260(3): 326. ISSN: 0362 2525.
NAL Call Number: 444.8J826
Descriptors: zebrafish embryo, development, genetics, correlation analysis, mathematical and computer techniques, genetic breeding, genetic techniques, early ontogeny, embryonic development, natural selection, phenotypic variation.

Schneider, P.N., D.C. Slusarski, and D.W. Houston (2006). **Role of the rgs (regulator of g-protein signaling) domain of axin in vertebrate axis formation**. *Developmental Biology* 295(1): 435. ISSN: 0012 1606.
NAL Call Number: 442.8D49

Descriptors: zebrafish, vertebrate axis formation, cell biology, development, regulation of axin formation.

Schoenebeck, J.J. and D. Yelon (2007). **Illuminating cardiac development: Advances in imaging add new dimensions to the utility of zebrafish genetics.** *Seminars in Cell and Developmental Biology* 18(1): 27-35. ISSN: 1084-9521.
NAL Call Number: QH573
Abstract: The use of the zebrafish as a model organism for the analysis of cardiac development is no longer proof-of-principle science. Over the last decade, the identification of a variety of zebrafish mutations and the subsequent cloning of mutated genes have revealed many critical regulators of cardiogenesis. More recently, increasingly sophisticated techniques for phenotypic characterization have facilitated analysis of the specific mechanisms by which key genes drive cardiac specification, morphogenesis, and function. Future enrichment of the arsenal of experimental strategies available for zebrafish should continue the yield of high returns from such a small source.
Descriptors: zebrafish embryos, heart embryology, microscopy methods, cell movement heart anatomy and histology, heart physiology, biological models.

Scholpp, S. and M. Brand (2003). **Integrity of the midbrain region is required to maintain the diencephalic-mesencephalic boundary in zebrafish no isthmus/pax2.1 mutants.** *Developmental Dynamics* 228(3): 313-22. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryology, DNA binding proteins genetics, diencephalon embryology, mesencephalon embryology, transcription factors genetics, cell division, developmental gene expression regulation genetics , in situ hybridization, neurons cytology, neurons physiology, pax2 transcription factor, proteins.

Scholpp, S., O. Wolf, M. Brand, and A. Lumsden (2006). **Hedgehog signalling from the zona limitans intrathalamica orchestrates patterning of the zebrafish diencephalon.** *Development* 133(5): 855-64. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Midway between the anterior neural border and the midbrain-hindbrain boundary, two well-known local signalling centres in the early developing brain, is a further transverse boundary with putative signalling properties -- the zona limitans intrathalamica (ZLI). Here, we describe formation of the ZLI in zebrafish in relation to expression of sonic hedgehog (shh) and tiggy-winkle hedgehog (twhh), and to development of the forebrain regions that flank the ZLI: the prethalamus and thalamus. We find that enhanced Hh signalling increases the size of prethalamic and thalamic gene expression domains, whereas lack of Hh signalling leads to absence of these domains. In addition, we show that shh and twhh display both unique and redundant functions during diencephalic patterning. Genetic ablation of the basal plate shows that Hh expression in the ZLI alone is sufficient for diencephalic differentiation. Furthermore, acquisition of correct prethalamic and thalamic gene expression is dependent on direct Hh signalling. We conclude that proper maturation of the diencephalon requires ZLI-derived Hh signalling.
Descriptors: zebrafish embryos, body patterning genetics, diencephalon embryology, trans activators metabolism, diencephalon cytology, diencephalon metabolism, embryo cytology, embryo metabolism, gene expression, Hedgehog proteins, mutation, signal transduction, thalamus cytology, thalamus embryology, thalamus metabolism, trans activation genetics, trans activators genetics, Wnt proteins metabolism.

Schonthaler, H.B., J.M. Lampert, J. von Lintig, H. Schwarz, R. Geisler, and S.C. Neuhauss (2005). **A mutation in the silver gene leads to defects in melanosome biogenesis and alterations in the visual system in the zebrafish mutant fading vision.** *Developmental Biology* 284(2): 421-36. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Forward genetic screens have been instrumental in defining molecular components of visual function. The zebrafish mutant fading vision (fdv) has been identified in such a screen due to defects in vision accompanied by hypopigmentation in the retinal pigment epithelium (RPE) and body melanocytes. The RPE forms the outer most layer of the retina, and its function is essential for vision. In fdv mutant larvae, the outer segments of photoreceptors are strongly reduced in length or absent due to defects in RPE cells. Ultrastructural analysis of RPE cells reveals dramatic cellular changes such as an absence of microvilli and vesicular inclusions. The retinoid profile is altered as judged by biochemical analysis, arguing for a partial block in visual pigment regeneration. Surprisingly, homozygous fdv vision mutants survive to adulthood and show, despite a persistence of the hypopigmentation, a partial recovery of retinal morphology. By positional cloning and subsequent morpholino knock-down, we identified a mutation in the silver gene as the molecular defect underlying the fdv phenotype. The Silver protein is required for intralumenal fibril formation in melanosomes by amylogenic cleavage. Our data reveal an unexpected link between melanosome biogenesis and the visual system, undetectable in cell culture.

Descriptors: zebrafish embryo, melanosomes physiology, point mutation, vision physiology, amino acid and base sequence, chromosomes developmental gene expression regulation, genetic markers, genome, homozygote, linkage genetics, melanocytes ultrastructure, melanosomes ultrastructure, molecular sequence data, vertebrate photoreceptors ultrastructure, pigment epithelium of eye ultrastructure, genetic polymorphism, protein sorting signals, tertiary protein structure, radiation hybrid mapping, DNA sequence analysis, protein sequence analysis, amino acid sequence homology, vision genetics, proteins chemistry.

Schottenfeld, J., J. Sullivan Brown, and R.D. Burdine (2007). **Zebrafish curly up encodes a Pkd2 ortholog that restricts left-side-specific expression of southpaw.** *Development* 134(8): 1605-15. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: The zebrafish mutation curly up (cup) affects the zebrafish ortholog of polycystic kidney disease 2, a gene that encodes the Ca(2+)-activated non-specific cation channel, Polycystin 2. We have characterized two alleles of cup, both of which display defects in organ positioning that resemble human heterotaxia, as well as abnormalities in asymmetric gene expression in the lateral plate mesoderm (LPM) and dorsal diencephalon of the brain. Interestingly, mouse and zebrafish pkd2(-/-) mutants have disparate effects on nodal expression. In the majority of cup embryos, the zebrafish nodal gene southpaw (spaw) is activated bilaterally in LPM, as opposed to the complete absence of Nodal reported in the LPM of the Pkd2-null mouse. The mouse data indicate that Pkd2 is responsible for an asymmetric calcium transient that is upstream of Nodal activation. In zebrafish, it appears that pkd2 is not responsible for the activation of spaw transcription, but is required for a mechanism to restrict spaw expression to the left half of the embryo. pkd2 also appears to play a role in the propagation of Nodal signals in the LPM. Based on morpholino studies, we propose an additional role for maternal pkd2 in general mesendoderm patterning.

Descriptors: zebrafish, developmental gene expression regulation, trpp cation channels metabolism, transforming growth factor beta biosynthesis, metabolism, alleles, body pat-

terning, mesoderm metabolism, mice, mutation, transforming growth factor beta genetics, proteins biosynthesisand genetics.

Schottenfeld, J. and R.D. Burdine (2006). **New insights into left-right patterning: the role of pkd2 in the zebrafish**. *Developmental Biology* 295(1): 442. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, development, molecular genetics, nervous system, neural coordination, phenotype.

Schweitzer, J., T. Becker, J. Lefebvre, M. Granato, M. Schachner, and C.G. Becker (2005). **Tenascin-C is involved in motor axon outgrowth in the trunk of developing zebrafish.** *Developmental Dynamics* 234(3): 550-66. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryo, axons metabolism, motor neurons metabolism, tenascin metabolism, amino acid sequence, axons chemistry, cell differentiation, molecular cloning, cysteine metabolism, complementary DNA genetics, epidermal growth factor genetics and metabolism, developmental gene expression regulation, immunohistochemistry, molecular sequence data, motor neurons chemistry, motor neurons cytology, mutation genetics, messenger RNA genetics, sequence alignment, amino acid and homology , tenascin chemistry and genetics, .

Scott, I.C., L.A. D'amico, H. Baier, and D.Y. Stainier (2004). **Analysis of myocardial differentiation in zebrafish.** *Developmental Biology* 271(2): 623. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, cardiovascular system, transport and circulation, molecular genetics, development, myocardial differentiation.

Seiler, C., O. Ben David, S. Sidi, O. Hendrich, A. Rusch, B. Burnside, K.B. Avraham, and T. Nicolson (2004). **Myosin VI is required for structural integrity of the apical surface of sensory hair cells in zebrafish.** *Developmental Biology* 272(2): 328-38. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Unconventional myosins have been associated with hearing loss in humans, mice, and zebrafish. Mutations in myosin VI cause both recessive and dominant forms of nonsyndromic deafness in humans and deafness in Snell's waltzer mice associated with abnormal fusion of hair cell stereocilia. Although myosin VI has been implicated in diverse cellular processes such as vesicle trafficking and epithelial morphogenesis, the role of this protein in the sensory hair cells remains unclear. To investigate the function of myosin VI in zebrafish, we cloned and examined the expression pattern of myosin VI, which is duplicated in the zebrafish genome. One duplicate, myo6a, is expressed in a ubiquitous pattern during early development and at later stages, and is highly expressed in the brain, gut, and kidney. myo6b, on the other hand, is predominantly expressed in the sensory epithelium of the ear and lateral line at all developmental stages examined. Both molecules have different splice variants expressed in these tissues. Using a candidate gene approach, we show that myo6b is satellite, a gene responsible for auditory/vestibular defects in zebrafish larvae. Examination of hair cells in satellite mutants revealed that stereociliary bundles are irregular and disorganized. At the ultrastructural level, we observed that the apical surface of satellite mutant hair cells abnormally protrudes above the epithelium and the membrane near the base of the stereocilia is raised. At later stages, stereocilia fused together. We conclude that zebrafish myo6b is required for maintaining the integrity of the apical surface of hair cells, suggesting a

conserved role for myosin VI in regulation of actin-based interactions with the plasma membrane.
Descriptors: zebrafish embryos, hair cells, inner ultrastructure, myosin heavy chains genetics, alternative splicing, amino acid sequence, cell membrane physiology, cell membrane ultrastructure, gene expression regulation, hair cells, inner physiology, molecular sequence data, mutation, myosin heavy chains metabolism, phylogeny and metabolism.

Seiler, C., K.C. Finger Baier, O. Rinner, Y.V. Makhankov, H. Schwarz, S.C. Neuhauss, and T. Nicolson (2005). **Duplicated genes with split functions: independent roles of protocadherin15 orthologues in zebrafish hearing and vision.** *Development* 132(3): 615-23. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: In the sensory receptors of both the eye and the ear, specialized apical structures have evolved to detect environmental stimuli such as light and sound. Despite the morphological divergence of these specialized structures and differing transduction mechanisms, the receptors appear to rely in part on a shared group of genes for function. For example, mutations in Usher (USH) genes cause a syndrome of visual and acoustic-vestibular deficits in humans. Several of the affected genes have been identified, including the USH1F gene, which encodes protocadherin 15 (PCDH15). Pcdh15 mutant mice also have both auditory and vestibular defects, although visual defects are not evident. Here we show that zebrafish have two closely related pcdh15 genes that are required for receptor-cell function and morphology in the eye or ear. Mutations in pcdh15a cause deafness and vestibular dysfunction, presumably because hair bundles of inner-ear receptors are splayed. Vision, however, is not affected in pcdh15a mutants. By contrast, reduction of pcdh15b activity using antisense morpholino oligonucleotides causes a visual defect. Optokinetic and electroretinogram responses are reduced in pcdh15b morpholino-injected larvae. In electron micrographs, morphant photoreceptor outer segments are improperly arranged, positioned perpendicular to the retinal pigment epithelium and are clumped together. Our results suggest that both cadherins act within their respective transduction organelles: Pcdh15a is necessary for integrity of the stereociliary bundle, whereas Pcdh15b is required for alignment and interdigitation of photoreceptor outer segments with the pigment epithelium. We conclude that after a duplication of pcdh15, one gene retained an essential function in the ear and the other in the eye.
Descriptors: zebrafish, cadherins genetics and metabolism, duplicate hearing and vision physiology, proteins metabolism, electrophysiology, fish gene duplication, developmental gene expression regulation, hearing genetics, electron microscopy, mutation genetics, phenotype, photoreceptors cytology, photoreceptors embryology, metabolism and ultrastructure, retina embryology, metabolism and ultrastructure, sensitivity and specificity, vision genetics.

Seiliez, I., B. Thisse, and C. Thisse (2006). **Foxa3 and goosecold promote anterior neural fate through inhibition of wnt8a activity before the onset of gastrulation**. *Developmental Biology* 290(1): 152-163. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Formation of the nervous system initially requires the acquisition of neural identity, which is achieved through the inhibition of epidermalizing factors. A regional patterning then takes place within the neural plate through the activity of caudalizing factors. These two processes are tightly regulated early in development by the dorsal organizer. Here, we show that, in zebrafish embryos, two transcription factors, FoxA3 and Goosecoid, coexpressed at the dorsal blastula margin, are required for the definition of anterior neural fate. Their inactivation results in deletions of anterior head structures associated with an increase of

Wnt8 activity at the dorsal blastula margin. These phenotypes can be fully rescued by overexpression of Wnt inhibitors or by inactivation of wnt8a. Altogether, foxA3 and goosecoid cooperate to promote formation of anterior neural tissue by protecting, as early as blastula stage, presumptive anterior neural cells from an irreversible caudalization by the posteriorizing factor Wnt8a. (c) 2005 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, nervous system, neural coordination, development, molecular genetics, gastrulation.

Seki, S., T. Kouya, T. Hara, D.M.J. Valdez, Jin Bo, M. Kasai, and K. Edashige (2007). **Exogenous expression of rat aquaporin-3 enhances permeability to water and cryoprotectants of immature oocytes in the zebrafish (*Danio rerio*)**. *Journal of Reproduction and Development* 53(3): 597-604. ISSN: 0916 8818.

NAL Call Number: SF1.K3

Abstract: Movement of water and cryoprotectants through the plasma membrane needs to be accelerated for successful cryopreservation of zebrafish oocytes/embryos, which are much larger than their mammalian counterparts. Aquaporin-3 is a water/solute channel that can transport not only water but also various cryoprotectants. In this study, we attempted to increase the permeability of immature zebrafish oocytes at stage III to water and cryoprotectants by exogenous expression of rat aquaporin-3. Immature zebrafish oocytes were injected with rat aquaporin-3 cRNA and cultured for 5-12 h. Permeability to water and cryoprotectants was then determined based on changes in the volumes of the oocytes in a hypertonic sucrose solution and various cryoprotectant solutions at 25 C. The permeability to water of the aquaporin-3 cRNA-injected oocytes was three times higher than that of intact and water-injected oocytes. The permeability of the aquaporin-3 cRNA-injected oocytes to ethylene glycol, glycerol, propylene glycol, and DMSO was also 2-4 times higher than that of intact oocytes. Thus, the permeability of immature zebrafish oocytes to water and cryoprotectants was enhanced by exogenous expression of aquaporin-3. Cryopreservation of teleost oocytes may be realized through a further increase in permeability. Copyright © 2008 CABI

Descriptors: zebrafish, aquaporin, cryopreservation, cryoprotectants, freezing, oocytes, permeability, plasma membranes, water, *Danio rerio*, rats.

Sekimizu, K., N. Nishioka, H. Sasaki, H. Takeda, R.O. Karlstrom, and A. Kawakami (2004). **The zebrafish iguana locus encodes Dzip1, a novel zinc-finger protein required for proper regulation of Hedgehog signaling.** *Development* 131(11): 2521-32. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Members of the Hedgehog (Hh) family of intercellular signaling molecules play crucial roles in animal development. Aberrant regulation of Hh signaling in humans causes developmental defects, and leads to various genetic disorders and cancers. We have characterized a novel regulator of Hh signaling through the analysis of the zebrafish midline mutant iguana (igu). Mutations in igu lead to reduced expression of Hh target genes in the ventral neural tube, similar to the phenotype seen in zebrafish mutants known to affect Hh signaling. Contradictory at first sight, igu mutations lead to expanded Hh target gene expression in somites. Genetic and pharmacological analyses revealed that the expression of Hh target genes in igu mutants requires Gli activator function but does not depend on Smoothened function. Our results show that the ability of Gli proteins to activate Hh target gene expression in response to Hh signals is generally reduced in igu mutants both in the neural tube and in somites. Although this reduced Hh signaling activity leads to a loss of Hh target gene expression in the neural tube, the same low levels of Hh signaling appear to be sufficient to

activate Hh target genes throughout somites because of different threshold responses to Hh signals. We also show that Hh target gene expression in igu mutants is resistant to increased protein kinase A activity that normally represses Hh signaling. Together, our data indicate that igu mutations impair both the full activation of Gli proteins in response to Hh signals, and the negative regulation of Hh signaling in tissues more distant from the source of Hh. Positional cloning revealed that the igu locus encodes Dzip1, a novel intracellular protein that contains a single zinc-finger protein-protein interaction domain. Overexpression of Igu/Dzip1 proteins suggested that Igu/Dzip1 functions in a permissive way in the Hh signaling pathway. Taken together, our studies show that Igu/Dzip1 functions as a permissive factor that is required for the proper regulation of Hh target genes in response to Hh signals.
Descriptors: zebrafish, carrier proteins metabolism, signal transduction, trans activators metabolism and metabolism, amino acid sequence, body patterning genetics, carrier proteins genetics developmental gene expression regulation, Hedgehog proteins, molecular sequence data, mutation, oncogene oncogene proteins metabolism, peptides genetics, peptides metabolism, quantitative trait loci, G protein Coupled receptors genetics, G protein Coupled receptors metabolism, somites, trans activators genetics, transcription factors genetics and metabolism, zinc fingers.

Semina, E.V., D.V. Bosenko, N.C. Zinkevich, K.A. Soules, D.R. Hyde, T.S. Vihtelic, G.B. Willer, R.G. Gregg, and B.A. Link (2006). **Mutations in laminin alpha 1 result in complex, lens-independent ocular phenotypes in zebrafish.** *Developmental Biology* 299(1): 63-77. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: We report phenotypic and genetic analyses of a recessive, larval lethal zebrafish mutant, bal(a69), characterized by severe eye defects and shortened body axis. The bal(a69) mutation was mapped to chromosome 24 near the laminin alpha 1 (lama1) gene. We analyzed the lama1 gene sequence within bal(a69) embryos and two allelic mutants, bal(arl) and bal(uw1). Missense (bal(a69)), nonsense (bal(arl)), and frameshift (bal(uw1)) alterations in lama1 were found to underlie the phenotypes. Extended analysis of bal(a69) ocular features revealed disrupted lens development with subsequent lens degeneration, focal cornea dysplasia, and hyaloid vasculature defects. Within the neural retina, the ganglion cells showed axonal projection defects and ectopic photoreceptor cells were noted at inner retinal locations. To address whether ocular anomalies were secondary to defects in lens differentiation, bal(a69) mutants were compared to embryos in which the lens vesicle was surgically removed. Our analysis suggests that many of the anterior and posterior ocular defects in bal(a69) are independent of the lens degeneration. Analysis of components of focal adhesion signaling complexes suggests that reduced focal adhesion kinase activation underlies the anterior segment dysgenesis in lama1 mutants. To assess adult ocular phenotypes associated with lama1 mutations, genetic mosaics were generated by transplanting labeled bal cells into ocular-fated regions of wild-type blastulas. Adult chimeric eyes displayed a range of defects including anterior segment dysgenesis and cataracts. Our analysis provides mechanistic insights into the developmental defects and ocular pathogenesis caused by mutations in laminin subunits.
Descriptors: zebrafish embryology, laminin deficiency and genetics, crystalline lens embryology, mutation genetics, amino acid sequence, anterior eye segment abnormalities, cytology and embryology, base sequence, cataract pathology, chromosomes genetics, molecular cloning, DNA mutational analysis, cytology, embryology, focal adhesions, developmental gene expression regulation, laminin chemistry, crystalline lens abnormalities ,cytology and surgery, molecular sequence data , mosaicism, phenotype, messenger RNA genetics and

metabolism, retinal ganglion cells cytology, retinal vessels abnormalities and embryology, zebrafish abnormalities.

Seok, S.H., J.H. Park, M.W. Baek, H.Y. Lee, D.J. Kim, and J.H. Park (2004). **Abnormal larvae development of zebrafish eggs fertilized by intracytoplasmic sperm injection.** *Korean Journal of Laboratory Animal Science* 20(3): 229-33. ISSN: 1225 813X.
Descriptors: zebrafish larvae, semen, fertilization, intracytoplasmic sperm injection, body fluids, body parts, reproduction, sexual reproduction.

Sepich, D.S., C. Calmelet, M. Kiskowski, and L. Solnica Krezel (2005). **Initiation of convergence and extension movements of lateral mesoderm during zebrafish gastrulation.** *Developmental Dynamics* 234(2): 279-92. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, gastrula pathology, gene expression regulation, mesoderm pathology, cell movement, chemotactic factors chemistry, chemotaxis, ectoderm metabolism, endoderm metabolism, gastrula metabolism, mesoderm metabolism, genetic models, movement, STAT3 transcription factor metabolism, signal transduction, time factors, Wnt proteins metabolism.

Seth, A., J. Culverwell, M. Walkowicz, S. Toro, J.M. Rick, S.C. Neuhauss, Z.M. Varga, and R.O. Karlstrom (2006). **Belladonna/(Ihx2) is required for neural patterning and midline axon guidance in the zebrafish forebrain.** *Development* 133(4): 725-35. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Some of the earliest axon pathways to form in the vertebrate forebrain are established as commissural and retinal axons cross the midline of the diencephalon and telencephalon. To better understand axon guidance in the forebrain, we characterized the zebrafish belladonna (bel) mutation, which disrupts commissural and retinal axon guidance in the forebrain. Using a positional cloning strategy, we determined that the bel locus encodes zebrafish Lhx2, a lim-homeodomain transcription factor expressed in the brain, eye and fin buds. We show that bel(Ihx2) function is required for patterning in the ventral forebrain and eye, and that loss of bel function leads to alterations in regulatory gene expression, perturbations in axon guidance factors, and the absence of an optic chiasm and forebrain commissures. Our analysis reveals new roles for Ihx2 in midline axon guidance, forebrain patterning and eye morphogenesis.
Descriptors: zebrafish embryology, axons physiology, body patterning, eye embryology, prosencephalon embryology, amino acid sequence, cell proliferation, diencephalon embryology and metabolism, eye cytology, Fgf metabolism, molecular sequence data , morphogenesis, mutation, nerve tissue proteins genetics and physiology, neuroglia physiology, signal transduction, telencephalon embryology and metabolism.

Shackel, N. (2007). **Zebrafish and the understanding of liver development: the emerging role of the Wnt pathway in liver biology.** *Hepatology* 45(2): 540-1. ISSN: 0270-9139.
Descriptors: zebrafish, liver, development, Wnt, pathway, liver, biology.

Shang, E.H., R.M. Yu, and R.S. Wu (2006). **Hypoxia affects sexdifferentiation and development, leading to a male-dominated population in zebrafish (*Danio rerio*).** *Environmental Science and Technology* 40(9): 3118-22. ISSN: 0013 936X.
NAL Call Number: TD420.A1E5
Abstract: Hypoxia is affecting thousands of square kilometers of water and has caused

declines in fish populations and major changes in aquatic communities worldwide. For the first time, we report that hypoxia can affect sex differentiation and sex development of zebrafish (Danio rerio), leading to a male-biased population in the F1 generation (74.4% +/- 1.7% males in the hypoxic groups versus 61.9% +/- 1.6% males in the normoxic groups, n = 5; $p < 0.05$, chi2 test). The increase in males was associated with downregulations of various genes controlling the synthesis of sex hormones (i.e., 3beta-HSD, CYP11A, CYP19A, and CYP19B) as well as an increase in the testosterone/estradiol ratio. The male-dominated populations caused by hypoxia will have reduced reproductive success, thereby threatening the sustainability of natural fish populations.

Descriptors: zebrafish, anoxia, sex differentiation physiology, DNA primers chemistry, complementary DNA metabolism, environment, estradiol metabolism, gene expression, reverse transcriptase PCR, sex characteristics, sex determination genetics, testosterone metabolism, chemical water pollutants.

Shankaran, S.S., D. Sieger, C. Schroter, C. Czepe, M.C. Pauly, M.A. Laplante, T.S. Becker, A.C. Oates, and M. Gajewski (2007). **Completing the set of h/E(spl) cyclic genes in zebrafish: her12 and her15 reveal novel modes of expression and contribute to the segmentation clock.** *Developmental Biology* 304(2): 615-32. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Somitogenesis is the key developmental process that lays down the framework for a metameric body in vertebrates. Somites are generated from the un-segmented presomitic mesoderm (PSM) by a pre-patterning process driven by a molecular oscillator termed the segmentation clock. The Delta-Notch intercellular signaling pathway and genes belonging to the hairy (h) and Enhancer of split (E(spl))-related (h/E(spl)) family of transcriptional repressors are conserved components of this oscillator. A subset of these genes, called cyclic genes, is characterized by oscillating mRNA expression that sweeps anteriorly like a wave through the embryonic PSM. Periodic transcriptional repression by H/E(spl) proteins is thought to provide a critical part of a negative feedback loop in the oscillatory process, but it is an open question how many cyclic h/E(spl) genes are involved in the somitogenesis clock in any species, and what distinct roles they might play. From a genome-wide search for h/E(spl) genes in the zebrafish, we previously estimated a total of five cyclic members. Here we report that one of these, the mHes5 homologue her15 actually exists as a very recently duplicated gene pair. We investigate the expression of this gene pair and analyse its regulation and activity in comparison to the paralogous her12 gene, and the other cyclic h/E(spl) genes in the zebrafish. The her15 gene pair and her12 display novel and distinct expression features, including a caudally restricted oscillatory domain and dynamic stripes of expression in the rostral PSM that occur at the future segmental borders. her15 expression stripes demarcate a unique two-segment interval in the rostral PSM. Mutant, morpholino, and inhibitor studies show that her12 and her15 expression in the PSM is regulated by Delta-Notch signaling in a complex manner, and is dependent on her7, but not her1 function. Morpholino-mediated her12 knockdown disrupts cyclic gene expression, indicating that it is a non-redundant core component of the segmentation clock. Over-expression of her12, her15 or her7 disrupts cyclic gene expression and somite border formation, and structure function analysis of Her7 indicates that DNA binding, but not Groucho-recruitment seems to be important in this process. Thus, the zebrafish has five functional cyclic h/E(spl) genes, which are expressed in a distinct spatial configuration. We propose that this creates a segmentation oscillator that varies in biochemical composition depending on position in the PSM.

Descriptors: zebrafish embryos, basic helix loop helix transcription factors physiology, repressor, amino acid sequence, basic helix loop helix transcription factors biosynthesis and

genetics, biological clocks, body patterning, developmental gene expression regulation, genome, mesoderm metabolism, molecular sequence data, messenger RNA biosynthesis, repressor proteins biosynthesis and genetics, metabolism, proteins biosynthesis.

Sharma, D., L. Holets, X. Zhang, and W.H. Kinsey (2005). **Role of Fyn kinase in signaling associated with epiboly during zebrafish development.** *Developmental Biology* 285(2): 462-76. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The function of Fyn kinase during zebrafish development through the blastula stage was investigated through the use of dominant-negative constructs designed to suppress the function of zebrafish c-Fyn. Microinjection of SH2 domain-containing fusion protein or mRNA encoding the mutated, catalytically inactive Fyn at 45 min post-insemination had no significant effect during cleavage and did not inhibit formation of the yolk syncitial layer. Smoothing of the enveloping cell layer at the midblastula transition occurred normally and expression of bon/mixer and mezzo, zygotic transcription factors indicated that activation of the zygotic genome did occur. Signaling pathways involved with axis determination such as beta-catenin, activin, and nodal appeared to function normally as evidenced by expression of boz, goosecoid, and mezzo. However, while formation of the yolk syncitial layer was normal, the marginal blastomeres failed to migrate toward the vegetal pole and epiboly did not occur, a phenotype similar but distinct from that resulting from suppression of c-Yes kinase. The block to development was prevented by co-injection of c-Fyn mRNA with the dominant-negative construct indicating that it was a specific effect. Injection of the dominant-negative mRNA into individual blastomeres indicated that the effect was exerted on the intrinsic ability of the individual blastomeres to respond to signals directing epiboly and not on the signals themselves. Analysis of the pattern of calcium signaling in experimental and control embryos demonstrated that the elevated [Ca2+]i characteristic of the marginal blastomeres was suppressed. Together, these observations indicate that Fyn kinase plays an important role in epiboly, possibly through its effects in calcium signaling.

Descriptors: zebrafish embryology, blastomeres physiology, calcium signaling physiology, cell movement physiology, embryonic development physiology, proto oncogene proteins C fyn metabolism, proteins metabolism, blastomeres metabolism, cell movement genetics, molecular cloning, ectoderm physiology, immunoprecipitation, in situ hybridization, mutagenesis, site directed.

Sharma, D. and W.H. Kinsey (2006). **Fertilization triggers localized activation of Src-family protein kinases in the zebrafish egg.** *Developmental Biology* 295(2): 604-14. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Fertilization triggers activation of Src-family kinases in eggs of various species including marine invertebrates and lower vertebrates. While immunofluorescence studies have localized Src-family kinases to the plasma membrane or cortical cytoplasm, no information is available regarding the extent to which these kinases are activated in different regions of the zygote. The objective of the present study was to detect the subcellular distribution of activated Src-family kinases in the fertilized zebrafish egg. An antibody specific for the active, non-phosphorylated form of Src-family PTKs was used to detect these activated kinases by immunofluorescence. The results demonstrate that Fyn, and possibly other Src family members are activated by dephosphorylation of the C-terminal tyrosine at fertilization. The activated Src-family kinases are asymmetrically distributed around the egg cortex with an area of higher kinase activity localized adjacent to the micropyle near the presumptive animal

pole. Fertilization initially caused elevation of kinase activity in the cytoplasm underlying the micropyle, but this quickly spread to involve the entire zygote cortex. Later, during egg activation, formation of the blastodisc involved concentration of active Src-family kinase in the blastodisc cortex. As cytokinesis began, activated Src-family kinases were no longer limited to the cortex, but became more evenly distributed in the clear apical cytoplasm of the blastomeres. The results demonstrate that the cortex of the zebrafish egg is functionally differentiated and that fertilization triggers localized activation of Src-family kinases at the point of sperm entry, which subsequently progresses through the entire egg cortex.
Descriptors: zebrafish eggs, fertilization physiology, ovum physiology, proto oncogene proteins C fyn metabolism, src family kinases metabolism , cytokinesis, enzyme activation, fluorescent antibody technique, ovum enzymology, phosphorylation, protein transport, tissue distribution.

Shaw, K., D. Castranova, V. Pham, M. Kamei, K. Kidd, B. Lo, J. TorresVasquez, A. Ruby, and B. Weinstein (2006). **Fused-somites-like mutants exhibit defects in trunk vessel patterning**. *Developmental Dynamics* 235(7): 1753-1760. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: We identified four mutants in two distinct loci exhibiting similar trunk vascular patterning defects in an F3 genetic screen for zebrafish vascular mutants. Initial vasculogenesis is not affected in these mutants, with proper specification and differentiation of endothelial cells. However, all four display severe defects in the growth and patterning of angiogenic vessels in the trunk, with ectopic branching and disoriented migration of intersegmental vessels. The four mutants are allelic to previously characterized mutants at the fused-somites (fss) and beamter (bea) loci, and they exhibit comparable defects in trunk somite boundary formation. The fss locus has been shown to correspond to tbx24; we show here that bea mutants are defective in the zebrafish dlC gene. Somitic expression of known vascular guidance factors efnb2a, sema3a1, and sema3a2 is aberrantly patterned in fss and bea mutants, suggesting that the vascular phenotype is due to loss of proper guidance cues provided by these factors. Copyright©Thomson Reuters 2009
Descriptors: zebrafish mutants, molecular genetics, biochemistry and molecular biophysics, phenotype development, vasculogenesis.

Sheela, S.G., W.C. Lee, W.W. Lin, and B.C. Chung (2005). **Zebrafish ftz-f1a (nuclear receptor 5a2) functions in skeletal muscle organization.** *Developmental Biology* 286(2): 377-90. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Fushi-tarazu factor 1a (Ftz-F1a, Ff1a, Nr5a2) is a nuclear receptor with diverse functions in many tissues. Here, we report the function of ff1a in zebrafish muscle differentiation. In situ hybridization revealed that ff1a mRNA was present in the adaxial and migrating slow muscle precursors and was down-regulated when slow muscle cells matured. This expression was under the control of hedgehog genes, expanded when hedgehog was increased and missing in mutants defective in genes in the Hedgehog pathway like you-too (yot), sonic you (syu), and u-boot (ubo). Blocking ff1a activity by injecting a deleted form of ff1a or an antisense ff1a morpholino oligo into fish embryos caused thinner and disorganized fibers of both slow and fast properties. Transient expression of ff1a in syu, ubo, and yot embryos led to more fibril bundles, even when slow myoblasts were transfated into fast properties. We showed that ff1a and prox1 complemented each other in slow myofibril assembly, but they did not affect the expression of each other. These results demonstrate that ff1a functions in both slow and fast muscle morphogenesis in response to Hedgehog signaling, and

this function parallels the activity of another slow muscle gene, prox1.
Descriptors: zebrafish embryology, homeodomain, morphogenesis, skeletal muscle growth and development, cytoplasmic receptors and nuclear physiology, transcription factors physiology, Hedgehog proteins, homeodomain fast twitch muscle fibers and slow twitch, messenger RNA analysis, cytoplasmic receptors and nuclear genetics, trans activators physiology, transcription factors genetics.

Shen, Y.C. and P.A. Raymond (2004). **Zebrafish cone-rod (crx) homeobox gene promotes retinogenesis.** *Developmental Biology* 269(1): 237-51. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The mammalian Cone-rod homeobox (Crx) gene is a divergent member of the Otx gene family known to be involved in differentiation and survival of retinal photoreceptors and photoentrainment of circadian rhythms. Zebrafish have two genes in the Otx5/crx orthology class, and we previously showed that crx can transactivate rhodopsin expression in vitro, and that otx5 (orthodenticle-related gene), but not crx, regulates expression of circadian genes in the pineal. Here, we show that zebrafish crx does not regulate expression of opsins and other photoreceptor-specific genes in the pineal. We further show that crx is expressed in proliferating retinal progenitors and may be involved in patterning the early optic primordium and in promoting the differentiation of retinal progenitors, including photoreceptors. These results suggest novel functions for zebrafish crx during retinal specification and differentiation.
Descriptors: zebrafish embryology, retina embryology, trans activators genetics, body patterning genetics and physiology, cell differentiation physiology, homeodomain proteins metabolism and genetics, neurons metabolism, retina metabolism, trans activators metabolism, metabolism.

Shepherd, I.T., J. Pietsch, S. Elworthy, R.N. Kelsh, and D.W. Raible (2004). **Roles for GFRalpha1 receptors in zebrafish enteric nervous system development.** *Development* 131(1): 241-9. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Components of the zebrafish GDNF receptor complex are expressed very early in the development of enteric nervous system precursors, and are already present as these cells begin to enter the gut and migrate caudally along its length. Both gfra1a and gfra1b as well as ret are expressed at this time, while gfra2 expression, the receptor component that binds the GDNF-related ligand neurturin, is not detected until the precursors have migrated along the gut. Gfra genes are also expressed in regions of the zebrafish brain and peripheral ganglia, expression domains conserved with other species. Enteric neurons are eliminated after injection with antisense morpholino oligonucleotides against ret or against both Gfra1 orthologs, but are not affected by antisense oligonucleotides against gfra2. Blocking GDNF signaling prevents migration of enteric neuron precursors, which remain positioned at the anterior end of the gut. Phenotypes induced by injection of antisense morpholinos against both Gfra orthologs can be rescued by introduction of mRNA for gfra1a or for gfra2, suggesting that GFRalpha1 and GFRalpha2 are functionally equivalent.
Descriptors: zebrafish embryos, nervous system embryology, proto oncogene receptor protein tyrosine kinases genetics, amino acid sequence, chickens, conserved sequence , physiology, glial cell line derived neurotrophic factor receptors, in situ hybridization, molecular sequence data, proto oncogene proteins chemistry and proteins C ret, receptor protein tyrosine kinases chemistry, sequence alignment, amino acid sequence homology, proteins.

Shi, X., D.V. Bosenko, N.S. Zinkevich, S. Foley, D.R. Hyde, E.V. Semina, and T.S. Vihtelic (2005). **Zebrafish pitx3 is necessary for normal lens and retinal development.** *Mechanisms of Development* 122(4): 513-27. ISSN: 0925-4773.

NAL Call Number: QH607.A1C4

Abstract: The human PITX3 gene encodes a bicoid-like homeodomain transcription factor associated with a variety of congenital ocular conditions, including anterior segment dysgenesis, Peter's anomaly, and cataracts. We identified a zebrafish pitx3 gene encoding a protein (Pitx3) that possesses 63% amino acid identity with human PITX3. The zebrafish pitx3 gene encompasses approximately 16.5kb on chromosome 13 and consists of four exons, which is similar to the genomic organization of other pitx genes. Expression of the zebrafish pitx3 gene was studied by in situ mRNA hybridization and RT-PCR. The pitx3 transcripts were detected throughout development with the greatest level of expression occurring in the developing lens and brain at 24hpf. In adults, the highest expression was detected in the eye. Morpholinos were used to knockdown expression of the Pitx3 protein and a control morpholino that contains five mismatched bases was used to confirm the specificity of the phenotypes. The morphants had small eyes, misshapen heads and reduced jaws and fins relative to controls. The morphants exhibited abnormalities in lens development and their retinas contained pyknotic nuclei accompanied by a reduction in the number of cells in different neuronal classes. This suggests the lens is required for retinal development or Pitx3 has an unexpected role in retinal cell differentiation or survival. These results demonstrate zebrafish pitx3 represents a true ortholog of the human PITX3 gene and the general function of the Pitx3 protein in lens development is conserved between mammals and the teleost fish.

Descriptors: zebrafish embryos, homeodomain proteins metabolism, crystalline lens embryology, crystalline lens metabolism, retina embryology and metabolism, actins metabolism, amino acid sequence, genetically modified, molecular cloning, embryology, metabolism, developmental gene expression regulation genetics, homeodomain proteins chemistry and genetics, immunohistochemistry, molecular sequence data, phenotype, sequence alignment.

Shi, X., Y. Luo, S. Howley, A. Dzialo, S. Foley , D.R. Hyde, and T.S. Vihtelic (2006). **Zebrafish foxe3: roles in ocular lens morphogenesis through interaction with pitx3.** *Mechanisms of Development* 123(10): 761-82. ISSN: 0925-4773.

NAL Call Number: QH607.A1C4

Abstract: Foxe3 is a winged helix/forkhead domain transcription factor necessary for mammalian and amphibian lens development. Human FOXE3 mutations cause anterior segment dysgenesis and cataracts. The zebrafish foxe3 cDNA was PCR amplified from 24 h post-fertilization (hpf) embryo cDNA. The zebrafish foxe3 gene consists of a single exon on chromosome 8 and encodes a 422 amino acid protein. This protein possesses 44% and 67% amino acid identity with the human FOXE3 and Xenopus FoxE4 proteins, respectively. A polyclonal antiserum was generated against a bacterial fusion protein containing the Foxe3 carboxyl terminus. The purified antiserum detects zebrafish Foxe3 on immunoblots, in embryo wholemounts, and frozen tissue sections. The zebrafish Foxe3 protein is first detected in the lens at 31hpf and is restricted to the nucleated cell population, including the epithelial and elongating fiber cells. Knockdown of Foxe3 protein using an antisense morpholino results in small lenses with multilayered epithelial cells and fiber cell dysmorphogenesis. The morphants posses normal retinas, although retinal cell proteins, including rhodopsin, are abnormally expressed in the morphant lens tissue. Functional interactions between foxe3 and pitx3 during lens development were assessed by RT-PCR and comparison of Foxe3 and Pitx3 protein expression in both foxe3 and pitx3 morphants. Immunoblots and immunohistochemistry reveal Pitx3 is expressed in the foxe3 morphant lens, while Pitx3 knockdown

results in the elimination of Foxe3 expression. These data demonstrate that Foxe3 is necessary for lens development in zebrafish and that foxe3 lies genetically downstream of pitx3 in a zebrafish lens development pathway.

Descriptors: zebrafish embryology, eye proteins metabolism, eye lens development, forkhead transcription factors metabolism, homeodomain proteins metabolism, crystalline lens embryology, morphogenesis, proteins metabolism, amino acid sequence, biological markers metabolism, eye proteins classification and genetics, forkhead transcription factors classification andgenetics , homeodomain crystalline lens anatomy, histology and metabolism, molecular sequence data, antisense oligonucleotides genetics and metabolism, phylogeny, sequence alignment, metabolism, proteins classification.

Shimada, N. and S.J. Moorman (2006). **Changes in gravitational force cause changes in gene expression in the lens of developing zebrafish.** *Developmental Dynamics* 235(10): 2686-94. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryos, eye development, crystalline lens metabolism, weightlessness, genetically modified, apoptosis genetics and physiology, Northern blotting, cell nucleus metabolism, developmental gene expression regulation genetics, green fluorescent proteins genetics and metabolism, HSP70 heat shock proteins genetics, metabolism and physiology, heat shock proteins genetics, metabolism and physiology, in situ hybridization methods, in situ nick end labeling, crystalline lens embryology, messenger RNA genetics and metabolism.

Shimada, N., G. Sokunbi, and S.J. Moorman (2005). **Changes in gravitational force affect gene expression in developing organ systems at different developmental times**. *Bio Med Central Developmental Biology* 5 ISSN: 1471-213X.

NAL Call Number: QH491.B63

Descriptors: zebrafish embryos, actin, embryonic development, freshwater fish, gene expression, neurons, promoters, *Danio rerio.*

Shimizu, T., Y.K. Bae, and M. Hibi (2006). **Cdx-Hox code controls competence for responding to Fgfs and retinoic acid in zebrafish neural tissue.** *Development* 133(23): 4709-19. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Fibroblast growth factor (Fgf) and retinoic acid (RA) signals control the formation and anteroposterior patterning of posterior hindbrain. They are also involved in development processes in other regions of the embryo. Therefore, responsiveness to Fgf and RA signals must be controlled in a context-dependent manner. Inhibiting the caudal-related genes cdx1a and cdx4 in zebrafish embryos caused ectopic expression of genes that are normally expressed in the posterior hindbrain and anterior spinal cord, and ectopic formation of the hindbrain motor and commissure neurons in the posteriormost neural tissue. Combinational marker analyses suggest mirror-image duplication in the Cdx1a/4-defective embryos, and cell transplantation analysis further revealed that Cdx1a and Cdx4 repress a posterior hindbrain-specific gene expression cell-autonomously in the posterior neural tissue. Expression of fgfs and retinaldehyde dehydrogenase 2 suggested that in the Cdx1a/4-defective embryos, the Fgf and RA signaling activities overlap in the posterior body and display opposing gradients, compared with those in the hindbrain region. We found that Fgf and RA signals were required for ectopic expression. Expression of the posterior hox genes hoxb7a, hoxa9a or hoxb9a, which function downstream of Cdx1a/4, or activator fusion genes of hoxa9a or hoxb9a (VP16-hoxa9a, VP16-hoxb9a) suppressed this loss-of-function phenotype. These

data suggest that Cdx suppresses the posterior hindbrain fate through regulation of the posterior hox genes; the posterior Hox proteins function as transcriptional activators and indirectly repress the ectopic expression of the posterior hindbrain genes in the posterior neural tissue. Our results indicate that the Cdx-Hox code modifies tissue competence to respond to Fgfs and RA in neural tissue.

Descriptors: zebrafish embryology, rhombencephalon embryology, Fgf pharmacology, developmental gene expression regulation, gene targeting, homeobox genes, homeodomain proteins genetics and metabolism, rhombencephalon drug effects and metabolism, signal transduction, spinal cord drug effects, embryology and metabolism, transcription factors deficiency, genetics and metabolism, tretinoin pharmacology, metabolism, proteins deficiency.

Shimizu, T., T. Yabe, O. Muraoka, S. Yonemura, S. Aramaki, K. Hatta, Y.K. Bae, H. Nojima , and M. Hibi (2005). **E-cadherin is required for gastrulation cell movements in zebrafish.** *Mechanisms of Development* 122(6): 747-63. ISSN: 0925-4773.

NAL Call Number: QH607.A1C4

Abstract: E-cadherin is a member of the classical cadherin family and is known to be involved in cell-cell adhesion and the adhesion-dependent morphogenesis of various tissues. We isolated a zebrafish mutant (cdh1(rk3)) that has a mutation in the e-cadherin/cdh1 gene. The mutation rk3 is a hypomorphic allele, and the homozygous mutant embryos displayed variable phenotypes in gastrulation and tissue morphogenesis. The most severely affected embryos displayed epiboly delay, decreased convergence and extension movements, and the dissociation of cells from the embryos, resulting in early embryonic lethality. The less severely affected embryos survived through the pharyngula stage and showed flattened anterior neural tissue, abnormal positioning and morphology of the hatching gland, scattered trigeminal ganglia, and aberrant axon bundles from the trigeminal ganglia. Maternal-zygotic cdh1(rk3) embryos displayed epiboly arrest during gastrulation, in which the enveloping layer (EVL) and the yolk syncytial layer but not the deep cells (DC) completed epiboly. A similar phenotype was observed in embryos that received antisense morpholino oligonucleotides (cdh1MO) against E-cadherin, and in zebrafish epiboly mutants. Complementation analysis with the zebrafish epiboly mutant weg suggested that cdh1(rk3) is allelic to half baked/weg. Immunohistochemistry with an anti-beta-catenin antibody and electron microscopy revealed that adhesion between the DCs and the EVL was mostly disrupted but the adhesion between DCs was relatively unaffected in the MZcdh1(rk3) mutant and cdh1 morphant embryos. These data suggest that E-cadherin-mediated cell adhesion between the DC and EVL plays a role in the epiboly movement in zebrafish.

Descriptors: zebrafish embryos, cadherins physiology, gastrula physiology, developmental gene expression regulation, mutation, alleles, base sequence, boron compounds pharmacology, cadherins genetics and metabolism, cell adhesion, cell movement, exons, immunohistochemistry, in situ hybridization, indoles pharmacology, electron microscopy, molecular sequence data, movement, neurons metabolism , oligonucleotides pharmacology, phenotype, nucleic acid sequence homology, temperature, zygote.

Shimoda, N., K. Yamakoshi, A. Miyake, and H. Takeda (2005). **Identification of a gene required for de novo DNA methylation of the zebrafish no tail gene.** *Developmental Dynamics* 233(4): 1509-16. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryology, DNA cytosine 5 methyltransferase genetics, DNA methylation, developmental gene expression regulation physiology, T-box domain proteins

genetics and metabolism, proteins genetics and metabolism, catalytic domain genetics, DNA cytosine 5 methyltransferase metabolism and physiology, molecular evolution, oligonucleotides.

Shin, D., C.H. Shin, J. Tucker, E.A. Ober, F. Rentzsch, K.D. Poss, M. Hammerschmidt, M.C. Mullins, and D.Y. Stainier (2007). **Bmp and Fgf signaling are essential for liver specification in zebrafish.** *Development* 134(11): 2041-50. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Based on data from in vitro tissue explant and ex vivo cell/bead implantation experiments, Bmp and Fgf signaling have been proposed to regulate hepatic specification. However, genetic evidence for this hypothesis has been lacking. Here, we provide in vivo genetic evidence that Bmp and Fgf signaling are essential for hepatic specification. We utilized transgenic zebrafish that overexpress dominant-negative forms of Bmp or Fgf receptors following heat-shock induction. These transgenes allow one to bypass the early embryonic requirements for Bmp and Fgf signaling, and also to completely block Bmp or Fgf signaling. We found that the expression of hhex and prox1, the earliest liver markers in zebrafish, was severely reduced in the liver region when Bmp or Fgf signaling was blocked just before hepatic specification. However, hhex and prox1 expression in adjacent endodermal and mesodermal tissues appeared unaffected by these manipulations. Additional genetic studies indicate that the endoderm maintains competence for Bmp-mediated hepatogenesis over an extended window of embryonic development. Altogether, these data provide the first genetic evidence that Bmp and Fgf signaling are essential for hepatic specification, and suggest that endodermal cells remain competent to differentiate into hepatocytes for longer than anticipated.
Descriptors: zebrafish embryology, hhex, prox1, expression, endodermal, mesodermal, tissue, Bmp, FGF, signaling, liver, hepatocytes.

Shin, J., J. Poling, H.C. Park, and B. Appel (2007). **Notch signaling regulates neural precursor allocation and binary neuronal fate decisions in zebrafish.** *Development* 134(10): 1911-20. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Notch signaling plays a well-described role in regulating the formation of neurons from proliferative neural precursors in vertebrates but whether, as in flies, it also specifies sibling cells for different neuronal fates is not known. Ventral spinal cord precursors called pMN cells produce mostly motoneurons and oligodendrocytes, but recent lineage-marking experiments reveal that they also make astrocytes, ependymal cells and interneurons. Our own clonal analysis of pMN cells in zebrafish showed that some produce a primary motoneuron and KA' interneuron at their final division. We investigated the possibility that Notch signaling regulates a motoneuron-interneuron fate decision using a combination of mutant, transgenic and pharmacological manipulations of Notch activity. We show that continuous absence of Notch activity produces excess primary motoneurons and a deficit of KA' interneurons, whereas transient inactivation preceding neurogenesis results in an excess of both cell types. By contrast, activation of Notch signaling at the neural plate stage produces excess KA' interneurons and a deficit of primary motoneurons. Furthermore, individual pMN cells produce similar kinds of neurons at their final division in mib mutant embryos, which lack Notch signaling. These data provide evidence that, among some postmitotic daughters of pMN cells, Notch promotes KA' interneuron identity and inhibits primary motoneuron fate, raising the possibility that Notch signaling diversifies vertebrate neuron type by mediating similar binary fate decisions.

Descriptors: zebrafish, developmental gene expression regulation, homeodomain proteins genetics and physiology, nerve tissue proteins genetics and physiology, neurons metabolism, Notch1 receptor genetics and physiology and physiology, genetically modified, cell lineage, developmental biology, immunohistochemistry, interneurons metabolism, neurological models, motor neurons metabolism, mutation, oligodendroglia cytology, signal transduction.

Shkumatava, A., S. Fischer, F. Muller, U. Strahle, and C.J. Neumann (2004). **Sonic Hedgehog, secreted by amacrine cells, acts as a short-range signal to direct differentiation and lamination in the zebrafish retina.** *Development* 131(16): 3849-58. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Neurogenesis in the zebrafish retina occurs in several waves of differentiation. The first neurogenic wave generates ganglion cells and depends on hedgehog (hh) signaling activity. Using transgenic zebrafish embryos that express GFP under the control of the sonic hedgehog (shh) promoter, we imaged the differentiation wave in the retina and show that, in addition to the wave in the ganglion cell layer, shh expression also spreads in the inner nuclear layer. This second wave generates amacrine cells expressing shh, and although it overlaps temporally with the first wave, it does not depend on it, as it occurs in the absence of ganglion cells. We also show that differentiation of cell types found in the inner and outer nuclear layers, as well as lamination of the retina, depends on shh. By performing mosaic analysis, we demonstrate that Shh directs these events as a short-range signal within the neural retina.
Descriptors: zebrafish embryos, amacrine cells metabolism, trans activators metabolism, amacrine cells embryology, cell differentiation physiology, reporter genes , Hedgehog proteins, photoreceptors embryology and physiology, signal transduction physiology.

Shoji, W., S. Isogai, M. Sato Maeda, M. Obinata, and J.Y. Kuwada (2003). **Semaphorin3a1 regulates angioblast migration and vascular development in zebrafish embryos.** *Development* 130(14): 3227-36. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Semaphorins are a large family of secreted and cell surface molecules that guide neural growth cones to their targets during development. Some semaphorins are expressed in cells and tissues beyond the nervous system suggesting the possibility that they function in the development of non-neural tissues as well. In the trunk of zebrafish embryos endothelial precursors (angioblasts) are located ventral and lateral to the somites. The angioblasts migrate medially and dorsally along the medial surface of the somites to form the dorsal aorta just ventral to the notochord. Here we show that in zebrafish Sema3a1 is involved in angioblast migration in vivo. Expression of sema3a1 in somites and neuropilin 1, which encodes for a component of the Sema3a receptor, in angioblasts suggested that Sema3a1 regulates the pathway of the dorsally migrating angioblasts. Antisense knockdown of Sema3a1 inhibited the formation of the dorsal aorta. Induced ubiquitous expression of sema3a1 in hsp70:(gfp) sema3a1(myc) transgenic embryos inhibited migration of angioblasts ventral and lateral to the somites and retarded development of the dorsal aorta, resulting in severely reduced blood circulation. Furthermore, analysis of cells that express angioblast markers following induced expression of sema3a1 or in a mutant that changes the expression of sema3a1 in the somites confirmed these results. These data implicate Sema3a1, a guidance factor for neural growth cones, in the development of the vascular system.
Descriptors: zebrafish, physiologic neovascularization, semaphorin 3a metabolism and physiology, genetically modified, Western blotting, cell movement, green fluorescent proteins,

in situ hybridization, luminescent proteins metabolism, mesoderm pathology, fluorescence microscopy, neuropilins metabolism, antisense oligonucleotides metabolism.

Shu, X., K. Cheng, N. Patel, F. Chen, E. Joseph, H.J. Tsai, and J.N. Chen (2003). **Na,K-ATPase is essential for embryonic heart development in the zebrafish.** *Development* 130(25): 6165-73. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Na,K-ATPase is an essential gene maintaining electrochemical gradients across the plasma membrane. Although previous studies have intensively focused on the role of Na,K-ATPase in regulating cardiac function in the adults, little is known about the requirement for Na,K-ATPase during embryonic heart development. Here, we report the identification of a zebrafish mutant, heart and mind, which exhibits multiple cardiac defects, including the primitive heart tube extension abnormality, aberrant cardiomyocyte differentiation, and reduced heart rate and contractility. Molecular cloning reveals that the heart and mind lesion resides in the alpha1B1 isoform of Na,K-ATPase. Blocking Na,K-ATPase alpha1B1 activity by pharmacological means or by morpholino antisense oligonucleotides phenocopies the patterning and functional defects of heart and mind mutant hearts, suggesting crucial roles for Na,K-ATPase alpha1B1 in embryonic zebrafish hearts. In addition to alpha1B1, the Na,K-ATPase alpha2 isoform is required for embryonic cardiac patterning. Although the alpha1B1 and alpha2 isoforms share high degrees of similarities in their coding sequences, they have distinct roles in patterning zebrafish hearts. The phenotypes of heart and mind mutants can be rescued by supplementing alpha1B1, but not alpha2, mRNA to the mutant embryos, demonstrating that alpha1B1 and alpha2 are not functionally equivalent. Furthermore, instead of interfering with primitive heart tube formation or cardiac chamber differentiation, blocking the translation of Na,K-ATPase alpha2 isoform leads to cardiac laterality defects.

Descriptors: zebrafish embryos, heart embryology, morphogenesis physiology, Na+ K+ exchanging ATPase genetics, base sequence, DNA primers, developmental gene expression regulation, essential genes, congenital heart defects enzymology and genetics, in situ hybridization, isoenzymes genetics and metabolism, molecular sequence data, mutation, Na+ K+ exchanging ATPase metabolism, PCR.

Shu, X., J. Huang, Y. Dong, J. Choi, A. Langenbacher , and J.N. Chen (2007). **Na,K-ATPase alpha2 and Ncx4a regulate zebrafish left-right patterning.** *Development* 134(10): 1921-30. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: A conserved molecular cascade involving Nodal signaling that patterns the laterality of the lateral mesoderm in vertebrates has been extensively studied, but processes involved in the initial break of left-right (LR) symmetry are just beginning to be explored. Here we report that Na,K-ATPase alpha2 and Ncx4a function upstream of Nodal signaling to regulate LR patterning in zebrafish. Knocking down Na,K-ATPase alpha2 and Ncx4a activity in dorsal forerunner cells (DFCs), which are precursors of Kupffer's vesicle (KV), is sufficient to disrupt asymmetric gene expression in the lateral plate mesoderm and randomize the placement of internal organs, indicating that the activity of Na,K-ATPase alpha2 and Ncx4a in DFCs/KV is crucial for LR patterning. High-speed videomicroscopy and bead implantation experiments show that KV cilia are immobile and the directional fluid flow in KV is abolished in Na,K-ATPase alpha2 and Ncx4a morphants, suggesting their essential role in KV ciliary function. Furthermore, we found that intracellular Ca(2+) levels are elevated in Na,K-ATPase alpha2 and Ncx4a morphants and that the defects in ciliary motility, KV fluid flow and placement of internal organs induced by their knockdown could be suppressed by

inhibiting the activity of Ca(2+)/calmodulin-dependent protein kinase II. Together, our data demonstrate that Na,K-ATPase alpha2 and Ncx4a regulate LR patterning by modulating intracellular calcium levels in KV and by influencing cilia function, revealing a previously unrecognized role for calcium signaling in LR patterning.

Descriptors: zebrafish embryos, developmental gene expression regulation, Na+ K+ exchanging ATPase physiology, body patterning, Ca2+ calmodulin dependent protein kinase metabolism, calcium signaling, cilia metabolism, molecular cloning, fluorescent dyes pharmacology, mesoderm metabolism, video microscopy, molecular sequence data, Na+ K+ exchanging ATPase biosynthesis, transforming growth factor beta metabolism, proteins biosynthesis.

Sidi, S., C. Goutel, N. Peyrieras, and F.M. Rosa (2003). **Maternal induction of ventral fate by zebrafish radar.** *Proceedings of the National Academy of Sciences of the United States of America* 100(6): 3315-20. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: In vertebrate embryos, maternal determinants are thought to preestablish the dorsoventral axis by locally activating zygotic ventral- and dorsal-specifying genes, e.g., genes encoding bone morphogenetic proteins (BMPs) and BMP inhibitors, respectively. Whereas the canonical Wntbeta-catenin pathway fulfills this role dorsally, the existence of a reciprocal maternal ventralizing signal remains hypothetical. Maternal noncanonical WntCa(2+) signaling may promote ventral fates by suppressing Wntbeta-catenin dorsalizing signals; however, whether any maternal determinant is directly required for the activation of zygotic ventral-specifying genes is unknown. Here, we show that such a function is achieved, in part, in the zebrafish embryo by the maternally encoded transforming growth factor beta (TGF-beta) signaling molecule, Radar. Loss-of-function experiments, together with epistasis analyses, identify maternal Radar as an upstream activator of bmps expression. Maternal induction of bmps by Radar is essential for zebrafish development as its removal results in larval-lethal dorsalized phenotypes. Double-morphant analyses further suggest that Radar functions through the TGF-beta receptor Alk8 to initiate the expression of bmp genes. Our results support the existence of a previously uncharacterized maternal ventralizing pathway. They might further indicate that maternal TGF-betaRdr and WntCa(2+) pathways complementarily specify ventral cell fates, with the former triggering bmps expression and the latter indirectly repressing genes encoding BMP antagonists.

Descriptors: zebrafish embryology, bone morphogenetic proteins genetics and physiology, proteins, base sequence, body patterning, embryonic induction, developmental gene expression regulation, in situ hybridization, mutation, phenotype, messenger RNA genetics and metabolism.

Sieger, D., D. Tautz, and M. Gajewski (2003). **The role of Suppressor of Hairless in Notch mediated signalling during zebrafish somitogenesis.** *Mechanisms of Development* 120(9): 1083-94. ISSN: 0925-4773.

NAL Call Number: QH607.A1C4

Abstract: Suppressor of Hairless (Su(H)) codes for a protein that interacts with the intracellular domain of Notch to activate the target genes of the Delta-Notch signalling pathway. We have cloned the zebrafish homologue of Su(H) and have analysed its function by morpholino mediated knockdown. While there are at least four notch and four delta homologues in zebrafish, there appears to be only one complete Su(H) homologue. We have analysed the function of Su(H) in the somitogenesis process and its influence on the expression of notch pathway genes, in particular her1, her7, deltaC and deltaD. The cyclic expression of

her1, her7 and deltaC in the presomitic mesoderm is disrupted by the Su(H) knockdown mimicking the expression of these genes in the notch1a mutant deadly seven. deltaD expression is similarly affected by Su(H) knockdown like deltaC but shows in addition an ectopic expression in the developing neural tube. The inactivation of Su(H) in a fss/tbx24 mutant background leads furthermore to a clear breakdown of cyclic her1 and her7 expression, indicating that the Delta-Notch pathway is required for the creation of oscillation and not only for the synchronisation between neighbouring cells. The strongest phenotypes in the Su(H) knockdown embryos show a loss of all somites posterior to the first five to seven ones. This phenotype is stronger than the known amorphic phenotypes for notch1 (des) or deltaD (aei) in zebrafish, but mimicks the knockout phenotype of RBP-Jkappa gene in the mouse, which is the homologue of Su(H). This suggests that there is some functional redundancy among the Notch and Delta genes. This fact that the first five to seven somites are only weakly affected by Su(H) knockdown indicates that additional genetic pathways may be active in the specification of the most anterior somites.

Descriptors: zebrafish embryology, membrane somites metabolism, base sequence, molecular cloning, complementary DNA genetics, developmental gene expression regulation, gene targeting, membrane proteins metabolism, molecular sequence data, nerve tissue proteins genetics and metabolism, Notch receptors, signal transduction, metabolism.

Sieger, D., B. Ackermann, C. Winkler, D. Tautz, and M. Gajewski (2006). **Her1 and her13.2 are jointly required for somitic border specification along the entire axis of the fish embryo**. *Developmental Biology* 293(1): 242-251. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Delta-Notch and FGF signaling are involved in the control of somitogenesis in zebrafish. her genes are generally known as downstream targets of Delta-Notch signaling, but the her13.2 gene from zebrafish has recently been shown to depend on FGF signaling only. We have here studied the functional role of her13.2 in conjunction with her genes that are under Delta-Notch control. We show that joint inactivation of her1 and her13.2 leads to a complete loss of all somitic borders, including the most anterior ones. This somitic phenotype is much stronger than would be expected from the effects of the inactivation of either gene alone. A joint inactivation of her13.2 and her7, which is a paralogue of her1, does not show this enhanced effect. Thus, our results confirm inferences from in vitro studies that her1 and her13.2 form specific heterodimers, which may directly be required for regulating further target genes. These two her genes thus constitute the link between Delta-Notch pathway and FGF signaling during entire somitogenesis. We show that this interaction is conserved in the rice fish medaka, as a joint inactivation of the respective orthologues leads also to the same phenotype as in zebrafish. In addition, our results suggest that the mechanisms for anterior and posterior somite formation are not principally different, although the anterior somites often seem more refractory to genetic perturbations. (c) 2006 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, molecular genetics, development, reproductive system, reproduction, phenotype, somitogenesis, somitic border specification.

Siekmann, A.F. and M. Brand (2005). **Distinct tissue-specificity of three zebrafish ext1 genes encoding proteoglycan modifying enzymes and their relationship to somitic onic Hedgehog signaling.** *Developmental Dynamics* 232(2): 498-505. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryos, developmental gene expression regulation, n acetylglucosaminyltransferases biosynthesis, proteoglycans metabolism, trans activators metabolism,

amino acid sequence, brain embryology, molecular cloning, *Drosophila*, embryonic development, Hedgehog proteins, in situ hybridization, molecular sequence data, mutation, n acetylglucosaminyltransferases physiology, open reading frames, phylogeny, RNA metabolism, signal transduction, time factors, tissue distribution, proteins chemistry.

Sirbu, I.O., L. Gresh, J. Barra, and G. Duester (2005). **Shifting boundaries of retinoic acid activity control hindbrain segmental gene expression**. *Development* 132(11): 2611-2622. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Retinoic acid (RA) generated by Raldh2 in paraxial mesoderm is required for specification of the posterior hindbrain, including restriction of Hoxb1 expression to presumptive rhombomere 4 (r4). Hoxb1 expression requires 3' and 5' RA response elements for widespread induction up to r4 and for r3/r5 repression, but RA has previously been detected only from r5-r8, and vHnf1 is required for repression of Hoxb1 posterior to r4 in zebrafish. We demonstrate in mouse embryos that an RA signal initially travels from the paraxial mesoderm to r3, forming a boundary next to the r2 expression domain of Cyp26a1 (which encodes an RA-degrading enzyme). After Hoxb1 induction, the RA boundary quickly shifts to r4/r5, coincident with induction of Cyp26c1 in r4. A functional role for Cyp26c1 in RA degradation was established through examination of RA-treated embryos. Analysis of Raldh2(-/-) and vHnf1(-/-) embryos supports a direct role for RA in Hoxb1 induction up to r4 and repression in r3/r5, as well as an indirect role for RA in Hoxb1 repression posterior to r4 via RA induction of vHnf1 up to the r4/r5 boundary. Our findings suggest that Raldh2 and Cyp26 generate shifting boundaries of RA activity, such that r3-r4 receives a short pulse of RA and r5-r8 receives a long pulse of RA. These two pulses of RA activity function to establish expression of Hoxb1 and vHnf1 on opposite sides of the r4/r5 boundary. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, mouse embryos, nervous system, neural coordination, development, molecular genetics, retinoic acid.

Sire, J.Y. and M.A. Akimenko (2004). **Scale development in fish: a review, with description of sonic Hedgehog (shh) expression in the zebrafish (*Danio rerio*).** *International Journal of Developmental Biology* 48(2-3): 233-247. ISSN: 0214 6282.

Abstract: In the first part of this paper we review current knowledge regarding fish scales, focusing on elasmoid scales, the only type found in two model species, the zebrafish and the *medaka*. After reviewing the structure of scales and their evolutionary origin, we describe the formation of the squamation pattern. The regularity of this process suggests a pre-patterning of the skin before scale initiation. We then summarise the dynamics of scale development on the basis of morphological observations. In the absence of molecular data, these observations support the existence of genetic cascades involved in the control of scale development. In the second part of this paper, we illustrate the potential that scale development offers as a model to study organogenesis mediated by epithelial-mesenchymal interactions. Using the zebrafish (*Danio rerio*), we have combined alizarin red staining, light and transmission electron microscopy and in situ hybridisation using an anti-sense RNA probe for the sonic hedgehog (shh) gene. Scales develop late in ontogeny (30 days post-fertilisation) and close to the epidermal cover. Only cells of the basal epidermal layer express shh. Transcripts are first detected after the scale papillae have formed. Thus, shh is not involved in the mechanisms controlling squamation patterning and scale initiation. As the scales enlarge, shh expression is progressively restricted to a subset of basal epidermal cells located in the region that overlies their posterior field. This pattern of expression suggests that shh may be involved in the control of scale

morphogenesis and differentiation in relationship with the formation of the epidermal fold in the posterior region.
Descriptors: zebrafish, *Danio rerio*, *medaka*, gene expression, sonic Hedgehog expression, role in scale development, scale development, organogenesis, epithelial-mesenchymal interactions.

Sivasubbu, S., D. Balciunas, A.E. Davidson, M.A. Pickart, S.B. Hermanson, K.J. Wangensteen, D.C. Wolbrink, and S.C. Ekker (2006). **Gene-breaking transposon mutagenesis reveals an essential role for histone H2afza in zebrafish larval development.** *Mechanisms of Development* 123(7): 513-29. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: We report a novel gene tagging, identification and mutagenicity ('gene-breaking') method for the zebrafish, *Danio rerio*. This modular approach consists of two distinct and separable molecular cassettes. The first is a gene-finding cassette. In this study, we employed a 3' gene-tagging approach that selectively 'traps' transcripts regardless of expression status, and we show that this cassette identifies both known and novel endogenous transcripts in transgenic zebrafish. The second is a transcriptional termination mutagenicity cassette assembled from a combination of a splice acceptor and polyadenylation signal to disrupt tagged transcripts upon integration into intronic sequence. We identified both novel and conserved loci as linked phenotypic mutations using this gene-breaking strategy, generating molecularly null mutations in both larval lethal and adult viable loci. We show that the Histone 2a family member z (H2afza) variant is essential for larval development through the generation of a lethal locus with a truncation of conserved carboxy-terminal residues in the protein. In principle this gene-breaking strategy is scalable for functional genomics screens and can be used in Sleeping Beauty transposon and other gene delivery systems in the zebrafish.
Descriptors: zebrafish mutants, gene tagging, DNA transposable elements genetics, histones genetics, insertional mutagenesis, genetically modified, histones physiology, larva genetics, larva growth and development.

Skinner, A. (2005). **Comments on variations of sperm release in three batches of zebra fish.** *Journal of Fish Biology* 67(1): 1-2. ISSN: 0022 1112.
NAL Call Number: QL614.J68
Descriptors: zebrafish males, *Danio rerio*, sperm , sperm release and motility, observations , reproductive behavior, spawning behavior observations.

Skromne, I., D. Thorsen, M. Hale, V.E. Prince, and R.K. Ho (2006). **Cdx determines the spinal cord in zebrafish by preventing rhombomere formation and inducing posterior hox gene expression in the caudal CNS**. *Developmental Biology* 295(1): 334-335. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, cell differentiation, central nervous system, developmental stages, embryology, freshwater fish, gene expression, morphogenesis, organogenesis, spinal cord, *Danio rerio.*

Slanchev, K., J. Stebler, G. de la Cueva Mendez, and E. Raz (2005). **Development without germ cells: the role of the germ line in zebrafish sex differentiation.** *Proceedings of the National Academy of Sciences of the United States of America* 102(11): 4074-9. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: The progenitors of the gametes, the primordial germ cells (PGCs) are typically specified early in the development in positions, which are distinct from the gonad. These cells

then migrate toward the gonad where they differentiate into sperms and eggs. Here, we study the role of the germ cells in somatic development and particularly the role of the germ line in the sex differentiation in zebrafish. To this end, we ablated the germ cells using two independent methods and followed the development of the experimental fish. First, PGCs were ablated by knocking down the function of dead end, a gene important for the survival of this lineage. Second, a method to eliminate the PGCs using the toxin-antitoxin components of the parD bacterial genetic system was used. Specifically, we expressed a bacterial toxin Kid preferentially in the PGCs and at the same time protected somatic cells by uniformly expressing the specific antidote Kis. Our results demonstrate an unexpected role for the germ line in promoting female development because PGC-ablated fish invariably developed as males.
Descriptors: zebrafish embryology, ovum physiology, sex determination genetics, spermatozoa physiology, bacterial bacterial proteins metabolism, diphtheria toxin pharmacology, *Escherichia coli* proteins genetics and metabolism, ovum drug effects, spermatozoa drug effects.

Smith, A., F. Avaron, D. Guay, B.K. Padhi, and M.A. Akimenko (2006). **Inhibition of BMP signaling during zebrafish fin regeneration disrupts fin growth and scleroblasts differentiation and function.** *Developmental Biology* 299(2): 438-54. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The zebrafish caudal fin provides a simple model to study molecular mechanisms of dermal bone regeneration. We previously showed that misexpression of Bone morphogenetic protein 2b (Bmp2b) induces ectopic bone formation within the regenerate. Here we show that in addition to bmp2b and bmp4 another family member, bmp6, is involved in fin regeneration. We further investigated the function of BMP signaling by ectopically expressing the BMP signaling inhibitor Chordin which caused: (1) inhibition of regenerate outgrowth due to a decrease of blastema cell proliferation and downregulation of msxb and msxC expression and (2) reduced bone matrix deposition resulting from a defect in the maturation and function of bone-secreting cells. We then identified targets of BMP signaling involved in regeneration of the bone of the fin rays. runx2a/b and their target col10a1 were downregulated following BMP signaling inhibition. Unexpectedly, the sox9a/b transcription factors responsible for chondrocyte differentiation were detected in the non-cartilaginous fin rays, sox9a and sox9b were not only differentially expressed but also differentially regulated since sox9a, but not sox9b, was downregulated in the absence of BMP signaling. Finally, this analysis revealed the surprising finding of the expression, in the fin regenerate, of several factors which are normally the signatures of chondrogenic elements during endochondral bone formation although fin rays form through dermal ossification, without a cartilage intermediate.
Descriptors: zebrafish fins, bone morphogenetic, cell differentiation, osteoblasts cytology, regeneration, anatomy and histology, bone morphogenetic proteins metabolism, cell proliferation, chondrocytes cytology, down regulation, glycoproteins biosynthesis, hmgb proteins biosynthesis, homeodomain proteins metabolism, intercellular signaling peptides and proteins biosynthesis, osteogenesis, signal transduction, transcription factors metabolism.

Smith, T.H., C.C. Dueck, A.A. Mhanni, and R.A. McGowan (2005). **Novel splice variants associated with one of the zebrafish dnmt3 genes.** *BioMed Central Developmental Biology* 5: 23. ISSN: 1471 213X.
NAL Call Number: QH491.B63
Abstract: BACKGROUND: DNA methylation and the methyltransferases are known to be important in vertebrate development and this may be particularly true for the Dnmt3

family of enzymes because they are thought to be the de novo methyltransferases. Mammals have three Dnmt3 genes; Dnmt3a, Dnmt3b, and Dnmt3L, two of which encode active enzymes and one of which produces an inactive but necessary cofactor. However, due to multiple promoter use and alternative splicing there are actually a number of dnmt3 isoforms present. Six different dnmt3 genes have recently been identified in zebrafish. RESULTS: We have examined two of the dnmt3 genes in zebrafish that are located in close proximity in the same linkage group and we find that the two genes are more similar to each other than they are to the other zebrafish dnmt3 genes. We have found evidence for the existence of several different splice variants and alternative splice sites associated with one of the two genes and have examined the relative expression of these genes/variants in a number of zebrafish developmental stages and tissues. CONCLUSION: The similarity of the dnmt3-1 and dnmt3-2 genes suggests that they arose due to a relatively recent gene duplication event. The presence of alternative splice and start sites, reminiscent of what is seen with the human DNMT3s, demonstrates strong parallels between the control/function of these genes across vertebrate species. The dynamic expression levels of these genes/variants suggest that they may well play a role in early development and this is particularly true for dnmt3-2-1 and dnmt3-1. dnmt3-2-1 is the predominantly expressed form prior to zygotic gene activation whereas dnmt3-1 predominates post zygotic gene activation suggesting a distinct developmental role for each.
Descriptors: zebrafish, Dnmt genes, alternative splicing, DNA cytosine 5 methyltransferase genetics, base sequence, epigenesis, genetic, gene expression regulation, isoenzymes, zygote.

Solomon, K.S., T. Kudoh, I.B. Dawid, and A. Fritz (2003). **Zebrafish foxi1 mediates otic placode formation and jaw development.** *Development* 130(5): 929-40. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The otic placode is a transient embryonic structure that gives rise to the inner ear. Although inductive signals for otic placode formation have been characterized, less is known about the molecules that respond to these signals within otic primordia. Here, we identify a mutation in zebrafish, hearsay, which disrupts the initiation of placode formation. We show that hearsay disrupts foxi1, a forkhead domain-containing gene, which is expressed in otic precursor cells before placodes become visible; foxi1 appears to be the earliest marker known for the otic anlage. We provide evidence that foxi1 regulates expression of pax8, indicating a very early role for this gene in placode formation. In addition, foxi1 is expressed in the developing branchial arches, and jaw formation is disrupted in hearsay mutant embryos.
Descriptors: zebrafish embryos, DNA binding proteins genetics, proteins metabolism, nuclear proteins, transcription factors metabolism, proteins metabolism, amino acid and base sequence, DNA binding proteins genetics, proteins chemistry and genetics, ear embryology, embryonic structures embryology, forkhead transcription factors, developmental gene expression regulation, in situ hybridization, mandible embryology, molecular sequence data, mutation, neural crest cytology, neural crest physiology, paired box transcription factors, phenotype, trans activators genetics and metabolism, transcription factors chemistry and genetics, anatomy and histology, proteins chemistry.

Solomon, K.S., J.M.J. Logsdon, and A. Fritz (2003). **Expression and phylogenetic analyses of three zebrafish FoxI class genes.** *Developmental Dynamics* 228(3): 301-7. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, developmental gene expression regulation genetics, phylogeny, RNA binding proteins genetics, amino acid sequence , conserved sequence, physiology, mice, molecular sequence data, morphogenesis, multigene family, RNA binding proteins

chemistry, rats, sequence alignment, amino acid sequence homology, zebrafish classification, proteins chemistry.

Sonawane, M., Y. Carpio, R. Geisler, H. Schwarz, H.M. Maischein, and C. Nuesslein Volhard (2005). **Zebrafish penner/lethal giant larvae 2 functions in hemidesmosome formation, maintenance of cellular morphology and growth regulation in the developing basal epidermis.** *Development* 132(14): 3255-65. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Epithelial cells are equipped with junctional complexes that are involved in maintaining tissue architecture, providing mechanical integrity and suppressing tumour formation as well as invasiveness. A strict spatial segregation of these junctional complexes leads to the polarisation of epithelial cells. In vertebrate epithelia, basally localised hemidesmosomes mediate stable adhesion between epithelial cells and the underlying basement membrane. Although components of hemidesmosomes are relatively well known, the molecular machinery involved in governing the formation of these robust junctions, remains elusive. Here, we have identified the first component of this machinery using a forward genetic approach in zebrafish as we show that the function of penner (pen)/lethal giant larvae 2 (lgl2) is necessary for hemidesmosome formation and maintenance of the tissue integrity in the developing basal epidermis. Moreover, in pen/lgl2 mutant, basal epidermal cells hyper-proliferate and migrate to ectopic positions. Of the two vertebrate orthologues of the Drosophila tumour suppressor gene lethal giant larvae, the function of lgl2 in vertebrate development and organogenesis remained unclear so far. Here, we have unravelled an essential function of lgl2 during development of the epidermis in vertebrates.
Descriptors: zebrafish, *Drosophila*, epidermis cytology, epidermis embryology, lethal genes, hemidesmosomes metabolism, tumor suppressor, proteins metabolism, amino acid sequence, *Drosophila* epidermis growth and development, interference microscopy, molecular sequence data, tumor suppressor proteins genetics.

Song, J., H.J. Kim, Z. Gong, N.A. Liu, and S. Lin (2007). **Vhnf1 acts downstream of Bmp, Fgf, and RA signals to regulate endocrine beta cell development in zebrafish.** *Developmental Biology* 303(2): 561-75. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Bmp, Fgf, and retinoic acid (RA) signals have been implicated as regulators of pancreas development. However, the integration of these signaling pathways in vivo is not fully understood. Variant hnf1 (Vhnf1) is a transcription factor involved in pancreas, liver, and kidney development and its mutation in zebrafish causes underdeveloped pancreas and liver. We investigated the signaling pathways that regulate vhnf1 expression during pancreas development. First, we showed that Bmp activity is required for vhnf1 expression in the endoderm. In chordin (a Bmp antagonist) morpholino (MO)-injected embryos, vhnf1 expression in endoderm and in endocrine beta cells is expanded. On the other hand, in alk8 (a type I TGFbeta receptor) MO-injected embryos, vhnf1 expression in the endoderm is significantly reduced. Second, we showed that Fgf signaling participates in regulation of pancreas development through the vhnf1 pathway. Third, we demonstrated that RA fails to rescue reduction of insulin expression in vhnf1 mutants, whereas overexpression of vhnf1 restores insulin expression that is repressed by treatment with a RA receptor inhibitor. And finally, we revealed that both Bmp and Fgf signals act genetically upstream of RA in directing pancreas development. Taken together, our data establish that vhnf1 acts downstream of the signaling pathways of RA, Bmp, and Fgf to regulate pancreas development in zebrafish.
Descriptors: zebrafish embryos, bone morphogenetic proteins metabolism, Fgf metabolism,

hepatocyte nuclear factor 1 beta metabolism, insulin secreting cells cytology and metabolism, tretinoin metabolism, base sequence, bone morphogenetic epistasis, genetic, Fgf genetics, developmental gene expression regulation drug effects, hepatocyte nuclear factor 1 beta genetics, insulin genetics, metabolism and secreting cells drug effects, oligodeoxyribonucleotides, antisense genetics, point mutation, signal transduction, tretinoin pharmacology.

Song, J., H. Kim, and S. Lin (2005). **Vhnf1 integrates bmp, ra, and fgf signaling pathways to regulate pancreas development in zebrafish.** *Developmental Biology* 283(2): 685. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, development, digestive system, ingestion and assimilation, endocrine system, chemical coordination and homeostasis, signaling pathway, pancreas development.

Song, M.H., N.L. Brown, and J.Y. Kuwada (2004). **The cfy mutation disrupts cell divisions in a stage-dependent manner in zebrafish embryos.** *Developmental Biology* 276(1): 194-206. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The zebrafish curly fry (cfy) mutation leads to embryonic lethality and abnormal cell divisions starting at 12-14 h postfertilization (hpf) during neural tube formation. The mitotic defect is seen in a variety of tissues including the central nervous system (CNS). In homozygous mutant embryos, mitoses are disorganized with an increase in mitotic figures throughout the developing neural tube. One consequence of aberrant mitoses in cfy embryos is an increase in cell death. Despite this, patterning of the early CNS is relatively unperturbed with distribution of the early, primary neurons indistinguishable from that of wild-type embryos. At later stages, however, the number of neurons was dramatically decreased throughout the CNS. The effect on neurons in older cfy embryos but not young ones correlates with the time of birth of neurons: primary neurons are born before the action of the cfy gene and later neurons after. Presumably, death of neuronal progenitors that divide beginning at the neural keel stage or death of their neuronal progeny accounts for the diminution of neurons in older mutant embryos. In addition, oligodendrocytes, which also develop late in the CNS, are greatly reduced in number in cfy embryos due to an apparent decrease in oligodendrocyte precursors. Genetic mosaic analysis demonstrates that the mutant phenotype is cell-autonomous. Furthermore, there are no obvious defects in apical/basal polarity within the neuroepithelium, suggesting that the cfy gene is not critical for epithelial polarity and that polarity defects are unlikely to account for the increased mitotic figures in mutants. These results suggest that the cfy gene regulates mitosis perhaps in a stage-dependent manner in vertebrate embryos.
Descriptors: zebrafish, cell cycle cell division genetics, developmental gene expression regulation, mutation, cell death homozygote, immunohistochemistry, in situ hybridization, mosaicism embryology, neurons physiology, oligodendroglia cytology, staining and labeling.

Songhet, P., D. Adzic, S. Reibe, and K.B. Rohr (2007). **Fgf1 is required for normal differentiation of erythrocytes in zebrafish primitive hematopoiesis.** *Developmental Dynamics* 236(3): 633-43. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Hematopoiesis in vertebrate development involves an embryonic, primitive wave and a later, definitive wave in which embryonic blood cells are replaced with adult blood cells. We here show that zebrafish fgf1 is involved in vivo in primitive hematopoiesis. Fibro-

blast growth factor-1 (FGF1) morpholino knockdown leads to abnormal accumulation of blood cells in the posterior intermediate cell mass at 32 hr postfertilization. Expression of the erythroid markers gata1 and ika, normally diminishing in differentiating erythrocytes at this stage, is maintained at abnormally high levels in primitive blood cells. The onset of erythrocyte differentiation as assessed by o-dianisidine staining is severely delayed. Most fgf1 morphants later recover to wild-type appearance, and primitive erythrocytes eventually differentiate. Zebrafish fgf1 is syntenic to human FGF1, which maps to a critically deleted region in human del(5q) syndrome posing an increased risk of leukemia to patients. As its knockdown in zebrafish changes expression of gata1, a gene involved in hematopoietic stem cell decisions, FGF1 should be considered to play a role in the pathogenesis of del(5q) syndrome.
Descriptors: zebrafish, cell differentiation physiology, erythropoiesis, fibroblast growth factor 1 physiology, cell differentiation genetics, erythrocytes cytology and metabolism, fibroblast growth factor 1 genetics, GATA1 transcription factor genetics and metabolism, developmental gene expression regulation, Ikaros transcription factor genetics, Ikaros transcription factor metabolism, phylogeny, reverse transcriptase PCR, metabolism.

Soules, K.A. and B.A. Link (2005). **Morphogenesis of the anterior segment in the zebrafish eye.** *BioMed Central Developmental Biology* 5: 12. ISSN: 1471 213X.
NAL Call Number: QH491.B63
Abstract: BACKGROUND: The ocular anterior segment is critical for focusing incoming light onto the neural retina and for regulating intraocular pressure. It is comprised of the cornea, lens, iris, ciliary body, and highly specialized tissue at the iridocorneal angle. During development, cells from diverse embryonic lineages interact to form the anterior segment. Abnormal migration, proliferation, differentiation, or survival of these cells contribute to diseases of the anterior segment such as corneal dystrophy, lens cataract, and glaucoma. Zebrafish represent a powerful model organism for investigating the genetics and cell biology of development and disease. To lay the foundation for genetic studies of anterior segment development, we have described the morphogenesis of this structure in zebrafish. RESULTS: As in other vertebrates, the zebrafish anterior segment derives from diverse origins including surface ectoderm, periocular mesenchyme, and neuroepithelium. Similarly, the relative timing of tissue differentiation in the anterior segment is also conserved with other vertebrates. However, several morphogenic features of the zebrafish anterior segment differ with those of higher vertebrates. These include lens delamination as opposed to invagination, lack of iris muscles and ciliary folds, and altered organization in the iridocorneal angle. In addition, substantial dorsal-ventral differences exist within the zebrafish anterior segment. CONCLUSION: Cumulatively, our anatomical findings provide a reference point to utilize zebrafish for genetic studies into the mechanisms of development and maintenance of the anterior segment.
Descriptors: zebrafish embryos, anterior eye segment growth and development, morphogenesis, anterior eye segment anatomy and histology and embryology, cornea growth and development epithelial cells, iris, crystalline lens growth and development.

Stadler, J.A., A. Shkumatava, W.H. Norton, M.J. Rau, R. Geisler, S. Fischer, and C.J. Neumann (2005). **Histone deacetylase 1 is required for cell cycle exit and differentiation in the zebrafish retina.** *Developmental Dynamics* 233(3): 883-9. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Histone acetylation is an important epigenetic mechanism for the control of

eukaryotic transcription. The histone deacetylase 1 (HDAC1) gene has been implicated in controlling the transcription of core cell cycle regulators, but the in vivo role of HDACs in cell cycle regulation is still poorly understood. Loss of HDAC1 activity causes underproliferation in several contexts during vertebrate development. In contrast, we show here that HDAC1 has the opposite effect in the zebrafish visual system, where loss of HDAC1 activity leads to failure of cells to exit the cell cycle in the retina and in the optic stalk. The effect of HDAC1 on cell cycle exit is cell-autonomous, and loss of HDAC1 in the retina leads to up-regulation of cyclin D and E transcripts. These results demonstrate that the in vivo role of HDAC1 in regulating cell cycle progression is region-specific, as HDAC1 promotes cell cycle exit in the retina but stimulates proliferation in other cellular contexts.
Descriptors: zebrafish embryology, cell cycle, cell differentiation, histone deacetylases metabolism, retina cytology, retina enzymology, metabolism, alleles, developmental gene expression regulation, histone deacetylases deficiency and genetics, retina embryology, proteins deficiency.

Stafford, D., R.J. White, M.D. Kinkel, A. Linville, T.F. Schilling, and V.E. Prince (2006). **Retinoids signal directly to zebrafish endoderm to specify insulin-expressing beta-cells.** *Development* 133(5): 949-56. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: During vertebrate development, the endodermal germ layer becomes regionalized along its anteroposterior axis to give rise to a variety of organs, including the pancreas. Genetic studies in zebrafish and mice have established that the signaling molecule retinoic acid (RA) plays a crucial role in endoderm patterning and promotes pancreas development. To identify how RA signals to pancreatic progenitors in the endoderm, we have developed a novel cell transplantation technique, using the ability of the SOX32 transcription factor to confer endodermal identity, to selectively target reagents to (or exclude them from) the endodermal germ layer of the zebrafish. We show that RA synthesized in the anterior paraxial mesoderm adjacent to the foregut is necessary for the development of insulin-expressing beta-cells. Conversely, RA receptor function is required in the foregut endoderm for insulin expression, but not in mesoderm or ectoderm. We further show that activation of RA signal transduction in endoderm alone is sufficient to induce insulin expression. Our results reveal that RA is an instructive signal from the mesoderm that directly induces precursors of the endocrine pancreas. These findings suggest that RA will have important applications in the quest to induce islets from stem cells for therapeutic uses.
Descriptors: zebrafish embryology, cell differentiation, endoderm cytology, insulin secreting cells cytology, pancreas embryology, retinoids metabolism, endoderm metabolism, high mobility group proteins metabolism, insulin secreting cells metabolism, mesoderm cytology and metabolism, pancreas cytology, pancreas metabolism and exocrine cytology, exocrine pancreas embryology and metabolism, retinoic acid receptors genetics, retinoic acid receptors physiology, signal transduction, transcription factors metabolism, metabolism.

Steffen, L.S., J.R. Guyon, E.D. Vogel, M.H. Howell, Y. Zhou, G.J. Weber, L.I. Zon, and L.M. Kunkel (2007). **The zebrafish runzel muscular dystrophy is linked to the titin gene.** *Developmental Biology* 309(2): 180-92. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Titin (also called connectin) acts as a scaffold for signaling proteins in muscle and is responsible for establishing and maintaining the structure and elasticity of sarcomeres in striated muscle. Several human muscular dystrophies and cardiomyopathies have previously been linked to mutations in the titin gene. This study reports linkage of the runzel

homozygous lethal muscular dystrophy in the zebrafish *Danio rerio* to a genomic interval containing the titin gene. Analysis of the genomic sequence suggests that zebrafish contain two adjacent titin loci. One titin locus lies within the genetic linkage interval and its expression is significantly reduced in runzel mutants by both immunofluorescence and protein electrophoresis. Morpholino downregulation of this same titin locus in wild-type embryos results in decreased muscle organization and mobility, phenocopying runzel mutants. Additional protein analysis demonstrates that, in wild-type zebrafish, titin isoform sizes are rapidly altered during the development of striated muscle, likely requiring a previously unrecognized need for vertebrate sarcomere remodeling to incorporate developmentally regulated titin isoforms. Decreases of affected titin isoforms in runzel mutants during this time correlate with a progressive loss of sarcomeric organization and suggest that the unaffected titin proteins are capable of sarcomerogenesis but not sarcomere maintenance. In addition, microarray analysis of the ruz transcriptome suggests a novel mechanism of dystrophy pathogenesis, involving mild increases in calpain-3 expression and upregulation of heat shock proteins. These studies should lead to a better understanding of titin's role in normal and diseased muscle.
Descriptors: zebrafish mutants, runzel, muscular dystrophy, titin gene, connectin, gene, calpain-3.

Stenkamp, D.L. and R.A. Frey (2003). **Extraretinal and retinal Hedgehog signaling sequentially regulate retinal differentiation in zebrafish.** *Developmental Biology* 258(2): 349-63. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Hedgehog (Hh) signaling is required for eye development in vertebrates; known roles in the zebrafish include regulation of eye morphogenesis and ganglion cell and photoreceptor differentiation. We employed a temporally selective Hh signaling knockdown strategy, by using antisense morpholino oligonucleotides or the teratogenic alkaloid cyclopamine, in order to dissect the separate roles of Hh signaling arising from specific sources. We also examined the eye phenotype of zebrafish slow muscle-omitted (smu) mutants, which lack a functional smoothened gene, encoding a component of the Hh signal transduction pathway. We find that Hh signaling from extraretinal sources is required for the initiation of retinal differentiation, but this involvement may be independent of the effects of Hh signaling on optic stalk development. We also find that Hh signals from ganglion cells participate in propagating expression of ath5, and we suggest that the effects of Hh signals from the retinal pigmented epithelium on photoreceptor differentiation may be mediated by the transcription factor rx1.
Descriptors: zebrafish embryology, G protein Coupled receptors, retina embryology, trans activators physiology, cell differentiation, DNA binding proteins genetics, DNA binding, gene targeting, growth substances genetics and physiology, Hedgehog proteins, mutation, antisense oligonucleotides genetics, antisense oligonucleotides pharmacology, phenotype, cell surface receptors genetics, cell surface receptors physiology, retina abnormalities, retina drug effects, retinal ganglion cells cytology and drug effects, signal transduction, trans activators antagonists and inhibitors, trans activators genetics.

Stetsyuk, V., B. Peers, A. Mavropoulos, V. Verbruggen, B. Thisse, C. Thisse, P. Motte, B. Duvillie, and R. Scharfmann (2007). **Calsenilin is required for endocrine pancreas development in zebrafish.** *Developmental Dynamics* 236(6): 1517-25. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryology, endocrine system embryology and metabolism, kv channel interactinG proteins metabolism, pancreas embryology, pancreas metabolism,

genetically modified, embryology, metabolism, developmental gene expression regulation, kv channel interactinG mutation genetics, pancreatic hormones metabolism, Notch receptors metabolism, signal transduction, tretinoin metabolism.

Stewart, R.A., B.L. Arduini, S. Berghmans, R.E. George, J.P. Kanki, P.D. Henion, and A.T. Look (2006). **Zebrafish foxd3 is selectively required for neural crest specification, migration and survival.** *Developmental Biology* 292(1): 174-88. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The vertebrate neural crest is a pluripotent cell population that generates a large variety of cell types, including peripheral neurons, cartilage and pigment cells. Mechanisms that control the patterning of the neural crest toward specific cell fates remain only partially understood. Zebrafish homozygous for the sympathetic mutation 1 (sym1) have defects in a subset of neural crest derivatives, such as peripheral neurons, glia and cartilage, but retain normal numbers of melanocytes. The sym1 mutation is a nucleotide deletion that disrupts the forkhead DNA-binding domain of the foxd3 gene, which encodes a conserved winged-helix transcription factor. We show that sym1 mutants have normal numbers of premigratory neural crest cells, but these cells express reduced levels of snai1b and sox10, implicating foxd3 as an essential regulator of these transcription factors in the premigratory neural crest. The onset of neural crest migration is also delayed in sym1 mutants, and there is a reduction in the number of migratory trunk neural crest cells, particularly along the medial migration pathway. TUNEL analysis revealed aberrant apoptosis localized to the hindbrain neural crest at the 15-somite stage, indicating a critical role for foxd3 in the survival of a subpopulation of neural crest cells. These results show that foxd3 selectively specifies premigratory neural crest cells for a neuronal, glial or cartilage fate, by inducing the expression of lineage-associated transcription factors in these cells and regulating their subsequent migration.
Descriptors: zebrafish embryos, cell movement physiology, forkhead transcription factors physiology, neural crest cytology, neural crest embryology, cell lineage genetics and physiology, cell movement genetics, survival genetics and survival physiology, forkhead transcription factors deficiency and genetics, neural crest physiology, sympathetic nervous system cytology and physiology, transcription factors biosynthesis and physiology, proteins deficiency and genetics.

Stickney, H.L., Y. Imai, B. Draper, C. Moens, and W.S. Talbot (2007). **Zebrafish bmp4 functions during late gastrulation to specify ventroposterior cell fates.** *Developmental Biology* 310(1): 71-84. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Bone morphogenetic proteins (BMPs) are key mediators of dorsoventral patterning in vertebrates and are required for the induction of ventral fates in fish and frogs. A widely accepted model of dorsoventral patterning postulates that a morphogenetic BMP activity gradient patterns cell fates along the dorsoventral axis. Recent work in zebrafish suggests that the role of BMP signaling changes over time, with BMPs required for global dorsoventral patterning during early gastrulation and for tail patterning during late gastrulation and early somitogenesis. Key questions remain about the late phase, including which BMP ligands are required and how the functions of BMPs differ during the early and late gastrula stages. In a screen for dominant enhancers of mutations in the homeobox genes vox and vent, which function in parallel to bmp signaling, we identified an insertion mutation in bmp4. We then performed a reverse genetic screen to isolate a null allele of bmp4. We report the characterization of these two alleles and demonstrate that BMP4 is required during the later phase of BMP signaling for the specification of ventroposterior cell fates. Our results

indicate that different bmp genes are essential at different stages. In addition, we present genetic evidence supporting a role for a morphogenetic BMP gradient in establishing mesodermal fates during the later phase of BMP signaling.
Descriptors: zebrafish embryology, alleles, bone morphogenetic gastrulation genetics, developmental gene expression regulation, body patterning genetics, bone morphogenetic proteins metabolism, cell differentiation genetics, mesoderm embryology, signal transduction, transforming growth factor beta genetics and metabolism, .

Stigloher, C., J. Ninkovic, M. Laplante, A. Geling, B. Tannhauser, S. Topp, H. Kikuta, T.S. Becker, C. Houart, and L. Bally Cuif (2006). **Segregation of telencephalic and eye-field identities inside the zebrafish forebrain territory is controlled by Rx3.** *Development* 133(15): 2925-35. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Anteroposterior patterning of the vertebrate forebrain during gastrulation involves graded Wnt signaling, which segregates anterior fields (telencephalon and eye) from the diencephalon. How the telencephalic and retinal primordia are subsequently subdivided remains largely unknown. We demonstrate that at late gastrulation the Paired-like homeodomain transcription factor Rx3 biases cell specification choices towards the retinal fate within a population of bipotential precursors of the anterior forebrain: direct cell tracing demonstrates that retinal precursors acquire a telencephalic fate in embryos homozygous for the rx3-null allele ckh(ne2611), characterized by an enlarged telencephalon and a lack of eyes. Chimera analyses further indicate that this function of Rx3 is cell autonomous. Transfating of the eye field in the absence of Rx3 function correlates with a substantial posterior expansion of expression of the Wnt antagonist Tlc and the winged-helix transcription factor Foxg1. These results suggest that the process segregating the telencephalic and eye fields is isolated from diencephalic patterning, and is mediated by Rx3.
Descriptors: zebrafish, eye embryology, homeodomain, prosencephalon embryology, telencephalon embryology, visual fields physiology, amino acid and base sequence, DNA primers, ethylnitrosourea pharmacology, gastrula physiology, gene deletion, homeodomain homeostasis, PCR.

Streit, D.P.J., R.P. Ribeiro, G.V. Moraes, and L.C. Capovilla (2004). **Criopreservacao de embrioes de peixes [Cryopreservation of fish embryos]**. *Revista Brasileira De Reproducao Animal* 28(1): 54-62. ISSN: 0102 0803.
NAL Call Number: QP251.R48
Abstract: Induced reproduction is used with success in several fish species. Fish spermatozoa cryopreservation is a biotechnology used with success, however the fish embryo freezing has not been elucidated. The *Brachydanio Danio rerio* is the species used as reference, because its physiology of embryonic development may be applied to all teleosts. The cryoprotectants used in studies on fish embryo freezing are glycerol, ethylene glycol, dimethyl sulfoxide, propylene glycol and methanol. Methanol is the cryoprotectant solution that presents the best results. The use of sugar associated with cryoprotectants has shown promising results. Fish embryos are extremely sensitive to chilling and tropical species are less tolerant to cold than temperate climate species. In fish, the morula stage has presented the best results in research on freezing of fish embryos. New techniques as partial removal of yolk and dechorionation has been researched as methods to improve fish embryo freezing. Studies on new techniques and antifreeze proteins have been investigated. The domain of this biotechnology will bring considerable progress for fish production, as well for preservation of species. Copyright © 2008 CABI

Descriptors: zebrafish embryos, biotechnology, cryopreservation, cryoprotectants, culture techniques, dimethyl sulfoxide, embryos, ethylene glycol, fish culture, fish eggs, freezing, glycerol, methanol, propylene glycol, reproduction, *Danio rerio*.
Language of Text: Portuguese.

Strickland, L.I. and D.R. Burgess (2004). **Pathways for membrane trafficking during cytokinesis**. *Trends in Cell Biology* 14(3): 115-118. ISSN: 0962 8924.
NAL Call Number: QH573.T73
Abstract: The molecular mechanisms underlying targeted deposition of new membrane at the advancing furrow of a dividing cell have long been intriguing to cell biologists. Three recent studies have made use of *Drosophila* cellularization to explore current questions in this field. These findings indicate that both the secretory pathway and endosomal recycling contribute membrane to the advancing furrow. Furthermore, new work reveals that vesicles derived from the Rab11 recycling endosome (RE) promote actin remodeling at the furrow. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryo, development, membranes, cell biology, actin remodeling, cytokinesis, embryogenesis, endosomal recycling, membrane deposition, molecular mechanisms, membrane trafficking: pathways, secretory pathway.

Sumanas, S., J.D. Larson, and M. Miller Bever (2003). **Zebrafish chaperone protein GP96 is required for otolith formation during ear development.** *Developmental Biology* 261(2): 443-55. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Chaperone proteins are considered to be fairly ubiquitous proteins that promote the correct folding and assembly of multiple newly synthesized proteins. While performing an embryonic screen in zebrafish using morpholino phosphorodiamidate oligonucleotides (MPOs), we identified a role for an endoplasmic reticulum chaperone protein family member, zebrafish GP96. Knockdown of GP96 resulted in a specific otolith formation defect during early ear development. Otolith precursor particles did not adhere to the kinocilia of the tether cells in the GP96-MPO-injected embryos, aggregating instead into a single clump. Although otolith development was abnormal, the patterning of the ear and the differentiation of tether cells and macular sensory and support cells was not affected. We have isolated and sequenced the full open reading frame of zebrafish GP96 and characterized its expression pattern. GP96 is expressed both maternally and zygotically. GP96 RNA is localized within the floorplate, hatching gland, and in the cells of the otic placode and otic vesicle, consistent with the function of GP96 in ear development. We conclude that the GP96 chaperone protein is involved in the otolith formation during normal ear development. This is the first report of a specific function during organism development being attributed to a chaperone class molecule.
Descriptors: zebrafish embryology, molecular chaperones metabolism, otolithic membrane embryology, amino acid sequence, body patterning physiology, molecular chaperones isolation and purification, molecular sequence data, oligonucleotides metabolism, sequence alignment.

Sumanas, S., B. Zhang, R. Dai, and S. Lin (2005). **15-zinc finger protein Bloody fingers is required for zebrafish morphogenetic movements during neurulation.** *Developmental Biology* 283(1): 85-96. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: A novel zebrafish gene bloody fingers (blf) encoding a 478 amino acid protein

containing fifteen C(2)H(2) type zinc fingers was identified by expression screening. As determined by in situ hybridization, blf RNA displays strong ubiquitous early zygotic expression, while during late gastrulation and early somitogenesis, blf expression becomes transiently restricted to the posterior dorsal and lateral mesoderm. During later somitogenesis, blf expression appears only in hematopoietic cells. It is completely eliminated in cloche, moonshine but not in vlad tepes (gata1) mutant embryos. Morpholino (MO) knockdown of the Blf protein results in the defects of morphogenetic movements. Blf-MO-injected embryos (morphants) display shortened and widened axial tissues due to defective convergent extension. Unlike other convergent extension mutants, blf morphants display a split neural tube, resulting in a phenotype similar to the human open neural tube defect spina bifida. In addition, dorsal ectodermal cells delaminate in blf morphants during late somitogenesis. We propose a model explaining the role of blf in convergent extension and neurulation. We conclude that blf plays an important role in regulating morphogenetic movements during gastrulation and neurulation while its role in hematopoiesis may be redundant.
Descriptors: zebrafish embyology, carrier, gastrula physiology, morphogenesis, amino acid sequence, body patterning, carrier developmental gene expression regulation, in situ hybridization, molecular sequence data, salivary proteins chemistry, salivary sequence alignment and homology, genetic transcription, proteins chemistry and proteins genetics.

Sun, C.K., S.W. Chu, S.Y. Chen, T.H. Tsai, T.M. Liu, C.Y. Lin, and H.J. Tsai (2004). **Higher harmonic generation microscopy for developmental biology**. *Journal of Structural Biology* 147(1): 19-30. ISSN: 1047 8477.
NAL Call Number: QH573.J68
Abstract: Optical higher harmonic generation, including second harmonic generation and third harmonic generation, leaves no energy deposition to its interacted matters due to an energy-conservation characteristic, providing the "noninvasiveness" nature desirable for biological studies. Combined with its nonlinearity, higher harmonic generation microscopy provides excellent three-dimensional (313) sectioning capability, offering new insights into the studies of embryonic morphological changes and complex developmental processes. By choosing a laser working in the biological penetration window, here we present a noninvasive in vivo light microscopy with sub-micron 3D resolution and millimeter penetration, utilizing endogenous higher harmonic generation signals in live specimens. Noninvasive imaging was performed in live zebrafish (Danio rerio) embryos. The complex developmental processes within > 1 mm-thick zebrafish embryos can be observed in vivo without any treatment. No optical damage was found even with high illumination after long-term observations and the examined embryos all developed normally at least to the larval stage. The excellent 3D resolution of the demonstrated technology allows us to capture the subtle developmental information on the cellular or subcellular levels occurring deep inside the live embryos and larvae. This technique can not only provide in vivo observation of the cytoarchitecture dynamics during embryogenesis with submicron resolution and millimeter penetration depth, but would also make strong impact in developmental and structural biology studies. Copyright 2003 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, methods and techniques, higher harmonic generation microscopy, imaging and microscopy techniques, light microscopy, and noninvasive imaging, cytoarchitecture, dynamics, embryogenesis.

Sun, Z., A. Amsterdam, G.J. Pazour, D.G. Cole, M.S. Miller, and N. Hopkins (2004). **A genetic screen in zebrafish identifies cilia genes as a principal cause of cystic kidney.** *Development* 131(16): 4085-93. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Polycystic kidney disease (PKD) is a common human genetic illness. It is characterized by the formation of multiple kidney cysts that are thought to result from over-proliferation of epithelial cells. Zebrafish larvae can also develop kidney cysts. In an insertional mutagenesis screen in zebrafish, we identified 12 genes that can cause cysts in the glomerular-tubular region when mutated and we cloned 10 of these genes. Two of these genes, vhnf1 (tcf2) and pkd2, are already associated with human cystic kidney diseases. Recently, defects in primary cilia have been linked to PKD. Strikingly, three out of the 10 genes cloned in this screen are homologues of *Chlamydomonas* genes that encode components of intraflagellar transport (IFT) particles involved in cilia formation. Mutation in a fourth blocks ciliary assembly by an unknown mechanism. These results provide compelling support for the connection between cilia and cystogenesis. Our results also suggest that lesions in genes involved in cilia formation and function are the predominant cause of cystic kidney disease, and that the genes identified here are excellent candidates for novel human PKD genes.

Descriptors: zebrafish embryos, larvae, kidney metabolism, polycystic kidney diseases genetics, cilia genetics and metabolism, kidney cytology, kidney embryology, molecular sequence data, insertional mutagenesis, mutation, polycystic kidney diseases embryology, polycystic kidney diseases metabolism, metabolism.

Sun, Z., J. Zhao, Y. Zhang, and A. Meng (2006). **Sp5l is a mediator of Fgf signals in anteroposterior patterning of the neuroectoderm in zebrafish embryo.** *Developmental Dynamics* 235(11): 2999-3006. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryology, body patterning genetics, Fgf genetics, developmental gene expression regulation, nervous system embryology, sp transcription factors physiology, ectoderm chemistry, ectoderm metabolism, embryonic development genetics, Fgf antagonists and inhibitors, nervous system chemistry, nervous system metabolism, messenger RNA analysis and metabolism, signal transduction, sp transcription factors analysis and genetics, proteins analysis and genetics.

Svetic, V., G.E. Hollway, S. Elworthy, T.R. Chipperfield, C. Davison, R.J. Adams, J.S. Eisen, P.W. Ingham, P.D. Currie, and R.N. Kelsh (2007). **Sdf1a patterns zebrafish melanophores and links the somite and melanophore pattern defects in choker mutants.** *Development* 134(5): 1011-22. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Pigment pattern formation in zebrafish presents a tractable model system for studying the morphogenesis of neural crest derivatives. Embryos mutant for choker manifest a unique pigment pattern phenotype that combines a loss of lateral stripe melanophores with an ectopic melanophore ;collar' at the head-trunk border. We find that defects in neural crest migration are largely restricted to the lateral migration pathway, affecting both xanthophores (lost) and melanophores (gained) in choker mutants. Double mutant and timelapse analyses demonstrate that these defects are likely to be driven independently, the collar being formed by invasion of melanophores from the dorsal and ventral stripes. Using tissue transplantation, we show that melanophore patterning depends upon the underlying somitic cells, the myotomal derivatives of which--both slow--and fast-twitch muscle fibres--are themselves significantly disorganised in the region of the ectopic collar. In addition, we uncover an aberrant pattern of expression of the gene encoding the chemokine Sdf1a in choker mutant homozygotes that correlates with each aspect of the melanophore pattern defect. Using morpholino

knock-down and ectopic expression experiments, we provide evidence to suggest that Sdf1a drives melanophore invasion in the choker mutant collar and normally plays an essential role in patterning the lateral stripe. We thus identify Sdf1 as a key molecule in pigment pattern formation, adding to the growing inventory of its roles in embryonic development.
Descriptors: zebrafish embryology, Cxc chemokines physiology and genetics, melanophores physiology, neural crest physiology, somites physiology, body patterning, cell movement, Cxc chemokines metabolism, melanophores cytology, muscle fibers, slow and fast twitch physiology, mutation, pigmentation, somites cytology.

Szeto, D.P. and D. Kimelman (2004). **Combinatorial gene regulation by Bmp and Wnt in zebrafish posterior mesoderm formation.** *Development* 131(15): 3751-60. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Combinatorial signaling is an important mechanism that allows the embryo to utilize overlapping signaling pathways to specify different territories. In zebrafish, the Wnt and Bmp pathways interact to regulate the formation of the posterior body. In order to understand how this works mechanistically, we have identified tbx6 as a posterior mesodermal gene activated by both of these signaling pathways. We isolated a genomic fragment from the tbx6 gene that recapitulates the endogenous tbx6 expression, and used this to ask how the Bmp and Wnt signaling pathways combine to regulate gene expression. We find that the tbx6 promoter utilizes distinct domains to integrate the signaling inputs from each pathway, including multiple Tcf/LEF sites and a novel Bmp-response element. Surprisingly, we found that overexpression of either signaling pathway can activate the tbx6 promoter and the endogenous gene, whereas inputs from both pathways are required for the normal pattern of expression. These results demonstrate that both Bmp and Wnt are present at submaximal levels, which allows the pathways to function combinatorially. We present a model in which overlapping Wnt and Bmp signals in the ventrolateral region activate the expression of tbx6 and other posterior mesodermal genes, leading to the formation of posterior structures.
Descriptors: zebrafish embryology, body patterning, bone morphogenetic proteins metabolism, developmental gene expression regulation, mesoderm physiology, proto oncogene proteins metabolism, signal transduction physiology, transcription factors metabolism, bone morphogenetic DNA mutational analysis, reporter genes, in situ hybridization, antisense oligonucleotides genetics and metabolism, promoter regions genetics, proto oncogene transcription factors genetics, Wnt proteins.
Notes: Comments: Erratum In: Development.2004 Aug; 131(6):4117
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Tabin, C. (2005). **Do we know anything about how left-right asymmetry is first established in the vertebrate embryo?** *Journal of Molecular Histology* 36(5): 317-323. ISSN: 1567 2379.
NAL Call Number: QH611.A1H58
Descriptors: zebrafish embryology, development, molecular genetics, asymmetric activity, left-right asymmetry.

Tada, S., T. Era, C. Furusawa, H. Sakurai, S. Nishikawa, M. Kinoshita, K. Nakao, T. Chiba, and S.I. Nishikawa (2005). **Characterization of mesendoderm: a diverging point of the definitive endoderm and mesoderm in embryonic stem cell differentiation culture.** *Development* 132(19): 4363-4374. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Bipotent mesendoderm that can give rise to both endoderm and mesoderm is an

established entity from *C elegans* to zebrafish. Although previous studies in mouse embryo indicated the presence of bi-potent mesendoderm cells in the organizer region, characterization of mesendoderm and its differentiation processes are still unclear. As bi-potent mesendoderm is implicated as the major precursor of definitive endoderm, its identification is also essential for exploring the differentiation of definitive endoderm. In this study, we have established embryonic stem (ES) cell lines that carry GFP gene in the goosecoid (Gsc) gene locus and have investigated the differentiation course of mesendodermal cells using Gsc expression as a marker. Our results show that mesendoderm is represented as a Gsc-GFP(+) E-cadherin(ECD)(+)PDGFR alpha(alpha R)(+) population and is selectively induced from ES cells under defined conditions containing either activin or nodal. Subsequently, it diverges to Gsc(+)ECD(+)alpha R- and Gsc(+)ECD(-)alpha R+ intermediates that eventually differentiate into definitive endoderm and mesodermal lineages, respectively. The presence of mesendodermal cells in nascent Gsc(+)ECD(+)alpha R+ population was also confirmed by single cell analysis. Finally, we show that the defined culture condition and surface markers developed in this study are applicable for obtaining pure mesendodermal cells and their immediate progenies from genetically unmanipulated ES cells. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, development, molecular genetics, endoderm, mesoderm, embryonic stem cell, bi-potent mesendoderm, GFP gene.

Takamiya, M. and J.A. Campos Ortega (2006). **Hedgehog signalling controls zebrafish neural keel morphogenesis via its level-dependent effects on neurogenesis.** *Developmental Dynamics* 235(4): 978-97. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, morphogenesis, nervous system embryology, signal transduction, trans activators metabolism, genetically modified, cell polarity Hedgehog proteins, in situ hybridization, microinjections, nervous system cytology, messenger RNA genetics and metabolism, metabolism.

Takechi, M., T. Hamaoka, and S. Kawamura (2003). **Fluorescence visualization of ultraviolet-sensitive cone photoreceptor development in living zebrafish.** *Federation of European Biochemical Societies Journal* 553(1-2): 90-4. ISSN: 1742 464X.
NAL Call Number: QP501.E8
Abstract: Cone photoreceptor cells of fish retinae are arranged in a highly organized fashion. However, the molecular mechanisms underlying photoreceptor development and retinal pattern formation are largely unknown. Here we established transgenic lines of zebrafish carrying green fluorescent protein (GFP) cDNA with the 5.5-kb upstream region of the ultraviolet-sensitive cone opsin gene (SWS1). In the transgenic fish, GFP gene expression proceeded in the same spatiotemporal pattern as SWS1 in the retinae of embryos. In the adult retina, GFP expression was observed throughout the short single cone (SSC) layer where SWS1 is specifically expressed. Therefore, the transgenic fish provides an excellent genetic background to study retinal pattern formation, photoreceptor determination and differentiation, and factors regulating these processes and SSC-specific expression of SWS1.
Descriptors: zebrafish embryology, retina cones embryology and metabolism, fluorescence microscopy methods, opsin genetics, metabolism, genetically modified, base sequence, cell differentiation, retina cones cytology, retina cones radiation effects, developmental gene expression regulation radiation effects, green fluorescent proteins, luminescent proteins genetics and metabolism, molecular sequence data, trans ultraviolet rays.

Takemoto, T., S. Natsuka, S. Nakakita, and S. Hase (2005). **Expression of complex-type N-glycans in developmental periods of zebrafish embryo.** *Glycoconjugate Journal* 22(1-2): 21-6. ISSN: 0282-0080.

NAL Call Number: QP752.G56

Abstract: As a first step to elucidate a role of N-glycans in development of vertebrates, we analyzed structures of the glycans expressed in early stages of zebrafish embryo. N-glycans were prepared from zebrafish embryos at several developmental stages followed by tagging with a fluorophore, 2-aminopyridine. The labeled glycans were analyzed by two modes of HPLC's. The comparison of the elution profiles of HPLC's unveil the change of the oligosaccharide structure during the development. These peaks were merely detected during 4-7 h after fertilization, however, increased from 12 h, and at 15 h a fairly amount of them was appeared. Structure analysis revealed that they were bianntenary complex-type N-glycans with or without fucose and/or bisecting N-acetylglucosamine residues. These results suggest that the complex-type N-glycans are concerned in some developmental event from segmentation period downward in zebrafish.

Descriptors: zebrafish, polysaccharides biosynthesis, metabolism , carbohydrate sequence, high pressure liquid chromatography molecular sequence data, polysaccharides isolation and purification.

Tallafuss, A. and L. Bally Cuif (2003). **Tracing of her5 progeny in zebrafish transgenics reveals the dynamics of midbrain-hindbrain neurogenesis and maintenance.** *Development* 130(18): 4307-23. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: The midbrain-hindbrain domain (MH) of the vertebrate embryonic neural tube develops in response to the isthmic organizer (IsO), located at the midbrain-hindbrain boundary (MHB). MH derivatives are largely missing in mutants affected in IsO activity; however, the potentialities and fate of MH precursors in these conditions have not been directly determined. To follow the dynamics of MH maintenance in vivo, we used artificial chromosome transgenesis in zebrafish to construct lines where egfp transcription is driven by the complete set of regulatory elements of her5, the first known gene expressed in the MH area. In these lines, egfp transcription faithfully recapitulates her5 expression from its induction phase onwards. Using the stability of GFP protein as lineage tracer, we first demonstrate that her5 expression at gastrulation is a selective marker of MH precursor fate. By comparing GFP protein and her5 transcription, we further reveal the spatiotemporal dynamics of her5 expression that conditions neurogenesis progression towards the MHB over time. Finally, we trace the molecular identity of GFP-positive cells in the acerebellar (ace) and no-isthmus (noi) mutant backgrounds to analyze directly fgf8 and pax2.1 mutant gene activities for their ultimate effect on cell fate. We demonstrate that most MH precursors are maintained in both mutants but express abnormal identities, in a manner that strikingly differs between the ace and noi contexts. Our observations directly support a role for Fgf8 in protecting anterior tectal and metencephalic precursors from acquiring anterior identities, while Pax2.1 controls the choice of MH identity as a whole. Together, our results suggest a model where an ordered MH pro-domain is identified at gastrulation, and where cell identity choices within this domain are subsequently differentially controlled by Fgf8 and Pax2.1 functions.

Descriptors: zebrafish embryos, cell lineage, developmental gene expression regulation, mesencephalon embryology, rhombencephalon embryology, transcription factors metabolism, proteins metabolism, genetically modified, basic helix loop helix transcription factors, body patterning, DNA binding proteins genetics, proteins genetics and metabolism, fibroblast growth factor 8, Fgf metabolism, reporter genes, green fluorescent proteins, luminescent

luminescent proteins metabolism, organizers, embryonic, pax2 transcription factor, regulatory sequences, nucleic acid, transcription factors genetics, genetic transcription, anatomy and histology, proteins genetics.

Tanaka, H., R. Maeda, W. Shoji, H. Wada, I. Masai, T. Shiraki, M. Kobayashi, R. Nakayama, and H. Okamoto (2007). **Novel mutations affecting axon guidance in zebrafish and a role for plexin signalling in the guidance of trigeminal and facial nerve axons.** *Development* 134(18): 3259-69. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: In zebrafish embryos, the axons of the posterior trigeminal (Vp) and facial (VII) motoneurons project stereotypically to a small number of target muscles derived from the first and second branchial arches (BA1, BA2). Use of the Islet1 (Isl1)-GFP transgenic line enabled precise real-time observations of the growth cone behaviour of the Vp and VII motoneurons within BA1 and BA2. Screening for N-ethyl-N-nitrosourea-induced mutants identified seven distinct mutations affecting different steps in the axonal pathfinding of these motoneurons. The class 1 mutations caused severe defasciculation and abnormal pathfinding in both Vp and VII motor axons before they reached their target muscles in BA1. The class 2 mutations caused impaired axonal outgrowth of the Vp motoneurons at the BA1-BA2 boundary. The class 3 mutation caused impaired axonal outgrowth of the Vp motoneurons within the target muscles derived from BA1 and BA2. The class 4 mutation caused retraction of the Vp motor axons in BA1 and abnormal invasion of the VII motor axons in BA1 beyond the BA1-BA2 boundary. Time-lapse observations of the class 1 mutant, vermicelli (vmc), which has a defect in the plexin A3 (plxna3) gene, revealed that Plxna3 acts with its ligand Sema3a1 for fasciculation and correct target selection of the Vp and VII motor axons after separation from the common pathways shared with the sensory axons in BA1 and BA2, and for the proper exit and outgrowth of the axons of the primary motoneurons from the spinal cord.
Descriptors: zebrafish embryology, axons physiology, embryonic development genetics, facial nerve embryology, cell surface receptors physiology, trigeminal nerve embryology, motor neurons physiology, mutation, cell surface receptors genetics, semaphorins physiology, proteins genetics.

Tawk, M., C. Araya, D.A. Lyons, A.M. Reugels, G.C. Girdler, P.R. Bayley, D.R. Hyde, M. Tada, and J.D. Clarke (2007). **A mirror-symmetric cell division that orchestrates neuroepithelial morphogenesis**. *Nature* 446(7137): 797-800. ISSN: 0028-0836.
NAL Call Number: 472 N21
Descriptors: zebrafish embryos, cell division, cytology, embryogenesis, embryonic development, epithelia, freshwater fish, morphogenesis, neural stem cells, neural tube, pattern formation, polarity, *Danio rerio.*

Tay, S.Y., P.W. Lngham, and S. Roy (2005). **A homologue of the *drosophila* kinesin-like protein Costal2 regulates Hedgehog signal transduction in the vertebrate embryo**. *Development* 132(4): 625-634. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Orthologues of nearly all of the core components of the Hedgehog signalling pathway, defined originally through genetic analysis in *Drosophila*, have now been discovered in vertebrates and shown to have highly conserved functions. The one striking exception to this rule is the kinesin-like protein Costal2, which plays a central role in controlling the activity of the zinc-finger-containing transcriptional regulator, Cubitus interruptus that modulates

all Hedgehog-dependent target gene expression, but whose involvement in Hedgehog signalling has not been demonstrated in vertebrates. We report the cloning of a kinesin-related gene from the zebrafish that in structure as well as function, appears to represent the first vertebrate orthologue of costal2. Using a combination of genetic and biochemical analysis, we provide evidence that as in *Drosophila*, zebrafish Costal2 acts principally as an intracellular repressor of signal transduction, in conjunction with Suppressor of Fused, another protein that negatively regulates signalling in Hedgehog-responsive cells. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, cell biology, molecular genetics, gene cloning, genetic techniques, genetic analysis, Hedgehog, Costa 12.

Tena, J.J., A. Neto, E. de la Calle-Mustienes, C. Bras-Pereira, F. Casares, and J.L. Gomez-Skarmeta (2007). **Odd-skipped genes encode repressors that control kidney development**. *Developmental Biology* 301(2): 518-531. ISSN: 0012-1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish, amphibiotic species, biological development, developmental stages, evolutionary conservation, freshwater fish, genes, genomes, kidney, kidneys, Malpighian tubules, repressors, territory, transcription, transcription factors, zinc finger proteins, Danio rerio, Drosophila, Xenopus, clawed frogs, zebra fish, g 07730 development & cell cycle, Q1 01324 reproduction and development.

Thatcher, E.J., A.S. Flynt, N. Li, J.R. Patton, and J.G. Patton (2007). **MiRNA expression analysis during normal zebrafish development and following inhibition of the Hedgehog and Notch signaling pathways.** *Developmental Dynamics* 236(8): 2172-80. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryos, developmental gene expression regulation, Hedgehog proteins antagonists and inhibitors, microRNAs genetics, Notch receptors antagonists and inhibitors, signal transduction, embryonic development genetics, gene expression profiling, messenger RNA analysis.

Theusch, E.V., K.J. Brown, and F. Pelegri (2006). **Separate pathways of RNA recruitment lead to the compartmentalization of the zebrafish germ plasm.** *Developmental Biology* 292(1): 129-41. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The maternal RNAs vasa, dead end, nanos1, and daz-like all become localized to the peripheral ends of the first and second cleavage furrows, where they form part of the zebrafish germ plasm. We show that aggregates of a first class of germ plasm components, which include dead end, nanos1, and vasa RNAs, are initially present in a wide cortical band at the animal pole. Aggregates containing these three RNAs appear to be associated with f-actin, which during the first cell cycle undergoes a microtubule-dependent movement towards the periphery as well as circumferential alignment. These cytoskeletal rearrangements lead to the further aggregation of particles containing these RNAs and their concomitant recruitment to the forming furrow. Aggregates containing a second class of germ plasm RNA components, which include the transcript for daz-like, translocate along the plane of the cortex towards the animal pole, where they are recruited to the germ plasm. After recruitment to the furrow, these two classes of RNAs occupy overlapping yet distinct regions of the germ plasm, and this arrangement is maintained during the early cleavage stages. Our observations suggest that separate pathways of RNA recruitment facilitate the compartmentalization of the zebrafish germ plasm.

Descriptors: zebrafish embryos, cell compartmentation genetics, cleavage stage, ovum metabolism, messenger RNA metabolism, signal transduction genetics, actins metabolism, cytoskeleton metabolism, RNA binding proteins biosynthesis, RNA binding proteins genetics.

Thorpe, C.J. and R.T. Moon (2004). **Nemo-like kinase is an essential co-activator of Wnt signaling during early zebrafish development.** *Development* 131(12): 2899-909. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Wnt/beta-catenin signaling regulates many aspects of early vertebrate development, including patterning of the mesoderm and neurectoderm during gastrulation. In zebrafish, Wnt signaling overcomes basal repression in the prospective caudal neurectoderm by Tcf homologs that act as inhibitors of Wnt target genes. The vertebrate homolog of Drosophila nemo, nemo-like kinase (Nlk), can phosphorylate Tcf/Lef proteins and inhibit the DNA-binding ability of beta-catenin/Tcf complexes, thereby blocking activation of Wnt targets. By contrast, mutations in a C. elegans homolog show that Nlk is required to activate Wnt targets that are constitutively repressed by Tcf. We show that overexpressed zebrafish nlk, in concert with wnt8, can downregulate two tcf3 homologs, tcf3a and tcf3b, that repress Wnt targets during neurectodermal patterning. Inhibition of nlk using morpholino oligos reveals essential roles in regulating ventrolateral mesoderm formation in conjunction with wnt8, and in patterning of the midbrain, possibly functioning with wnt8b. In both instances, nlk appears to function as a positive regulator of Wnt signaling. Additionally, nlk strongly enhances convergent/extension phenotypes associated with wnt11/silberblick, suggesting a role in modulating cell movements as well as cell fate.

Descriptors: zebrafish embryos, body patterning physiology, brain embryology, developmental gene expression regulation genetics, mitogen activated protein kinases genetics, mitogen activated protein kinases metabolism, proto oncogene, proteins, amino acid sequence, conserved sequence, ectoderm physiology, physiology, gastrula physiology, enzymologic gene expression regulation genetics, in situ hybridization, molecular sequence data, morphogenesis, nervous system embryology, PCR, sequence alignment and homology, Wnt proteins.

Thorpe, C.J., G. Weidinger, and R.T. Moon (2005). **Wnt/beta-catenin regulation of the Sp1-related transcription factor sp5l promotes tail development in zebrafish.** *Development* 132(8): 1763-72. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Tail formation in vertebrates involves the specification of a population of multipotent precursors, the tailbud, which will give rise to all of the posterior structures of the embryo. Wnts are signaling proteins that are candidates for promoting tail outgrowth in zebrafish, although which Wnts are involved, what genes they regulate, and whether Wnts are required for initiation or maintenance steps in tail formation has not been resolved. We show here that both wnt3a and wnt8 are expressed in the zebrafish tailbud and that simultaneous inhibition of both wnt3a and wnt8 using morpholino oligonucleotides can completely block tail formation. In embryos injected with wnt3a and wnt8 morpholinos, expression of genes in undifferentiated presomitic mesoderm is initiated, but not maintained. To identify genes that might function downstream of Wnts in tail formation, a DNA microarray screen was conducted, revealing that sp5l, a member of the Sp1 family of zinc-finger transcription factors, is activated by Wnt signaling. Moreover, we show that sp5l expression in the developing tail is dependent on both wnt3a and wnt8 function. Supporting a role for sp5l in tail

formation, we find that inhibition of sp5l strongly enhances the effects of wnt3a inhibition, and overexpression of sp5l RNA is able to completely restore normal tail development in wnt3a morphants. These data place sp5l downstream of wnt3a and wnt8 in a Wnt/beta-catenin signaling pathway that controls tail development in zebrafish.
Descriptors: zebrafish embryos, cytoskeletal proteins metabolism, developmental gene expression regulation, intercellular signaling peptides and proteins metabolism, signal transduction physiology, tail embryology, trans activators metabolism, proteins metabolism, in situ hybridization, mesoderm metabolism, oligonucleotide array sequence analysis, oligonucleotides, tail metabolism, Wnt proteins, beta catenin.

Thorpe, J.L., M. Doitsidou, S.Y. Ho, E. Raz, and S.A. Farber (2004). **Germ cell migration in zebrafish is dependent on HMGCoA reductase activity and prenylation.** *Developmental Cell* 6(2): 295-302. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Hydroxymethylglutaryl coenzyme A reductase (HMGCoAR) is required for isoprenoid and cholesterol biosynthesis. In Drosophila, reduced HMGCoAR activity results in germ cell migration defects. We show that pharmacological HMGCoAR inhibition alters zebrafish development and germ cell migration. Embryos treated with atorvastatin (Lipitor) exhibited germ cell migration defects and mild morphologic abnormalities. The effects induced by atorvastatin were completely rescued by prior injection of mevalonate, the product of HMGCoAR activity, or the prenylation precursors farnesol and geranylgeraniol. In contrast, squalene, a cholesterol intermediate further down the pathway, failed to rescue statin-induced defects. Moreover, pharmacologic inhibition of geranylgeranyl transferase 1 (GGT1) protein prenylation activity also resulted in abnormal germ cell migration. Thus, our pharmacological inhibition-and-rescue approach provided detailed information about the elements of isoprenoid biosynthesis that contribute to germ cell migration. Together with data from Drosophila (Santos and Lehmann, this issue), our results highlight a conserved role for protein geranylgeranylation in this context.
Descriptors: zebrafish embryos, cell movement physiology, germ cells physiology, hydroxymethylglutaryl coa reductases physiology, protein isoprenylation physiology, anticholesteremic agents pharmacology, cell movement drug effects and genetics, Cxc chemokines metabolism, diterpenes pharmacology, drug dose response relationship, drug interactions, embryo cytology, drug effects and metabolism, enzyme inhibitors pharmacology, farnesol pharmacology, germ cells drug effects and enzymology, heptanoic acids pharmacology, hydroxymethylglutaryl coa reductases genetics, mevalonic acid pharmacology, microinjections methods, morpholines pharmacology, pyrroles pharmacology, messenger RNA metabolism, somites drug effects, time factors, gamma glutamyltransferase metabolism.

Thorsen, D. and M. Hale (2005). **Development of zebrafish (*Danio rerio*) pectoral fin musculature.** *Journal of Morphology* 266(2): 241-255. ISSN: 0362 2525.
NAL Call Number: 444.8J826
Descriptors: zebrafish larva to adult, *Danio rerio*, fins, pectoral fin musculature, skeletal musculature, development and functional significance, development, swimming.

Thummel, R., S. Bai, M.P.J. Sarras, P. Song, J. McDermott, J. Brewer, M. Perry, X. Zhang, D.R. Hyde, and A.R. Godwin (2006). **Inhibition of zebrafish fin regeneration using in vivo electroporation of morpholinos against fgfr1 and msxb.** *Developmental Dynamics* 235(2): 336-46. ISSN: 1058 8388.
NAL Call Number: QL801.A4

Descriptors: zebrafish, homeodomain proteins metabolism, fibroblast receptors growth factor genetics, fibroblast receptors growth factor metabolism, regeneration physiology, electroporation.

Thummel, R., C.T. Burket, J.L. Brewer, M.P.J. Sarras, L. Li, M. Perry, J.P. McDermott, B. Sauer, D.R. Hyde, and A.R. Godwin (2005). **Cre-mediated site-specific recombination in zebrafish embryos.** *Developmental Dynamics* 233(4): 1366-77. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, integrases, site directed mutagenesis genetic recombination, genetically modified, physiology, reporter genes, HSP70 heat shock mosaicism, recombinant fusion proteins genetics, beta galactosidase genetics.

Thummel, R., L. Li, C. Tanase, M.P.J. Sarras, and A.R. Godwin (2004). **Differences in expression pattern and function between zebrafish hoxc13 orthologs: recruitment of Hoxc13b into an early embryonic role.** *Developmental Biology* 274(2): 318-33. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Vertebrate Hox genes are generally believed to initiate expression at the primitive streak or early neural plate stages. The timing and spatial restrictions of the Hox expression patterns during these stages correlate well with their demonstrated role in axial patterning. Here we demonstrate that one zebrafish hoxc13 ortholog, hoxc13a, has an expression pattern in the developing tail bud that is consistent with the gene playing a role in axial patterning. However, the second hoxc13 ortholog, hoxc13b, is maternally expressed and is detectable in every cell of early cleavage embryos through gastrulae. In addition, both transcript and protein are detectable at these stages. At 19 h post fertilization (hpf), hoxc13b expression is up-regulated in the tail bud, becoming restricted to the tail bud by 24 hpf. Importantly, by 24 hpf, hoxc13b morphants show a specific developmental delay, which can be rescued by co-injecting synthetic capped hoxc13a or hoxc13b message. These data suggest some functional divergence due to altered expression patterns of the two hoxc13 orthologs after duplication. Further characterization of the hoxc13b morphant delay reveals that it is biphasic in nature, with the first phase of the delay occurring before gastrulation, suggesting a new role for vertebrate Hox genes before their conserved role in axial patterning. The extent of the delay does not change through 20 hpf; however, an additional delay emerges at this time. Notably, this second phase of the delay correlates with hoxc13b expression pattern becoming restricted to the tail bud.
Descriptors: zebrafish embryos, body patterning, developmental gene expression regulation, homeodomain proteins metabolism, protein isoforms metabolism, proteins metabolism, amino acid and base sequence, homeodomain proteins chemistry and in situ hybridization, mice, molecular sequence data, antisense oligonucleotides genetics and metabolism, protein isoforms genetics, sequence alignment.

Thummel, R., C.T. Burket, M.P. Sarras, B. Sauer, D.R. Hyde, and A.R. Godwin (2006). **Cre-mediated site-specific recombination in zebrafish embryos**. *Developmental Biology* 295(1): 371-372. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, methods and techniques, molecular genetics, cre-mediated site specific recombination.

Tian, J., C. Yam, G. Balasundaram, H. Wang, A. Gore, and K. Sampath (2003). **A temperature-sensitive mutation in the nodal-related gene cyclops reveals that the floor plate is induced during gastrulation in zebrafish.** *Development* 130(14): 3331-42. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The floor plate, a specialized group of cells in the ventral midline of the neural tube of vertebrates, plays crucial roles in patterning the central nervous system. Recent work from zebrafish, chick, chick-quail chimeras and mice to investigate the development of the floor plate have led to several models of floor-plate induction. One model suggests that the floor plate is formed by inductive signalling from the notochord to the overlying neural tube. The induction is thought to be mediated by notochord-derived Sonic hedgehog (Shh), a secreted protein, and requires direct cellular contact between the notochord and the neural tube. Another model proposes a role for the organizer in generating midline precursor cells that produce floor plate cells independent of notochord specification, and proposes that floor plate specification occurs early, during gastrulation. We describe a temperature-sensitive mutation that affects the zebrafish Nodal-related secreted signalling factor, Cyclops, and use it to address the issue of when the floor plate is induced in zebrafish. Zebrafish cyclops regulates the expression of shh in the ventral neural tube. Although null mutations in cyclops result in the lack of the medial floor plate, embryos homozygous for the temperature-sensitive mutation have floor plate cells at the permissive temperature and lack floor plate cells at the restrictive temperature. We use this mutant allele in temperature shift-up and shift-down experiments to answer a central question pertaining to the timing of vertebrate floor plate induction. Abrogation of Cyc/Nodal signalling in the temperature-sensitive mutant embryos at various stages indicates that the floor plate in zebrafish is induced early in development, during gastrulation. In addition, continuous Cyclops signalling is required through gastrulation for a complete ventral neural tube throughout the length of the neuraxis. Finally, by modulation of Nodal signalling levels in mutants and in ectopic overexpression experiments, we show that, similar to the requirements for prechordal plate mesendoderm fates, uninterrupted and high levels of Cyclops signalling are required for induction and specification of a complete ventral neural tube.
Descriptors: zebrafish embryos, mutation, neural crest embryology, transforming growth factor beta genetics and physiology, alleles, COS cells, chromosome mapping, gastrula cytology, genotype, homozygote, in situ hybridization, intracellular signaling peptides and proteins, fluorescence microscopy, genetic models, neural crest cytology, neurons cytology, messenger RNA metabolism, signal transduction, temperature, proteins.

Tidyman, W.E., A.J. Sehnert, A. Huq, J. Agard, F. Deegan, D.Y. Stainier, and C.P. Ordahl (2003). **In vivo regulation of the chicken cardiac troponin T gene promoter in zebrafish embryos.** *Developmental Dynamics* 227(4): 484-96. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, chickens genetics, molecular evolution, developmental gene expression regulation, troponin T genetics, genetically modified, base sequence, binding sites genetics, cultured cells, chick embryo, enhancer elements genetics, gene components, green fluorescent proteins, in situ hybridization, luciferases, luminescent proteins, microinjections, muscles embryology, mutation genetics, plasmids, promoter regions genetics, .

Tingaud Sequeira, A., J. Forgue, M. Andre, and P.J. Babin (2006). **Epidermal transient down-regulation of retinol-binding protein 4 and mirror expression of apolipoprotein Eb and estrogen receptor 2a during zebrafish fin and scale development.** *Developmental Dynamics* 235(11): 3071-9. ISSN: 1058 8388.

NAL Call Number: QL801.A4
Descriptors: zebrafish, epidermis growth and development, developmental gene expression regulation, morphogenesis genetics, apolipoproteins e analysis and genetics, carrier proteins analysis and genetics, down regulation, epidermis chemistry and drug effects, larva growth and development and metabolism, morphogenesis drug effects, messenger RNA analysis and metabolism, estrogen receptorsanalysis and genetics, thiourea analogs and derivatives and pharmacology, genetic transcription drug effects, up regulation, proteins analysis and genetics.

Toro, S. and Z.M. Varga (2007). **Equivalent progenitor cells in the zebrafish anterior preplacodal field give rise to adenohypophysis, lens, and olfactory placodes.** *Seminars in Cell and Developmental Biology* 18(4): 534-42. ISSN: 1084-9521.
NAL Call Number: QH573
Abstract: Embryonic organizing centers secrete signaling molecules that instruct target cells about their position and future identity. Information about cell position in relation to sources of instructive signals and about precursor cell lineages is key to our understanding of developmental processes that restrict cell potency and determine cell fate. We review adenohypophysis, lens, and olfactory placode formation and how gene expression patterns, cell positions, and cell fates in the anterior neural plate and anterior placodal field correlate in zebrafish and other vertebrates. Single cell lineage analysis in zebrafish suggests that the majority of preplacodal cells might be specified for pituitary, lens, or olfactory placode by the end of gastrulation.
Descriptors: zebrafish embryos, crystalline lens embryology, neural plate embryology, anterior pituitary gland embryology, body patterning physiology, cell lineage physiology, cell movement physiology, developmental gene expression regulation, neurons physiology.

Torres, V.J., A.D. Gitler, S.D. Fraser, J.D. Berk, V.N. Pham, M.C. Fishman, S. Childs, J.A. Epstein, and B.M. Weinstein (2004). **Semaphorin-plexin signaling guides patterning of the developing vasculature**. *Developmental Cell* 7(1): 117-123. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Major vessels of the vertebrate circulatory system display evolutionarily conserved and reproducible anatomy, but the cues guiding this stereotypic patterning remain obscure. In the nervous system, axonal pathways are shaped by repulsive cues provided by ligands of the semaphorin family that are sensed by migrating neuronal growth cones through plexin receptors. We show that proper blood vessel pathfinding requires the endothelial receptor PlexinD1 and semaphorin signals, and we identify mutations in plexinD1 in the zebrafish vascular patterning mutant out of bounds. These results reveal the fundamental conservation of repulsive patterning mechanisms between axonal migration in the central nervous system and vascular endothelium during angiogenesis. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, cardiovascular system, development, videomicroscopy, imaging and microscopy techniques, vascular endothelium, semaphorin-plexin D1, vascular patterning mutant.

Tribulo, C., M.J. Aybar, V.H. Nguyen, M.C. Mullins, and R. Mayor (2003). **Regulation of msx genes by a bmp gradient is essential for neural crest specification.** *Development* 130(26): 6441-6452. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Descriptors: zebrafish embryo, development, molecular genetics, nervous system, neural

coordination, in situ hybridization, genetic techniques, dorsoventral patterning, gene expression, gene regulation.

Trinh, L.A., D. Meyer, and D.Y. Stainier (2003). **The Mix family homeodomain gene bonnie and clyde functions with oTher components of The Nodal signaling pathway to regulate neural patterning in zebrafish.** *Development* 130(20): 4989-98. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Mix family homeodomain proteins, such as Xenopus Mixer and zebrafish Bonnie and clyde (Bon), have been shown to regulate the formation of the endoderm and are likely to be transcriptional mediators of Nodal signaling. Here, we show that, in addition to its previously described role in endoderm formation, Bon also regulates the anteroposterior patterning of the neuroectoderm. bon-mutant embryos exhibit an anterior reduction of the neural plate. By using targeted injection of antisense morpholino oligonucleotides, we demonstrate that Bon is required in the axial mesoderm for anterior neural development. Consistent with these results, bon-mutant embryos show defects in axial mesoderm gene expression starting at mid-gastrulation stages. In addition, genetic analyses demonstrate a functional interaction during neural patterning between bon and two components of the Nodal signaling pathway, the nodal-related gene squint (sqt) and forkhead box H1 [foxh1; mutant locus schmalspur (sur)]. bon-/-;sqt-/- and bon-/-;sur-/- embryos exhibit neural patterning defects that are much more severe than those seen in the single mutants, suggesting that these genes function in parallel in this process. We also show that the severity of the neural patterning defects in the single- and double-mutant embryos correlates with the degree of reduction in expression of the Wnt antagonist gene dickkopf 1. Furthermore, bon-/-;sqt-/- and bon-/-;sur-/- embryos exhibit identical morphological and gene expression defects, suggesting, in part, that bon, sqt and sur (foxh1) play overlapping roles in neural patterning. Taken together, these results provide evidence for a complex genetic network in which bon functions both downstream of, and possibly in parallel to, Nodal signaling to regulate neural patterning via the modulation of mesendodermal gene expression.
Descriptors: zebrafish embryology, homeodomain nervous system embryology, transforming growth factor beta metabolism, proteins, body patterning physiology, homeodomain proteins metabolism, intercellular signaling peptides and proteins, signal transduction physiology.

Trinh, L.A. and D.Y. Stainier (2004). **Fibronectin regulates epithelial organization during myocardial migration in zebrafish.** *Developmental Cell* 6(3): 371-82. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Several genes have been implicated in heart tube formation, yet we know little about underlying cellular mechanisms. We analyzed the cellular architecture of the migrating myocardial precursors, and find that they form coherent epithelia that mature as they move medially. Mutant analyses indicate that the cardia bifida locus natter (nat) is required for the integrity of the myocardial epithelia. We positionally cloned nat and show that it encodes Fibronectin. During myocardial migration, Fibronectin is deposited at the midline between the endoderm and endocardial precursors, and laterally around the myocardial precursors. Further analyses show that Fibronectin deposition at the midline is required for the timely migration of myocardial precursors, but dispensable for the migration process itself. In the complete absence of Fibronectin, adherens junctions between myocardial precursors do not form properly, suggesting that cell-substratum interactions are required for epithelial organization. These data suggest that myocardial migration is dependent on epithelial integrity.
Descriptors: zebrafish embryo, cell movement physiology, epithelial cells physiology,

fibronectins physiology, heart embryology, myoblasts physiology, myocardium cytology, body patterning genetics and physiology, cell adhesion genetics and physiology, chromosome mapping methods, cytoskeletal proteins metabolism, DNA mutational analysis fibronectins genetics, developmental gene expression regulation, genotype, green fluorescent proteins, immunohistochemistry, in situ hybridization methods, luminescent proteins metabolism, microinjections methods, myosin light chains metabolism, antisense oligonucleotides metabolism, protein kinase C metabolism, somites metabolism, trans activators metabolism, proteins, beta catenin.

Tsai, W.B., X. Zhang, D. Sharma, W. Wu, and W.H. Kinsey (2005). **Role of Yes kinase during early zebrafish development.** *Developmental Biology* 277(1): 129-41. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: We have identified the Yes kinase in zebrafish eggs and investigated its role in development of the zebrafish embryo. In situ hybridization as well as immunofluorescence techniques demonstrated that Yes kinase is maternally expressed and is localized to the cortical region of the unfertilized egg. Fertilization resulted in concentration of Yes kinase to the blastodisc where it continued to be localized to the blastoderm cells through cleavage, gastrulation, and later development. Yes kinase activity was found to decrease abruptly at fertilization, then increase progressively during epiboly, and was maintained at high levels throughout gastrulation. The role of Yes kinase in development was tested by treating embryos with chemical protein tyrosine kinase (PTK) inhibitors such as 4-amino-5-(4-chlorophenyl)-7-(t-butyl) pyrazolo[3,4-d] pyrimidine (PP2) and by injection of antisense morpholinos. Both treatments resulted in the arrest of development at the beginning of the epiboly. Co-immunoprecipitation studies demonstrated that Yes kinase participates in a stable complex with focal adhesion kinase (FAK), which is phosphorylated in vitro. These results demonstrate that Yes kinase plays an important role in epiboly and indicate that Yes kinase participates in signaling by focal adhesion kinase during early development.
Descriptors: zebrafish embryos, embryonic development, proto oncogene, src family kinases physiology, amino acid sequence, chemistry, molecular sequence data, ovum chemistry, proto oncogene proteins analysis, proto oncogene proteins C yes, src family kinases analysis.

Tsang, M., S. Maegawa, A. Kiang, R. Habas, E. Weinberg, and I.B. Dawid (2004). **A role for MKP3 in axial patterning of the zebrafish embryo.** *Development* 131(12): 2769-79. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Fibroblast growth factors (FGFs) are secreted molecules that can activate the RAS/mitogen-activated protein kinase (MAPK) pathway to serve crucial functions during embryogenesis. Through an in situ hybridization screen for genes with restricted expression patterns during early zebrafish development, we identified a group of genes that exhibit similar expression patterns to FGF genes. We report the characterization of zebrafish MAP kinase phosphatase 3 (MKP3; DUSP6 - Zebrafish Information Network), a member of the FGF synexpression group, showing that it has a crucial role in the specification of axial polarity in the early zebrafish embryo. MKP3 dephosphorylates the activated form of MAPK, inhibiting the RAS/MAPK arm of the FGF signaling pathway. Gain- and loss-of-function studies reveal that MKP3 is required to limit the extent of FGF/RAS/MAPK signaling in the early embryo, and that disturbing this inhibitory pathway disrupts dorsoventral patterning at the onset of gastrulation. The earliest mkp3 expression is restricted to the future dorsal region of the embryo where it is initiated by a maternal beta-catenin signal, but soon after its initiation, mkp3 expression comes under the control of FGF signaling. Thus, mkp3 encodes a feedback

attenuator of the FGF pathway, the expression of which is initiated at an early stage so as to ensure correct FGF signaling levels at the time of axial patterning.
Descriptors: zebrafish, body patterning physiology, physiology, developmental gene expression regulation genetics, protein tyrosine phosphatase genetics, amino acid sequence, conserved sequence, Fgf genetics and physiology, in situ hybridization, molecular sequence data, reverse transcriptase PCR, sequence alignment and homology.

Tsend Ayush, E., L.A. O'Sullivan, F.S. Grutzner, S.M. Onnebo, R.S. Lewis, M.L. Delbridge, J.A. Marshall Graves, and A.C. Ward (2005). **RBMX gene is essential for brain development in zebrafish.** *Developmental Dynamics* 234(3): 682-8. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, brain embryology, brain metabolism, nuclear proteins metabolism, RNA binding proteins metabolism, amino acid sequence, embryology, metabolism, developmental gene expression regulation, molecular sequence data, nuclear proteins chemistry and genetics, phylogeny, messenger RNA genetics, RNA binding proteins chemistry and genetics, sequence alignment, amino acid sequence homology, proteins chemistry and genetics.

Tucker, J.A. and M.C. Mullins (2005). **Temporal progression of BMP signaling in dorsoventral patterning of the early zebrafish embryo.** *Developmental Biology* 283(2): 620. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, development, molecular genetics, cell fate, gastrulation, dorsoventral patterning, BMp.

Tyurina, O.V., B. Guner, E. Popova, J. Feng, A.F. Schier, J.D. Kohtz, and R.O. Karlstrom (2005). **Zebrafish Gli3 functions as both an activator and a repressor in Hedgehog signaling.** *Developmental Biology* 277(2): 537-56 . ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Hedgehog (Hh) signaling regulates cell differentiation and patterning in a wide variety of embryonic tissues. In vertebrates, at least three Gli transcription factors (Gli1, Gli2, and Gli3) are involved in Hh signal transduction. Comparative studies have revealed divergent requirements for Gli1 and Gli2 in zebrafish and mouse. Here, we address the question of whether Gli3 function has also diverged in zebrafish and analyze the regulatory interactions between Hh signaling and Gli activity. We find that zebrafish Gli3 has an early function as an activator of Hh target genes that overlaps with Gli1 activator function in the ventral neural tube. In vitro reporter analysis shows that Gli3 cooperates with Gli1 to activate transcription in the presence of high concentrations of Hh. During late somitogenesis stages, Gli3 is required as a repressor of the Hh response. Gli3 shares this repressor activity with Gli2 in the dorsal spinal cord, hindbrain, and midbrain, but not in the forebrain. Consistently, zebrafish Gli3 blocks Gli1-mediated activation of a reporter gene in the absence of Hh in vitro. In the eye, Gli3 is also required for proper ath5 expression and the differentiation of retinal ganglion cells (RGCs). These results reveal a conserved role for Gli3 in vertebrate development and uncover novel regional functions and regulatory interactions among gli genes.
Descriptors: zebrafish embryology, DNA binding proteins genetics, proteins metabolism, developmental gene expression regulation, nerve tissue proteins metabolism, signal transduction physiology, trans activators metabolism, transcription factors metabolism, amino acid and base sequence, cell differentiation physiology, central nervous system embryology and metabolism, cluster analysis, DNA primers, DNA binding proteins genetics, eye metabo-

lism, growth substances metabolism, Hedgehog proteins, in situ hybridization, kruppel like transcription factors, microinjections, molecular sequence data, nerve tissue antisense oligonucleotides, polymorphism, single stranded conformational, sequence alignment, DNA sequence analysis, transcription factors genetics.

Udvadia, A.J. and E. Linney (2003). **Windows into development: historic, current, and future perspectives on transgenic zebrafish.** *Developmental Biology* 256(1): 1-17. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The recent explosion of transgenic zebrafish lines in the literature demonstrates the value of this model system for detailed in vivo analysis of gene regulation and morphogenetic movements. The optical clarity and rapid early development of zebrafish provides the ability to follow these events as they occur in live, developing embryos. This article will review the development of transgenic technology in zebrafish as well as the current and future uses of transgenic zebrafish to explore the dynamic environment of the developing vertebrate embryo.
Descriptors: zebrafish, genetically modified, developmental biology history, developmental biology trends, developmental gene expression regulation, gene targeting history and trends, genetic engineering history, genetic engineering trends, 20th century hisrory, 21st century history.

Uemura, O., Y. Okada, H. Ando, M. Guedj, S.I. Higashijima, T. Shimazaki, N. Chino, H. Okano, and H. Okamoto (2005). **Comparative functional genomics revealed conservation and diversification of three enhancers of the Is11 gene for motor and sensory neuron-specific expression**. *Developmental Biology* 278(2): 587-606. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Islet-1 (Isl1) is a member of the Isl1 family of LIM-homeodomain transcription factors (LIM-HD) that is expressed in a defined subset of motor and sensory neurons during vertebrate ernbryogenesis. To investigate how this specific expression of isl1 is regulated, we searched for enhancers of the isl1 gene that are conserved in vertebrate evolution. Initially, two enhancer elements, CREST1 and CREST2, were identified downstream of the isl1 locus in the genomes of fugu, chick, mouse, and human by BLAST searching for highly similar elements to those originally identified as motor and sensory neuron-specific enhancers in the zebrafish genome. The combined action of these elements is sufficient for completely recapitulating the subtype-specific expression of the isl1 gene in motor neurons of the mouse spinal cord. Furthermore, by direct comparison of the upstream flanking regions of the zebrafish and human isl1 genes, we identified another highly conserved noncoding element, CREST3, and subsequently C3R, a similar element to CREST3 with two CDP CR1 recognition motifs, in the upstream regions of all other isl1 family members. In mouse and human, CRESTs are located as far as more than 300 kb away from the isl1 locus, while they are much closer to the isl1 locus in zebrafish. Although all of zebrafisli CREST2, CREST3, and C3R activate gene expression in the sensory neurons of zebrafish, CREST2 of mouse and human does not have the sequence necessary for sensory neuron-specific expression. Our results revealed both a remarkable conservation of the regulatory elements regulating subtype-specific gene expression in motor and sensory neurons and the dynamic process of reorganization of these elements whereby each element increases the level of cell-type specificity by losing redundant functions with the other elements during vertebrate evolution.
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Descriptors: zebrafish, development, molecular genetics, embryogenesis, CREST1, enhancers, Is11 gene.

Ulrich, F., M.L. Concha, P.J. Heid, E. Voss, S. Witzel, H. Roehl, M. Tada, S.W. Wilson, R.J. Adams, D.R. Soll, and C.P. Heisenberg (2003). **Slb/Wnt11 controls hypoblast cell migration and morphogenesis at the onset of zebrafish gastrulation.** *Development* 130(22): 5375-84. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: During vertebrate gastrulation, highly coordinated cellular rearrangements lead to the formation of the three germ layers, ectoderm, mesoderm and endoderm. In zebrafish, silberblick (slb)/wnt11 regulates normal gastrulation movements by activating a signalling pathway similar to the Frizzled-signalling pathway, which establishes epithelial planar cell polarity (PCP) in *Drosophila*. However, the cellular mechanisms by which slb/wnt11 functions during zebrafish gastrulation are still unclear. Using high-resolution two-photon confocal imaging followed by computer-assisted reconstruction and motion analysis, we have analysed the movement and morphology of individual cells in three dimensions during the course of gastrulation. We show that in slb-mutant embryos, hypoblast cells within the forming germ ring have slower, less directed migratory movements at the onset of gastrulation. These aberrant cell movements are accompanied by defects in the orientation of cellular processes along the individual movement directions of these cells. We conclude that slb/wnt11-mediated orientation of cellular processes plays a role in facilitating and stabilising movements of hypoblast cells in the germ ring, thereby pointing at a novel function of the slb/wnt11 signalling pathway for the regulation of migratory cell movements at early stages of gastrulation.

Descriptors: zebrafish embryology, cell movement physiology, gastrula metabolism, glycoproteins metabolism, embryonic induction physiology, endoderm, Wnt proteins, proteins.

Ulrich, F., M. Krieg, E.M. Schoetz, V. Link, I.A. Castanon, V. Schnabel, A. Taubenberger, D. Mueller, P. Puech, and C.P. Heisenberg (2005). **Wnt11 functions in gastrulation by controlling cell cohesion through Rab5c and E-cadherin**. *Developmental Cell* 9(4): 555-564. ISSN: 1534 5807.

NAL Call Number: QH573.C42

Abstract: Wnt11 plays a central role in tissue morphogenesis during vertebrate gastrulation, but the molecular and cellular mechanisms by which Wnt11 exerts its effects remain poorly understood. Here, we show that Wnt11 functions during zebrafish gastrulation by regulating the cohesion of mesodermal and endodermal (mesendodermal) progenitor cells. Importantly, we demonstrate that Wnt11 activity in this process is mediated by the GTPase Rab5, a key regulator of early endocytosis, as blocking Rab5c activity in wild-type embryos phenocopies slb/wnt11 mutants, and enhancing Rab5c activity in slb/wnt11 mutant embryos rescues the mutant phenotype. In addition, we find that Wnt11 and Rab5c control the endocytosis of E-cadherin and are required in mesendodermal cells for E-cadherin-mediated cell cohesion. Together, our results suggest that Wnt11 controls tissue morphogenesis by modulating E-cadherin-mediated cell cohesion through Rab5c, a novel mechanism of Wnt signaling in gastrulation. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, blood and lymphatics, transport and circulation, enzymology, population genetics, population studies, phenotype, gastrulation.

Notes: (DLC)2002242061.

Ungos, J.M., R.O. Karlstrom, and D.W. Raible (2003). **Hedgehog signaling is directly required for the development of zebrafish dorsal root ganglia neurons.** *Development* 130(22): 5351-62. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Hedgehog (Hh) signal transduction is directly required in zebrafish DRG precursors for proper development of DRG neurons. Zebrafish mutations in the Hh signaling pathway result in the absence of DRG neurons and the loss of expression of neurogenin1 (ngn1), a gene required for determination of DRG precursors. Cell transplantation experiments demonstrate that Hh acts directly on DRG neuron precursors. Blocking Hh pathway activation at later stages of embryogenesis with the steroidal alkaloid, cyclopamine, further reveals that the requirement for a Hh signal response in DRG precursors correlates with the onset of ngn1 expression. These results suggest that Hh signaling may normally promote DRG development by regulating expression of ngn1 in DRG precursors.
Descriptors: zebrafish embryos, spinal ganglia embryology, neurons metabolism, trans activators metabolism, proteins, basic helix loop helix transcription factors, ganglia, spinal drug effects, Hedgehog proteins, nerve tissue proteins biosynthesis, nerve tissue neural crest embryology, neurons drug effects, signal transduction drug effects and physiology, spinal cord embryology, teratogens pharmacology, trans activators drug effects, transcription factors biosynthesis and genetics, veratrum alkaloids pharmacology.

Urtishak, K.A., M. Choob, X. Tian, N. Sternheim, W.S. Talbot, E. Wickstrom, and S.A. Farber (2003). **Targeted gene knockdown in zebrafish using negatively charged peptide nucleic acid mimics.** *Developmental Dynamics* 228(3): 405-13. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, mutation genetics, peptide nucleic acids pharmacology, RNA genetics, base sequence, morpholines, nucleic acid denaturation and hybridization, peptide nucleic acids chemistry, thermodynamics.

Urven, L.E., T. Yabe, and F. Pelegri (2006). **A role for non-muscle myosin II function in furrow maturation in the early zebrafish embryo.** *Journal of Cell Science* 119(Pt 20): 4342-52. ISSN: 0021-9533.
NAL Call Number: QH301.J6
Abstract: Cytokinesis in early zebrafish embryos involves coordinated changes in the f-actin- and microtubule-based cytoskeleton, and the recruitment of adhesion junction components to the furrow. We show that exposure to inhibitors of non-muscle myosin II function does not affect furrow ingression during the early cleavage cycles but interferes with the recruitment of pericleavage f-actin and cortical beta-catenin aggregates to the furrow, as well as the remodeling of the furrow microtubule array. This remodeling is in turn required for the distal aggregation of the zebrafish germ plasm. Embryos with reduced myosin activity also exhibit at late stages of cytokinesis a stabilized contractile ring apparatus that appears as a ladder-like pattern of short f-actin cables, supporting a role for myosin function in the disassembly of the contractile ring after furrow formation. Our studies support a role for myosin function in furrow maturation that is independent of furrow ingression and which is essential for the recruitment of furrow components and the remodeling of the cytoskeleton during cytokinesis.
Descriptors: zebrafish embryos, myosin Type II physiology, actins metabolism, azepines pharmacology, cell aggregation drug effects, cytokinesis drug effects, cytoskeleton drug effects and metabolism, dead box RNA helicases genetics, cytology, drug effects, metabolism, developmental gene expression regulation genetics, heterocyclic compounds with 4 or more rings pharmacology, in situ hybridization, myosin Type II antagonists and inhibitors, myosin light chain kinase antagonists and inhibitors, naphthalenes pharmacology, messenger RNA genetics and metabolism, beta catenin metabolism.

Van der heyden, C., F. Allizard, J.Y. Sire, and A. Huysseune (2005). **Tooth development in vitro in two teleost fish, the cichlid Hemichromis bimaculatus and the cyprinid *Danio rerio.*** *Cell and Tissue Research* 321(3): 375-389. ISSN: 0302 766X.

NAL Call Number: QH581.A1Z4

Abstract: A technique for organotypic in vitro culture with serum-free medium was tested for its appropriateness to mimic normal odontogenesis in the cichlid fish *Hemichromis bimaculatus* and the zebrafish *Danio rerio*. Serial semithin sections were observed by light microscopy to collect data on tooth patterning and transmission electron microscopy was used to compare cellular and extracellular features of tooth germs developing in vitro with the situation in vivo. Head explants of *H. bimaculatus* from 120 h post-fertilization (hPF) to 8.5 days post-fertilization (dPF) and of zebrafish from 45 hPF to 79 hPF and adults kept in culture for 3, 4 or 7 days revealed that tooth germs developed in vitro from explants in which the buccal or pharyngeal epithelium was apparently undifferentiated and, when present at the time of explantation, they continued their development up to a stage of attachment. In addition, the medium allowed the morphogenesis and cytodifferentiation of the tooth germs similar to that observed in vivo and the establishment of a dental pattern (place and order of tooth appearance and of attachment) that mimicked that in vivo. Organotypic culture in serum-free conditions thus provides us with the means of studying epithelial-mesenchymal interactions during tooth development in teleost fish and of analysing the genetic control of either mandibular or pharyngeal tooth development and replacement in these polyphyodont species. Importantly, it allows heads from embryonically lethal (zebrafish) mutants or from early lethal knockdown experiments to develop beyond the point at which the embryos normally die. Such organotypic culture in serum-free conditions could therefore become a powerful tool in developmental studies and open new perspectives for craniofacial research.

Descriptors: zebrafish, *Danio rerio*, *Hemichromis bimaculatus*, laboratory culture, serum free organotypic in vitro culture, suitability as odontogenesis study system, evaluation , teeth , tooth development, development, germs.

Van der Meer, D.L., I.J. Marques, J.T. Leito, J. Besser, J. Bakkers, E. Schoonheere, and C.P. Bagowski (2006). **Zebrafish cypher is important for somite formation and heart development.** *Developmental Biology* 299(2): 356-72. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Mammalian CYPHER (Oracle, KIA0613), a member of the PDZ-LIM family of proteins (Enigma/LMP-1, ENH, ZASP/Cypher, RIL, ALP, and CLP-36), has been associated with cardiac and muscular myopathies. Targeted deletion of Cypher in mice is neonatal lethal possibly caused by myopathies. To further investigate the role of cypher in development, we have cloned the zebrafish orthologue. We present here the gene, domain structure, and expression pattern of zebrafish cypher during development. Cypher was not present as a maternal mRNA and was absent during early development. Cypher mRNA was first detected at the 3-somite stage in adaxial somites, and as somites matured, cypher expression gradually enveloped the whole somite. Later, cypher expression was also found in the heart, in head and jaw musculature, and in the brain. We further identified 13 alternative spliced forms of cypher from zebrafish heart and skeletal muscle tissue, among them a very short form containing the PDZ domain but lacking the ZM (ZASP-like) motif and the LIM domains. Targeted gene knock-down experiments using cypher antisense morpholinos led to severe defects, including truncation of the embryo, deformation of somites, dilatation of the pericardium, and thinning of the ventricular wall. The phenotype could be rescued by a cypher form, which contains the PDZ domain and the ZM motif, but lacks all three LIM domains. These findings indicate that a PDZ domain protein is important for normal somite forma-

tion and in normal heart development. Treatment of zebrafish embryos with cyclopamine, which disrupts hedgehog signaling, abolished cypher expression in 9 somite and 15-somite stage embryos. Taken together, our data suggest that cypher may play a role downstream of sonic hedgehog, in a late stage of somite development, when slow muscle fibers differentiate and migrate from the adaxial cells.

Descriptors: zebrafish embryos, heart embryology, homeodomain proteins metabolism, somites physiology, proteins metabolism, alternative splicing, amino acid sequence, genetically modified, heart physiology, homeodomain molecular sequence data, skeletal muscle embryology and metabolism, myocardium metabolism, organ specificity, tertiary protein structure, messenger RNA, stored metabolism, proteins genetics.

Van Raay, T.J., R.J. Coffey, and L. Solnica Krezel (2007). **Zebrafish Naked1 and Naked2 antagonize both canonical and non-canonical Wnt signaling.** *Developmental Biology* 309(2): 151-68. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Wnt signaling controls a wide range of developmental processes and its aberrant regulation can lead to disease. To better understand the regulation of this pathway, we identified zebrafish homologues of Naked Cuticle (Nkd), Nkd1 and Nkd2, which have previously been shown to inhibit canonical Wnt/beta-catenin signaling. Zebrafish nkd1 expression increases substantially after the mid-blastula transition in a pattern mirroring that of activated canonical Wnt/beta-catenin signaling, being expressed in both the ventrolateral blastoderm margin and also in the axial mesendoderm. In contrast, zebrafish nkd2 is maternally and ubiquitously expressed. Overexpression of Nkd1 or Nkd2a suppressed canonical Wnt/beta-catenin signaling at multiple stages of early zebrafish development and also exacerbated the cyclopia and axial mesendoderm convergence and extension (C&E) defect in the non-canonical Wnt/PCP mutant silberblick (slb/wnt11). Thus, Nkds are sufficient to antagonize both canonical and non-canonical Wnt signaling. Reducing Nkd function using antisense morpholino oligonucleotides resulted in increased expression of canonical Wnt/beta-catenin target genes. Finally, reducing Nkd1 function in slb mutants suppressed the axial mesendoderm C&E defect. These data indicate that zebrafish Nkd1 and Nkd2 function to limit both canonical and non-canonical Wnt signaling.

Descriptors: zebrafish, NakeD1, Naked2, canonical, non-canonical, Wnt signaling, Naked cuticle, cyclopia, axial mesendoderm .

Vanderlaan, G., O.V. Tyurina, R.O. Karlstrom, and A. Chandrasekhar (2005). **Gli function is essential for motor neuron induction in zebrafish.** *Developmental Biology* 282(2): 550-70. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The Gli family of zinc-finger transcription factors mediates Hedgehog (Hh) signaling in all vertebrates. However, their roles in ventral neural tube patterning, in particular motor neuron induction, appear to have diverged across species. For instance, cranial motor neurons are essentially lost in zebrafish detour (gli1(-)) mutants, whereas motor neuron development is unaffected in mouse single gli and some double gli knockouts. Interestingly, the expression of some Hh-regulated genes (ptc1, net1a, gli1) is mostly unaffected in the detour mutant hindbrain, suggesting that other Gli transcriptional activators may be involved. To better define the roles of the zebrafish gli genes in motor neuron induction and in Hh-regulated gene expression, we examined these processes in you-too (yot) mutants, which encode dominant repressor forms of Gli2 (Gli2(DR)), and following morpholino-mediated knockdown of gli1, gli2, and gli3 function. Motor neuron induction at all axial

levels was reduced in yot (gli2(DR)) mutant embryos. In addition, Hh target gene expression at all axial levels except in rhombomere 4 was also reduced, suggesting an interference with the function of other Glis. Indeed, morpholino-mediated knockdown of Gli2(DR) protein in yot mutants led to a suppression of the defective motor neuron phenotype. However, gli2 knockdown in wild-type embryos generated no discernable motor neuron phenotype, while gli3 knockdown reduced motor neuron induction in the hindbrain and spinal cord. Significantly, gli2 or gli3 knockdown in detour (gli1(-)) mutants revealed roles for Gli2 and Gli3 activator functions in ptc1 expression and spinal motor neuron induction. Similarly, gli1 or gli3 knockdown in yot (gli2(DR)) mutants resulted in severe or complete loss of motor neurons, and of ptc1 and net1a expression, in the hindbrain and spinal cord. In addition, gli1 expression was greatly reduced in yot mutants following gli3, but not gli1, knockdown, suggesting that Gli3 activator function is specifically required for gli1 expression. These observations demonstrate that Gli activator function (encoded by gli1, gli2, and gli3) is essential for motor neuron induction and Hh-regulated gene expression in zebrafish.
Descriptors: zebrafish embryology, embryonic induction physiology, developmental gene expression regulation, motor neurons physiology, signal transduction physiology, trans activators metabolism, transcription factors genetics, genotype, Hedgehog proteins, immunohistochemistry, multigene family genetics, mutation genetics, antisense oligonucleotides, cell surface receptors metabolism, veratrum alkaloids, metabolism.

VanStry, M., A. Kazlauskas, S.L. Schreiber, and K. Symes (2005). **Distinct effectors of platelet-derived growth factor receptor-alpha signaling are required for cell survival during embryogenesis**. *Proceedings of the National Academy of Sciences of the United States of America* 102(23): 8233-8238. ISSN: 0027 8424.
NAL Call Number: 500N21P
Abstract: Platelet-derived growth factor receptor (PDGFR) signaling is essential for normal embryonic development in many organisms, including frog, mouse, zebrafish, and sea urchin. The mode of action of PDGFR signaling during early development is poorly understood, however, mostly because inhibition of signaling through either the PDGFR alpha or PDGFR beta is embryonic lethal. In *Xenopus* embryos, disruption of PDGFR alpha signaling causes migrating anterior mesoderm cells to lose direction and undergo apoptosis through the mitochondrial pathway. To understand the mechanism of PDGFRa function in this process, we have analyzed all known effector-binding sites in vivo. By using a chemical inducer of dimerization to activate chimera PDGFR alpha s, we have identified a role for phospholipase C gamma (PLC gamma) in protecting cells from death, PDGFR alpha-mediated cell survival requires PLC gamma and phosphaticlylinositol 3-kinase signaling, and that PDGFR alpha with binding sites for these two signaling factors is sufficient for this activity. Other effectors of PDGFR alpha signaling, Shf, SHP-2, and Crk, are not required for this process. Thus, our findings show that PDGFR alpha signaling through PLC gamma and phosphatidylinositol 3-kinase has a protective role in preventing apoptosis in early development. Furthermore, we demonstrate that small molecule inducers of dimerization provide a powerful system to manipulate receptor function in developing embryos. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, cell biology, cell survival, embryogenesis.

Varga, M., S. Maegawa, G. Bellipanni, and E.S. Weinberg (2007). **Chordin expression, mediated by Nodal and FGF signaling, is restricted by redundant function of two beta-catenins in the zebrafish embryo.** *Mechanisms of Development* 124(9-10): 775-91. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: Using embryos transgenic for the TOP-GFP reporter, we show that the two

zebrafish beta-catenins have different roles in the organizer and germ-ring regions of the embryo. beta-Catenin-activated transcription in the prospective organizer region specifically requires beta-catenin-2, whereas the ventrolateral domain of activated transcription is abolished only when both beta-catenins are inhibited. chordin expression during zebrafish gastrulation has been previously shown in both axial and paraxial domains, but is excluded from ventrolateral domains. We show that this gene is expressed in paraxial territories adjacent to the domain of ventrolateral beta-catenin-activated transcription, with only slight overlap, consistent with the now well-known inhibitory effects of Wnt8 on dorsal gene expression. Eliminating both Wnt8/beta-catenin signaling and organizer activity by inhibition of expression of the two beta-catenins results in massive ectopic circumferential expression of chordin and later, by formation of a distinctive embryonic phenotype ('ciuffo') that expresses trunk and anterior neural markers with correct relative anteroposterior patterning. We show that chordin expression is required for this neural gene expression. The Nodal gene squint has been shown to be necessary for optimal expression of chordin and is sufficient in some contexts for its expression. However, chordin is not normally expressed in the ventrolateral germ-ring despite robust expression of squint in this domain. We show the ectopic circumferential expression of chordin and other dorsal genes to be completely dependent on Nodal and FGF signaling, and to be independent of a functional organizer. We propose that whereas the axial domain of chordin expression is formed by cells that are derived from the organizer, the paraxial domain is the result of axial-derived anti-Wnt signals, which relieve the repression that otherwise is set by the Wnt8/beta-catenin/vox,vent pathway on latent germ-ring Nodal/FGF-activated expression.

Descriptors: zebrafish embryos, Fgf physiology, glycoproteins biosynthesis, glycointercellular signaling peptides and proteins biosynthesis, intercellular signaling peptides and signal transduction physiology, transforming growth factor beta physiology, metabolism, beta catenin physiology, genetically modified, developmental gene expression regulation physiology, reporter genes, glycoproteins metabolism, intercellular signaling peptides and proteins metabolism, organizers, embryonic physiology, RNA 5' terminal oligopyrimidine sequence genetics, proteins antagonists and inhibitors, beta catenin antagonists and inhibitors.

Veien, E.S., M.J. Grierson, R.S. Saund, and R.I. Dorsky (2005). **Expression pattern of zebrafish tcf7 suggests unexplored domains of Wnt/beta-catenin activity.** *Developmental Dynamics* 233(1): 233-9. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryos, cytoskeletal proteins metabolism, intercellular signaling peptides and proteins metabolism, trans activators metabolism, amino acid sequence, basic helix loop helix transcription factors, binding sites, DNA binding proteins genetics, proteins metabolism, lymphoid enhancer bindingfactor 1, mice, molecular sequence data, organ specificity, repressor proteins metabolism, sequence alignment, trans activators genetics, transcription factors metabolism, Wnt proteins, beta catenin.

Verreijdt, L., M. Debiais Thibaud, V. Borday Birraux, C. Van der Heyden, J.Y. Sire, and A. Huysseune (2006). **Expression of the dlx gene family during formation of the cranial bones in the zebrafish (*Danio rerio*): differential involvement in the visceral skeleton and braincase.** *Developmental Dynamics* 235(5): 1371-89. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryology, homeodomain skull embryology, transcription factors genetics, homeodomain proteins biosynthesis, multigene family , osteogenesis genetics, skull

metabolism and physiology, transcription factors biosynthesis, metabolism, proteins biosynthesis.

Vihtelic, T.S., Y. Yamamoto, S.S. Springer, W.R. Jeffery, and D.R. Hyde (2005). **Lens opacity and photoreceptor degeneration in the zebrafish lens opaque mutant.** *Developmental Dynamics* 233(1): 52-65. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish opaque lens mutant, cataract genetics, crystalline lens abnormalities, photoreceptors physiology, cataract metabolism, immunohistochemistry, crystalline lens metabolism and transplantation, electron microscopy, opsin metabolism, photoreceptors abnormalities.

Villablanca, E.J., A. Pistocchi, F.A. Court, F. Cotelli, C. Bordignon, M.L. Allende, C. Traversari, and V. Russo (2007). **Abrogation of prostaglandin E2/EP4 signaling impairs the development of rag1+ lymphoid precursors in the thymus of zebrafish embryos.** *Journal of Immunology* 179(1): 357-64. ISSN: 0022-1767.
NAL Call Number: 448.8J8232
Abstract: PGE(2) is involved in a wide variety of physiological and pathological processes; however, deciphering its role in early mammalian development has been difficult due to the maternal contribution of PGE(2). To overcome this limitation we have investigated the role of PGE(2) during T cell development in zebrafish. In this study, we show that zebrafish ep4a, a PGE(2) receptor isoform of EP4, is expressed at 26 h postfertilization in the dorsal aorta-posterior cardinal vein joint region, which has a high homology with the mammal aorta-gonad-mesonephros area and where definitive hemopoiesis arises. Furthermore, it is expressed in the presumptive thymus rudiment by 48 h postfertilization. Supplementation of PGE(2) results in a strong increase in rag1 levels and cell proliferation in the thymus. In contrast, the inhibition of PGE(2) production, as well as EP4 blockade, abrogates the expression of rag1 in the thymus and that of the lymphoid precursor marker ikaros, not only in the dorsal aorta-posterior cardinal vein joint region but also in the newly identified caudal hemopoietic tissue without affecting early hemopoietic (scl, gata2) and erythropoietic (gata1) markers. These results identify ep4a as the earliest thymus marker and define a novel role for the PGE(2)/EP4 pathway in controlling T cell precursor development in zebrafish.
Descriptors: zebrafish, T cell development, cell differentiation immunology, homeodomain proteins biosynthesis, lymphocyte subsets metabolism, receptors, prostaglandin e physiology, signal transduction immunology, stem cells metabolism, thymus gland immunology, genetically modified, cell proliferation, dinoprostone antagonists and inhibitors and physiology, hematopoiesis immunology, lymphocyte subsets cytology, lymphopoiesis immunology, protein isoforms antagonists and inhibitors and physiology, receptors, prostaglandin e antagonists and inhibitors, stem cells cytology, thymus gland cytology, thymus gland embryology , up regulation immunology, zebrafish immunology.

Villablanca, E.J., A. Renucci, D. Sapede, V. Lec, F. Soubiran, P.C. Sandoval, C. Dambly Chaudiere, A. Ghysen, and M.L. Allende (2006). **Control of cell migration in the zebrafish lateral line: implication of the gene "tumour-associated calcium signal transducer," tacstd.** *Developmental Dynamics* 235(6): 1578-88 . ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, calcium signaling genetics, cell movement genetics, membrane glyco amino acid sequence, antigens, neoplasm genetics, calcium signaling physiology, cell adhe-

sion molecules genetics, cell movement physiology, membrane glycoproteins metabolism, molecular sequence data and physiology.

Villefranc, J.A., J. Amigo, and N.D. Lawson (2007). **Gateway compatible vectors for analysis of gene function in the zebrafish.** *Developmental Dynamics* 236(11): 3077-87. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, gateway compatible, vectors, plasmids, pcS-based plasmids, N-terminal, c-terminal, fusion proteins, mRNA.

Vitebsky, A., R. Reyes, M.J. Sanderson, W.C. Michel, and K.E. Whitlock (2005). **Isolation and characterization of the laure olfactory behavioral mutant in the zebrafish, *Danio rerio*.** *Developmental Dynamics* 234(1): 229-42. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: juvenile zebrafish, Laure (Ire) mutant, behavior physiology, cysteine metabolism, smell genetics, calcium binding protein, vitamin D dependent metabolism, electrophysiology, fluorescent antibody technique, afferent neurons cytology and metabolism, olfactory bulb abnormalities, cytology and metabolism, smell physiology, metabolism.

Vogeli, K.M., S.W. Jin, G.R. Martin, and D.Y. Stainier (2006). **A common progenitor for haematopoietic and endothelial lineages in the zebrafish gastrula.** *Nature* 443(7109): 337-9. ISSN: 0028-0836.
NAL Call Number: 472 N21
Abstract: It has been proposed that haematopoietic and endothelial cells share a common progenitor, termed the haemangioblast. This idea was initially conceived as a result of the observation that these two cell types develop in close proximity to each other within the embryo. Support for this hypothesis was provided by studies on single-cell-derived colonies that can produce both haematopoietic and endothelial cells in vitro. Although these data point towards the existence of a common progenitor for these two lineages, the presence of a bipotential progenitor cell has yet to be demonstrated in vivo. Through the construction of single-cell-resolution fate maps of the zebrafish late blastula and gastrula, we demonstrate that individual cells can give rise to both haematopoietic and endothelial cells. These bipotential progenitors arise along the entire extent of the ventral mesoderm and contribute solely to haematopoietic and endothelial cells. We also find that only a subset of haematopoietic and endothelial cells arise from haemangioblasts. The endothelial descendants of the haemangioblasts all clustered in a specific region of the axial vessels regardless of the location of their progenitors. Our results provide in vivo evidence supporting the existence of the haemangioblast and reveal distinct features of this cell population.
Descriptors: zebrafish, cell lineage, cytology, endothelial cells cytology, gastrula cytology, hematopoietic stem cells cytology, zebrafish blood, blastula cytology, embryology.

Von Hofsten, J., A. Larsson, and P.E. Olsson (2005). **Novel steroidogenic factor-1 homolog (ff1d) is coexpressed with anti-Mullerian hormone (AMH) in zebrafish.** *Developmental Dynamics* 233(2): 595-604. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryology, DNA binding proteins genetics , proteins metabolism, developmental gene expression regulation, glycoproteins metabolism, testicular hormones metabolism, transcription factors metabolism, amino acid sequence, DNA binding proteins genetics , proteins chemistry, genetics and metabolism, glycohomeodomain proteins, molecu-

lar sequence data, ovary metabolism, phylogeny, cytoplasmic receptors and nuclear, sequence alignment, testicular hormones genetics, testis metabolism, transcription factors chemistry, transcription factors genetics, proteins chemistry and genetics.

Von Trotha, J.W., J.A. Campos Ortega, and A.M. Reugels (2006). **Apical localization of ASIP/PAR-3:EGFP in zebrafish neuroepithelial cells involves the oligomerization domain CR1, the PDZ domains, and the C-terminal portion of the protein.** *Developmental Dynamics* 235(4): 967-977. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryos, cell polarity, green fluorescent proteins metabolism, neuroepithelial cells metabolism, proteins metabolism, amino acid sequence, *Caenorhabditis elegans* proteins chemistry, genetics and metabolism microinjections, confocal microscopy, video microscopy, molecular sequence data, protein isoforms chemistry, protein isoforms genetics and metabolism, tertiary protein structure, messenger RNA genetics and metabolism, recombinant fusion proteins chemistry and metabolism, proteins chemistry.

Wada, H., M. Iwasaki, T. Sato, I. Masai, Y. Nishiwaki, H. Tanaka, A. Sato, Y. Nojima, and H. Okamoto (2005). **Dual roles of zygotic and maternal Scribble1 in neural migration and convergent extension movements in zebrafish embryos.** *Development* 132(10): 2273-85. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: In the developing vertebrate hindbrain, the characteristic trajectory of the facial (nVII) motor nerve is generated by caudal migration of the nVII motor neurons. The nVII motor neurons originate in rhombomere (r) 4, and migrate caudally into r6 to form the facial motor nucleus. In this study, using a transgenic zebrafish line that expresses green fluorescent protein (GFP) in the cranial motor neurons, we isolated two novel mutants, designated landlocked (llk) and off-road (ord), which both show highly specific defects in the caudal migration of the nVII motor neurons. We show that the landlocked locus contains the gene scribble1 (scrb1), and that its zygotic expression is required for migration of the nVII motor neurons mainly in a non cell-autonomous manner. Taking advantage of the viability of the llk mutant embryos, we found that maternal expression of scrb1 is required for convergent extension (CE) movements during gastrulation. Furthermore, we show a genetic interaction between scrb1 and trilobite(tri)/strabismus(stbm) in CE. The dual roles of the scrb1 gene in both neuronal migration and CE provide a novel insight into the underlying mechanisms of cell movement in vertebrate development.
Descriptors: zebrafish embryos, cell movement physiology, facial nerve embryology, developmental gene expression regulation, membrane proteins genetics and metabolism, motor neurons cytology, proteins genetics and metabolism, base sequence, cell movement genetics, chromosome mapping, genetic crosses, DNA primers, gastrula metabolism, green fluorescent proteins metabolism, immunohistochemistry, in situ hybridization, molecular sequence data, mutation genetics, antisense oligonucleotides, reverse transcriptase PCR, rhombencephalon embryology, DNA sequence analysis.

Wada, H., H. Tanaka, S. Nakayama, M. Iwasaki, and H. Okamoto (2006). **Frizzled3a and Celsr2 function in the neuroepithelium to regulate migration of facial motor neurons in the developing zebrafish hindbrain.** *Development* 133(23): 4749-59. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Migration of neurons from their birthplace to their final target area is a crucial step in brain development. Here, we show that expression of the off-limits/frizzled3a (olt/

fz3a) and off-road/celsr2 (ord/celsr2) genes in neuroepithelial cells maintains the facial (nVII) motor neurons near the pial surface during their caudal migration in the zebrafish hindbrain. In the absence of olt/fz3a expression in the neuroepithelium, nVII motor neurons extended aberrant radial processes towards the ventricular surface and mismigrated radially to the dorsomedial part of the hindbrain. Our findings reveal a novel role for these genes, distinctive from their already known functions, in the regulation of the planar cell polarity (i.e. preventing integration of differentiated neurons into the neuroepithelial layer). This contrasts markedly with their reported role in reintegration of neuroepithelial daughter cells into the neuroepithelial layer after cell division.

Descriptors: zebrafish embryology, cadherins genetics, frizzled receptors genetics, rhombencephalon embryology, genetically modified, cadherins metabolism, cell movement genetics, cell movement physiology, frizzled receptors metabolism, developmental gene expression regulation, motor neurons cytology and metabolism, mutation, neuroepithelial cells cytology and metabolism, rhombencephalon metabolism.

Wada, N., Y. Javidan, S. Nelson, T.J. Carney, R.N. Kelsh, and T.F. Schilling (2005). **Hedgehog signaling is required for cranial neural crest morphogenesis and chondrogenesis at the midline in the zebrafish skull.** *Development* 132(17): 3977-88. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Neural crest cells that form the vertebrate head skeleton migrate and interact with surrounding tissues to shape the skull, and defects in these processes underlie many human craniofacial syndromes. Signals at the midline play a crucial role in the development of the anterior neurocranium, which forms the ventral braincase and palate, and here we explore the role of Hedgehog (Hh) signaling in this process. Using sox10:egfp transgenics to follow neural crest cell movements in the living embryo, and vital dye labeling to generate a fate map, we show that distinct populations of neural crest form the two main cartilage elements of the larval anterior neurocranium: the paired trabeculae and the midline ethmoid. By analyzing zebrafish mutants that disrupt sonic hedgehog (shh) expression, we demonstrate that shh is required to specify the movements of progenitors of these elements at the midline, and to induce them to form cartilage. Treatments with cyclopamine, to block Hh signaling at different stages, suggest that although requirements in morphogenesis occur during neural crest migration beneath the brain, requirements in chondrogenesis occur later, as cells form separate trabecular and ethmoid condensations. Cell transplantations indicate that these also reflect different sources of Shh, one from the ventral neural tube that controls trabecular morphogenesis and one from the oral ectoderm that promotes chondrogenesis. Our results suggest a novel role for Shh in the movements of neural crest cells at the midline, as well as in their differentiation into cartilage, and help to explain why both skeletal fusions and palatal clefting are associated with the loss of Hh signaling in holoprosencephalic humans.

Descriptors: zebrafish embryos, chondrogenesis, neural crest embryology, signal transduction, skull embryology, trans activators metabolism, carrier proteins genetics and metabolism, cartilage embryology and metabolism, developmental gene expression regulation, green fluorescent proteins genetics and metabolism, Hedgehog proteins, high mobility group proteins genetics and metabolism, morphogenesis, neural crest metabolism, skull metabolism, trans activators genetics and metabolism.

Wagner, D.S., R. Dosch, K.A. Mintzer, A.P. Wiemelt, and M.C. Mullins (2004). **Maternal control of development at the midblastula transition and beyond: mutants from the zebrafish II.** *Developmental Cell* 6(6): 781-90. ISSN: 1534 5807.

NAL Call Number: QH573.C42

Abstract: Many maternal factors in the oocyte persist in the embryo. They are required to initiate zygotic transcription but also function beyond this stage, where they interact with zygotic gene products during embryonic development. In a four-generation screen in the zebrafish, we identified 47 maternal-effect and five paternal-effect mutants that manifest their phenotypes at the time of, or after, zygotic genome activation. We propagated a subset of 13 mutations that cause developmental arrest at the midblastula transition, defects in cell viability, embryonic morphogenesis, and establishment of the embryonic body plan. This diverse group of mutants, many not previously observed in vertebrates, demonstrates a substantial maternal contribution to the "zygotic" period of embryogenesis and a surprising degree of paternal control. These mutants provide powerful tools to dissect the maternal and paternal control of vertebrate embryogenesis.
Descriptors: zebrafish, blastula metabolism, embryology , developmental gene expression regulation genetics, mutation genetics, blastula cytology, body patterning genetics, cell differentiation genetics, cell lineage genetics, cell survival genetics, cytology, phenotype, sex differentiation genetics, sex factors, zygote cytology and metabolism.

Wakahara, T., N. Kusu, H. Yamauchi, I. Kimura, M. Konishi, A. Miyake, and N. Itoh (2007). **Fibin, a novel secreted lateral plate mesoderm signal, is essential for pectoral fin bud initiation in zebrafish.** *Developmental Biology* 303(2): 527-35. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: We identified a novel secreted protein, fibin, in zebrafish, mice and humans. We inhibited its function in zebrafish embryos by injecting antisense fibin morpholino oligonucleotides. A knockdown of fibin function in zebrafish resulted in no pectoral fin bud initiation and abolished the expression of tbx5, which is involved in the specification of pectoral fin identification. The lack of pectoral fins in fibin-knockdown embryos was partially rescued by injection of fibin RNA. fibin was expressed in the lateral plate mesoderm of the presumptive pectoral fin bud regions. Its expression region was adjacent to that of tbx5. fibin expression temporally preceded tbx5 expression in presumptive pectoral fin bud regions, and not abolished in tbx5-knockdown presumptive fin bud regions. In contrast, fibin expression was abolished in retinoic acid signaling-inhibited or wnt2b-knockdown presumptive fin bud regions. These results indicate that fibin is a secreted signal essential for pectoral fin bud initiation in that it potentially acts downstream of retinoic acid and wnt signaling and is essential for tbx5 expression. The present findings have revealed a novel secreted lateral plate mesoderm signal essential for fin initiation in the lateral plate mesoderm.
Descriptors: zebrafish embryology, intracellular signaling peptides and proteins metabolism, amino acid and base sequence, developmental gene expression regulation, glycoproteins genetics and metabolism, intracellular signaling peptides and proteins antagonists, inhibitors, proteins deficiency and mesoderm metabolism, mice, molecular sequence data, oligodeoxyribonucleotides, antisense genetics, recombinant proteins metabolism, amino acid sequence homology, signal transduction, T-box domain proteins genetics and metabolism, Wnt proteins genetics and metabolism, proteins antagonists and inhibitors, proteins deficiency and genetics.

Walker, M.B., C.T. Miller, J. Coffin Talbot, D.W. Stock, and C.B. Kimmel (2006). **Zebrafish furin mutants reveal intricacies in regulating Endothelin1 signaling in craniofacial patterning.** *Developmental Biology* 295(1): 194-205. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Endothelin1 (Edn1) signaling promotes ventral character to the facial skeleton. In zebrafish edn1 mutants, the ventral jaw structures are severely reduced and fused to their

dorsal counterparts, with a loss of joints that normally form at an intermediate dorsal-ventral position. Loss of function at another locus, sturgeon, also yields joint losses, but only mild reductions in the ventral jaw structures. We show that sturgeon encodes one of two orthologs of Furin present in zebrafish, and that both furin genes may function partially redundantly to activate Edn1 signaling. Supporting this hypothesis, early expression of edn1-dependent genes is downregulated in sturgeon (furinA) mutants. Later in development, expression of most of these genes recovers to near wild-type levels in furinA mutants but not in edn1 mutants. The recovery explains the less severe furinA mutant skeletal phenotype and suggests that late gene expression is dependent on a critical level of Edn1 signaling not present in the more severe edn1 mutants. However, expression defects in the intermediate joint-forming domains in both mutants persist, explaining the joint losses observed later in both mutants. We further show that in both mutants the arches fail to correctly undergo ventral elongation before skeletogenesis begins and propose a model in which this failure is largely responsible for the loss of an Edn1-dependent compartmentation of the arch into the intermediate and ventral domains.

Descriptors: zebrafish embryology, body patterning physiology, endothelin 1 metabolism, furin metabolism, proteins metabolism, amino acid sequence, basic helix loop helix transcription factors genetics and metabolism, chromosome mapping, metabolism, endothelin 1 genetics , extremities embryology, furin genetics, gene duplication, developmental gene expression regulation, hmgb proteins genetics and metabolism, head embryology, homeodomain proteins genetics and metabolism, molecular sequence data, mutation, signal transduction, transcription factors genetics and metabolism, proteins genetics.

Walker, M.B., C.T. Miller, M.E. Swartz, J.K. Eberhart, and C.B. Kimmel (2007). **Phospholipase C, beta 3 is required for Endothelin1 regulation of pharyngeal arch patterning in zebrafish.** *Developmental Biology* 304(1): 194-207. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Genetic and pharmacological studies demonstrate that Endothelin1 (Edn1) is a key signaling molecule for patterning the facial skeleton in fish, chicks, and mice. When Edn1 function is reduced early in development the ventral lower jaw and supporting structures are reduced in size and often fused to their dorsal upper jaw counterparts. We show that schmerle (she) encodes a zebrafish ortholog of Phospholipase C, beta 3 (Plcbeta3) required in cranial neural crest cells for Edn1 regulation of pharyngeal arch patterning. Sequencing and co-segregation demonstrates that two independent she (plcbeta3) alleles have missense mutations in conserved residues within the catalytic domains of Plcbeta3. Homozygous plcbeta3 mutants are phenotypically similar to edn1 mutants and exhibit a strong arch expression defect in Edn1-dependent Distalless (Dlx) genes as well as expression defects in several Edn1-dependent intermediate and ventral arch domain transcription factors. plcbeta3 also genetically interacts with edn1, supporting a model in which Edn1 signals through a G protein-coupled receptor to activate Plcbeta3. Mild skeletal defects occur in plcbeta3 heterozygotes, showing the plcbeta3 mutations are partially dominant. Through a morpholino-mediated deletion in the N-terminal PH domain of Plcbeta3, we observe a partial rescue of facial skeletal defects in homozygous plcbeta3 mutants, supporting a hypothesis that an intact PH domain is necessary for the partial dominance we observe. In addition, through mosaic analyses, we show that wild-type neural crest cells can efficiently rescue facial skeletal defects in homozygous plcbeta3 mutants, demonstrating that Plcbeta3 function is required in neural crest cells and not other cell types to pattern the facial skeleton.

Descriptors: zebrafish embryos, body patterning physiology, branchial region embryology, endothelin 1 metabolism, developmental gene expression regulation, isoenzymes genetics,

neural crest metabolism, phospholipase C genetics, base sequence, branchial region enzymology, DNA primers, isoenzymes metabolism, confocal microscopy, molecular sequence data, missense mutation genetics, neural crest embryology, phospholipase C metabolism, DNA sequence analysis, signal transduction genetics.

Wallace, K.N., S. Akhter, E.M. Smith, K. Lorent, and M. Pack (2005). **Intestinal growth and differentiation in zebrafish.** *Mechanisms of Development* 122(2): 157-73. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: Intestinal development in amniotes is driven by interactions between progenitor cells derived from the three primary germ layers. Genetic analyses and gene targeting experiments in zebrafish offer a novel approach to dissect such interactions at a molecular level. Here we show that intestinal anatomy and architecture in zebrafish closely resembles the anatomy and architecture of the mammalian small intestine. The zebrafish intestine is regionalized and the various segments can be identified by epithelial markers whose expression is already segregated at the onset of intestinal differentiation. Differentiation of cells derived from the three primary germ layers begins more or less contemporaneously, and is preceded by a stage in which there is rapid cell proliferation and maturation of epithelial cell polarization. Analysis of zebrafish mutants with altered epithelial survival reveals that seemingly related single gene defects have different effects on epithelial differentiation and smooth muscle and enteric nervous system development.
Descriptors: zebrafish, developmental gene expression regulation, intestines embryology, growth and development, antimetabolites pharmacology, body patterning, bromodeoxyuridine pharmacology, cell differentiation, cell proliferation, enteric nervous system embryology, enteric nervous system growth and development, epithelial cells cytology, embryology and growth and development, horseradish peroxidase pharmacology, immunohistochemistry, in situ hybridization, intestines metabolism, smooth muscle cytology and metabolism, mutation, neurons metabolism, phenotype, RNA metabolism, time factors, zebrafish.

Wallace, K.N., A.C. Dolan, C. Seiler, E.M. Smith, S. Yusuff, L. Chaille Arnold, B. Judson, R. Sierk, C. Yengo, H.L. Sweeney, and M. Pack (2005). **Mutation of smooth muscle myosin causes epithelial invasion and cystic expansion of the zebrafish intestine.** *Developmental Cell* 8(5): 717-26. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Zebrafish meltdown (mlt) mutants develop cystic expansion of the posterior intestine as a result of stromal invasion of nontransformed epithelial cells. Positional cloning identified zebrafish smooth muscle myosin heavy chain (myh11) as the responsible gene. The mlt mutation constitutively activates the Myh11 ATPase, which disrupts smooth muscle cells surrounding the posterior intestine. Adjacent epithelial cells ectopically express metalloproteinases, integrins, and other genes implicated in human cancer cell invasion. Knockdown and pharmacological inhibition of these genes restores intestinal structure in mlt mutants despite persistent smooth muscle defects. These data identify an essential role for smooth muscle signaling in the maintenance of epithelial architecture and support gene expression analyses and other studies that identify a role for stromal genes in cancer cell invasion. Furthermore, they suggest that high-throughput screens to identify regulators of cancer cell invasion may be feasible in zebrafish.
Descriptors: zebrafish, intestines growth and development, myosin heavy chains genetics, myosin heavy chains metabolism, amino acid and base sequence, DNA genetics, epithelium growth and development, in situ hybridization, molecular sequence data, smooth muscle

growth and development and metabolism, mutation, phenotype, amino acid sequence homology, signal transduction, growth and development.

Wallace, K.N. and M. Pack (2003). **Unique and conserved aspects of gut development in zebrafish.** *Developmental Biology* 255(1): 12-29. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Although the development of the digestive system of humans and vertebrate model organisms has been well characterized, relatively little is known about how the zebrafish digestive system forms. We define developmental milestones during organogenesis of the zebrafish digestive tract, liver, and pancreas and identify important differences in the way the digestive endoderm of zebrafish and amniotes is organized. Such differences account for the finding that the zebrafish digestive system is assembled from individual organ anlagen, whereas the digestive anlagen of amniotes arise from a primitive gut tube. Despite differences of organ morphogenesis, conserved molecular programs regulate pharynx, esophagus, liver, and pancreas development in teleosts and mammals. Specifically, we show that zebrafish faust/gata-5 is a functional ortholog of gata-4, a gene that is essential for the formation of the mammalian and avian foregut. Further, extraembryonic gata activity is required for this function in zebrafish as has been shown in other vertebrates. We also show that a loss-of-function mutation that perturbs sonic hedgehog causes defects in the development of the esophagus that parallel those associated with targeted disruption of this gene in mammals. Perturbation of sonic hedgehog also affects zebrafish liver and pancreas development, and these effects occur in a reciprocal fashion, as has been described during mammalian liver and ventral pancreas development. Together, these data define aspects of digestive system development necessary for the characterization of zebrafish mutants. Given the similarities of teleost and mammalian digestive physiology and anatomy, these findings have implications for developmental and evolutionary studies as well as research of human diseases, such as diabetes, liver cirrhosis, and cancer.
Descriptors: zebrafish embryos, digestive system embryology, developmental gene expression regulation, alleles, cell polarity, digestive system cytology, digestive system drug effects, growth, development esophagus embryology, ethanol pharmacology, genetic markers, Hedgehog proteins, liver embryology, morphogenesis, mutation, pancreas embryology, pharynx embryology, messenger RNA genetics and metabolism, signal transduction genetics, trans activators genetics, trans activators metabolism, transcription factors genetics and metabolism, veratrum alkaloids pharmacology and metabolism.

Walpita, C.N., S. Van der Geyten, E. Rurangwa, and V.M. Darras (2007). **The effect of 3,5,3'-triiodothyronine supplementation on zebrafish (*Danio rerio*) embryonic development and expression of iodothyronine deiodinases and thyroid hormone receptors.** *General and Comparative Endocrinology* 152(2-3): 206-14. ISSN: 0016-6480.
NAL Call Number: 444.8G28
Abstract: The importance of thyroid hormones (TH) for embryonic development has long been shown in many vertebrates, but is not yet established in pre-hatch teleost models despite the presence of TH, TH receptors and iodothyronine deiodinases. Lack of data about the dynamics of TH metabolism in embryonic stages of fish does not allow to speculate about the involvement and/or role of TH in fish embryonic development. We therefore set up an experiment to examine the effect of 3,5,3'-triiodothyronine (T(3)) on zebrafish (*Danio rerio*) embryonic development and on the expression of some thyroid hormone-regulated genes as measured by real-time PCR. Maternally derived thyroxine (T(4)) and T(3) were detected throughout embryonic development and total levels remained stable. Thyroid

hormone receptor (TR) alpha and beta mRNA were found to be present in early embryos. After an initial fall, TRalpha mRNA levels in the control group increased gradually from 12h post fertilization (HPF) onwards. TRbeta mRNA levels of control embryos were relatively stable during embryonic development, but increased around the hatching period. We also quantified type I (D1) and type II (D2) deiodinase mRNA expression in zebrafish embryos. D1 mRNA levels in the control group gradually increased during development while D2 levels were kept at a low and stable level until hatching. At 75 HPF, a fivefold increase of D2 expression was observed compared to embryonic levels. Exogenous T(3) added to the water (5nM) was taken up by the embryos, causing increased pigmentation and accelerated hatching. T(3) treatment significantly up regulated TRalpha mRNA levels at 48 HPF, while D2 mRNA was significantly down regulated at 75 HPF. Neither TRbeta nor D1 mRNA levels seemed responsive to the treatment. Taken together, these data show that during embryonic development zebrafish already have the necessary regulatory machinery for TH activation and signaling, and that T(3) treatment at that stage indeed influence embryonic development.

Descriptors: zebrafish embryos, iodide peroxidase genetics, thyroid gland drug effects, thyroid gland embryology, thyroid hormone receptors alpha genetics and beta genetics, triiodothyronine pharmacology, developmental gene expression regulation drug effects and physiology , enzymologic gene expression regulation drug effects, enzymologic gene expression regulation physiology, pigmentation drug effects, pigmentation physiology, messenger RNA metabolism, thyroid gland enzymology, triiodothyronine blood, zebrafish.

Walshe, J. and I. Mason (2003). **Unique and combinatorial functions of Fgf3 and Fgf8 during zebrafish forebrain development.** *Development* 130(18): 4337-49. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Complex spatiotemporal expression patterns of fgf3 and fgf8 within the developing zebrafish forebrain suggest their involvement in its regionalisation and early development. These factors have unique and combinatorial roles during development of more posterior brain regions, and here we report similar findings for the developing forebrain. We show that Fgf8 and Fgf3 regulate different aspects of telencephalic development, and that Fgf3 alone is required for the expression of several telencephalic markers. Within the diencephalon, Fgf3 and Fgf8 act synergistically to pattern the ventral thalamus, and are implicated in the regulation of optic stalk formation, whereas loss of Fgf3 alone results in defects in ZLI development. Forebrain commissure formation was abnormal in the absence of either Fgf3 or Fgf8; however, most severe defects were observed in the absence of both. Defects were observed in patterning of both the midline territory, within which the commissures normally form, and neuronal populations, whose axons comprise the commissures. Analysis of embryos treated with an FGFR inhibitor suggests that continuous FGF signalling is required from gastrulation stages for normal forebrain patterning, and identifies additional requirements for FGFR activity.

Descriptors: zebrafish, Fgf metabolism, developmental gene expression regulation, prosencephalon embryology, proto oncogene proteins metabolism, proteins, axons physiology, biological markers, body patterning, cell death, cell differentiation, cell division, fibroblast growth factor 3, fibroblast growth factor 8, Fgf genetics, in situ hybridization, morphogenesis, antisense oligonucleotides metabolism, prosencephalon growth, development and physiology, proto oncogene fibroblast receptors growth factor metabolism, signal transduction physiology.

Walshe, J. and I. Mason (2003). **Fgf signalling is required for formation of cartilage in the head.** *Developmental Biology* 264(2): 522-536. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish head embryology, development, nervous system, neural coordination, skeletal system, movement and support, craniofacial syndrome, bone disease, cartilage, morphogenesis.

Walton, R.Z., A.E. Bruce, H.E. Olivey, K. Najib, V. Johnson, J.U. Earley, R.K. Ho, and E.C. Svensson (2006). **Fog1 is required for cardiac looping in zebrafish.** *Developmental Biology* 289(2): 482-93. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: To further our understanding of FOG gene function during cardiac development, we utilized zebrafish to examine FOG's role in the early steps of heart morphogenesis. We identified fragments of three fog genes in the zebrafish genomic database and isolated full-length coding sequences for each of these genes by using a combination of RT-PCR and 5'-RACE. One gene was similar to murine FOG-1 (fog1), while the remaining two were similar to murine FOG-2 (fog2a and fog2b). All Fog proteins were able to physically interact with GATA4 and function as transcriptional co-repressors. Whole-mount in situ hybridization revealed fog1 expression in the heart, the hematopoietic system, and the brain, while fog2a and fog2b expression was restricted to the brain. Injection of zebrafish embryos with a morpholino directed against fog1 resulted in embryos with a large pericardial effusion and an unlooped heart tube. This looping defect could be rescued by co-injection of mRNA encoding murine FOG-1, but not by mRNA encoding FOG-1 lacking the FOG repression motif. Taken together, these results demonstrate the importance of FOG proteins for zebrafish cardiac development and suggest a previously unappreciated role for FOG proteins in heart looping that is dependent on the FOG repression motif.
Descriptors: zebrafish embryology, heart embryology, transcription factors genetics, metabolism, base sequence, brain embryology and metabolism, conserved sequence, GATA4 transcription factor metabolism, heart physiology, mice, molecular sequence data, mutation, nuclear proteins genetics and metabolism, protein isoforms genetics and metabolism, sequence homology, transcription factors metabolism, transfection.

Wang, H., J. Kesinger, Y. Tang, B. Frank, and M. Centola (2004). **Identification of putative target genes in the zebrafish mbl/axin1 mutant by microarray analysis.** *Developmental Biology* 271(2): 595. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish mutant, nervous system, neural coordination, development, molecular genetics, microarray analysis, genetic techniques, circadian oscillation.

Wang, L., X.F. Liang, W.Q. Liao, and T.H. Zhou (2006). **Research progress on cell apoptosis during embryonic development of the zebrafish (*Danio rerio*).** *Yi Chuan; Hereditas/ Zhongguo Yi Chuan Xue Hui Bian Ji* 28(8): 1009-14. ISSN: 0253-9772.
Abstract: Cells have a built-in death program through the regulation of genes. Apoptosis (programmed cell death) plays a very important role in the development of the vertebrate embryo. The zebrafish (*Danio rerio*) has become a useful and ideal model in molecular developmental biology for in vivo study on the function of apoptosis in morphogenesis, sex differentiation and so on, and has already been garnering more and more attention. At present, much progress has been made regarding apoptotic pathways in zebrafish development. Caspases and other regulatory apoptotic genes have been cloned successfully.

Moreover, transgenic zebrafish embryos have been induced to undergo apoptosis in vivo to study apoptotic signaling pathways and the abnormal morphological changes during embryogenesis. Transgenic zebrafish has provided a powerful tool for illuminating the interaction between regulatory apoptotic genes and vertebrate development.
Descriptors: transgenic zebrafish embryos, apoptosis, development, morphogenesis.
Language of Text: Chinese.

Wang Li, F., Zhang Ying, Shao Ming, and W. Zhang Hong (2007). **Spatiotemporal expression of the creatine metabolism related genes agat, gamt and ct1 during zebrafish embryogenesis**. *International Journal of Developmental Biology* 51(3): 247-253. ISSN: 0214 6282.
Abstract: Glycine amidinotransferase (AGAT or GATM), guanidinoacetate methyltransferase (GAMT) and creatine transporter (CT1) are three proteins involved in the synthesis and uptake of creatine. The expression patterns of these three genes were examined in zebrafish embryos by whole mount in situ hybridization followed by histological sectioning. Expression of agat first appeared in the yolk syncytial layer (YSL) at the gastrula stage and was progressively up regulated during gastrulation. As development proceeds, agat was expressed in the mature somites during the segmentation stage and in the liver at 48 hpf. gamt showed a similar expression pattern to that of agat during embryogenesis. It was first detected in the center of the yolk from the cleavage to the gastrula stage. At the bud stage, its expression shifted to the YSL. gamt was also transiently expressed in the mature somites from 16 hpf to 24 hpf and became strongly expressed in the liver and in epithelial cells of the gut at 48 hpf. ct1 was initially uniformly expressed from the cleavage to the early segmentation stage; it was then strongly expressed in all the somites till 30 hpf and in the gut of 48 hpf embryos. However, ct1 transcripts also appeared in the central nervous system during the segmentation stage, but not in the YSL, the yolk or the liver. Our data reveal for the first time distinct and unique patterns of expression of the creatine metabolism genes agat, gamt and ct1 during zebrafish embryogenesis. Copyright © 2008 CABI
Descriptors: zebrafish embryology, biological development, cleavage, creatinine, embryogenesis, embryos, gene expression, genes, metabolism, morphogenesis, ontogeny, *Danio rerio*.

Wang, X., A. Emelyanov, V. Korzh, and Z. Gong (2003). **Zebrafish atonal homologue zath3 is expressed during neurogenesis in embryonic development.** *Developmental Dynamics* 227(4): 587-92. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryology, central nervous system embryology, DNA binding proteins genetics, proteins metabolism, gene expression profiling, nerve tissue proteins metabolism, proteins metabolism, amino acid sequence, basic helix loop helix transcription factors, chromosome mapping, DNA primers, complementary DNA genetics, DNA binding proteins genetics, helix loop helix motifs genetics, in situ hybridization, molecular sequence data, nerve tissue DNA sequence analysis.

Wang, X.G. and L. Orban (2007). **Anti-Mullerian hormone and 11 beta-hydroxylase show reciprocal expression to that of aromatase in the transforming gonad of zebrafish males.** *Developmental Dynamics* 236(5): 1329-38. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryology, aromatase genetics, glycosteroid 11 beta hydroxylase genetics, testicular hormones genetics, amino acid sequence, genetically modified, developmental gene expression regulation, green fluorescent in situ hybridization, molecular sequence data, ovary embryology, growth, development and metabolism, messenger RNA

genetics and metabolism, amino acid sequence homology, sex characteristics, sex differentiation genetics, signal transduction, testis embryology, growth, development and metabolism, testosterone analogs, derivatives and metabolism, zebrafish growth and development, metabolism.

Wang, Y., L. Qian, Y. Dong, Q. Jiang, Y. Gui, T.P. Zhong, and H. Song (2006). **Myocyte-specific enhancer factor 2A is essential for zebrafish posterior somite development.** *Mechanisms of Development* 123(10): 783-91. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: Somite development is governed tightly by genetic factors. In the large-scale mutagenesis screens of zebrafish, no mutations were linked to myocyte enhancer factor 2A (MEF2A) locus. In this study, we find that MEF2A knock-down embryos display a downward tail curvature and have U-shaped posterior somites. Furthermore, we demonstrate that MEF2A is required for Hedgehog signaling. MEF2A inhibition results in induction of apoptosis in the posterior somites. We further find that Hedgehog signaling can negatively regulate MEF2A expression in the somites. Microarray studies reveal a number of genes that are differentially expressed in the MEF2A morphants. Our studies suggest that MEF2A is essential for zebrafish posterior somite development.
Descriptors: zebrafish embryology, myogenic regulatory factors metabolism, somites physiology , apoptosis, body patterning, gene expression profiling, Hedgehog proteins genetics and metabolism, myogenic regulatory factors genetics, oligonucleotide array sequence analysis, antisense oligonucleotides genetics and metabolism, signal transduction physiology.

Wang, Y., S. Zhang, and M.S. Sawant (2004). **Expression of nitric oxide synthase in the developing eye of zebrafish *Danio rerio*.** *Chinese Journal of Oceanology and Limnology* 22(4): 399-402. ISSN: 0254-4059.
Descriptors: zebrafish embryos, developmental stages, eyes, freshwater fish, optics, photoreceptors, retinas, Staining, *Danio rerio*.

Ward, A.B., R.M. Warga, and V.E. Prince (2007). **Origin of the zebrafish endocrine and exocrine pancreas.** *Developmental Dynamics* 236(6): 1558-69. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryology, exocrine pancreas embryology, liver cytology, liver embryology, exocrine pancreas cytology, stem cells.

Waxman, J.S. (2005). **Regulation of the early expression patterns of the zebrafish Dishevelled-interacting proteins Dapper1 and Dapper2.** *Developmental Dynamics* 233(1): 194-200. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryology, gene expression regulation physiology, intracellular signaling peptides and proteins metabolism, proteins metabolism, signal transducing adaptor proteins, cytoskeletal proteins metabolism, gastrula metabolism, intracellular signaling peptides and molecular sequence data, organizers, embryonic metabolism, phosphoproteins metabolism, signal transduction physiology, trans activators metabolism, beta catenin.

Waxman, J.S., A.M. Hocking, C.L. Stoick, and R.T. Moon (2004). **Zebrafish Dapper1 and Dapper2 play distinct roles in Wnt-mediated developmental processes.** *Development* 131(23): 5909-21. ISSN: 0950 1991.
NAL Call Number: QL951.D38

Abstract: Wnt signaling pathways in vertebrates use the phosphoprotein Dishevelled (Dvl). The cellular responses to Wnt signaling may in part be modulated by Dvl-associated proteins, including Dapper (Dpr). We have cloned and characterized the zebrafish Dpr paralogs Dpr1 and Dpr2. Loss-of-function studies reveal that endogenous Dpr1 but not Dpr2 is required to enhance Wnt/beta-catenin activity in zebrafish embryos that are hypomorphic for Wnt8. Conversely, Dpr2 but not Dpr1 is required for normal convergence extension movements in embryos that are hypomorphic for Stbm or Wnt11, supporting a functional interaction of Dpr2 with Wnt/Ca2+-PCP signaling. In gain-of-function experiments, Dpr1 but not Dpr2 induces Wnt/beta-catenin target genes. Dpr1 synergizes with zebrafish Dvl2, and with the Dvl-interacting kinases CK1epsilon, Par1 and CK2, in activating target genes. We conclude that two Dvl-associated paralogs, Dpr1 and Dpr2, participate in distinct Wnt-dependent developmental processes.
Descriptors: zebrafish embryos, carrier, developmental gene expression regulation, intercellular signaling peptides and proteins metabolism, genetics and physiology, neoplasm, nuclear and physiology, signal transducing adaptor proteins, calcium metabolism, cell line, molecular cloning, cytoskeletal proteins metabolism, genome, immunohistochemistry, immunoprecipitation, phylogeny, reverse transcriptase PCR, signal transduction, trans activators metabolism, transfection, Wnt proteins, Xenopus proteins, beta catenin.

Waxman, J.S. and D. Yelon (2007). **Comparison of the expression patterns of newly identified zebrafish retinoic acid and retinoid X receptors.** *Developmental Dynamics* 236(2): 587-95. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Retinoic acid (RA) signaling is important for multiple aspects of embryonic development and tissue homeostasis. Heterodimers of retinoic acid receptors (RARs) and retinoid X receptors (RXRs) transduce RA signaling. It is not yet clear how the diversity of receptor combinations relates to the diversity of functions for RA. The expression patterns of three zebrafish RARs and four RXRs were reported recently. Here, we identify an additional RAR, a zebrafish RARgamma paralog, and two additional RXRs, duplicates of the previously identified RXRalpha and RXRgamma. Thus, the zebrafish genome contains duplicates of each RAR and RXR gene. All zebrafish RAR and RXR paralogs have overlapping and distinct areas of expression, as might be expected for duplicate genes in the process of diverging in function. By representing what is potentially the complete set of zebrafish RARs and RXRs, this study provides a valuable reference for future functional studies of the individual zebrafish RARs and RXRs.
Descriptors: zebrafish embryology, gene expression profiling, phylogeny, retinoic acid receptors metabolism, retinoid X receptors metabolism and genetics, amino acid and base sequence, molecular cloning, cluster analysis, in situ hybridization, molecular sequence data, retinoic acid receptors genetics, reverse transcriptase PCR, DNA sequence analysis, metabolism.

Webb, A.E., J. Sanderford, D. Frank, W.S. Talbot, W. Driever, and D. Kimelman (2007). **Laminin alpha5 is essential for the formation of the zebrafish fins.** *Developmental Biology* 311(2): 369-82. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The vertebrate fin fold, the presumptive evolutionary antecedent of the paired fins, consists of two layers of epidermal cells extending dorsally and ventrally over the trunk and tail of the embryo, facilitating swimming during the embryonic and larval stages. Development of the fin fold requires dramatic changes in cell shape and adhesion during early

development, but the proteins involved in this process are completely unknown. In a screen of mutants defective in fin fold morphogenesis, we identified a mutant with a severe fin fold defect, which also displays malformed pectoral fins. We find that the cause of the defect is a non-sense mutation in the zebrafish lama5 gene that truncates laminin alpha5 before the C-terminal laminin LG domains, thereby preventing laminin alpha5 from interacting with its cell surface receptors. Laminin is mislocalized in this mutant, as are the membrane-associated proteins, actin and beta-catenin, that normally form foci within the fin fold. Ultrastructural analysis revealed severe morphological abnormalities and defects in cell-cell adhesion within the epidermis of the developing fin fold at 36 hpf, resulting in an epidermal sheet that can not extend away from the body. Examining the pectoral fins, we find that the lama5 mutant is the first zebrafish mutant identified in which the pectoral fins fail to make the transition from an apical epidermal ridge to an apical fold, a transformation that is essential for pectoral fin morphogenesis. We propose that laminin alpha5, which is concentrated at the distal ends of the fins, organizes the distal cells of the fin fold and pectoral fins in order to promote the morphogenesis of the epidermis. The lama5 mutant provides novel insight into the role of laminins in the zebrafish epidermis, and the molecular mechanisms driving fin formation in vertebrates.
Descriptors: zebrafish embryos, laminin alpha5, essential, formation, pectoral fin, lama5 mutant, apical fold, epidermis.

Webb, J.F. and J.E. Shirey (2003). **Postembryonic development of the cranial lateral line canals and neuromasts in zebrafish.** *Developmental Dynamics* 228(3): 370-85. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryology, bone and bones embryology , skull embryology, body patterning, mandible embryology and ultrastructure, scanning electron microscopy, morphogenesis.

Webb, S.E. and A.L. Miller (2006). **Ca2+ signaling and early embryonic patterning during the blastula and gastrula periods of zebrafish and *Xenopus* development.** *Biochimica Et Biophysica Acta* 1763(11): 1192-208. ISSN: 0006 3002.
NAL Call Number: 381B522
Abstract: It has been proposed that Ca(2+) signaling, in the form of pulses, waves and steady gradients, may play a crucial role in key pattern forming events during early vertebrate development [L.F. Jaffe, Organization of early development by calcium patterns, BioEssays 21 (1999) 657-667; M.J. Berridge, P. Lipp, M.D. Bootman, The versatility and universality of calcium signaling, Nat. Rev. Mol. Cell Biol. 1 (2000) 11-21; S.E. Webb, A.L. Miller, Calcium signalling during embryonic development, Nat. Rev. Mol. Cell Biol. 4 (2003) 539-551]. With reference to the embryos of zebrafish (*Danio rerio*) and the frog, *Xenopus laevis*, we review the Ca(2+) signals reported during the Blastula and Gastrula Periods. This developmental window encompasses the major pattern forming events of epiboly, involution, and convergent extension, which result in the establishment of the basic germ layers and body axes [C.B. Kimmel, W.W. Ballard, S.R. Kimmel, B. Ullmann, T.F. Schilling, Stages of embryonic development of the zebrafish, Dev. Dyn. 203 (1995) 253-310]. Data will be presented to support the suggestion that propagating waves (both long and short range) of Ca(2+) release, followed by sequestration, may play a crucial role in: (1) Coordinating cell movements during these pattern forming events and (2) Contributing to the establishment of the basic embryonic axes, as well as (3) Helping to define the morphological boundaries of specific tissue domains and embryonic structures, including future organ anlagen [E. Gilland, A.L. Miller, E. Karplus, R. Baker, S.E. Webb, Imaging of multicellular large-scale rhythmic

calcium waves during zebrafish gastrulation, Proc. Natl. Acad. Sci. USA 96 (1999) 157-161; J.B. Wallingford, A.J. Ewald, R.M. Harland, S.E. Fraser, Calcium signaling during convergent extension in Xenopus, Curr. Biol. 11 (2001) 652-661]. The various potential targets of these Ca(2+) transients will also be discussed, as well as how they might integrate with other known pattern forming pathways known to modulate early developmental events (such as the Wnt/Ca(2+)pathway; [T.A. Westfall, B. Hjertos, D.C. Slusarski, Requirement for intracellular calcium modulation in zebrafish dorsal-ventral patterning, Dev. Biol. 259 (2003) 380-391]).

Descriptors: zebrafish embryos, blastula metabolism, calcium signaling, gastrula metabolism, *Xenopus* embryology, blastula cytology, gastrula cytology, *Xenopus* metabolism, metabolism.

Webb, S.E. and A.L. Miller (2006). **Ca2+ signaling during vertebrate somitogenesis**. *Acta Pharmacologica Sinica* 27(7): 781-790. ISSN: 1671 4083.

Abstract: A variety of Ca2+ signals, in the form of intercellular pulses and waves, have been reported to be associated with the various sequential stages of somitogenesis: from convergent extension and the formation of the paraxial mesoderm; during the patterning of the paraxial mesoderm to establish segmental units; throughout the formation of the morphological boundaries that delineate the segmental units, and finally from within the maturing somites as they undergo subsequent development and differentiation. Due to both the technical challenges presented in imaging intact, developing embryos, and the subtle nature of the Ca2+ transients generated, they have proved to be difficult to visualize. However, a combination of cultured cell preparations and improvements in explant and whole embryo imaging techniques has begun to reveal a new and exciting class of developmental Ca2+ signals. In this chapter, we review the small, but expanding, number of reports in the literature and attempt to identify common characteristics of the somitogenic Ca2+ transients, such as their mode of generation, as well as their spatial and temporal features. This may help to elucidate the significance and function of these intriguing Ca2+ transients and thus integrate them into the complex signaling networks that orchestrate early developmental events.
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Descriptors: zebrafish embryos, development, cell differentiation, vertebrate somitogenesis, Ca+2 signaling, paraxial mesoderm.

Wehman, A.M., W. Staub, and H. Baier (2007). **The anaphase-promoting complex is required in both dividing and quiescent cells during zebrafish development.** *Developmental Biology* 303(1): 144-56. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The anaphase-promoting complex/cyclosome (APC/C) regulates multiple stages of the cell cycle, most prominently mitosis. We describe zebrafish with mutations in two APC/C subunits, Cdc16 and Cdc26, whose phenotypes reveal a multifaceted set of defects resulting from the gradual depletion of the APC/C. First, loss of the APC/C in dividing cells results in mitotic arrest, followed by apoptosis. This defect becomes detectable in different organs at different larval ages, because the subunits of the APC/C are maternally deposited, are unusually stable, and are depleted at uneven rates in different tissues. Second, loss of the APC/C in quiescent or differentiated cells results in improper re-entry into the cell cycle, again in an apparently tissue-specific manner. This study is the first demonstration of both functions of the APC/C in a vertebrate organism and also provides an illustration of the surprisingly complex effects that essential, maternally supplied factors can have on the growing animal over a period of 10 days or longer.

Descriptors: zebrafish embryology, cell cycle physiology, mitosis physiology, ubiquitin protein ligase complexes genetics, ubiquitin protein ligase complexes metabolism, base sequence, cell cycle chromosome mapping , cyclin B, DNA primers, immunohistochemistry, molecular sequence data, retina embryology, DNA sequence analysis.

Wei, X., Y. Cheng, Y. Luo, X. Shi, S. Nelson, and D.R. Hyde (2004). **The zebrafish Pard3 ortholog is required for separation of the eye fields and retinal lamination.** *Developmental Biology* 269(1): 286-301. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The vertebrate retina develops from a sheet of neuroepithelial cells. Because adherens and tight junctions are critical for epithelial and neuronal differentiation in a variety of eukaryotic systems, we examined the role of Par-3, a PDZ scaffold protein that is critical in cellular membrane junction formation. We cloned the zebrafish Par-3 ortholog (pard3), which encodes two Pard3 proteins (150 and 180 kDa) that differ in their carboxyl-terminus. Immunohistochemistry revealed that Pard3 localized to the apical region of the retinal and brain neuroepithelium, partially overlapping the adherens junction-associated actin bundles. After retinal lamination, the Pard3 protein was restricted to the outer limiting membrane and the outer and inner plexiform layers in the retina. Reducing Pard3 expression with antisense morpholinos caused loss of the retinal pigmented epithelia, disruption of retinal lamination, and cell death in the ventral diencephalon, which resulted in cyclopia. Overexpressing Pard3 by injection of wild-type pard3 mRNA resulted in cyclopia and eyeless embryos. Thus, Pard3 plays a critical role in the origination and separation of zebrafish eye fields and retinal lamination.
Descriptors: zebrafish, carrier carrier proteins metabolism, cell adhesion molecules, retina embryology, proteins metabolism, amino acid sequence, brain embryology, brain metabolism, molecular sequence data, mutation, retina metabolism.

Weigel, D. and E. Izaurralde (2006). **A tiny helper lightens the maternal load**. *Cell* 124(6): 1117-1118. ISSN: 0092 8674.
NAL Call Number: QH573.C42
Descriptors: zebrafish embryos, development, molecular genetics, maternal mRNAs, mid-blastula, embryogenesis.

Weinstein, B.M. (2004). **Imaging developing blood vessels in the zebrafish**. *Federation of American Societies for Experimental Biology Journal* 18(4-5): Abst. 16.2. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: The regulation of blood vessel formation is a subject of intense research study, with profound potential implications for human health. Efforts to develop new pro- or anti-angiogenic therapies depend partly on a detailed understanding of the normal mechanisms of blood vessel formation. The zebrafish, a small tropical freshwater fish, possesses a unique combination of features that make it particularly well suited for studying blood vessel formation during embryonic development. We have developed new tools for studying and imaging blood vessel formation in the zebrafish. These include a confocal microangiography method that we have used to derive a complete 3-D descriptive atlas of the vascular anatomy of the zebrafish embryo and larva, and transgenic zebrafish expressing green fluorescent protein in the vasculature, permitting real-time visualization of the dynamics of blood vessel growth and development in living animals. We will show how these tools, and the zebrafish model, provide an unprecedented opportunities for imaging, describing, and experimentally analyzing the development of the vertebrate vasculature. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryo, blood formation, cardiovascular system, transport and circulation, development, 3 dimensional imaging, imaging and microscopy techniques.

Wen, C., Z. Zhang, W. Ma, M. Xu, Z. Wen, and J. Peng (2005). **Genome-wide identification of female-enriched genes in zebrafish.** *Developmental Dynamics* 232(1): 171-9. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Characteristic differences in morphology, physiology, and behavior between a male and female are correlated to the differential selection of sex-dependent transcriptomes. By using a cDNA array carrying approximately 9,000 zebrafish unique genes, we identified a group of genes whose expression are enriched in the female fish. A subset of these genes have been confirmed and further grouped as egg-enriched genes, as both somatic- and egg-enriched genes or as somatic-enriched genes by means of RNA gel blot hybridization. Most importantly, a significant proportion of these genes are either functionally unknown or are novel genes. Thus, future studies of this group of genes will help us greatly to understand more about sex-determination and sex-related physiology and behavior. In addition, comparison of zebrafish female-enriched genes with that in *Drosophila*, we found that only germline genes are shared between vertebrate and invertebrate, suggesting that the process of oogenesis is highly conserved during the evolution.
Descriptors: zebrafish, unique genes, molecular evolution, gene expression regulation, genome, sex factors, proteins biosynthesis, molecular cloning, complementary DNA metabolism, *Drosophila*, gene expression profiling, nucleic acid hybridization, oligonucleotide array sequence analysis, RNA metabolism.

Wendik, B., E. Maier, and D. Meyer (2004). **Zebrafish mnx genes in endocrine and exocrine pancreas formation.** *Developmental Biology* 268(2): 372-83 . ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The pancreas consists of two components, which exert distinct homeostatic function, an endocrine part that secretes hormones including insulin and an exocrine part that produces digestive enzymes. In mouse, one of the factors essential for development of the pancreas is the Mnx-class homeobox transcription factor Hb9. Genetic studies showed that Hb9 is required for both initial morphogenesis of the pancreas as well as subsequent differentiation of insulin-producing beta-cells [Nat. Genet. 23 (1999) 71; Nat. Genet. 23 (1999) 67]. To get a better understanding of what role mnx genes play in pancreas development, we isolated and characterized mnx genes in the model organism zebrafish. We found one gene with homology to hb9 orthologs and two that display homology to the related chicken mnr2. Embryonic expression of the zebrafish mnx genes is very dynamic and is detected in derivatives of all three germ layers. Endodermal expression of hb9 takes place in the early gut endoderm and, later, in the endocrine pancreas and the swim bladder. In addition, one of the mnr2 genes, mnr2a, shows expression in an endodermal cell population that is initially intermingled with insulin-positive cells and that later becomes restricted to the exocrine pancreas. In knockdown studies using antisense morpholinos, we show that hb9 is essential for differentiation of the insulin-producing beta-cells but unlike mouse Hb9 is not needed for early morphogenesis of the pancreas. In contrast, mnr2a is required during late morphogenesis of the exocrine pancreas. In summary, our data suggest a tissue-specific mnx-expression code in the zebrafish pancreas and they reveal a novel role of an mnr2-related gene.
Descriptors: zebrafish embryology, homeodomain Islets of Langerhans embryology, transcription factors genetics, air sacs metabolism, amino acid sequence, homeodomain proteins metabolism, molecular sequence data, transcription factors metabolism.

Wenzlau, J.M., P.J. Garl, P. Simpson, K.R. Stenmark, J. West, K.B. Artinger, R.A. Nemenoff, and M.C.M. Weiser-Evans (2006). **Embryonic growth-associated protein is one subunit of a novel n-terminal acetyltransferase complex essential for embryonic vascular development**. *Circulation Research* 98(6): 846-855. ISSN: 0009 7330.

Abstract: N-terminal protein acetylation, catalyzed by N-terminal acetyltransferases (NATs) recognizing distinct N-terminal sequences, is gaining recognition as an essential regulator of normal cell function, but little is known of its role in vertebrate development. We previously cloned a novel gene, embryonic growth-associated protein (EGAP), the expression of which is associated with rapid vascular smooth muscle cell proliferation during development. We show herein EGAP is the mammalian/zebrafish homologue of yeast Mak10p, one subunit of the yeast NatC complex, and describe the cloning of its binding partners Mak3 and Mak31. The EGAP NAT forms a functional complex in mammalian cells, is evolutionarily conserved, and developmentally regulated. It is widely but not ubiquitously expressed during early zebrafish development but undetectable in later developmental stages. We demonstrate EGAP-and Mak3-deficient zebrafish fail to develop because of, in part, decreased cell proliferation, increased apoptosis, and poor blood vessel formation contributing to embryonic lethality. We examined the role of target of rapamycin (TOR), a highly conserved protein kinase controlling cell growth, as a physiological target of EGAP NAT acetylation. Compared with controls, TOR expression and signaling is significantly reduced in EGAP morphants. Pharmacological inhibition of TOR with rapamycin phenocopied the EGAP morpholino oligonucleotide-induced growth and vessel defects. Overexpression of constitutively active TOR rescued EGAP morphants, suggesting TOR is a direct or indirect endogenous substrate of the EGAP NAT complex. These data suggest the EGAP NAT complex is an essential regulatory enzyme controlling the function of a subset of proteins required for embryonic growth control and vessel development. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, development, molecular genetics, enzymology, apoptosis, embryonic vascular development.

Wester, P.W. (2003). **Development and validation of a test method forthe identification of endocrine disrupting chemicals in the environment**. *Identification of Endocrine Disruptive Effects in the Aquatic Environment : a Partial Life Cycle Assay in Zebrafish.,* Bilthoven, Netherlands

Descriptors: zebrafish test organism, aquatic environment, environmental impact, estrogens, hormones, fishes, *Danio rerio*, life cycle, water pollution, environment, hormones, pollution, sex hormones.

Westfall, T.A., B. Hjertos, and D.C. Slusarski (2003). **Requirement for intracellular calcium modulation in zebrafish dorsal-ventral patterning.** *Developmental Biology* 259(2): 380-91. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The phosphoinositide (PI) cycle is an important signal transduction pathway that, upon activation, generates intracellular second messengers and leads to calcium release. To determine whether PI cycle-mediated intracellular calcium release is required for body plan formation, we systematically dissect PI cycle function in the zebrafish (*Danio rerio*). We inhibit PI cycle function at three different steps and deplete internal calcium stores, demonstrating an impact on endogenous calcium release and Wnt/beta-catenin signaling. Inhibition of endogenous calcium modulation induces hyperdorsalized phenotypes in a dose-dependent manner. Ectopic dorsal-signaling centers are generated in PI cycle-inhibited embryos as demonstrated by altered beta-catenin subcellular localization and ectopic expression of beta-catenin target genes. These results provide evidence that modulation of calcium

release is critical for early embryonic patterning and acts by influencing the stabilization of beta-catenin protein.
Descriptors: zebrafish embryology, body patterning genetics, calcium metabolism, cytoskeletal proteins drug effects, cytoskeletal proteins genetics and proteins metabolism, diphosphonates pharmacology, dose response relationship, drug enzyme inhibitors pharmacology, estrenes pharmacology, developmental gene expression regulation, phenotype, phosphatidylinositols metabolism, phospholipase C antagonists and inhibitors, pyrrolidinones pharmacology, signal transduction, thapsigargin pharmacology, trans activators drug effects, proteins, beta catenin.

Whitlock, K.E., K.M. Smith, H. Kim, and M.V. Harden (2005). **A role for foxd3 and sox10 in the differentiation of gonadotropin-releasing hormone (GnRH) cells in the zebrafish *Danio rerio.*** *Development* 132(24): 5491-502. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Gonadotropin-releasing hormone (GnRH) is found in a wide range of vertebrate tissues, including the nervous system. In general, GnRH has two functions: endocrine, acting as a releasing hormone; and neuromodulatory, affecting neural activity in the peripheral and central nervous system. The best understood population of GnRH cells is that of the hypothalamus, which is essential for reproduction. Less well understood are the populations of GnRH cells found in the terminal nerve and midbrain, which appear to be neuromodulatory in function. The GnRH-containing cells of the midbrain are proposed to arise from the mesencephalic region of the neural tube. Previously, we showed that neuromodulatory GnRH cells of the terminal nerve arise from cranial neural crest. To test the hypothesis that neuromodulatory GnRH cells of the midbrain also arise from neural crest, we used gene knockdown experiments in zebrafish to disrupt neural crest development. We demonstrate that decrement of the function of foxd3 and/or sox10, two genes important for the development and specification of neural crest, resulted in a reduction and/or loss of GnRH cells of the midbrain, as well as a reduction in the number of terminal nerve GnRH cells. Therefore, our data support a neural crest origin for midbrain GnRH cells. Additionally, we demonstrate that knockdown of kallmann gene function resulted in the loss of endocrine GnRH cells of the hypothalamus, but not of neuromodulatory GnRH cells of the midbrain and terminal nerve, thus providing additional evidence for separate pathways controlling the development of neuromodulatory and endocrine GnRH cells.
Descriptors: zebrafish embryo, carrier, cell differentiation physiology, forkhead transcription factors physiology, gonadotropin releasing hormone metabolism, high mobility group, mesencephalon cytology, neural crest cytology, carrier cranial nerves cytology and embryology, cytology, physiology, forkhead transcription factors genetics, high mobility group hypothalamus cytology and embryology, mesencephalon embryology, mutation, neural crest embryology, metabolism.

Whitlock, K.E., C.D. Wolf, and M.L. Boyce (2003). **Gonadotropin-releasing hormone (GnRH) cells arise from cranial neural crest and adenohypophyseal regions of the neural plate in the zebrafish, *Danio rerio.*** *Developmental Biology* 257(1): 140-52. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The olfactory placodes generate the primary sensory neurons of the olfactory sensory system. Additionally, the olfactory placodes have been proposed to generate a class of neuroendocrine cells containing gonadotropin-releasing hormone (GnRH). GnRH is a multifunctional decapeptide essential for the development of secondary sex characteristics in vertebrates as well as a neuromodulator within the central nervous system. Here, we

show that endocrine and neuromodulatory GnRH cells arise from two separate, nonolfactory regions in the developing neural plate. Specifically, the neuromodulatory GnRH cells of the terminal nerve arise from the cranial neural crest, and the endocrine GnRH cells of the hypothalamus arise from the adenohypophyseal region of the developing anterior neural plate. Our findings are consistent with cell types generated by the adenohypophysis, a source of endocrine tissue in vertebrate animals, and by neural crest, a source of cells contributing to the cranial nerves. The adenohypophysis arises from a region of the anterior neural plate flanked by the olfactory placode fields at early stages of development, and premigratory cranial neural crest lies adjacent to the caudal edge of the olfactory placode domain [Development 127 (2000), 3645]. Thus, the GnRH cells arise from tissue closely associated with the developing olfactory placode, and their different developmental origins reflect their different functional roles in the adult animal.
Descriptors: zebrafish embryology, gonadotropin releasing hormone metabolism, neural crest metabolism, carbocyanines, head embryology, immunohistochemistry, mutation, anterior pituitary gland embryology, anterior pituitary gland metabolism, staining and labeling, transcription factors genetics and.

Wiellette, E.L. and H. Sive (2004). **Early requirement for fgf8 function during hindbrain pattern formation in zebrafish.** *Developmental Dynamics* 229(2): 393-9. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, Fgf metabolism, rhombencephalon embryology and metabolism, body patterning drug effects and physiology, cytology, drug effects, metabolism, fibroblast growth factor 3, fibroblast growth factor 8, Fgf antagonists and inhibitors, developmental gene expression regulation drug effects, in situ hybridization, morphogenesis drug effects and physiology, antisense oligonucleotides pharmacology, signal transduction drug effects and physiology, somites drug effects, somites metabolism.

Wiellette, E.L. and H. Sive (2003). **Vhnf1 and Fgf signals synergize to specify rhombomere identity in the zebrafish hindbrain.** *Development* 130(16): 3821-9. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Vertebrate hindbrain segmentation is a highly conserved process but the mechanism of rhombomere determination is not well understood. Recent work in the zebrafish has shown a requirement for fibroblast growth factor (Fgf) signaling and for the transcription factor variant hepatocyte nuclear factor 1 (vhnf1) in specification of rhombomeres 5 and 6 (r5+r6). We show here that vhnf1 functions in two ways to subdivide the zebrafish caudal hindbrain domain (r4-r7) into individual rhombomeres. First, vhnf1 promotes r5+r6 identity through an obligate synergy with Fgf signals to activate valentino and krox20 expression. Second, vhnf1 functions independently of Fgf signals to repress hoxb1a expression. Although vhnf1 is expressed in a broad posterior domain during gastrulation, it promotes the specification of individual rhombomeres. This is achieved in part because vhnf1 gives cellular competence to respond to Fgf signals in a caudal hindbrain-specific manner.
Descriptors: zebrafish, DNA binding proteins genetics, proteins metabolism, Fgf metabolism, developmental gene expression regulation, nuclear proteins metabolism, rhombencephalon embryology, signal transduction physiology, transcription factors metabolism, proteins, DNA binding proteins genetics, epistasis, genetic, Fgf genetics, hepatocyte nuclear factor 1 beta, homeodomain proteins genetics and metabolism, in situ hybridization, mafb transcription factor , morphogenesis, nerve tissue nerve tissue proteins metabolism, nuclear antisense oligonucleotides genetics, antisense oligonucleotides metabolism, rhombencephalon anatomy, histology and physiology, transcription factors genetics.

Wiellette, E. and H. Sive (2004). **Combinatorial gene function in the embryonic zebrafish hindbrain.** *Developmental Biology* 271(2): 588. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, nervous system, neural coordination, development, molecular genetics.

Wilm, T.P. and L. Solnica Krezel (2005). **Essential roles of a zebrafish prdm1/blimp1 homolog in embryo patterning and organogenesis.** *Development* 132(2): 393-404. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: During vertebrate development the dorsal gastrula or Spemann-Mangold organizer orchestrates axis formation largely by limiting the ventralizing and posteriorizing activity of bone morphogenetic proteins (BMPs). In mouse and *Xenopus laevis*, genes encoding the zinc finger transcriptional repressor Prdm1/Blimp1 (PR domain containing 1, with ZNF domain; previously named B lymphocyte-induced maturation protein 1) were recently shown to be expressed in the visceral endoderm and anterior endomesoderm, respectively, and the prechordal plate of gastrula stage embryos. Later in development Prdm1/Blimp1 is expressed in many other tissues, including pharyngeal arches, limb buds, otic vesicles, photoreceptor cell layer, slow muscle and cloaca. Based on misexpression and dominant-negative studies, Prdm1/Blimp1 was proposed to promote anterior endomesoderm and head development in *Xenopus laevis*. Here we report the isolation and functional characterization of zebrafish prdm1 exhibiting a dynamic and evolutionarily conserved expression pattern. Misexpression of prdm1 inhibits the formation of dorsoanterior structures and reduces expression of chordin, which encodes a BMP antagonist. Conversely, interference with Prdm1 translation using antisense morpholino oligonucleotides, increases chordin expression, while reducing expression of Bmp genes, and consequently dorsalizing the embryo. At the end of the gastrula period, prdm1 morphant embryos have enlarged animal-vegetal and anteroposterior embryonic axes. This altered embryo morphology is associated with augmented extension movements of dorsal tissues and normal posterior migration of ventral tissues. Additionally, Prdm1 activity is essential for proper development of slow muscle, the photoreceptor cell layer, branchial arches and pectoral fins. Our studies reveal essential roles for prdm1 in limiting the function of the gastrula organizer and regulating cell fate specification and morphogenetic processes in precise correspondence with its intricate expression pattern.
Descriptors: zebrafish, repressor, transcription factors physiology, amino acid sequence, bone morphogenetic proteins metabolism, cell lineage, cell movement, molecular cloning, DNA binding proteins genetics, proteins, endoderm metabolism, expressed sequence tags, gastrula metabolism, dominant genes, glycoproteins metabolism, in situ hybridization, intercellular signaling peptides and proteins metabolism, mesoderm metabolism, mice, molecular sequence data, nuclear proteins, phenotype, protein biosynthesis, repressor proteins genetics and metabolism, amino acid sequence homology, signal transduction, transcription factors genetics and metabolism, *Xenopus*, zebrafish, proteins.

Wilson, A.L., Y.C. Shen, S.G. Babb Clendenon, J. Rostedt, B. Liu, K.F. Barald, J.A. Marrs, and Q. Liu (2007). **Cadherin-4 plays a role in the development of zebrafish cranial ganglia and lateral line system.** *Developmental Dynamics* 236(3): 893-902. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: We previously reported that cadherin-4 (also called R-cadherin) was expressed by the majority of the developing zebrafish cranial and lateral line ganglia. Cadherin-4 (Cdh4) function in the formation of these structures in zebrafish was studied using morpholino

antisense technology. Differentiation of the cranial and lateral line ganglia and lateral line nerve and neuromasts of the cdh4 morphants was analyzed using multiple neural markers. We found that a subset of the morphant cranial and lateral line ganglia were disorganized, smaller, with reduced staining, and/or with altered shape compared to control embryos. Increased cell death in the morphant ganglia likely contributed to these defects. Moreover, cdh4 morphants had shorter lateral line nerves and a reduced number of neuromasts, which was likely caused by disrupted migration of the lateral line primordia. These results indicate that Cdh4 plays a role in the normal formation of the zebrafish lateral line system and a subset of the cranial ganglia.

Descriptors: zebrafish, cadherins genetics, ganglia metabolism, lateral line system metabolism, cadherins physiology, cell adhesion molecules genetics, cell adhesion molecules physiology, cell differentiation genetics and physiology, ganglia embryology, developmental gene expression regulation, in situ hybridization , lateral line system embryology, reverse transcriptase PCR.

Wilson, J. and A.S. Tucker (2004). **Fgf and Bmp signals repress the expression of Bapx1 in the mandibular mesenchyme and control the position of the developing jaw joint**. *Developmental Biology* 266(1): 138-150. ISSN: 0012-1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish, bone morphogenetic proteins, cell differentiation, cell fate, developmental genetics, embryonic development, fibroblast growth factor, gene expression, genetics, Jaw, mouth parts, musculoskeletal system, pattern formation, *Danio rerio*, Bapx1 gene, bone morphogenetic proteins, fibroblast growth factor, jaw joint.

Wingert, R.A., A. Brownlie, J.L. Galloway, K. Dooley, P. Fraenkel, J.L. Axe, A.J. Davidson, B. Barut, L. Noriega, X. Sheng, Y. Zhou, and L.I. Zon (2004). **The chianti zebrafish mutant provides a model for erythroid-specific disruption of transferrin receptor 1.** *Development* 131(24): 6225-35. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: Iron is a crucial metal for normal development, being required for the production of heme, which is incorporated into cytochromes and hemoglobin. The zebrafish chianti (cia) mutant manifests a hypochromic, microcytic anemia after the onset of embryonic circulation, indicative of a perturbation in red blood cell hemoglobin production. We show that cia encodes tfr1a, which is specifically expressed in the developing blood and requisite only for iron uptake in erythroid precursors. In the process of isolating zebrafish tfr1, we discovered two tfr1-like genes (tfr1a and tfr1b) and a single tfr2 ortholog. Abrogation of tfr1b function using antisense morpholinos revealed that this paralog was dispensable for hemoglobin production in red cells. tfr1b morphants exhibited growth retardation and brain necrosis, similar to the central nervous system defects observed in the Tfr1 null mouse, indicating that tfr1b is probably used by non-erythroid tissues for iron acquisition. Overexpression of mouse Tfr1, mouse Tfr2, and zebrafish tfr1b partially rescued hypochromia in cia embryos, establishing that each of these transferrin receptors are capable of supporting iron uptake for hemoglobin production in vivo. Taken together, these data show that zebrafish tfr1a and tfr1b share biochemical function but have restricted domains of tissue expression, and establish a genetic model to study the specific function of Tfr1 in erythroid cells.

Descriptors: zebrafish embryos, erythroid cells cytology, iron metabolism, phylogeny, transferrin receptor metabolism, amino acid sequence, cytology, metabolism, erythroid cells metabolism, developmental gene expression regulation genetics, hemoglobins metabolism, molecular sequence data, mutation genetics, ransferrin receptor genetics, metabolism.

Wohlgemuth, S.L., B.D. Crawford, and D.B. Pilgrim (2007). **The myosin co-chaperone UNC-45 is required for skeletal and cardiac muscle function in zebrafish.** *Developmental Biology* 303(2): 483-92. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The assembly of myosin into higher order structures is dependent upon accessory factors that are often tissue-specific. UNC-45 acts as such a molecular chaperone for myosin in the nematode Caenorhabditis elegans, in both muscle and non-muscle contexts. Although vertebrates contain homologues of UNC-45, their requirement for muscle function has not been assayed. We identified a zebrafish gene, unc45b, similar to a mammalian unc-45 homologue, expressed exclusively in striated muscle tissue, including the somites, heart and craniofacial muscle. Morpholino-oligonucleotide-mediated knockdown of unc45b results in paralysis and cardiac dysfunction. This paralysis is correlated with a loss of myosin filaments in the sarcomeres of the trunk muscle. Morphants lack circulation, heart looping and display severe cardiac and yolk-sac edema and also demonstrate ventral displacement of several jaw cartilages. Overall, this confirms a role for unc45b in zebrafish motility consistent with a function in myosin thick filament assembly and stability and uncovers novel roles for this gene in the function and morphogenesis of the developing heart and jaw. These results suggest that Unc45b acts as a chaperone that aids in the folding of myosin isoforms required for skeletal, cranial and cardiac muscle contraction.

Descriptors: zebrafish embryology, muscle, myosins physiology, base sequence, craniofacial abnormalities genetics, heart embryology and physiology, mesoderm metabolism, molecular sequence data, muscle skeletal muscle embryology, skeletal muscle physiology, mutation, myosins genetics, oligodeoxyribonucleotides, antisense genetics.

Won, M., H. Ro, H.C. Park, K.E. Kim, T.L. Huh, C.H. Kim, and M. Rhee (2006). **Dynamic expression patterns of zebrafish 1G5 (1G5z), a calmodulin kinase-like gene in the developing nervous system.** *Developmental Dynamics* 235(3): 835-42. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Abstract: Evolutionarily well-conserved Ca(2+)/calmodulin-dependent protein kinase (CaMK) proteins are known for their role as Ca(2+) signaling mediators. 1G5 encodes a CaMK like protein, which belongs to a calmodulin (CaM) kinase gene family. Here, we report the isolation of zebrafish homologue of mammalian 1G5, which we named 1G5z. 1G5z is composed of three major domains: (1) an N-terminal serine/threonine kinase domain, (2) a central calmodulin-binding domain, and (3) a C-terminal alanine-rich domain, the 1G5z-specific domain. 1G5z shares 83 approximately 84% homology with other vertebrate 1G5 proteins. Spatiotemporal expression studies found that 1G5z is expressed by means of zygotic transcription and appears in various neuronal tissues from the 20-somite stage. 1G5z transcripts are more regionalized in the brain and spinal cord at 24 hr postfertilization (hpf). At 35 hpf, 1G5z transcripts are exclusively present in the anterior trunk spinal cord as well as in the hindbrain, tegmentum, hypothalamus, and telencephalon. This expression pattern lasts until 48 hpf but ceases in the trunk. At 72 hpf, 1G5z is abundantly transcribed particularly in the specific region of the tectum and eye. We further observed that the number of 1G5z-positive cells is dramatically increased in the mindbomb mutant embryos but abolished in the trigeminal ganglion and caudal trunk sensory neuron of the neurogenin1 morphant at 24 hpf. In addition, bromodeoxyuridine staining further confirmed that the 1G5z-positive cells were postmitotic sensory and interneurons.

Descriptors: zebrafish embryos, Ca2+ calmodulin dependent protein kinase genetics, nervous system embryology, amino acid sequence, Ca2+ calmodulin dependent protein kinase analysis and chemistry, molecular cloning, enzymology, molecular sequence data,

nervous system enzymology, afferent neurons cytology, afferent neurons enzymology, tertiary protein structure, metabolism, proteins analysis and chemistry.

Wood, A.W. and P.J. Schlueter (2006). **Functional divergence of duplicate type-1 IgF receptors in zebrafish primordial germ cell development.** *Biology of Reproduction*: 94-95. ISSN: 0006 3363.
NAL Call Number: QL876.B5
Descriptors: zebrafish germ cells, molecular genetics, molecular biophysics, reproductive system, phenotype, cell apoptosis, cell migration, functional divergence.

Wright, G.J., J.D. Leslie, L. Ariza McNaughton, and J. Lewis (2004). **Delta proteins and MAGI proteins: an interaction of Notch ligands with intracellular scaffolding molecules and its significance for zebrafish development.** *Development* 131(22): 5659-69. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Delta proteins activate Notch through a binding reaction that depends on their extracellular domains; but the intracellular (C-terminal) domains of the Deltas also have significant functions. All classes of vertebrates possess a subset of Delta proteins with a conserved ATEV* motif at their C termini. These ATEV Deltas include Delta1 and Delta4 in mammals and DeltaD and DeltaC in the zebrafish. We show that these Deltas associate with the membrane-associated scaffolding proteins MAGI1, MAGI2 and MAGI3, through a direct interaction between the C termini of the Deltas and a specific PDZ domain (PDZ4) of the MAGIs. In cultured cells and in subsets of cells in the intact zebrafish embryo, DeltaD and MAGI1 are co-localized at the plasma membrane. The interaction and the co-localization can be abolished by injection of a morpholino that blocks the mRNA splicing reaction that gives DeltaD its terminal valine, on which the interaction depends. Embryos treated in this way appear normal with respect to some known functions of DeltaD as a Notch ligand, including the control of somite segmentation, neurogenesis, and hypochord formation. They do, however, show an anomalous distribution of Rohon-Beard neurons in the dorsal neural tube, suggesting that the Delta-MAGI interaction may play some part in the control of neuron migration.
Descriptors: zebrafish embryology, membrane proteins metabolism, brain cytology, brain metabolism, central nervous system cytology, central nervous system embryology and metabolism, conserved sequence, cytology, embryology, metabolism, developmental gene expression regulation, guanylate kinase, ligands, membrane proteins chemistry and genetics, nucleoside phosphate kinase chemistry and genetics, nucleoside phosphate kinase metabolism, peptide fragments metabolism, protein binding, RNA splicing genetics, Notch receptors, valine genetics and metabolism, proteins chemistry and genetics.

Wu, C.T. and S.J. Lee (2005). **Profilin II is required for proper embryogenesis in zebrafish**. *Journal of the Fisheries Society of Taiwan* 32(1): 13. ISSN: 0379-4180.
NAL Call Number: SH135.T3
Descriptors: zebrafish, abnormalities, body shape, body size, cell division, defects, developmental stages, embryonic development, freshwater fish, gene expression, proteins, *Danio rerio*, *Teleostei.*

Wu, S., L. Page, and N.M. Sherwood (2006). **Brain regionalization and eye development: a role for GNRH in zebrafish embryo**. *Developmental Biology* 295(1): 371. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryo, nervous system, neural coordination, development, molecu-

lar genetics, sense organs, sensory reception, reverse transcriptase PCR, genetic techniques, in situ hybridization, genetic techniques.

Xiao, T., T. Roeser, W. Staub, and H. Baier (2005). **A GFP-based genetic screen reveals mutations that disrupt the architecture of the zebrafish retinotectal projection.** *Development* 132(13): 2955-67. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: The retinotectal projection is a premier model system for the investigation of molecular mechanisms that underlie axon pathfinding and map formation. Other important features, such as the laminar targeting of retinal axons, the control of axon fasciculation and the intrinsic organization of the tectal neuropil, have been less accessible to investigation. In order to visualize these processes in vivo, we generated a transgenic zebrafish line expressing membrane-targeted GFP under control of the brn3c promoter/enhancer. The GFP reporter labels a distinct subset of retinal ganglion cells (RGCs), which project mainly into one of the four retinorecipient layers of the tectum and into a small subset of the extratectal arborization fields. In this transgenic line, we carried out an ENU-mutagenesis screen by scoring live zebrafish larvae for anatomical phenotypes. Thirteen recessive mutations in 12 genes were discovered. In one mutant, ddl, the majority of RGCs fail to differentiate. Three of the mutations, vrt, late and tard, delay the orderly ingrowth of retinal axons into the tectum. Two alleles of drg disrupt the layer-specific targeting of retinal axons. Three genes, fuzz, beyo and brek, are required for confinement of the tectal neuropil. Fasciculation within the optic tract and adhesion within the tectal neuropil are regulated by vrt, coma, bluk, clew and blin. The mutated genes are predicted to encode molecules essential for building the intricate neural architecture of the visual system.

Descriptors: zebrafish embryology, reporter genes, green fluorescent retina embryology , retinal ganglion cells metabolism, superior colliculus embryology, DNA binding proteins genetics, DNA binding proteins genetics, proteins metabolism, enhancer elements genetics, genetic screening methods, green fluorescent proteins metabolism, confocal microscopy, retina abnormalities, superior colliculus abnormalities, transcription factor brn 3, transcription factor brn 3c, transcription factors genetics and metabolism.

Xie, J. and S. Fisher (2005). **Twisted gastrulation enhances BMP signaling through Chordin dependent and independent mechanisms**. *Development* 132(2): 383-391. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: BMP signaling is modulated by a number of extracellular proteins, including the inhibitor Chordin, Tolloid-related enzymes (Tld), and the interacting protein Twisted Gastrulation (Tsg). Although in vitro studies have demonstrated Chordin cleavage by Tld enzymes, its significance as a regulatory mechanism in vivo has not been established in vertebrates. In addition, Tsg has been reported in different contexts to either enhance or inhibit BMP signaling through its interactions with Chordin. We have used the zebrafish gastrula to carry out structure/function studies on Chordin, by making versions of Chordin partially or wholly resistant to Tld cleavage and introducing them into chordin-deficient embryos. We examined the cleavage products generated in vivo from wild-type and altered Chordins, and tested their efficacy as BMP inhibitors in the embryo. We demonstrate that Tld cleavage is crucial in restricting Chordin function in vivo, and is carried out by redundant enzymes in the zebrafish gastrula. We also present evidence that partially cleaved Chordin is a stronger BMP inhibitor than the full-length protein, suggesting a positive role for Tld in regulating Chordin. We find that depletion of the embryo for Tsg leads to decreased BMP signaling,

and to increased levels of Chordin. Finally, we show that Tsg also enhances BMP signaling in the absence of Chordin, and its depletion can partially rescue the chordin mutant phenotype, demonstrating that important components of the BMP signaling pathway remain unidentified. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, development, enzymology, molecular biophysics, twisted gastrulation, chordin, mechanisms, Chordin.

Xiong, B., Y. Rui, M. Zhang, K. Shi, S. Jia, T. Tian, K. Yin, H. Huang, S. Lin, X. Zhao, Y. Chen, Y.G. Chen, S.C. Lin, and A. Meng (2006). **Tob1 controls dorsal development of zebrafish embryos by antagonizing maternal beta-catenin transcriptional activity.** *Developmental Cell* 11(2): 225-38. ISSN: 1534 5807.

NAL Call Number: QH573.C42

Abstract: Maternal beta-catenin and Nodal signals are essential for the formation of the dorsal organizer, which, in turn, induces neural and other dorsal tissue development in vertebrate embryos. Tob (Transducer of ErbB2) proteins possess antiproliferative properties and are known to influence BMP signaling, but their relationship to other signaling pathways and to embryonic patterning in general was unclear. In this study, we demonstrate that zebrafish tob1a is required for correct dorsoventral patterning. Mechanistically, Tob1a inhibits beta-catenin transcriptional activity by physically associating with beta-catenin and preventing the formation of beta-catenin/LEF1 complexes. Although Tob1a can also inhibit the transcriptional activity of the Nodal effector Smad3, its role in limiting dorsal development is executed primarily by antagonizing the beta-catenin signal. We further demonstrate that Tob family members across species share similar biochemical properties and biological activities.

Descriptors: zebrafish embryos, spinal nerve roots drug effects, spinal nerve roots growth and development, genetic transcription drug effects, beta catenin antagonists and inhibitors, embryology, metabolism, protein bindingdrug effects, protein binding physiology, signal transduction drug effects, signal transduction physiology, Smad3 protein antagonists, inhibitors and metabolism, spinal nerve roots physiology, transforming growth factor beta antagonists and inhibitors and metabolism, proteins pharmacology, beta catenin genetics and physiology.

Xu, X., T.M. Han, and S.L. Amacher (2005). **A novel conserved gene, paladin, is dynamically expressed in the midline and paraxial mesoderm of zebrafish embryos.** *Developmental Biology* 283(2): 659. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish embryos, development, molecular genetics, paladin, gene, mesoderm.

Yabe, T., X. Ge, and F. Pelegri (2007). **The zebrafish maternal-effect gene cellular atoll encodes The centriolar component sas-6 and defects in its paternal function promote whole genome duplication.** *Developmental Biology* 312(1): 44-60. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: A female-sterile zebrafish maternal-effect mutation in cellular atoll (cea) results in defects in the initiation of cell division starting at the second cell division cycle. This phenomenon is caused by defects in centrosome duplication, which in turn affect the formation of a bipolar spindle. We show that cea encodes the centriolar coiled-coil protein Sas-6, and that zebrafish Cea/Sas-6 protein localizes to centrosomes. cea also has a genetic paternal contribution, which when mutated results in an arrested first cell division followed by normal cleavage. Our data supports the idea that, in zebrafish, paternally inherited centrosomes

are required for the first cell division while maternally derived factors are required for centrosomal duplication and cell divisions in subsequent cell cycles. DNA synthesis ensues in the absence of centrosome duplication, and the one-cycle delay in the first cell division caused by cea mutant sperm leads to whole genome duplication. We discuss the potential implications of these findings with regards to the origin of polyploidization in animal species. In addition, the uncoupling of developmental time and cell division count caused by the cea mutation suggests the presence of a time window, normally corresponding to the first two cell cycles, which is permissive for germ plasm recruitment.

Descriptors: zebrafish embryology, centrioles metabolism, chromosomal proteins, non histone genetics, gene duplication, genome, alleles, amino acid sequence, chromosomal proteins, non histone chemistry, cleavage stage, ovum cytology, cytology, embryonic development, fathers, developmental gene expression regulation, mitosis, mitotic spindle apparatus metabolism, genetic models, molecular sequence data, mothers, mutant proteins metabolism, mutation genetics, ploidies, protein transport, spermatozoa cytology, proteins chemistry.

Yamaguchi, M., N. Tonou Fujimori, A. Komori, R. Maeda, Y. Nojima, H. Li, H. Okamoto, and I. Masai (2005). **Histone deacetylase 1 regulates retinal neurogenesis in zebrafish by suppressing Wnt and Notch signaling pathways.** *Development* 132(13): 3027-43. ISSN: 0950 1991.

NAL Call Number: QL951.D38

Abstract: In the developing vertebrate retina, progenitor cells initially proliferate but begin to produce postmitotic neurons when neuronal differentiation occurs. However, the mechanism that determines whether retinal progenitor cells continue to proliferate or exit from the cell cycle and differentiate is largely unknown. Here, we report that histone deacetylase 1 (Hdac1) is required for the switch from proliferation to differentiation in the zebrafish retina. We isolated a zebrafish mutant, ascending and descending (add), in which retinal cells fail to differentiate into neurons and glial cells but instead continue to proliferate. The cloning of the add gene revealed that it encodes Hdac1. Furthermore, the ratio of the number of differentiating cells to that of proliferating cells increases in proportion to Hdac activity, suggesting that Hdac proteins regulate a crucial step of retinal neurogenesis in zebrafish. Canonical Wnt signaling promotes the proliferation of retinal cells in zebrafish, and Notch signaling inhibits neuronal differentiation through the activation of a neurogenic inhibitor, Hairy/Enhancer-of-split (Hes). We found that both the Wnt and Notch/Hes pathways are activated in the add mutant retina. The cell-cycle progression and the upregulation of Hes expression in the add mutant retina can be inhibited by the blockade of Wnt and Notch signaling, respectively. These data suggest that Hdac1 antagonizes these pathways to promote cell-cycle exit and the subsequent neurogenesis in zebrafish retina. Taken together, these data suggest that Hdac1 functions as a dual switch that suppresses both cell-cycle progression and inhibition of neurogenesis in the zebrafish retina.

Descriptors: zebrafish embryos, down regulation physiology, histone deacetylases metabolism, intercellular signaling peptides and proteins metabolism, membrane proteins metabolism, retina embryology, signal transduction genetics, proteins metabolism, amino acid sequence, cell cycle genetics and proteins metabolism, cell differentiation physiology, cell proliferation, cyclin D1 metabolism, cyclin dependent kinase inhibitor p27, down regulation genetics, growth inhibitors genetics and physiology, histone deacetylases genetics, membrane proteins antagonists and inhibitors, molecular sequence data, mutation, Notch receptors, retina cytology, tumor suppressor proteins metabolism, Wnt proteins, proteins genetics.

Yamamoto, Y. and M. Oelgeschlaeger (2004). **Regulation of bone morphogenetic proteins in early embryonic development**. *Naturwissenschaften* 91(11): 519-534. ISSN: 0028 1042.
NAL Call Number: 474 N213
Abstract: Bone morphogenetic proteins (BMPs), a large subgroup of the TGF-beta family of secreted growth factors, control fundamental events in early embryonic development, organogenesis and adult tissue homeostasis. The plethora of dose-dependent cellular processes regulated by BMP signalling demand a tight regulation of BMP activity. Over the last decade, a number of proteins have been identified that bind BMPs in the extracellular space and regulate the interaction of BMPs with their cognate receptors, including the secreted BMP antagonist Chordin. In the early vertebrate embryo, the localized secretion of BMP antagonists from the dorsal blastopore lip establishes a functional BMP signalling gradient that is required for the determination of the dorsoventral - or back to belly - body axis. In particular, inhibition of BMP activity is essential for the formation of neural tissue in the development of vertebrate and invertebrate embryos. Here we review recent studies that have provided new insight into the regulation of BMP signalling in the extracellular space. In particular, we discuss the recently identified Twisted gastrulation protein that modulates, in concert with metalloproteinases of the Tolloid family, the interaction of Chordin with BMP and a family of proteins that share structural similarities with Chordin in the respective BMP binding domains. In addition, genetic and functional studies in zebrafish and frog provide compelling evidence that the secreted protein Sizzled functionally interacts with the Chd - BMP pathway, despite being expressed ventrally in the early gastrula-stage embryo. These intriguing discoveries may have important implications, not only for our current concept of early embryonic patterning, but also for the regulation of BMP activity at later developmental stages and tissue homeostasis in the adult. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, development, embryonic development, organogenesis, tissue homeostasis.

Yamashita, M., K. Uchino, Y. Taguchi, S. Imamura, D. Uchida, T. Yabu, M. Hojo and N. Ojima (2003). **Stress response and apoptosis in zebrafish embryos.** *Aquatic Genomics: Steps Toward a Great Future,* Springer-Verlag Tokyo Inc.: Bunkyo-ku, Tokyo, 113-0033, Japan, p. 195-206. ISBN: 4431703314.
NAL Call Number: QL638.99 .A67
Descriptors: zebrafish embryos, development, molecular genetics, gamma ray irradiation, genetic techniques, apoptosis, embyrogenesis , stress conditions, stress response.

Yan, Y.L., J. Willoughby, D. Liu, J.G. Crump, C. Wilson, C.T. Miller, A. Singer, C. Kimmel, M. Westerfield, and J.H. Postlethwait (2005). **A pair of Sox: distinct and overlapping functions of zebrafish sox9 co-orthologs in craniofacial and pectoral fin development.** *Development* 132(5): 1069-83. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: Understanding how developmental systems evolve after genome amplification is important for discerning the origins of vertebrate novelties, including neural crest, placodes, cartilage and bone. Sox9 is important for the development of these features, and zebrafish has two co-orthologs of tetrapod SOX9 stemming from an ancient genome duplication event in the lineage of ray-fin fish. We have used a genotype-driven screen to isolate a mutation deleting sox9b function, and investigated its phenotype and genetic interactions with a sox9a null mutation. Analysis of mutant phenotypes strongly supports the interpretation that ancestral gene functions partitioned spatially and temporally between Sox9 co-orthologs. Distinct subsets of the craniofacial skeleton, otic placode and pectoral appendage express

each gene, and are defective in each single mutant. The double mutant phenotype is additive or synergistic. Ears are somewhat reduced in each single mutant but are mostly absent in the double mutant. Loss-of-function animals from mutations and morpholino injections, and gain-of-function animals injected with sox9a and sox9b mRNAs showed that sox9 helps regulate other early crest genes, including foxd3, sox10, snai1b and crestin, as well as the cartilage gene col2a1 and the bone gene runx2a; however, tfap2a was nearly unchanged in mutants. Chondrocytes failed to stack in sox9a mutants, failed to attain proper numbers in sox9b mutants and failed in both morphogenetic processes in double mutants. Pleiotropy can cause mutations in single copy tetrapod genes, such as Sox9, to block development early and obscure later gene functions. By contrast, subfunction partitioning between zebrafish co-orthologs of tetrapod genes, such as sox9a and sox9b, can relax pleiotropy and reveal both early and late developmental gene functions.
Descriptors: zebrafish embryos, developmental gene expression regulation, high mobility group proteins biosynthesis and physiology, transcription factors biosynthesis and physiology , genetically modified, body patterning, bone and bones embryology, cartilage embryology, cell death, chondrocytes metabolism, ear embryology, embryonic development, molecular evolution, extremities, gene deletion, genotype, in situ hybridization, in situ nick end labeling, genetic models, mutation, phenotype, tertiary protein structure, messenger RNA metabolism, time factors.

Yang, C.T. and S.L. Johnson (2006). **Small molecule-induced ablation and subsequent regeneration of larval zebrafish melanocytes.** *Development* 133(18): 3563-73. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: We developed a method to efficiently ablate a single cell type, the zebrafish melanocyte, and study the mechanisms of its regeneration. We found that a small molecule, (2-morpholinobutyl)-4-thiophenol (MoTP), specifically ablates zebrafish larval melanocytes or melanoblasts, and that this melanocytotoxicity is dependent on tyrosinase activity, which presumably converts MoTP to cytotoxic quinone species. Following melanocyte ablation by MoTP treatment, we demonstrate by BrdU incorporation experiments that regenerated melanocytes are derived from the division of otherwise quiescent melanocyte precursors or stem cells. We further show that larval melanocyte regeneration requires the kit receptor tyrosine kinase. Our results suggest that a small number of melanocyte precursors or stem cells unevenly distributed in larvae are drawn upon to reconstitute the larval melanocyte population following melanocyte ablation by MoTP.
Descriptors: zebrafish, melanocytes drug effects, morpholines pharmacology, phenols pharmacology and toxicity, sulfhydryl compounds toxicity, cell differentiation drug effects and genetics, cell division drug effects, cell division genetics, cell survival drug effects and genetics, cultured cells, developmental gene expression regulation drug effects, in situ hybridization methods, larva cytology, larva drug effects, larva metabolism, melanocytes cytology and metabolism, monophenol monooxygenase metabolism, morpholines chemistry, mutation genetics, phenols chemistry, stem cells cytology, drug effects and metabolism, sulfhydryl compounds chemistry.

Yang, J.F., Y.X. Cao, Z.H. Yang, and W.C. Liu (2005). **Androgenesis in zebrafish (*Danio rerio*).** *Fisheries Science/Shuichan Kexue* 24(2): 18-21. ISSN: 1003-1111.
Descriptors: zebrafish, androgenesis, diploids, fish eggs, fishery sciences, freshwater fish, genomes, heat shock, mutations, ultraviolet radiation, *Danio rerio*.

Yang, Z., H. Jiang, F. Zhao, D.B. Shankar, K.M. Sakamoto, M.Q. Zhang, and S. Lin (2007). **A highly conserved regulatory element controls hematopoietic expression of GATA-2 in zebrafish.** *BioMed Central Developmental Biology* 7: 97. ISSN: 1471 213X.
NAL Call Number: QH491.B63
Abstract: BACKGROUND: GATA-2 is a transcription factor required for hematopoietic stem cell survival as well as for neuronal development in vertebrates. It has been shown that specific expression of GATA-2 in blood progenitor cells requires distal cis-acting regulatory elements. Identification and characterization of these elements should help elucidating transcription regulatory mechanisms of GATA-2 expression in hematopoietic lineage. RESULTS: By pair-wise alignments of the zebrafish genomic sequences flanking GATA-2 to orthologous regions of fugu, mouse, rat and human genomes, we identified three highly conserved non-coding sequences in the genomic region flanking GATA-2, two upstream of GATA-2 and another downstream. Using both transposon and bacterial artificial chromosome mediated germline transgenic zebrafish analyses, one of the sequences was established as necessary and sufficient to direct hematopoietic GFP expression in a manner that recapitulates that of GATA-2. In addition, we demonstrated that this element has enhancer activity in mammalian myeloid leukemia cell lines, thus validating its functional conservation among vertebrate species. Further analysis of potential transcription factor binding sites suggested that integrity of the putative HOXA3 and LMO2 sites is required for regulating GATA-2/GFP hematopoietic expression. CONCLUSION: Regulation of GATA-2 expression in hematopoietic cells is likely conserved among vertebrate animals. The integrated approach described here, drawing on embryological, transgenesis and computational methods, should be generally applicable to analyze tissue-specific gene regulation involving distal DNA cis-acting elements.
Descriptors: zebrafish embryos, GATA2 transcription factor genetics, developmental gene expression regulation, regulatory elements, transcriptional genetics, tumor cell line, conserved sequence, green fluorescent proteins, hematopoietic stem cells, transfection.

Yaniv, K., S. Isogai, D. Castranova, L. Dye, J. Hitomi, and B.M. Weinstein (2006). **Live imaging of lymphatic development in the zebrafish.** *Nature Medicine* 12(6): 711-6. ISSN: 1078-8956.
NAL Call Number: RB113
Abstract: The lymphatic system has become the subject of great interest in recent years because of its important role in normal and pathological processes. Progress in understanding the origins and early development of this system, however, has been hampered by difficulties in observing lymphatic cells in vivo and in performing defined genetic and experimental manipulation of the lymphatic system in currently available model organisms. Here, we show that the optically clear developing zebrafish provides a useful model for imaging and studying lymphatic development, with a lymphatic system that shares many of the morphological, molecular and functional characteristics of the lymphatic vessels found in other vertebrates. Using two-photon time-lapse imaging of transgenic zebrafish, we trace the migration and lineage of individual cells incorporating into the lymphatic endothelium. Our results show lymphatic endothelial cells of the thoracic duct arise from primitive veins through a novel and unexpected pathway.
Descriptors: zebrafish embryology, diagnostic imaging, lymphatic system anatomy and histology, lymphatic system embryology, lymphatic system growth and development, anatomy and histology, growth and development, genetically modified anatomy and histology, genetically modified embryology and growth and development, cell lineage, endothelial cells cytology and physiology, recombinant fusion proteins genetics and metabolism, time factors and metabolism.

Yee, N.S., K. Lorent, and M. Pack (2005). **Exocrine pancreas development in zebrafish.** *Developmental Biology* 284(1): 84-101. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Although many of the genes that regulate development of the endocrine pancreas have been identified, comparatively little is known about how the exocrine pancreas forms. Previous studies have shown that exocrine pancreas development may be modeled in zebrafish. However, the timing and mechanism of acinar and ductal differentiation and morphogenesis have not been described. Here, we characterize zebrafish exocrine pancreas development in wild type and mutant larvae using histological, immunohistochemical and ultrastructural analyses. These data allow us to identify two stages of zebrafish exocrine development. During the first stage, the exocrine anlage forms from rostral endodermal cells. During the second stage, proto-differentiated progenitor cells undergo terminal differentiation followed by acinar gland and duct morphogenesis. Immunohistochemical analyses support a model in which the intrapancreatic ductal system develops from progenitors that join to form a contiguous network rather than by branching morphogenesis of the pancreatic epithelium, as described for mammals. Contemporaneous appearance of acinar glands and ducts in developing larvae and their disruption in pancreatic mutants suggest that common molecular pathways may regulate gland and duct morphogenesis and differentiation of their constituent cells. By contrast, analyses of mind bomb mutants and jagged morpholino-injected larvae suggest that Notch signaling principally regulates ductal differentiation of bipotential exocrine progenitors.

Descriptors: zebrafishembryology, cell differentiation physiology, morphogenesis physiology, pancreas embryology, signal transduction physiology, calcium binding endoderm physiology, immunohistochemistry, in situ hybridization, intercellular signaling peptides and proteins, membrane electron transmission microscopy, mutagenesis, pancreas ultrastructure, ubiquitin protein ligases genetics.

Yeo, S.Y., M. Kim, H.S. Kim, T.L. Huh, and A.B. Chitnis (2007). **Fluorescent protein expression driven by her4 regulatory elements reveals the spatiotemporal pattern of Notch signaling in the nervous system of zebrafish embryos.** *Developmental Biology* 301(2): 555-67. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Notch activation inhibits neuronal differentiation during development of the nervous system; however, the dynamic role of Notch signaling in individual cell lineages remains poorly understood. We have characterized 3.4 kb 5'-regulatory sequence of a Notch target gene, her4, and used it to drive fluorescent gene expression in transgenic lines where the spatiotemporal pattern of Notch activation can be examined in vivo. The 3.4 kb her4 promoter contains five predicted Su(H) binding sites of which two proximal sites were confirmed to be required for Notch-mediated transcriptional activation. Without Notch, Su(H) effectively represses transcription regulated by the promoter. Analyses of transgenic zebrafish showed that while the expression of proneural genes and Notch activation were both critical for endogenous her4 expression, reporter gene expression was primarily regulated by Notch activity. This study also showed that her4 may be differently regulated in sensory cranial ganglia, where Notch activity is not essential for her4 expression and where Su(H) may repress her4 expression. The establishment of a reporter line with Notch-Su(H)-dependent fluorescent gene expression provides a tool to explore the complex role of Notch signaling in the development of vertebrate nervous system.

Descriptors: zebrafish embryology, basic helix loop helix transcription factors metabolism, neurons metabolism, Notch receptors metabolism, regulatory elements, transcriptional

genetics, signal transduction, metabolism, genetically modified, base sequence, basic helix loop helix transcription factors genetics, cell differentiation, embryology, metabolism, ganglia embryology and metabolism, developmental gene expression regulation, reporter green fluorescent proteins genetics and metabolism, heterozygote, luciferases genetics and metabolism, nerve tissue proteins genetics and metabolism, neurons cytology, promoter regions genetics, Notch receptors genetics, somites metabolism, proteins genetics.

Yeo, S.Y., T. Miyashita, C. Fricke, M.H. Little, T. Yamada, J.Y. Kuwada, T.L. Huh, C.B. Chien, and H. Okamoto (2004). **Involvement of Islet-2 in the Slit signaling for axonal branching and defasciculation of the sensory neurons in embryonic zebrafish.** *Mechanisms of Development* 121(4): 315-24. ISSN: 0925-4773.
NAL Call Number: QH607.A1C4
Abstract: In *Drosophila melanogaster*, Slit acts as a repulsive cue for the growth cones of the commissural axons which express a receptor for Slit, Roundabout (Robo), thus preventing the commissural axons from crossing the midline multiple times. Experiments using explant culture have shown that vertebrate Slit homologues also act repulsively for growth cone navigation and neural migration, and promote branching and elongation of sensory axons. Here, we demonstrate that overexpression of Slit2 in vivo in transgenic zebrafish embryos severely affected the behavior of the commissural reticulospinal neurons (Mauthner neurons), promoted branching of the peripheral axons of the trigeminal sensory ganglion neurons, and induced defasciculation of the medial longitudinal fascicles. In addition, Slit2 overexpression caused defasciculation and deflection of the central axons of the trigeminal sensory ganglion neurons from the hindbrain entry point. The central projection was restored by either functional repression or mutation of Robo2, supporting its role as a receptor mediating the Slit signaling in vertebrate neurons. Furthermore, we demonstrated that Islet-2, a LIM/homeodomain-type transcription factor, is essential for Slit2 to induce axonal branching of the trigeminal sensory ganglion neurons, suggesting that factors functioning downstream of Islet-2 are essential for mediating the Slit signaling for promotion of axonal branching.
Descriptors: zebrafish, axons metabolism, glycoproteins metabolism, homeodomain proteins metabolism, nerve tissue proteins metabolism, afferent neurons metabolism, signal transduction physiology, transcription factors metabolism, proteins metabolism, genetically modified, reporter genes, glyconerve tissue proteins genetics, messenger RNA metabolism, immunologic receptors genetics and metabolism, recombinant fusion proteins, trigeminal ganglion metabolism.

Yin, C. and L. Solnica Krezel (2007). **Convergence and extension movements affect dynamic notochord-somite interactions essential for zebrafish slow muscle morphogenesis.** *Developmental Dynamics* 236(10): 2742-56. ISSN: 1058-8388.
NAL Call Number: QL801.A4
Descriptors: wild-type and mutant zebrafish, convergence, extension, notochord-somite, interaction, muscle, morphogenesis, myotome surface.

Yin, C. and L. Solnica Krezel (2007). **Convergence and extension movements mediate the specification and fate maintenance of zebrafish slow muscle precursors.** *Developmental Biology* 304(1): 141-55. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: During vertebrate gastrulation, concurrent inductive events and cell movements fashion the body plan. Convergence and extension (C&E) gastrulation movements narrow the vertebrate embryonic body mediolaterally while elongating it rostrocaudally. Segmented

somites are shaped and positioned by C&E alongside the notochord and differentiate into skeleton, fast, and slow muscles during somitogenesis. In zebrafish, simultaneous inactivation of non-canonical Wnt signaling components Knypek and Trilobite strongly impairs C&E gastrulation movements. Here we show that knypek;trilobite double mutants exhibit a severe deficit in slow muscles and their precursor, adaxial cells, revealing essential roles of C&E movements in adaxial cell development. Adaxial cells become distinguishable in the presomitic mesoderm during late gastrulation by their expression of myogenic factors and axial-adjacent position. Using cell tracing analyses and genetic manipulations, we demonstrate that C&E movements regulate the number of prospective adaxial cells specified during gastrulation by determining the size of the interface between the inductive axial and target presomitic tissues. During segmentation, when the range of Hedgehog signaling from the axial tissue declines, tight apposition of prospective adaxial cells to the notochord, which is achieved by convergence movements, is necessary for their continuous Hedgehog reception and fate maintenance. We provide direct evidence to show that the deficiency of adaxial cells in knypek;trilobite double mutants is due to impaired C&E movements, rather than an alteration in Hedgehog signal and its reception, or a cell-autonomous requirement for Knypek and Trilobite in adaxial cell development. Our results underscore the significance of precise coordination between cell movements and inductive tissue interactions during cell fate specification.

Descriptors: zebrafish embryology, cell differentiation physiology, cell movement physiology, heparan sulfate proteoglycans genetics, morphogenesis physiology, skelatal muscle embryology, heparan sulfate proteoglycans metabolism, fluorescence microscopy, skeletal muscle cytology, mutation genetics.

Yokoi, H., A. Shimada, M. Carl, S. Takashima, D. Kobayashi, T. Narita, T. Jindo, T. Kimura , T. Kitagawa, T. Kage, A. Sawada, K. Naruse, S. Asakawa, N. Shimizu, H. Mitani, A. Shima , M. Tsutsumi, H. Hori, J. Wittbrodt, Y. Saga, Y. Ishikawa, K. Araki, and H. Takeda (2007). **Mutant analyses reveal different functions of fgfr1 in medaka and zebrafish despite conserved ligand-receptor relationships.** *Developmental Biology* 304(1): 326-37. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Medaka (*Oryzias latipes*) is a small freshwater teleost that provides an excellent developmental genetic model complementary to zebrafish. Our recent mutagenesis screening using medaka identified headfish (hdf) which is characterized by the absence of trunk and tail structures with nearly normal head including the midbrain-hindbrain boundary (MHB). Positional-candidate cloning revealed that the hdf mutation causes a functionally null form of Fgfr1. The fgfr1hdf is thus the first fgf receptor mutant in fish. Although FGF signaling has been implicated in mesoderm induction, mesoderm is induced normally in the fgfr1hdf mutant, but subsequently, mutant embryos fail to maintain the mesoderm, leading to defects in mesoderm derivatives, especially in trunk and tail. Furthermore, we found that morpholino knockdown of medaka fgf8 resulted in a phenotype identical to the fgfr1hdf mutant, suggesting that like its mouse counterpart, Fgf8 is a major ligand for Fgfr1 in medaka early embryogenesis. Intriguingly, Fgf8 and Fgfr1 in zebrafish are also suggested to form a major ligand-receptor pair, but their function is much diverged, as the zebrafish fgfr1 morphant and zebrafish fgf8 mutant acerebellar (ace) only fail to develop the MHB, but develop nearly unaffected trunk and tail. These results provide evidence that teleost fish have evolved divergent functions of Fgf8-Fgfr1 while maintaining the ligand-receptor relationships. Comparative analysis using different fish is thus invaluable for shedding light on evolutionary diversification of gene function.

Descriptors: zebrafish embryology, body patterning genetics, mesoderm physiology, *Oryzias* genetics, type 1 fibroblast growth factor receptors genetics, amino acid sequence, chromosome mapping, cluster analysis, computational biology, DNA primers, Fgf genetics and metabolism, in situ hybridization, microspheres, molecular sequence data, mutation genetics, oligonucleotides, *Oryzias* embryology, phylogeny, type 1 fibroblast growth factor receptors metabolism, species specificity, proteins genetics and metabolism.

Yoshikawa, S., E. Norcom, H. Nakamura, R.W. Yee, and X.C. Zhao (2007). **Transgenic analysis of the anterior eye-specific enhancers of the zebrafish gelsolin-like 1 (gsnl1) gene.** *Developmental Dynamics* 236(7): 1929-38. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, genetically modified, anterior eye segment metabolism, enhancer elements genetics, gelsolin genetics, promoter regions genetics, metabolism.

Yoshimura, Y., K. Zama, S. Sato, M. Masuda, M. Ishibashi, Y. Hayashi, Y. Hori, N. Okino, Y. Hirabayashi, and M. Ito (2006). **Biological significance of ceramide metabolism in development of nervous and vascular systems in zebrafish**. *Chemistry and Physics of Lipids* 143(1-2): 65. ISSN: 0009 3084.
NAL Call Number: QP751.C4
Descriptors: zebrafish, cardiovascular system, transport and circulation, metabolism, development, nervous system, neural coordination, apoptosis, cell differentiation, cell growth arrest, ceramide metabolism.

Yu, H.H. and C.B. Moens (2005). **Semaphorin signaling guides cranial neural crest cell migration in zebrafish.** *Developmental Biology* 280(2): 373-85. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Cranial neural crest cells (NCCs) migrate into the pharyngeal arches in three primary streams separated by two cranial neural crest (NC)-free zones. Multiple tissues have been implicated in the guidance of cranial NCC migration; however, the signals provided by these tissues have remained elusive. We investigate the function of semaphorins (semas) and their receptors, neuropilins (nrps), in cranial NCC migration in zebrafish. We find that genes of the sema3F and sema3G class are expressed in the cranial NC-free zones, while nrp2a and nrp2b are expressed in the migrating NCCs. sema3F/3G expression is expanded homogeneously in the head periphery through which the cranial NCCs migrate in lzr/pbx4 mutants, in which the cranial NC streams are fused. Antisense morpholino knockdown of Sema3F/3G or Nrp2 suppresses the abnormal cranial NC phenotype of lzr/pbx4 mutants, demonstrating that aberrant Sema3F/3G-Nrp2 signaling is responsible for this phenotype and suggesting that repulsive Sema3F/3G-Npn2 signaling normally contributes to the guidance of migrating cranial NCCs. Furthermore, global over-expression of sema3Gb phenocopies the aberrant cranial NC phenotype of lzr/pbx4 mutants when endogenous Sema3 ligands are knocked down, consistent with a model in which the patterned expression of Sema3 ligands in the head periphery coordinates the migration of Nrp-expressing cranial NCCs.
Descriptors: zebrafish, developmental gene expression regulation, neural crest cytology, semaphorins metabolism, cell lineage, cell movement, in situ hybridization, ligands, membrane proteins metabolism, mosaicism, mutation, nerve tissue proteins metabolism, neuropilins metabolism, phenotype, messenger RNA metabolism, signal transduction.

Zampolla, T., D.M. Rawson, and T. Zhang (2006). **Development of new viability assessment methods for zebrafish (*Danio rerio*) oocytes**. *Cryobiology* 53(3): 418. ISSN: 0011-2240.
NAL Call Number: QH324.C7
Descriptors: zebrafish, cryobiology, cryopreservation, freshwater fish, methodology, oocytes, *Danio rerio*.

Zanella, C., B. Rizzi, C. Melani, M. Campana, P. Bourgine, K. Mikula, N. Peyrieras, and A. Sarti (2007). **Segmentation of cells from 3-d confocal images of live zebrafish embryo.** *Engineering in Medicine and Biology Magazine* 1: 6027-30. ISSN: 0739 5175.
Abstract: In this paper, we use partial-differential-equation-based segmentation to accurately extract the shapes of membranes and nuclei from time lapse confocal microscopy images, taken throughout early Zebrafish embryogenesis. This strategy is a prerequisite for an accurate quantitative analysis of cell shape and morphodynamics during organogenesis and is the basis for an integrated understanding of biological processes. This data will also serve for the measurement of the variability between individuals in a population. The segmentation of cellular structures is achieved by first using an edge-preserving image filtering method for noise reduction and then applying an algorithm for cell shape reconstruction based on the Subjective Surfaces technique.
Descriptors: zebrafish embryo, partial-differential-equation-based segmentation, shapes of membranes, nuclei, time lapse, confocal microscopy images.

Zchut, S., R. Creton, and L. Kochilas (2005). **Role of histone deacetylase 3 in zebrafish heart development and growth.** *Developmental Biology* 283(2): 607. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish embryos, cardiovascular system development, transport and circulation, enzymology, cardiac hypoplasia, heart disease, molecular analysis, genetic techniques, expression pattern.

Zecchin, E., A. Conigliaro, N. Tiso, F. Argenton, and M. Bortolussi (2005). **Expression analysis of jagged genes in zebrafish embryos.** *Developmental Dynamics* 233(2): 638-45. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish embryology, calcium binding developmental gene expression regulation genetics, calcium binding proteins metabolism, intercellular signaling peptides and proteins, membrane proteins, phylogeny, metabolism.

Zecchin, E., A. Filippi, F. Biemar, N. Tiso, S. Pauls, E. Ellertsdottir, L. Gnugge, M. Bortolussi, W. Driever, and F. Argenton (2007). **Distinct delta and jagged genes control sequential segregation of pancreatic cell types from precursor pools in zebrafish.** *Developmental Biology* 301(1): 192-204. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The different cell types of the vertebrate pancreas arise asynchronously during organogenesis. Beta-cells producing insulin, alpha-cells producing glucagon, and exocrine cells secreting digestive enzymes differentiate sequentially from a common primordium. Notch signaling has been shown to be a major mechanism controlling these cell-fate choices. So far, the pleiotropy of Delta and Jagged/Serrate genes has hindered the evaluation of the roles of specific Notch ligands, as the phenotypes of knock-out mice are lethal before complete pancreas differentiation. Analyses of gene expression and experimental manipulations of zebrafish embryos allowed us to determine individual contributions of Notch ligands to

pancreas development. We have found that temporally distinct phases of both endocrine and exocrine cell type specification are controlled by different delta and jagged genes. Specifically, deltaA knock-down embryos lack alpha cells, similarly to mib (Delta ubiquitin ligase) mutants and embryos treated with DAPT, a gamma secretase inhibitor able to block Notch signaling. Conversely, jagged1b morphants develop an excess of alpha-cells. Moreover, the pancreas of jagged2 knock-down embryos has a decreased ratio of exocrine-to-endocrine compartments. Finally, overexpression of Notch1a-intracellular-domain in the whole pancreas primordium or specifically in beta-cells helped us to refine a model of pancreas differentiation in which cells exit the precursor state at defined stages to form the pancreatic cell lineages, and, by a feedback mediated by different Notch ligands, limit the number of other cells that can leave the precursor state.

Descriptors: zebrafish embryology, calcium binding intercellular signaling peptides and membrane pancreas embryology, Notch receptors physiology, base sequence, cell differentiation physiology, DNA primers, pancreas cytology, signal transduction.

Zecchin, E., A. Mavropoulos, N. Devos, A. Filippi, N. Tiso, D. Meyer, B. Peers, M. Bortolussi, and F. Argenton (2004). **Evolutionary conserved role of ptf1a in the specification of exocrine pancreatic fates.** *Developmental Biology* 268(1): 174-184. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: We have characterized and mapped the zebrafish ptf1a gene, analyzed its embryonic expression, and studied its role in pancreas development. In situ hybridization experiments show that from the 12-somite stage to 48 hpf, ptf1a is dynamically expressed in the spinal cord, hindbrain, cerebellum, retina, and pancreas of zebrafish embryos. Within the endoderm, ptf1a is initially expressed at 32 hpf in the ventral portion of the pdx1 expression domain; ptf1a is expressed in a subset of cells located on the left side of the embryo posteriorly to the liver primordium and anteriorly to the endocrine islet that arises from the posterodorsal pancreatic anlage. Then the ptf1a expression domain buds giving rise to the anteroventral pancreatic anlage that grows posteriorly to eventually engulf the endocrine islet. By 72 hpf, ptf1a continues to be expressed in the exocrine compartment derived from the anteroventral anlage. Morpholino-induced ptf1a loss of function suppresses the expression of the exocrine markers, while the endocrine markers in the islet are unaffected. In mind bomb (mib) mutants, in which delta-mediated notch signalling is defective (Dev. Cell 4 (2003) 67), ptf1a is normally expressed. In addition, the slow-muscle-omitted (smu) mutants that lack expression of endocrine markers because of a defective hedgehog signalling (Curr. Biol. 11(2001) 1358) exhibit normal levels of ptf1a. This indicates that hedgehog signaling plays a different genetic role in the specification of the anteroventral (mostly exocrine) and posterodorsal (endocrine) pancreatic anlagen. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, digestive system: ingestion and assimilation, endocrine system: chemical coordination and homeostasis, evolution and adaptation, molecular genetics, pancreas, endocrine, exocrine, Hedgehog signaling.

Zeng, X.X., T.P. Wilm, D.S. Sepich, and L. Solnica Krezel (2007). **Apelin and its receptor control heart field formation during zebrafish gastrulation.** *Developmental Cell* 12(3): 391-402. ISSN: 1534 5807.

NAL Call Number: QH573.C42

Abstract: The vertebrate heart arises during gastrulation as cardiac precursors converge from the lateral plate mesoderm territories toward the embryonic midline and extend rostrally to form bilateral heart fields. G protein-coupled receptors (GPCRs) mediate functions of the nervous and immune systems; however, their roles in gastrulation remain largely unex-

plored. Here, we show that the zebrafish homologs of the Agtrl1b receptor and its ligand, Apelin, implicated in physiology and angiogenesis, control heart field formation. Zebrafish gastrulae express agtrl1b in the lateral plate mesoderm, while apelin expression is confined to the midline. Reduced or excess Agtrl1b or Apelin function caused deficiency of cardiac precursors and, subsequently, the heart. In Apelin-deficient gastrulae, the cardiac precursors converged inefficiently to the heart fields and showed ectopic distribution, whereas cardiac precursors overexpressing Apelin exhibited abnormal morphology and rostral migration. Our results implicate GPCR signaling in movements of discrete cell populations that establish organ rudiments during vertebrate gastrulation.
Descriptors: zebrafish embryos, chemokines metabolism, gastrula metabolism, heart embryology, intercellular signaling peptides and proteins metabolism, cardiac myoblastsmetabolism, organogenesis genetics, G protein Coupled receptors metabolism, body patterning physiology, cell differentiation physiology, cell movement physiology, chemokines genetics, embryonic development physiology, gastrula cytology, developmental gene expression regulation physiology, intercellular signaling peptides and mesoderm physiology, cardiac myoblastscytology, cardiac myocytes cytology and metabolism, G protein Coupled receptors genetics, *Xenopus* proteins genetics and metabolism, zebrafish.

Zeng, Z., X. Liu, S. Seebah, and Z. Gong (2005). **Faithful expression of living color reporter genes in transgenic medaka under two tissue-specific zebrafish promoters.** *Developmental Dynamics* 234(2): 387-92. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Descriptors: zebrafish, promoter and reporter genes, genetic techniques, transgenic medaka lines, *Oryzias* genetics, promoter regions genetics, amino acid sequence, genetically modified, cardiac myosins genetics, molecular cloning, conserved sequence, complementary DNA metabolism, green fluorescent proteins metabolism, in situ hybridization, keratin 8, keratins genetics, luminescent proteins metabolism, fluorescence microscopy, molecular sequence data, skeletal muscle metabolism, myosin light chains genetics, RNA chemistry, amino acid sequence homology, species specificity, tissue distribution, transgenes.

Zhang, C., T. Basta, L. Hernandez Lagunas, P. Simpson, D.L. Stemple, K.B. Artinger, and M.W. Klymkowsky (2004). **Repression of nodal expression by maternal B1-type SOXs regulates germ layer formation in Xenopus and zebrafish.** *Developmental Biology* 273(1): 23-37. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: B1-type SOXs (SOXs 1, 2, and 3) are the most evolutionarily conserved subgroup of the SOX transcription factor family. To study their maternal functions, we used the affinity-purified antibody antiSOX3c, which inhibits the binding of *Xenopus* SOX3 to target DNA sequences [Development. 130(2003)5609]. The antibody also cross-reacts with zebrafish embryos. When injected into fertilized *Xenopus* or zebrafish eggs, antiSOX3c caused a profound gastrulation defect; this defect could be rescued by the injection of RNA encoding SOX3DeltaC-EnR, a SOX3-engrailed repression domain chimera. In antiSOX3c-injected *Xenopus* embryos, normal animal-vegetal patterning of mesodermal and endodermal markers was disrupted, expression domains were shifted toward the animal pole, and the levels of the endodermal markers SOX17 and endodermin increased. In Xenopus, SOX3 acts as a negative regulator of Xnr5, which encodes a nodal-related TGFbeta-family protein. Two nodal-related proteins are expressed in the early zebrafish embryo, squint and cyclops; anti-SOX3c-injection leads to an increase in the level of cyclops expression. In both *Xenopus* and zebrafish, the antiSOX3c phenotype was rescued by the injection of RNA encoding the nodal

inhibitor Cerberus-short (CerS). In *Xenopus*, antiSOX3c's effects on endodermin expression were suppressed by injection of RNA encoding a dominant negative version of Mixer or a morpholino against SOX17alpha2, both of which act downstream of nodal signaling in the endoderm specification pathway. Based on these data, it appears that maternal B1-type SOX functions together with the VegT/beta-catenin system to regulate nodal expression and to establish the normal pattern of germ layer formation in Xenopus. A mechanistically conserved system appears to act in a similar manner in the zebrafish.

Descriptors: zebrafish embryos, body patterning physiology, DNA binding proteins genetics, proteins metabolism, developmental gene expression regulation physiology, germ layers metabolism, high mobility group proteins metabolism, transforming growth factor beta antagonists and inhibitors, *Xenopus* embryology, proteins, amino acid sequence, antibodies pharmacology, antibodies physiology, body patterning drug effects, DNA binding proteins genetics, enzyme repression drug effects, developmental gene expression regulation drug effects, germ layers physiology , high mobility group immunohistochemistry , in situ hybridization, intercellular signaling peptides and proteins, intracellular signaling peptides and proteins, molecular sequence data, plasmids genetics, proteins pharmacology, reverse transcriptase PCR, transcription factors metabolism, transforming growth factor beta metabolism, *Xenopus* proteins metabolism.

Zhang, C., Q. Li, C.H. Lim, X. Qiu, and Y.J. Jiang (2007). **The characterization of zebrafish antimorphic mib alleles reveals that Mib and Mind bomb-2 (Mib2) function redundantly.** *Developmental Biology* 305(1): 14-27. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Both mind bomb (mib) and mind bomb-2 (mib2) encode RING E3 ubiquitin ligases that promote Delta ubiquitylation and endocytosis in Notch activation. Detailed morphological and molecular examinations revealed that zebrafish mib(ta52b) (missense mutation in the C-terminal RING Finger (RF), M1013R) and mib(m132) (nonsense mutation resulting in a truncated protein that loses all three RFs, C785stop) are strong and weak antimorphic alleles, respectively, compared to the null allele, mib(tfi91) (nonsense mutation resulting in a truncated protein of only 60 amino acids, Y60stop). Zebrafish mib2 ortholog was identified in this study. Zebrafish Mib and Mib2 are colocalized in transfected cells and function redundantly in regulating Notch signaling in embryos. Mib(ta52b) and Mib(m132) have a dosage-dependent dominant-negative effect, at least, on Mib2, which is a molecular basis for the antimorphic phenotypes. It was also shown that Notch signaling negatively regulates mib expression in a Su(H)-dependent manner, forming a negative feedback loop in modulating Notch activation.

Descriptors: zebrafish embryos, alleles, ubiquitin protein ligases genetics, molecular cloning, embryo metabolism, gene expression regulation physiology, immunohistochemistry, in situ hybridization, mutation genetics, antisense oligonucleotides, Notch receptors metabolism, signal transduction genetics, ubiquitin protein ligases metabolism.

Zhao, C. and J. Malicki (2007). **Genetic defects of pronephric cilia in zebrafish.** *Mechanisms of Development* 124(7-8): 605-16. ISSN: 0925-4773.

NAL Call Number: QH607.A1C4

Abstract: Cilia play key roles in many aspects of embryogenesis and adult physiology in vertebrates. Past genetic screens in zebrafish identified numerous defects of ciliogenesis, including several mutations in the components of the intraflagellar transport machinery. In contrast to previous studies, here we describe a collection of mutants that affect subpopulations of cilia. Mutant embryos are characterized by a shortening and an abnormal movement

of kidney cilia, and in one case also a reduction of cilia length in the Kupffer's vesicle. In contrast to that, the cilia of sensory neurons, including photoreceptor cells, hair cells, and olfactory sensory cells, appear grossly intact. Motility defects of pronephric cilia vary in mutant strains from complete paralysis to an increased frequency of movement, and are associated with left-right asymmetry defects. While ciliary ultrastructure is normal in most mutants, one of the mutant loci is essential for the formation of proper microtubule architecture in the axoneme of pronephric cilia. Mutants characterized in this study reveal intriguing genetic differences between subpopulations of embryonic cilia, and provide an opportunity to study several aspects of cilia structure and function.
Descriptors: zebrafish embryos and adults, kidney cilia, Kupffer's vesicle, gentics, defects, sensory neurons, photoreceptor cells, pronephric.

Zhao, C., J. Qi, and A. Meng (2005). **Characterization and expression pattern of two zebrafish atf7 genes.** *Developmental Dynamics* 233(3): 1157-62. ISSN: 1058 8388.
NAL Call Number: QL801.A4
Abstract: Members of the ATF/CREB (activating transcription factor/cAMP-responsive element binding protein) transcription factor family play diverse roles in controlling cell proliferation, apoptosis, and oncogenesis, as well as in embryonic development of vertebrates. We identified two zebrafish orthologs of human ATF7 gene: atf7a and atf7b. Whole-mount in situ hybridization shows that zebrafish atf7a is first expressed in the notochord precursors at 80% epiboly stage and then in the developing notochord during segmentation. The expression of atf7a is positively regulated by ntl, flh, and spr2, which are involved in development of the notochord. In contrast, atf7b is maternally expressed and during embryogenesis its mRNA is ubiquitously distributed, showing an expression pattern similar to that of mammalian Atf7.
Descriptors: zebrafish embryology, DNA binding proteins genetics, developmental gene expression regulation genetics, trans activators genetics, amino acid sequence, molecular cloning, DNA binding proteins genetics, proteins chemistry, embryology and metabolism, homeodomain molecular sequence data, phylogeny, physical chromosome mapping, sequence alignment, sp3 transcription factor, T-box domain trans activators chemistry, transcription factors genetics, proteins chemistry.

Zhu, Y., D. Song, N.T. Tran, and N. Nguyen (2007). **The effects of The members of growth hormone family knockdown in zebrafish development.** *General and Comparative Endocrinology* 150(3): 395-404. ISSN: 0016-6480.
NAL Call Number: 444.8G28
Abstract: Growth hormone (GH), prolactin (PRL), and somatolactin (SL) are members of GH/PRL superfamily. These hormones are involved in the regulation of an array of physiological processes, including growth, lactation, and osmoregulation. While recent evidence has shown the GH, PRL, and SL gene transcripts and protein products are expressed during early zebrafish development, their functions at this time of embryogenesis remain unknown. In the current study, antisense morpholino oligonucleotide (MO) inhibition of gh, prl, and sl gene translation was used to examine the effects of gene knockdown on hormone function in zebrafish development. We observed that PRL, SLalpha and SLbeta MO treatment all affected development. PRL MO-treated embryos showed defects in gas bladder inflation, reduced head and eye size, shorter body length and fewer melanophores than untreated controls, whereas SLalpha and SLbeta MO-treated embryos were only defective in gas bladder inflation, GH MO-inhibition of GH specific translation did not lead to any discernable morphological changes within 10 days post fertilization (dpf). The effects of PRL knockdown

were further verified using a second PRL morpholino antisense and by a rescue experiment with in vitro transcribed prl mRNA containing 5 nucleotide mismatch within the PRL-MO binding region. These results provide the first evidence that members of the GH/PRL superfamily play a role in proper development of various structures including the head, eyes, melanophores and the gas bladder in zebrafish.

Descriptors: zebrafish embryos, morphogenesis physiology, pituitary hormones metabolism, air sacs embryology eye embryology, fish fish proteins metabolism, gene silencing, glycoglycoproteins metabolism, growth hormone genetics and metabolism, head embryology, morphogenesis genetics, antisense oligonucleotides, pituitary hormones genetics, prolactin genetics and metabolism.

Zinkevich, N.S., D.V. Bosenko, B.A. Link, and E.V. Semina (2006). **Laminin alpha 1 gene is essential for normal lens development in zebrafish.** *BioMed Central Developmental Biology* 6: 13. ISSN: 1471 213X.

NAL Call Number: QH491.B63

Abstract: Laminins represent major components of basement membranes and play various roles in embryonic and adult tissues. The functional laminin molecule consists of three chains, alpha, beta and gamma, encoded by separate genes. There are twelve different laminin genes identified in mammals to date that are highly homologous in their sequence but different in their tissue distribution. The laminin alpha -1 gene was shown to have the most restricted expression pattern with strong expression in ocular structures, particularly in the developing and mature lens. RESULTS: We identified the zebrafish lama1 gene encoding a 3075-amino acid protein (lama1) that possesses strong identity with the human LAMA1. Zebrafish lama1 transcripts were detected at all stages of embryo development with the highest levels of expression in the developing lens, somites, nervous and urogenital systems. Translation of the lama1 gene was inhibited using two non-overlapping morpholino oligomers that were complementary to sequences surrounding translation initiation. Morphant embryos exhibited an arrest in lens development and abnormalities in the body axis length and curvature. CONCLUSION: These results underline the importance of the laminin alpha 1 for normal ocular development and provide a basis for further analysis of its developmental roles.

Descriptors: zebrafish embryos, laminin physiology, crystalline lens embryology, amino acid sequence, metabolism, laminin antagonists and inhibitors, laminin genetics, crystalline lens metabolism, molecular sequence data, phenotype, phylogeny, sequence alignment, metabolism, proteins antagonists, inhibitors and genetics.

Ziv, L. and Y. Gothilf (2006). **Circadian time-keeping during early stages of development**. *Proceedings of the National Academy of Sciences of the United States of America* 103(11): 4146-4151. ISSN: 0027 8424.

NAL Call Number: 500N21P

Abstract: The zebrafish pineal gland is a photoreceptive organ containing an intrinsic central circadian oscillator, which drives daily rhythms of gene expression and the melatonin hormonal signal. Here we investigated the effect of light, given at early developmental stages before pineal gland formation, on the pineal circadian oscillator. Embryos that were exposed to light at 0-6, 10-13, or 10-16 h after fertilization exhibited clock-controlled rhythms of arylalkylamine-N-acetyltransferase (zfaanat2) mRNA in the pineal gland during the third and fourth day of development. This rhythm was absent in embryos that were placed in continuous dark within 2 h after fertilization (before blastula stage). Differences in the phases of these rhythms indicate that they are determined by the time of illumination. Light

treatments at these stages also caused a transient increase in period2 mRNA levels, and the development of zfaanat2 mRNA rhythm was abolished by PERIOD2 knock-down. These results indicate that light exposure at early developmental stages, and light-induced expression of period2, are both required for setting the phase of the circadian clock. The 24-h rhythm is then maintained throughout rapid proliferation and, remarkably, differentiation.
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Descriptors: zebrafish embryos, nervous system, neural coordination, molecular genetics, development, endocrine system, chemical coordination and homeostasis, biosynchronization, pineal gland development, melatonin, circadian rhythm, light dark cycle, photo period.

Cardiology

Ahmed, H. and G.R. Vasta (2006). **Knock-down of galectin-1-like proteins in zebrafish *(danio rerio)* reveals a muscle and heart developmental phenotype**. *Glycobiology* 16(11): 1134. ISSN: 0959 6658.
NAL Call Number: QP552.G59G593
Descriptors: zebrafish, cardiovascular system, molecular genetics, muscular system, neural coordination, cell differentiation, phenotype, expression pattern, circulation, morphological defect.

Auman, H.J., H. Coleman, H.E. Riley, F. Olale, H.-J. Tsai, and D. Yelon (2007). **Functional modulation of cardiac form through regionally confined cell shape changes**. *Public Library of Science: Biology* 5(3) ISSN: 1544-9173.
NAL Call Number: QH301.P56
Descriptors: zebrafish, blood circulation, cell morphology, circulatory system, heart, morphogenesis, *Danio rerio*.

Ayers, M., F. Moore, and B. Bagatto (2005). **University of Akron searching for critical windows in the development of the zebrafish cardiovascular system**. *Integrative and Comparative Biology* 45(6): 1107. ISSN: 1540 7063.
NAL Call Number: QL1.I67
Descriptors: zebrafish, cardiovascular system, circulation, development, environmental hypoxia sensitivity.

Bagatto, B. and W. Burggren (2006). **A three-dimensional functional assessment of heart and vessel development in the larva of the zebrafish (*Danio rerio*).** *Physiological and Biochemical Zoology* 79(1): 194-201. ISSN: 1522-2152.
NAL Call Number: QL1.P52
Abstract: There has been considerable recent interest in the development of the circulation in the zebrafish. Optical techniques typically used to visualize changes in heart size allow measurement of stroke volume during early vertebrate development, but this approach is complicated in zebrafish larvae because of the heart's irregular shape and its significant change in morphology during the first 6 d of development. By use of a three-dimensional integration of the early zebrafish heart and vessels, we have greatly reduced measurement error of stroke volume and cardiac output and have determined the cross-sectional growth of major vessels in the developing zebrafish larvae. A dramatic 500%-600% increase in cardiac output (from 10 to 50-60 nL min(-1)) occurs on days 5 and 6 postfertilization in *Danio rerio*. Cross-sectional area of key vessels (dorsal artery, caudal artery, dorsal vein) as well as between-individual variation significantly decreased over the first 6 d of development. Associated with the decrease in cross-sectional area is a significant increase in red blood cell velocity on days 5 and 6 postfertilization. Together, the three-dimensional data of the cardiac and vascular systems have shown that the most profound physiological and developmental changes occur in days 5 and 6, which corresponds with the appearance of the adult form of the heart and the transition from diffusive to convective O2 supply to internal tissues.
Descriptors: zebrafish, blood vessels growth and development, heart growth and development, larva anatomy and histology, larva growth and development, blood vessels anatomy and histology, cardiac output physiology, heart anatomy and histology, heart physiology, larva physiology.

Boudoulas, K.D., P.G. Burgon, L. Wang, D. Conner, L. Zon, A. Pizard, J.T. Shin, C. Macrae, J.G. Seidman, and C.E. Seidman (2005). **C6p: a novel cardiac protein that interacts with lamin A/C**. *Circulation* 112(17, Suppl. S): U242. ISSN: 0009 7322.
NAL Call Number: RC681.A1C8
Descriptors: zebrafish, cardiovascular system, molecular genetics, molecular biophysics, congestive heart failure, heart disease, etiology, dilated cardiomyopathy, yeast two hybrid screening, nuclear localization.

Burns, C.G. and C.A. MacRae (2006). **Purification of hearts from zebrafish embryos.** *BioTechniques* 40(3): 274-278 . ISSN: 0736 6205.
NAL Call Number: RB37.1B56
Descriptors: zebrafish heart embryology, heart anatomy and histology, organ culture techniques methods, specimen handling methods, zebrafish anatomy and histology .

Burns, C.G., D.J. Milan, E.J. Grande, W. Rottbauer, C.A. MacRae, and M.C. Fishman (2005). **High-throughput assay for small molecules that modulate zebrafish embryonic heart rate.** *Nature Chemical Biology* 1(5): 263-4. ISSN: 1552-4450.
Abstract: To increase the facility and throughput of scoring phenotypic traits in embryonic zebrafish, we developed an automated micro-well assay for heart rate using automated fluorescence microscopy of transgenic embryos expressing green fluorescent protein in myocardium. The assay measures heart rates efficiently and accurately over a large linear dynamic range, and it rapidly characterizes dose dependence and kinetics of small molecule-induced changes in heart rate. This is the first high-throughput micro-well assay for organ function in an intact vertebrate.
Descriptors: zebrafish, biological assay methods, cardiovascular agents toxicity, green fluorescent proteins analysis, heart drug effects, heart rate physiology, heart rate drug effects, dose response relationship, preclinical drug evaluation methods, embryo physiology, feasibility studies, heart embryology, myocardium metabolism, time factors.

Chun, C.Z., H.J. Tsai, and T.T. Chen (2006). **Trout Ea4- or human Eb-peptide of pro-IGF-I disrupts heart, red blood cell, and vasculature development in zebrafish embryos.** *Molecular Reproduction and Development* 73(9): 1112-21. ISSN: 1040-452X.
NAL Call Number: QP251.M64
Abstract: E-peptide of the pro-insulin-like growth factor (pro-IGF)-I is produced by proteolytic cleavage of the pro-hormone in post-translational processing. Introduction of a transgene encoding a secreted form of rtEa4- or hEb-peptide into newly fertilized zebrafish (*Danio rerio*) eggs by electroporation or microinjection resulted in embryos with abnormal cardiovascular features and reduced red blood cells and vasculature. Two different phenocopies of heart developmental defects were observed: (i) Group I embryos exhibited heart development arrested at the heart muscle stage and (ii) group II embryos exhibited heart development arrested at the heart tube stage. Both groups of embryos also exhibited reduction of red blood cells and vasculature. The mRNA levels of genes essential for heart development (GATA 5 and NKX2.5), hematopoiesis (GATA 1 and GATA 2), and vasculogenesis (VEGF) in normal and defective embryos were determined by quantitative real-time RT-PCR at 36 hr post-fertilization (hpf). Significant reduction of GATA 5, NKX2.5, GATA 1, GATA 2, and VEGF mRNA levels was observed in both groups of defective embryos. These results suggest that overexpression of rtEa4 or hEb transgene in zebrafish embryos disrupts heart development, hematopoiesis, and vasculogenesis by reducing the levels of GATA 5, NKX2.5, GATA 1, GATA 2, and VEGF mRNA.
Descriptors: zebrafish, embryo abnormalities, blood supply, erythrocytes metabolism, heart embryology, insulin like growth factor Imetabolism, protein precursors metabolism, trout ,

genetically modified, erythrocytes cytology, GATA transcription factors genetics , developmental gene expression regulation , hematopoiesis, homeodomain insulin like growth factor I genetics, myocardium metabolism, peptide fragments genetics, peptide fragments metabolism, protein precursors genetics, messenger RNA genetics, recombinant fusion proteins metabolism, vascular endothelialgrowth factor A genetics, metabolism.

Ebert, A.M., G.L. Hume, K.S. Warren, N.P. Cook, C.G. Burns, M.A. Mohideen, G. Siegal, D. Yelon, M.C. Fishman, and D.M. Garrity (2005). **Calcium extrusion is critical for cardiac morphogenesis and rhythm in embryonic zebrafish hearts.** *Proceedings of the National Academy of Sciences of the United States of America* 102(49): 17705-710. ISSN: 0027 8424.

NAL Call Number: 500N21P

Abstract: Calcium entry into myocytes drives contraction of the embryonic heart. To prepare for the next contraction, myocytes must extrude calcium from intracellular space via the Na+/Ca2+ exchanger (NCX1) or sequester it into the sarcoplasmic reticulum, via the sarcoplasmic reticulum Ca2+-ATPase2 (SERCA2). In mammals, defective calcium extrusion correlates with increased intracellular calcium levels and may be relevant to heart failure and sarcoplasmic dysfunction in adults. We report here that mutation of the cardiac-specific NCX1 (NCX1h) gene causes embryonic lethal cardiac arrhythmia in zebrafish tremblor (tre) embryos. The tre ventricle is nearly silent, whereas the atrium manifests a variety of arrhythmias including fibrillation. Calcium extrusion defects in tre mutants correlate with severe disruptions in sarcomere assembly, whereas mutations in the L-type calcium channel that abort calcium entry do not produce this phenotype. Knockdown of SERCA2 activity by morpholino-mediated translational inhibition or pharmacological inhibition causes embryonic lethality due to defects in cardiac contractility and morphology but, in contrast to tre mutation, does not produce arrhythmia. Analysis of intracellular calcium levels indicates that homozygous tre embryos develop calcium overload, which may contribute to the degeneration of cardiac function in this mutant. Thus, the inhibition of NCX1h versus SERCA2 activity differentially affects the pathophysiology of rhythm in the developing heart and suggests that relative levels of NCX1 and SERCA2 function are essential for normal development.

Descriptors: zebrafish, calcium metabolism, heart embryology, heart physiopathology, morphogenesis physiology, myocardial contraction physiology, amino acid sequence, calcium pharmacology, calcium transporting ATPases genetics and metabolism, heart drug effects, electron, transmission microscopy, molecular sequence data, mutation genetics, sarcoplasmic reticulum, sequence alignment, sequence homology, sodium calcium exchanger chemistry and metabolism, sodium calcium exchanger genetics.

Forouhar, A.S., J.R. Hove, C. Calvert, J. Flores, H. Jadvar, and M. Gharib (2004). **Electrocardiographic characterization of embryonic zebrafish.** *Public Library of Science: One* 5: 3615-7 . ISSN: 0739-5175.

Abstract: The zebrafish (*Danio rerio*) has emerged as one of the primary experimental models of developmental cardiovascular research. Recent progress in flow visualization techniques along with the existing genetic map of the species has made zebrafish amenable to a variety of experiments relating cardiac developmental structure and function. One essential tool in establishing the proper functioning of the heart is the electrocardiogram (ECG). This study presents a methodology whereby the ECGs of embryonic zebrafish could be used in assessing the electrophysiological consequences of genetically-, mechanically-, or pharmacokinetically-induced cardiac perturbations. Five day post-fertilization (dpf) embryos produced repeating bimodal ECGs with clearly distinguished atrial (P) and ventricular (R) depolarization waves. P-R intervals along with P-P intervals are cited.

Descriptors: zebrafish, day post-fertilization, dpf, embryos, bimodal ECGs, atrial (p), ventricular (r), depolarization, waves, p-r intervals, p-p intervals, electrocardiaogram.

Forouhar, A.S., M. Liebling, A. Hickerson, A. Nasiraei-Moghaddam, H.J. Tsai, J.R. Hove, S.E. Fraser, M.E. Dickinson, and M. Gharib (2006). **The embryonic vertebrate heart tube is a dynamic suction pump**. *Science* 312(5774): 751-753. ISSN: 0036 8075.
NAL Call Number: 470Sci2
Abstract: The embryonic vertebrate heart begins pumping blood long before the development of discernable chambers and valves. At these early stages, the heart tube has been described as a peristaltic pump. Recent advances in confocal laser scanning microscopy and four-dimensional visualization have warranted another look at early cardiac structure and function. We examined the movement of cells in the embryonic zebrafish heart tube and the flow of blood through the heart and obtained results that contradict peristalsis as a pumping mechanism in the embryonic heart. We propose a more likely explanation of early cardiac dynamics in which the pumping action results from suction due to elastic wave propagation in the heart tube. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, cardiovascular system, elastic wave propagation, early cardiac dynamics.

Fraser, S.D., M.C. Fishman, and S. Childs (2003). **Guidance of angiogenesis in zebrafish.** *Molecular and Cellular Proteomics* 2(9): 801. ISSN: 1535 9476.
Descriptors: zebrafish, cardiovascular, development , molecular genetics, angiogenesis.

Hansen, C., A. Stauffer, and J. Robishaw (2006). **G protein receptor regulation of zebrafish cardiac development**. *Journal of the Pennsylvania Academy of Science* 79: 110. ISSN: 1044 6753.
Descriptors: zebrafish, cardiovascular system: transport and circulation, development, molecular genetics, cardiac edema, heart disease cloning, genetic techniques, whole mount RNA in situ hybridization, histology and cytology techniques.

Heideman, W., D.S. Antkiewicz, S.A. Carney, and R.E. Peterson (2005). **Zebrafish and cardiac toxicology.** *Cardiovascular Toxicology* 5(2): 203-14. ISSN: 1530 7905.
Abstract: Model systems are a mainstay in toxicological research. Zebrafish are rapidly becoming an important model organism for studying vertebrate development. The advantages of zebrafish: short reproductive cycle, production of numerous transparent, synchronously developing embryos, low cost, and standardization make zebrafish an attractive model for toxicologists as well. The use of these fish to study heart development has moved forward very rapidly, laying the groundwork for studying the effects of chemicals on cardiac development and function. Here we describe approaches that can be used to study cardiac toxicity in developing zebrafish, focusing on examples where zebrafish embryos have been especially useful in understanding the impact of specific toxicants on heart development and function.
Descriptors: zebrafish, heart diseases chemically induced, chemical water pollutants toxicity, gene expression regulation drug effects, heart diseases genetics, heart diseases pathology , heart diseases physiopathology.

Ho, Y.L., Y.H. Lin, I.J. Tsai, F.J. Hsieh, and H.J. Tsai (2007). **In vivo assessment of cardiac morphology and function in heart-specific green fluorescent zebrafish.** *Journal of the Formosan Medical Association* 106(3): 181-6. ISSN: 0929-6646.
Abstract: BACKGROUND/PURPOSE: The zebrafish (*Danio rerio*) is a new animal model for cardiac research. Zebrafish possessing a green fluorescent heart facilitates the dynamic observation of cardiac development, morphology, and function in vivo. However, the effect of an

excessive expression of green fluorescent protein (GFP) in cardiac muscle on the heart function of zebrafish has not been reported. METHODS: We cloned a 1.6 kb polymerase chain reaction (PCR) product containing the upstream sequence (870 bp), exon 1 (39 bp), intron 1 (682 bp), and exon 2 (69 bp) of the zebrafish cardiac myosin light chain 2 gene. A germ line-transmitted zebrafish possessing a green fluorescent heart was generated by injecting this PCR product fused with the GFP gene with ends consisting of inverted terminal repeats of an adeno-associated virus. RESULTS: Green fluorescence was intensively and specifically expressed in the myocardial cells located around both the heart chambers. Two lines with different GFP expression were bred (A26 and A277). The luminance of A277 was brighter than that of A26 (1.7-fold). The 4 days postfertilization (dpf) cardiac function and morphology were similar between these two groups. However, the 8 dpf cardiac growth seemed to be retarded in the A277 group. The 8 dpf heart rate, stroke volume, and cardiac output were also significantly lower in the A277 group. CONCLUSION: Excess expression of GFP seems to exert some detrimental effects on zebrafish hearts.
Descriptors: zebrafish, green fluorescent heart anatomy and histology, heart physiology, genetically modified, immunohistochemistry.

Hove, J.R., R.W. Koester, A.S. Forouhar, G. Acevedo-Bolton, S.E. Fraser, and M. Gharib (2003). **Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis**. *Nature* 421(6919): 172-177. ISSN: 0028-0836.
NAL Call Number: 472 N21
Descriptors: zebrafish, blood circulation, blood flow, body organs, cell morphology, development, embryogenesis, embryonic development, gene expression , heart, Reynolds number, shear stress, vortices, *Danio rerio*, cardiogenesis, epigenetics, flow-induced forces, inheritance.

Hove, J.R. (2006). **Quantifying cardiovascular flow dynamics during early development**. *Pediatric Research* 60(1): 6-13. ISSN: 0031 3998.
Abstract: The relationship between developing biologic tissues and their dynamic fluid environments is intimate and complex. Increasing evidence supports the notion that these embryonic flow-structure interactions influence whether development will proceed normally or become pathogenic. Genetic, pharmacological, or surgical manipulations that alter the flow environment can thus profoundly influence morphologic and functional cardiovascular phenotypes. Functionally deficient phenotypes are particularly poorly described as there are few imaging tools with sufficient spatial and temporal resolution to quantify most intra-vital flows. The ability to visualize biofluids flow in vivo would be of great utility in functionally phenotyping model animal systems and for the elucidation of the mechanisms that underlie flow-related mechano-sensation and transduction in living organisms. This review summarizes the major methodological advances that have evolved for the quantitative characterization of intra-vital fluid dynamics with an emphasis on assessing cardiovascular flows in vertebrate model organisms. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, cardiovascular system, transport and circulation, positron emission tomography, doppler ultrasonography , digital particle image velocimetry, phase contrast magnetic resonance, orthogonal polarization spectral imaging, ultrasound biomicroscopy doppler, cardiovascular flow dynamics.

Incardona, J., M. Carls, T. Collier, and N. Scholz (2006). **Cardiovascular toxicity of petrogenic polycyclic aromatic hydrocarbons is aryl hydrocarbon receptor-independent**. *Marine Environmental Research* 62(Suppl. S): S73-S74. ISSN: 0141 1136.
NAL Call Number: QH545.W3M36
Descriptors: zebrafish, cardiovascular system, transport and circulation, toxicology, marine

ecology, ecology, environmental sciences, cardiac dysfunction, heart disease, edema, craniofacial defect, nervous system disease.

Jagadeeswaran, P., M. Cykowski, and B. Thattaliyath (2004). **Vascular occlusion and thrombosis in zebrafish.** *Methods in Cell Biology* 76: 489-500. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, embolism physiopathology, thrombosis physiopathology, blood coagulation tests methods, blood platelets physiology, blood vessels physiopathology , cell adhesion physiology, larva genetics, larva physiology, fluorescence microscopy, mutation, thrombosis genetics, wounds and injuries physiopathology.

Keegan, B.R., J.L. Feldman, G. Begemann, P.W. Ingham, and D. Yelon (2005). **Retinoic acid signaling restricts the cardiac progenitor pool**. *Science* 307(5707): 247-249. ISSN: 0036 8075.
NAL Call Number: 470Sci2
Abstract: Organogenesis begins with specification of a progenitor cell population, the size of which provides a foundation for the organ's final dimensions. Here, we present a new mechanism for regulating the number of progenitor cells by limiting their density within a competent region. We demonstrate that retinoic acid signaling restricts cardiac specification in the zebrafish embryo. Reduction of retinoic acid signaling causes formation of an excess of cardiomyocytes, via fate transformations that increase cardiac progenitor density within a multipotential zone. Thus, retinoic acid signaling creates a balance between cardiac and noncardiac identities, thereby refining the dimensions of the cardiac progenitor pool. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular biophysics, cardiovascular system, development, cardiac specification, organogenesis, retinoic acid signaling, zebrafish embryo.

Kirby, M.L. and A. Grimes (2006). **Cardiac arterial pole development is conserved in vertebrates**. *Developmental Biology* 295(1): 419. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, biochemistry, development, cardiovascular system.

Langenbacher, A.D., Y. Dong, X. Shu, J. Choi, D.A. Nicoll, J.I. Goldhaber, K.D. Philipson, and J.N. Chen (2005). **Mutation in sodium-calcium exchanger 1 (NCX1) causes cardiac fibrillation in zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 102(49): 17699-704. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: Cardiac fibrillation, a form of cardiac arrhythmia, is the most common cause of embolic stroke and death associated with heart failure. The molecular mechanisms underlying cardiac fibrillation are largely unknown. Here we report a zebrafish model for cardiac fibrillation. The hearts of zebrafish tremblor (tre) mutants exhibit chaotic movements and fail to develop synchronized contractions. Calcium imaging showed that normal calcium transients are absent in tre cardiomyocytes, and molecular cloning of the tre mutation revealed that the tre locus encodes the zebrafish cardiac-specific sodium-calcium exchanger (NCX) 1, NCX1h. Forced expression of NCX1h or other calcium-handling molecules restored synchronized heartbeats in tre mutant embryos in a dosage-dependent manner, demonstrating the critical role of calcium homeostasis in maintaining embryonic cardiac function. By creating mosaic zebrafish embryos, we showed that sporadic NCX1h-null cells were not sufficient to disrupt normal cardiac function, but clustered wild-type cardiomyocytes contract in unison in tre mutant hearts. These data signify the essential role of calcium homeostasis and NCX1h in establishing rhythmic contraction in the embryonic

zebrafish heart.
Descriptors: zebrafish embryonic heart, arrhythmia genetics, arrhythmia physiopathology, mutation genetics, sodium calcium exchanger genetics, sodium calcium exchanger metabolism, base sequence, calcium metabolism, embryology, metabolism, homeostasis, molecular sequence data, myocardial contraction genetics, myocardial contraction physiology, phenotype, phylogeny, RNA genetics.

Langheinrich, U., G. Vacun, and T. Wagner (2003). **Zebrafish embryos express an orthologue of HERG and are sensitive toward a range of QT-prolonging drugs inducing severe arrhythmia.** *Toxicology and Applied Pharmacology* 193(3): 370-82. ISSN: 0041-008X.
NAL Call Number: 391.8T662
Abstract: A wide range of drugs has been shown to prolong the QT interval of the electrocardiogram by blocking the pore-forming subunit of the rapidly activating delayed rectifier K+ channel, HERG (ether-a-go-go-related gene), sometimes leading to life-threatening arrhythmia. In this paper we describe cloning, sequence, and expression of the zebrafish orthologue of HERG, Zerg. Further, we studied effects of Zerg inhibition in zebrafish embryos caused by drugs or by an antisense approach. Zerg is expressed specifically in both heart chambers of zebrafish embryos, is composed of six transmembrane domains, and shows an especially high degree of amino acid conservation in the S6 and pore domain (99% identity). Several QT-prolonging drugs added to the bathing medium elicited bradycardia and arrhythmia in zebrafish embryos. The arrhythmia induced ranged from an atrioventricular 2:1 block, the ventricle beating half as often as the atrium, to more severe irregular arrhythmia with higher concentrations of the drugs. These effects were highly specific, reproducible, and rapid, e.g., 10 microM astemizole caused a 2:1 heartbeat within a minute after addition of the compound in all the embryos studied. Morpholino antisense oligonucleotides targeting Zerg were injected into zebrafish embryos and elicited similar dose-sensitive and specific arrhythmia as the QT-prolonging drugs, suggesting an evolutionarily conserved role for Erg in regulating heartbeat rate and rhythm. Further, we identified a mutation in the Per-Arnt-Sim domain of the Zerg channel in the breakdance mutant, also characterized by a 2:1 atrioventricular block. In conclusion, the zebrafish could be a tractable model organism for the study of Erg function and modulation but might also have a value in the field of cardiovascular pharmacology, e.g., as an early preclinical model for testing drugs under development for potential QT prolongation.
Descriptors: zebrafish embryos, cation transport proteins, heart rate drug effects, long QT syndrome chemically induced, pharmaceutical preparations adverse effects, potassium channels biosynthesis, potassium channels, voltage gated, arrhythmia chemically induced, base sequence, molecular cloning, embryo, , embryo, , ether a go go potassium channels, heart embryology, models, molecular sequence data, messenger RNA analysis, reverse transcriptase PCR, sequence alignment, metabolism.

Lepilina, A., A.N. Coon, K. Kikuchi, J.E. Holdway, R.W. Roberts, C.G. Burns, and K.D. Poss (2006). **A dynamic epicardial injury response supports progenitor cell activity during zebrafish heart regeneration.** *Cell* 127(3): 607-19. ISSN: 0092 8674.
NAL Call Number: QH573.C42
Abstract: Zebrafish possess a unique yet poorly understood capacity for cardiac regeneration. Here, we show that regeneration proceeds through two coordinated stages following resection of the ventricular apex. First a blastema is formed, comprised of progenitor cells that express precardiac markers, undergo differentiation, and proliferate. Second, epicardial tissue surrounding both cardiac chambers induces developmental markers and rapidly expands, creating a new epithelial cover for the exposed myocardium. A subpopulation of these epicardial cells undergoes

epithelial-to-mesenchymal transition (EMT), invades the wound, and provides new vasculature to regenerating muscle. During regeneration, the ligand fgf17b is induced in myocardium, while receptors fgfr2 and fgfr4 are induced in adjacent epicardial-derived cells. When fibroblast growth factors (Fgf) signaling is experimentally blocked by expression of a dominant-negative Fgf receptor, epicardial EMT and coronary neovascularization fail, prematurely arresting regeneration. Our findings reveal injury responses by myocardial and epicardial tissues that collaborate in an Fgf-dependent manner to achieve cardiac regeneration.

Descriptors: zebrafish, heart physiology, pericardium injuries, regeneration physiology, stem cells physiology , genetically modified, outbred strains, cell differentiation, heart injuries pathology, heart injuries physiopathology, heart ventricles pathology, heart ventricles physiopathology, heart ventricles surgery, cardiovascular model, myocardium metabolism , myocardium pathology, regeneration genetics.

Notes:

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Li, Y.X., M. Zdanowicz, L. Young, D. Kumiski, L. Leatherbury, and M.L. Kirby (2003). **Cardiac neural crest in zebrafish embryos contributes to myocardial cell lineage and early heart function.** *Developmental Dynamics* 226(3): 540-50. ISSN: 1058 8388.

NAL Call Number: QL801.A4

Descriptors: zebrafish embryos, heart embryology, myocardium cytology, neural crest embryology, cell lineage physiology, cell movement physiology, heart physiology, heart ventricles cytology, heart ventricles physiology, neural crest cytology.

Liebling, M., A.S. Forouhar, M.A. Gharib, S.E. Fraser, and M.E. Dickinson (2005). **Four-dimensional cardiac imaging in living embryos via postacquisition synchronization of nongated slice sequences**. *Journal of Biomedical Optics* 10(5) ISSN: 1083 3668.

Abstract: Being able to acquire, visualize, and analyze 3D time series (4D data) from living embryos makes it possible to understand complex dynamic movements at early stages of embryonic development. Despite recent technological breakthroughs in 2D dynamic imaging, confocal microscopes remain quite slow at capturing optical sections at successive depths. However, when the studied motion is periodic-such as for a beating heart-a way to circumvent this problem is to acquire, successively, sets of 2D+time slice sequences at increasing depths over at least one time period and later rearrange them to recover a 3D+time sequence. In other imaging modalities at macroscopic scales, external gating signals, e.g., an electro-cardiogram, have been used to achieve proper synchronization. Since gating signals are either unavailable or cumbersome to acquire in microscopic organisms, we have developed a procedure to reconstruct volumes based solely on the information contained in the image sequences. The central part of the algorithm is a least-squares minimization of an objective criterion that depends on the similarity between the data from neighboring depths. Owing to a wavelet-based multiresolution approach, our method is robust to common confocal microscopy artifacts. We validate the procedure on both simulated data and in vivo measurements from living zebrafish embryos. (c) 2005 society of Photo-Optical Instrumentation Engineers. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, cardiovascular system, transport and circulation, confocal microscopy, imaging and microscopy techniques, electrocardiography, four dimensional cardiac imaging, dynamic movement, postacquisition synchronization, nongated slice sequence, optical section, wavelet based multiresolution.

Ma, X., V.M. Reddy, and R.K. Riemer (2005). **Notch signaling is involved in development of pulmonary arteriovenous malformations after cavopulmonary anastomosis**. *Circulation* 112(17, Suppl. S): U58. ISSN: 0009 7322.
NAL Call Number: RC681.A1C8
Descriptors: zebrafish, cardiovascular system, respiratory system, respiration, pulmonary vascular arteriovenous malformation, vascular disease, respiratory system disease, immunostaining, gene expression microarray, genetic techniques, immunohistochemical staining, histology and cytology techniques, immunologic techniques, quantitative real time PCR, cavopulmonary anastomsis.

Malone, M.H., N. Sciaky, L. Stalheim, K.M. Hahn, E. Linney, and G.L. Johnson (2007). **Laser-scanning velocimetry: a confocal microscopy method for quantitative measurement of cardiovascular performance in zebrafish embryos and larvae.** *BioMed Central Biotechnology* 7: 40. ISSN: 1472 6750.
NAL Call Number: TP248.13.B532
Abstract: BACKGROUND: The zebrafish *Danio rerio* is an important model system for drug discovery and to study cardiovascular development. Using a laser-scanning confocal microscope, we have developed a non-invasive method of measuring cardiac performance in zebrafish embryos and larvae that obtains cardiovascular parameters similar to those obtained using Doppler echocardiography in mammals. A laser scan line placed parallel to the path of blood in the dorsal aorta measures blood cell velocity, from which cardiac output and indices of vascular resistance and contractility are calculated. RESULTS: This technique, called laser-scanning velocimetry, was used to quantify the effects of pharmacological, developmental, and genetic modifiers of cardiac function. Laser-scanning velocimetry was applied to analyze the cardiovascular effects of morpholino knockdown of osmosensing scaffold for MEKK3 (OSM), which when mutated causes the human vascular disease cerebral cavernous malformations. OSM-deficient embryos had a constricted aortic arch and markedly increased peak cell velocity, a characteristic indicator of aortic stenosis. CONCLUSION: These data validate laser-scanning velocimetry as a quantitative tool to measure cardiovascular performance for pharmacological and genetic analysis in zebrafish, which requires no specialized equipment other than a laser-scanning confocal microscope.
Descriptors: zebrafish , heart physiology, confocal microscopy methods, aminobenzoic acids pharmacology, aorta drug effects, aorta embryology, aorta physiology, blood flow velocity drug effects, Western blotting, drug effects, metabolism, physiology, developmental gene expression regulation, genetic complementation test, heart embryology, larva cytology, larva genetics, larva physiology, microfilament microfilament proteins metabolism, microfilament, microinjections, morpholines pharmacology, mutation, myocardium cytology, myocardium metabolism, messenger RNA administration and dosage, messenger RNA genetics, messenger RNA metabolism, reproducibility of results.

Matsui, T., A. Raya, C. Callol Massot, Y. Kawakami, I. Oishi, C. Rodriguez Esteban, and J.C. Belmonte (2007). **Miles-apart-Mediated regulation of cell-fibronectin interaction and myocardial migration in zebrafish.** *Nature Clinical Practice. Cardiovascular Medicine* 4(Suppl 1): S77-82. ISSN: 1743-4297.
Abstract: The migration of myocardial precursor cells towards the embryonic midline underlies the formation of the heart tube and is a key process of heart organogenesis. The zebrafish mutation miles-apart (mil), which affects the gene encoding a sphingosine-1-phosphate receptor, is characterized by defective migration of myocardial precursor cells and results in the formation of two laterally positioned hearts, a condition known as cardia bifida. The mechanism that disrupts myocardial migration in mil mutants remains largely unclear. To investigate how mil regulates this process, here we analyze the interactions between mil and other mediators of myocardial

migration. We show that mil function is associated with the other known cardia bifida locus, natter/fibronectin (nat/fn), which encodes fibronectin, a major component of the extracellular matrix, in the control of myocardial migration. By using a primary culture system of embryonic zebrafish cells, we also show that signaling from the sphingosine-1-phosphate receptor regulates cell-fibronectin interactions in zebrafish. In addition, localized inhibition and activation of cell-fibronectin interactions during the stages of myocardial migration reveal that the temporal regulation of cell-fibronectin interaction by mil is required for proper myocardial migration. Our study reveals novel functional links between sphingosine-1-phosphate receptor signaling and cell-fibronectin interaction in the control of myocardial migration during zebrafish heart organogenesis.

Descriptors: zebrafish, fibronectins physiology, cardiac myocytes physiology, organogenesis physiology, receptors, lysosphingolipid genetics, cell communication, cell movement, genotype, mutation, organogenesis genetics.

Moeller, H., A. Jenny, H.-J. Schaeffer, T. Schwarz-Romond, M. Mlodzik, M. Hammerschmidt, and W. Birchmeier (2006). **Diversin regulates heart formation and gastrulation movements in development**. *Proceedings of the National Academy of Sciences, USA* 103(43): 15900-15905. ISSN: 0027-8424.

NAL Call Number: 500N21P

Descriptors: zebrafish, ankyrin, dishevelled protein, embryogenesis, embryonic development, gastrulation, guanosinetriphosphatase, heart, mutation, polarity, signal transduction, Wnt protein, catenin, *Danio rerio*, *Drosophila*.

Moore, F.B.-G., M. Hosey, and B. Bagatto (2006). **Cardiovascular system in larval zebrafish responds to developmental hypoxia in a family specific manner**. *Frontiers in Zoology* 3 ISSN: 1742-9994.

NAL Call Number: QL1F76

Descriptors: zebrafish, circulatory system, environmental factors, erythrocytes, fish physiology, heart, hypoxia, larval development, *Danio rerio*.

Poss, K.D. (2007). **Getting to the heart of regeneration in zebrafish.** *Seminars in Cell and Developmental Biology* 18(1): 36-45. ISSN: 1084-9521.

NAL Call Number: QH573

Abstract: A scientific and clinical prerogative of the 21st century is to stimulate the regenerative ability of the human heart. While the mammalian heart shows little or no natural regeneration in response to injury, certain non-mammalian vertebrates possess an elevated capacity for cardiac regeneration. Adult zebrafish restore ventricular muscle removed by surgical resection, events that involve little or no scarring. Recent studies have begun to reveal cellular and molecular mechanisms of this regenerative process that have exciting implications for human cardiac biology and disease.

Descriptors: zebrafish, heart physiology, surgical resection, regeneration genetics, amphibia physiology, mammals physiology, biological models.

Raya, A., A. Consiglio, Y. Kawakami, C. Rodriguez Esteban, and J.C. Izpisua Belmonte (2004). **The zebrafish as a model of heart regeneration.** *Cloning and Stem Cells* 6(4): 345-51. ISSN: 1536 2302.

NAL Call Number: QH442.2.C57

Abstract: Regeneration is a complex biological process by which animals can restore the shape, structure and function of body parts lost after injury, or after experimental amputation. Only a

few species of vertebrates display the capacity to regenerate body parts during adulthood. In the case of the heart, newts display a remarkable ability to regenerate large portions of myocardium after amputation, although the mechanisms underlying this process have not been addressed. Recently, it has been shown that adult zebrafish can also regenerate their hearts, thus offering new possibilities for experimentally approaching this fascinating biological phenomenon. The first insights into heart regeneration gained by studying this model organism are reviewed here.
Descriptors: zebrafish, heart physiology, models, regeneration physiology.

Raya, A., C.M. Koth, D. Buscher, Y. Kawakami, T. Itoh, R.M. Raya, G. Sternik, H.J. Tsai, C. Rodriguez Esteban, and J.C. Izpisua Belmonte (2003). **Activation of Notch signaling pathway precedes heart regeneration in zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 100(Suppl 1): 11889-95. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: Several vertebrates display the ability to regenerate parts of their body after amputation. During this process, differentiated cells reenter the cell cycle and proliferate to generate a mass of undifferentiated cells. Repatterning mechanisms act on these cells to eventually shape a regenerated tissue or organ that replaces the amputated one. Experiments with regenerating limbs/fins in newts and zebrafish have shown that members of the Msx family of homeodomain-containing transcription factors play key roles during blastema formation and patterning. Here we show that adult zebrafish have a remarkable capacity to regenerate the heart in a process that involves up-regulation of msxB and msxC genes. We present evidence indicating that heart regeneration involves the execution of a specific genetic program, rather than redeployment of a cardiac development program. Preceding Msx activation, there is a marked increase in the expression of notch1b and deltaC, which we show are also up-regulated during fin regeneration. These data suggest a role for the Notch pathway in the activation of the regenerative response. Taken together, our results underscore the use of zebrafish as a model for investigating the process of regeneration in particular and the biology of stem cells in general. Advances in these fields will undoubtedly aid in the implementation of strategies for regenerative medicine.
Descriptors: zebrafish, heart physiology, membrane, regeneration genetics, regeneration physiology, genetically modified, heart growth and development, homeodomain in situ hybridization, membrane models, Notch receptors, signal transduction, transcription factors genetics, up regulation.

Rottbauer, W., G. Wessels, T. Dahme, S. Just, N. Trano, D. Hassel, C.G. Burns, H.A. Katus, and M.C. Fishman (2006). **Cardiac myosin light chain-2 - a novel essential component of thick- myofilament assembly and contractility of the heart**. *Circulation Research* 99(3): 323-331. ISSN: 0009 7330.
Abstract: Although it is well known that mutations in the cardiac regulatory myosin light chain-2 (mlc-2) gene cause hypertrophic cardiomyopathy, the precise in vivo structural and functional roles of MLC-2 in the heart are only poorly understood. We have isolated a mutation in zebrafish, tell tale heart (tel(m225)), which selectively perturbs contractility of the embryonic heart. By positional cloning, we identified tel to encode the zebrafish mlc-2 gene. In contrast to mammals, zebrafish have only 1 cardiac-specific mlc-2 gene, which we find to be expressed in atrial and ventricular cardiomyocytes during early embryonic development, but also in the adult heart. Accordingly, loss of zMLC-2 function cannot be compensated for by upregulation of another mlc-2 gene. Surprisingly, ultrastructural analysis of tel cardiomyocytes reveals complete absence of organized thick myofilaments. Thus, our findings provide the first in vivo evidence that cardiac MLC-2 is required for thick-filament stabilization and contractility in the vertebrate heart. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, muscular system, movement and support, cardiovascular system, transport and circulation, heart contractility.

Schwerte, T., C. Prem, A. Mairosl, and B. Pelster (2006). **Development of the sympatho-vagal balance in the cardiovascular system in zebrafish (*Danio rerio*) characterized by power spectrum and classical signal analysis.** *Journal of Experimental Biology* 209(Pt 6): 1093-1100. ISSN: 0022-0949.

NAL Call Number: 442.8B77

Abstract: The development of sympatho-vagal control of cardiac activity was analyzed in zebrafish (*Danio rerio*) larvae from 2 to 15 days post fertilization (d.p.f.) by pharmacological studies as well as by assessing short term heart rate variability. Changes in heart rate in response to cholinergic and adrenergic receptor stimulation or inhibition were investigated using in situ preparations and digital video-microscopic techniques. The data revealed that the heart responded to adrenergic stimulation starting at 4 d.p.f. and to cholinergic stimulation starting at 5 d.p.f. Atropine application resulted in an increase in heart rate beyond 12 d.p.f., while the inhibitory effect of cholinergic stimulation ceased at this time of development. Adrenergic inhibition (propranolol) reduced heart rate for the first time at 5 d.p.f., but the reduction was only very small (3.8%). Between 5 and 12 d.p.f. propranolol application always resulted in a minor reduction in heart rate, but because the effect was so small it was not always significant. Because the presence of an adrenergic or cholinergic tone may influence the stability of heart rate, we analyzed short-term heart rate variability (HRV). The frequency band width of heart rate variability revealed that HRV increased between 4 d.p.f. and 15 d.p.f. From 13 to 15 d.p.f. atropine reduced the frequency band width of HRV, whereas the combination of atropine and propranolol effectively reduced the frequency band width between 11 and 15 d.p.f. Classical power spectrum analysis using electrocardiograms is not possible in tiny zebrafish larvae and juveniles. It was therefore performed using optical methods, recording cardiac movement and cardiotachograms calculated from these measurements. Whereas heart movements contained frequency components characterizing HRV, the cardiotachogram did not show typical frequency spectra as known from other species.

Descriptors: zebrafish, cardiovascular system innervation, heart rate physiology, sympathetic nervous system physiology, vagus nerve physiology, atropine pharmacology, heart rate drug effects, propranolol pharmacology, sympathetic nervous system drug effects, vagus nerve drug effects.

Serluca, F.C. and M.C. Fishman (2006). **Big, bad hearts: from flies to man**. *Proceedings of the National Academy of Sciences of the United States of America* 103(11): 3947-3948. ISSN: 0027 8424.

NAL Call Number: 500N21P

Descriptors: zebrafish, molecular genetics, cardiovascular system, transport and circulation, evolution and adaptation, cardiomyopathy, heart disease, etiology, genetics, optical coherence tomography, imaging and microscopy techniques, contractility, integrative physiology.

Shin, J.T., E.V. Pomerantsev, K.D. Bloch, and C. Macrae (2003). **Quantitative cardiovascular physiology reveals a role for soluble guanylate cyclase in the developing zebrafish.** *Circulation* 108(17 Supplement): IV-12-IV-13. ISSN: 0009 7322.

NAL Call Number: RC681.A1C8

Descriptors: zebrafish, cardiovascular system, transport and circulation, development, guanylate cyclase, ventricular performance index, cardiac output.

Sidi, S. and F.M. Rosa (2004). **Mechanotransduction of hemodynamic forces regulates organogenesis**. *Medecine Sciences* 20(5): 557-561. ISSN: 0767 0974.

Abstract: Endothelial cells (EC) of the vertebrate cardiovascular system (CVS) are bona fide, yet enigmatic mechanoreceptors. When cultured in vitro and exposed to fluid forces, EC modify their physiological behaviour at the structural, metabolical and gene expression levels in response to the mechanical stimulus. However, as a direct consequence of the hypoxic bias (and often the lethality) that results from blocking blood flow in most animal systems, the in vivo role of EC mechanosensation (ECMS) remains poorly understood. The zebrafish has recently emerged as an alternative genetic model for the study of vertebrate development. Its striking ability to survive until larval stages in the absence of blood circulation circumveys the usual caveats that are inherent to CVS research, and offers the exciting opportunity to dissect the function of ECMS in vivo. Two groups have already uncovered an essential role for ECMS in zebrafish organogenesis, particularly in heart morphogenesis. In embryos in which intracardiac blood flow is genetically or physically compromised, several features of the normally developing heart, including valve formation, are specifically disrupted. In addition, impressive imaging studies of zebrafish hemodynamics demonstrate that the shear stress exerted upon the cardiac endothelium is largely in the range of the stimulus that in vitro activates cytoskelettal remodeling and gene expression changes in EC. Hence the cardiac phenotypes observed in vivo may indeed directly result from a lack of ECMS-dependent EC activity. These data shed first light on the role of ECMS in vivo. Notably, they also suggest that a number of human congenital cardiomyopathies may arise through abnormal fetal hemodynamics and/or EC sensory activity. Finally, these discoveries reinforce the too often neglected role of epigenetic factors (in this case, fluid forces) in the regulation of animal development. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, cardiovascular system: transport and circulation, development, molecular genetics, cardiomyopathy, congestive heart disease, cytoskeletal remodeling, gene expression, hemodynamics, organogenesis.

Language of Text: French.

Smith, L.E. and K.S. Warren (2004). **Adenosinergic regulation of heart rate in wildtype and slow mo mutant zebrafish embryos.** *Abstracts of Papers American Chemical Society* 227(Part 1): U422. ISSN: 0065 7727.

NAL Call Number: 381Am33Pa

Descriptors: zebrafish, cardiovascular system, transport and circulation, heart rate, receptor blockade, pacemaker current, receptor function, receptor stimulation, cardiac pacing, adenosinergic regulation, smo mutant.

Stringer, S.E. (2006). **The role of heparan sulphate proteoglycans in angiogenesis**. *Biochemical Society Transactions* 34(Part 3): 451-453. ISSN: 0300 5127.

NAL Call Number: QD415.A1B58

Abstract: The presence of HS (heparan sulphate) proteoglycans on the cell surface and in the extracellular environment is critical to many physiological processes including the growth of new blood vessels from pre-existing vasculature (angiogenesis). A plethora of growth factors and their receptors, extracellular matrix molecules and enzymes bind to specific sites on the HS sugar chain. For example, HIS proteoglycans have profound effects on the bioactivity of the key angiogenic factor VEGF (vascular endothelial growth factor) (VEGF(165)), affecting its diffusion, half-life and interaction with its tyrosine kinase receptors. A number of HS structural features that mediate the specific binding of VEGF(165), including sulphation requirements, have been determined. in parallel, zebrafish embryos were used as a vertebrate model system to study the role in vascular development of the biosynthetic enzymes that create these specific binding sites on

HS. It was discovered that knockdown of one of the HS 6-0-sulphotransferases in zebrafish with morpholino antisense oligonucleotides reduced vascular branching and corresponded to changes in the HS structure. The roles of the extracellular 6-0-sulphatase enzymes, the sulfs, in vascular development are now being investigated. Both oligosaccharides and small molecule biosynthetic enzyme inhibitors could be valuable HS-based strategies for controlling aberrant angiogenesis in diseases as diverse as cancer and heart disease. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, enzymology, cardiovascular system, transport and circulation, angiogenesis, vascular development.

Yelon, D., N. Glickman, and H.J. Tsai (2006). **Endocardium directs cardiomyocyte movement during heart tube assembly**. *Federation of American Societies for Experimental Biology Journal* 20(4, Part 1): A438. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: Heart formation requires the union of bilateral populations of cardiomyocytes and their reorganization into a simple tube. Little is known about the morphogenetic mechanisms regulating these processes. Using time-lapse confocal microscopy to track individual cardiomyocyte movements in the zebrafish embryo, we identify two morphologically and genetically separable phases of cell movement that coordinate heart tube assembly. First, all cardiomyocytes undergo coherent medial movement. Next, peripherally located cardiomyocytes change their direction of movement, angling toward the endocardium and thereby establishing the initial circumference of the nascent heart tube. These two phases of cardiomyocyte behavior are independently regulated; furthermore, we find that myocardial-endocardial interactions influence the second phase, regulating the induction, direction, and duration of cardiomyocyte movement. Thus, the endocardium plays a critical early role in cardiac morphogenesis, organizing cardiomyocytes into a configuration appropriate for tube assembly. Together, our data reveal a dynamic cellular mechanism by which tissue interactions establish organ architecture. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, muscular system: movement and support, cardiovascular system, transport and circulation, time lapse confocal microscopy, imaging and microscopy techniques, cardiac morphogenesis, cellular mechanism, cardiomyocyte movement.

Yuan, S. and E.M. Joseph (2004). **The small heart mutation reveals novel roles of Na+/K+-ATPase in maintaining ventricular cardiomyocyte morphology and viability in zebrafish.** *Circulation Research* 95(6): 595-603. ISSN: 0009 7330.
NAL Call Number: RC681.A1A57137
Abstract: Forward genetic screens in zebrafish have been used to identify mutations in genes with important roles in organogenesis. One of these mutants, small heart, develops a diminutive and severely malformed heart and multiple developmental defects of the brain, ears, eyes, and kidneys. Using a positional cloning approach, we identify that the mutant gene encodes the zebrafish Na+/K+-ATPase alpha1B1 protein. Disruption of Na+/K+-ATPase alpha1B1 function via morpholino "knockdown" or pharmacological inhibition with ouabain phenocopies the mutant phenotype, in a dose-dependent manner. Heterozygosity for the mutation sensitizes embryos to ouabain treatment. Our findings present novel genetic and morphological details on the function of the Na+/K+-ATPase alpha1B1 in early cardiac morphogenesis and the pathogenesis of the small heart malformation. We demonstrate that the reduced size of the mutant heart is caused by dysmorphic ventricular cardiomyocytes and an increase in ventricular cardiomyocyte apoptosis. This study provides a new insight that Na+/K+-ATPase alpha1B1 is required for maintaining ventricular cardiomyocyte morphology and viability.
Descriptors: zebrafish, Na+ K+ exchanging ATPase physiology, and, drug induced abnormali-

ties embryology, multiple embryonic, multiple abnormalities enzymology and genetics, apoptosis genetics, brain abnormalities and embryology, chemically induced eye abnormalities, eye abnormalities embryology and genetics, lethal genes, congenital heart defects embryology, kidney abnormalities, morphogenesis genetics, morpholines toxicity, mutagenesis, cardiac myocytes drug effects, cardiac myocytes enzymology, cardiac myocytes ultrastructure, oligodeoxyribonucleotides, antisense pharmacology, oligodeoxyribonucleotides, antisense toxicity, otolithic membrane abnormalities, otolithic membrane embryology, ouabain pharmacology and toxicity, and metabolism, proteins antagonists and inhibitors.

Genetics

Abe, S., Wang PiLin, F. Takahashi, and E. Sasaki (2005). **Structural analysis of cDNAs coding for 4SNc-Tudor domain protein from fish and their expression in yellowtail organs**. *Marine Biotechnology* 7(6): 677-686. ISSN: 1436 2228.

NAL Call Number: TP248.27.M37M37

Abstract: We cloned complementary DNAs for 4SNc-Tudor protein (SN4TDR) from yellowtail *(Seriola quinqueradiata*), torafugu (*Takifugu rubripes*), and zebrafish (*Danio rerio*). This protein contains 4 staphylococcal nuclease domains at the N terminus followed by a Tudor domain. We also identified the 4SNc-Tudor proteins highly homologous to that in yellowtail from the Takifugu genomic database. According to the smart database, these fish proteins had an overlapping Tudor domain (smart00333) with a complete 5 SNc domain (smart00318). In addition, 2 possible translation start sites were observed at the 5' sequences in all 3 fish species. Northern blot analysis of different yellowtail organs showed that the full SN4TDR messenger RNA was approximately 4000 nucleotides long and that its expression was highest in liver and gallbladder, being about 2 to 5 times higher than in kidney, brain, ovary, and gills, and exceedingly low in spleen, heart, and muscle. A minor 2000-nucleotide transcript observed in kidney, spleen, and gallbladder, was attributable to an alternatively spliced variant of this gene. Total proteins extracted from yellowtail liver were fractionated by heparin affinity column chromatography and separated by sodium dodecylsulfate polyacrylamide gel electrophoresis. Analyses by SDS-PAGE and liquid chromatography with tandem mass spectroscopy identified the polypeptide encoded by SN4TDR as a single molecule of 102 kDa. Copyright © 2008 CABI

Descriptors: zebrafish, alternative splicing, complementary DNA, DNA cloning, gene expression, genes, genomes, linkage, messenger RNA, nucleases, nucleotide sequences, proteins, translation, *Danio rerio*, *Seriola quinqueradiata*, *Takifugu rubripes*.

Abe, T., T. Ishikawa, T. Masuda, K. Mizusawa, T. Tsukamoto, H. Mitani, T. Yanagisawa, T. Todo, and M. Iigo (2006). **Molecular analysis of Dec1 and Dec2 in the peripheral circadian clock of zebrafish photosensitive cells.** *Biochemical and Biophysical Research Communications* 351(4): 1072-7. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: To elucidate the roles of DEC1 and DEC2, basic helix-loop-helix transcription factors, in the circadian clock of photosensitive zebrafish peripheral cells, zebrafish Dec1 and Dec2 (zDec1 and zDec2) were cloned and their functions and expression patterns were examined in BRF41, a zebrafish cell line. zDEC1 and zDEC2 have high sequence similarity to mammalian counterparts and the molecular phylogenetic analysis of the zDEC1 and zDEC2 sequences reflected the predicted pattern of species classification. zDEC1 and zDEC2 inhibited zCLOCK1:zBMAL3 mediated transcription as CRY1a. zDec1 and zDec2 mRNA showed robust circadian oscillation in BRF41 cells. However, zDec1 and zDec2 mRNA was not strongly induced by exposure to light. These results indicate that zDec1 and zDec2 are involved in the circadian clock mechanism in photosensitive zebrafish peripheral cells by suppressing CLOCK/BMAL-induced gene expression and that the feedback loops of zDEC1 and zDEC2 may be interlocked with the PER/CRY core circadian feedback loops.

Descriptors: zebrafish, basic helix loop helix transcription factors physiology, circadian rhythm genetics, gene expression regulation, basic helix loop helix transcription factors genetics, circadian rhythm radiation effects, molecular cloning, light, phylogeny, genetic suppression, trans activators genetics, genetic transcription radiation effects, proteins genetics.

Acosta, J., Y. Carpio, I. Borroto, O. Gonzalez, and M.P. Estrada (2005). **Myostatin gene silenced by RNAi show a zebrafish giant phenotype.** *Journal of Biotechnology* 119(4): 324-31. ISSN: 0168-1656.

NAL Call Number: QH442.J69

Abstract: Myostatin is a member of the transforming growth factor-beta (TGF-beta) family that functions as a negative regulator of skeletal muscle development and growth. Recently, it has been reported that the transgenic zebrafish expressing myostatin prodomain exhibited an increased number of fiber in skeletal muscle. Other novel results suggest that myostatin plays a mayor role during myogenesis, apart from inhibition of proliferation as well as differentiation. We have investigated the ability of double-stranded RNA (dsRNA) to inhibit myostatin function in the zebrafish. By microinjection dsRNA, corresponding to biologically active C-terminal domain from aminoacid 268 to end codon of tilapia myostatin protein, we produced an increased body mass in treated fish. The dsRNA injection in early development stage in zebrafish produced hyperplasia or hypertrophy. In addition, the interference of gene function showed a strong dependence on the amount of dsRNA.

Descriptors: zebrafish, skeletal muscle pathology and physiopathology, RNA interference, transforming growth factor beta genetics and metabolism, body weight genetics, gene silencing, hyperplasia genetics and metabolism, hypertrophy genetics and metabolism, organ size genetics, phenotype.

Adolf, B., G. Bellipanni, V. Huber, and L. Bally Cuif (2004). **Atoh1.2 and beta3.1 are two new bHLH-encoding genes expressed in selective precursor cells of the zebrafish anterior hindbrain.** *Gene Expression Patterns* 5(1): 35-41. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Transcription factors of the bHLH class play crucial roles in neurogenesis by controlling the location and timing of neuronal commitment and differentiation, as well as influencing neuronal identity. Proneural bHLH factors belong to the Olig, Neurogenin, NeuroD, Achaete-scute and Atonal subfamilies, and are expressed in partially overlapping or complementary patterns within the vertebrate embryonic neural tube. The combinatorial expression of these factors likely drives the generic and cell type-specific properties of neurogenesis throughout the nervous system. As an approach towards identifying a complete set of vertebrate proneural factors, we report here the isolation of two new zebrafish neural bHLH gene members, atoh1.2 and beta3.1. Among other sites, both are expressed in the late embryonic and early larval anterior hindbrain. In this territory we demonstrate that atoh1.2 and beta3.1 are transcribed in distinct precursors, further highlighting the subdivision of anterior zebrafish hindbrain into subdomains of bHLH expression.

Descriptors: zebrafish, rhombencephalon embryology, stem cells metabolism, transcription factors genetics, amino acid sequence, basic helix loop helix transcription factors, immunohistochemistry, in situ hybridization, molecular sequence data, rhombencephalon metabolism, transcription factors biosynthesis, metabolism, proteins biosynthesis.

Adolph, K.W. (2004). **The zebrafish metaxin 3 gene (mtx3): cDNA and protein structure, and comparison to zebrafish metaxins 1 and 2.** *Gene* 330: 67-73. ISSN: 0378-1119.

NAL Call Number: QH442.A1G4

Abstract: The metaxin genes of zebrafish have been investigated by determining the sequences of metaxin cDNAs and analyzing the translated amino acid sequences. A zebrafish cDNA corresponding to a third metaxin gene was identified. Zebrafish cDNAs representing metaxins 1 and 2, previously described in human and mouse, were also identified. The zebrafish metaxin genes are designated mtx1, mtx2 and mtx3, following zebrafish nomenclature guidelines. The zebrafish

metaxin 3 (ZMTX3) cDNA codes for a protein of 313 amino acids (MW 35,208), while the ZMTX1 and ZMTX2 cDNAs specify proteins of 317 residues (MW 35,906) and 274 residues (MW 30,852), respectively. Alignment of the ZMTX3 and ZMTX1 amino acid sequences revealed 40% identities, while 26% identities were found for the ZMTX3/ZMTX2 alignment. A phylogenetic tree showed that the metaxins share a common ancestry, with the grouping of the zebrafish sequences with the homologous human and mouse sequences. Analysis of the domain structure of the zebrafish metaxins uncovered a glutathione S-transferase (GST) domain for each protein and, in addition, a thioredoxin-like domain for ZMTX2. A region of transmembrane helices was found near the C-terminus for the ZMTX1 protein. In addition, regions of alpha helix were seen to be the predominant feature of zebrafish metaxin secondary structure, particularly for ZMTX2 and ZMTX3. The ZMTX3 cDNA sequence has the greatest homology to a human sequence at cytogenetic location 5q14.1, close to the thrombospondin 4 gene (THBS4). Also, the mouse metaxin 3 homologue is adjacent to Thbs4 at 13C3.
Descriptors: zebrafish, mice, membrane amino acid sequence, complementary DNA chemistry and genetics, molecular sequence data, phylogeny, sequence alignment, DNA sequence analysis, amino acid sequence homology.

Agaba, M., D.R. Tocher, C.A. Dickson, J.R. Dick, and A.J. Teale (2004). **Zebrafish cDNA encoding multifunctional fatty acid elongase involved in production of eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids**. *Marine Biotechnology* 6(3): 251-261. ISSN: 1436 2228.
NAL Call Number: TP248.27.M37M37
Abstract: Enzymes that increase the chain length of fatty acids are essential for biosynthesis of highly unsaturated fatty acids. The gLELO gene encodes a protein involved in the elongation of polyunsaturated fatty acids in the fungus Mortierella alpina. A search of the GenBank database identified several expressed sequence tag sequences, including one obtained from zebrafish (*Danio rerio*), with high similarity to gLELO. The full-length transcript ZfELO, encoding a polypeptide of 291 amino acid residues, was isolated from zebrafish liver cDNA. The predicted amino acid sequence of the open reading frame shared high similarity with the elongases of *Caenorhabditis elegans* and human. When expressed in *Saccharomyces cerevisiae*, the zebrafish open reading frame conferred the ability to lengthen the chain of a range of C18, C20, and C22 polyunsaturated fatty acids, indicating not only that biosynthesis of 22:6n-3 from 18:3n-3 via a 24-carbon intermediate is feasible, but also that one elongase enzyme can perform all three elongation steps required. The zebrafish enzyme was also able to elongate monounsaturated and saturated fatty acids, and thus demonstrates a greater level of promiscuity in terms of substrate use than any elongase enzyme described previously. Copyright © 2008 CABI
Descriptors: zebrafish, amino acid sequences, biosynthesis, complementary DNA, docosahexaenoic acid, eicosapentaenoic acid, enzymes, monoenoic fatty acids, open reading frames, polyenoic fatty acids, saturated fatty acids, *Danio rerio.*

Agathon, A., C. Thisse, and B. Thisse (2003). **The molecular nature of the zebrafish tail organizer.** *Nature* 424(6947): 448-52. ISSN: 0028-0836.
NAL Call Number: 472 N21
Abstract: Based on grafting experiments, Mangold and Spemann showed the dorsal blastopore lip of an amphibian gastrula to be able to induce a secondary body axis. The equivalent of this organizer region has been identified in different vertebrates including teleosts. However, whereas the graft can induce ectopic head and trunk, endogenous and ectopic axes fuse in the posterior part of the body, raising the question of whether a distinct organizer region is necessary for tail development. Here we reveal, by isochronic and heterochronic transplantation, the existence of a tail organizer deriving from the ventral margin of the zebrafish embryo, which is independent

of the dorsal Spemann organizer. Loss-of-function experiments reveal that bone morphogenetic protein (BMP), Nodal and Wnt8 signalling pathways are required for tail development. Moreover, stimulation of naive cells by a combination of BMP, Nodal and Wnt8 mimics the tail-organizing activity of the ventral margin and induces surrounding tissues to become tail. In contrast to induction of the vertebrate head, known to result from the triple inhibition of BMP, Nodal and Wnt, here we show that induction of the tail results from the triple stimulation of BMP, Nodal and Wnt8 signalling pathways.
Descriptors: zebrafish embryos, bone morphogenetic proteins metabolism, organizers, embryonic physiology, tail embryology, transforming growth factor beta metabolism, bone morphogenetic proteins antagonists and inhibitors, bone morphogenetic proteins pharmacology, cytoskeletal proteins, embryonic induction drug effects, proteins antagonists and inhibitors, proteins pharmacology, messenger RNA genetics and metabolism, signal transduction drug effects, tail drug effects, tail metabolism, transforming growth factor beta pharmacology, Wnt proteins, metabolism, proteins antagonists and inhibitors and pharmacology.

Ahmad, N., S. Long, and M. Rebagliati (2004). **A southpaw joins the roster: the role of the zebrafish nodal-related gene southpaw in cardiac Left-Right asymmetry.** *Trends in Cardiovascular Medicine* 14(2): 43-9. ISSN: 1050-1738.
Abstract: The establishment of the left-right (LR) asymmetry of the zebrafish heart involves at least two discrete events: cardiac jogging and cardiac looping. The nodal-related gene southpaw is required for both aspects of heart LR asymmetry as well as for LR patterning of other visceral organs. Reductions in southpaw activity abolish the normal LR biases of jogging and looping and uncouple the normal correlation between jogging and looping polarities. These loss-of-function experiments also reveal that southpaw is required for organizing at least the initial phase of diencephalic LR asymmetry and suggest that heart and brain LR patterning may be coordinated by southpaw signaling originating within the anterior, left lateral plate mesoderm.
Descriptors: zebrafish embryos, body patterning genetics, congenital heart defects genetics, embryonic induction genetics, fetal heart growth and development, fetal proteins genetics and physiology, heart embryology, congenital heart defects embryology , morphogenesis genetics, transcription factors genetics and physiology.

Ahmed, H., S.J. Du, N. O'Leary, and G.R. Vasta (2004). **Biochemical and molecular characterization of galectins from zebrafish (*Danio rerio*): notochord-specific expression of a prototype galectin during early embryogenesis.** *Glycobiology* 14(3): 219-32. ISSN: 0959-6658.
NAL Call Number: QP552.G59G593
Abstract: Galectins are a family of beta-galactoside-binding lectins that on synthesis are either translocated into the nucleus or released to the extracellular space. Their developmentally regulated expression, extracellular location, and affinity for extracellular components (such as laminin and fibronectin) suggest a role in embryonic development, but so far this has not been unequivocally established. Zebrafish constitute an ideal model for developmental studies because of their external fertilization, transparent embryos, rapid growth, and availability of a large collection of mutants. As a first step in addressing the biological roles in zebrafish embryogenesis, we identified and characterized members of the three galectin types: three protogalectins (Drgal1-L1, Drgal1-L2, Drgal1-L3), one chimera galectin (Drgal3), and one tandem-repeat galectin (Drgal9-L1). Like mammalian prototype galectin-1, Drgal1-L2 preferentially binds to N-acetyllactosamine. Genomic structure of Drgal1-L2 revealed four exons, with the exon-intron boundaries conserved with the mammalian galectin-1. Interestingly, this gene also encodes an alternatively spliced form of Drgal1-L2 that lacks eight amino acids near the carbohydrate-binding domain. Zebrafish galectins exhibited distinct patterns of temporal expression during embryo development. Drgal1-

L2 is expressed postbud stage, and its expression is strikingly specific to the notochord. In contrast, Drgal1-L1 is expressed maternally in the oocytes. Drgal1-L3, Drgal3, and Drgal9-L1 are expressed both maternally and zygotically, ubiquitously in the adult tissues. The distinct temporal and spatial patterns of expression of members of the zebrafish galectin repertoire suggest that each may play distinct biological roles during early embryogenesis.
Descriptors: zebrafish , galectins genetics, galectins metabolism, amino acid and base sequence, carbohydrate metabolism, molecular cloning, complementary DNA genetics, exons genetics, galectins chemistry, isolation and purification, introns genetics, laminin metabolism, molecular sequence data, organ specificity, protein isoforms chemistry, protein isoforms genetics, protein isoforms isolation, purification and metabolism, messenger RNA genetics and metabolism, sequence alignment.

Airhart, M.J., B. Miller, D. Lee, T. Conner, M. Miller, and R. Skalko (2005). **Movement disorders and neurochemical changes in zebrafish larvae after bath exposure to fluoxetine (prozac)**. *Birth Defects Research* 73(5): 371. ISSN: 1542 0752.
NAL Call Number: QL991.T4
Descriptors: zebrafish larvae, pharmacology, molecular biophysics, neural coordination, pregnancy, movement disorder.

Airhart, M.J., B. Miller, D. Lee, T. Wilson, M. Miller, and R.G. Skalko (2006). **Effects of serotonin depletion on locomotor development in zebrafish**. *Birth Defects Research* 76(5): 334. ISSN: 1542 0752.
NAL Call Number: QL991.T4
Descriptors: zebrafish, pharmacology, neural coordination, locomotor development.

Alberti, M., U. Kausch, S. Haindl, R. Leibiger, J. Budczies, M. Seifert, and B. Hock (2005). **Gene expression patterns - a tool for bioanalysis**. *International Journal of Environmental Analytical Chemistry* 85(9/11): 589-608. ISSN: 0306 7319.
Abstract: Gene expression patterns are efficient tools to elucidate responses to hormones as well as endocrine disruptors at the molecular level. Male zebrafish (*Danio rerio*) were exposed to 17 beta -estradiol (E2). Transcriptome analysis was first carried out by quantitative polymerase chain reaction (PCR) of the gene coding for vitellogenin (vtg1) in relation to the housekeeping gene ef1 alpha . A significant increase in the expression of vtg1 was found at concentrations >100 ng L-1 E2. Then DNA microarray experiments were performed to examine the expression levels of the zebrafish genome. A total of 210 sequences were regulated in male zebrafish exposed to 500 ng L-1 E2. They include genes involved in the oestrogen pathway, such as the gene coding for the oestrogen receptor, and in embryonic development, such as homeo box genes. A complete list of up- and down-regulated genes and sequences is provided in this article. Copyright © 2008 CABI
Descriptors: zebrafish, DNA, estradiol, gene expression, genes, male animals, molecular genetics, nucleotide sequences, transcriptome analysis, estrogenic properties, toxic substances, vitellogenins, *Danio rerio.*

Alberti, M., U.l.f. Kausch, S. Haindl, and M. Seifert (2005). **Gene expression analysis for exposure to estrogenic substances**. *Acta Hydrochimica Et Hydrobiologica* 33(1): 38-44. ISSN: 0323-4320.
Descriptors: zebrafish, DNA, exposure, fish, freshwater fish, gene expression, PCR, polypeptides, proteins, sex hormones, water pollution effects, *Danio rerio*, effects of pollution, monitoring, control and remediation.

Albertson, R.C. and T.D. Kocher (2006). **Genetic and developmental basis of cichlid trophic diversity**. *Heredity* 97(3): 211-221. ISSN: 0018-067X.
NAL Call Number: 443.8H42
Descriptors: zebrafish, bone morphogenetic protein 4, evolution, feeding apparatus, freshwater fish, genetic diversity, growth factors, growth rate, jaw, molecular modelling, phylogenetics, trophic relationships, cichlidae, *Danio rerio*, cichlids, taxonomy, systematics and evolutionary genetics, ecosystem and ecology studies, genetics and evolution.
Notes: A review.

Alestrom, P., P. Collas, J. Torgersen, M. Liang, A. Arenal and R. Nourizadeh Lillabadi (2005). **Gene transfer to germline and somatic tissues of zebrafish**. T.J. Pandian, C.A. Strussmann and M.P. Marian *Fish Genetics and Aquaculture Biotechnology,* Science Publishers, Inc.: Enfield, USA , p. 21-28. ISBN: 1578083729.
NAL Call Number: SH155.5.F57
Abstract: The development of vehicles driving foreign DNA into the cell nucleus is essential for effective gene transfer applications. We have previously reported that the use of Nuclear Localization Signal (NLS) peptides non-covalently complexed to plasmid DNA promotes the nuclear uptake of DNA and results in increased integration and germline transmission frequencies of the transgene. To achieve germline integration, inheritance and expression of single copy luciferase reporter gene in zebrafish, 10, 100 or 1000 copies of plasmid DNA complexed with NLS were used. As little as 10 DNA-NLS complexes (0.06 fg plasmid DNA) are adequate to produce germline-transgenic zebrafish bearing a single copy of the transgene. DNA transferred to zebrafish (*Danio rerio*) skin by gene gun revealed that already one hour after biolistic transfer of 1 micro g reporter gene construct to zebrafish skin, 100% of the cells in the target area exhibited GFP gene activity. The risk for accidental germline uptake of DNA molecules delivered to dermis by a gene gun was shown to be below the detection level in a screen of 250 000 zebrafish sperm cells investigated by fluorescence in situ hybridization (FISH) at 15 or 30 days post delivery of 1 micro g plasmid vector. Copyright © 2008 CABI
Descriptors: transgenic zebrafish, animal models, DNA, gene expression, gene transfer, genetic engineering and vectors, germ line, *Danio rerio*.

Alestrom, P., J.L. Holter, and R. Nourizadeh Lillabadi (2006). **Zebrafish in functional genomics and aquatic biomedicine.** *Trends in Biotechnology* 24(1): 15-21. ISSN: 0167-7799.
NAL Call Number: TA166.T72
Abstract: The zebrafish (*Danio rerio*) has many features that make it an ideal model for the study of developmental biology. It is small and easy to contain, it has transparent embryos, it is easy to breed and its early development is well characterized; these same characteristics have also made it an ideal vertebrate model in the areas of biomedicine and biotechnology. In aquaculture, the need for a well-characterized fish model has been satisfied by the zebrafish owing to the availability of functional genomics and molecular biology data to facilitate studies of growth, reproduction, meat quality and disease biology, with the corresponding development of vaccines and therapies. Zebrafish are also increasingly used in toxicogenomics to analyze the effects of toxins and pollutants in the environment, and for creating biomonitors that emit alarm signals when a toxic compound is detected. As detailed in this review, the zebrafish is a versatile and well-characterized model with applications in many fields of study.
Descriptors: zebrafish, genomics methods, biological markers , biotechnology methods, disease models, environmental monitoring, fish diseases prevention and control, toxicogenetics methods.
Notes: A review.

Alexandre, D., A. Lee, A. Duong, S. Ekker, and J. Waschek (2005). **Expression analysis of pac(1)r and pacap genes in zebrafish embryos**. *Regulatory Peptides* 130(3): 155. ISSN: 0167 0115.
NAL Call Number: QP552.P4
Descriptors: zebrafish, nervous system, development, molecular genetics, histology and cytology techniques, genetic techniques.
Notes: Electronic resource.

Aligianis, I., N.V. Morgan, M. Mione, C. Johnson, E. Rosser, R.C. Hennekam, G. Adams, R.C. Trembath, D.T. Pilz, N. Stoodley, A.T. Moore, S. Wilson, and E.R. Maher (2006). **Mutation in rab3 gtpase-activating protein (rab3gap) noncatalytic subunit in a kindred with martsolf syndrome**. *American Journal of Human Genetics* 78(4): 702-707. ISSN: 0002 9297.
NAL Call Number: QH 431. A1A 54
Abstract: We identified a homozygous missense mutation in the noncatalytic subunit (RAB3GAP2) of RAB3GAP that results in abnormal splicing in a family with congenital cataracts, hypogonadism, and mild mental retardation (Martsolf syndrome). Recently, mutations in the catalytic subunit of RAB3GAP (RAB3GAP1), a key regulator of calcium-mediated hormone and neurotransmitter exocytosis, were reported in Warburg micro syndrome, a severe neurodevelopmental condition with overlapping clinical features. RAB3GAP is a heterodimeric protein that consists of a catalytic subunit and a noncatalytic subunit encoded by RAB3GAP1 and RAB3GAP2, respectively. We performed messenger RNA-expression studies of RAB3GAP1 and RAB3GAP2 orthologues in *Danio rerio* embryos and demonstrated that, whereas developmental expression of rab3gap1 was generalized (similar to that reported elsewhere in mice), rab3gap2 expression was restricted to the central nervous system. These findings are consistent with RAB3GAP2 having a key role in neurodevelopment and may indicate that Warburg micro and Martsolf syndromes represent a spectrum of disorders. However, we did not detect RAB3GAP2 mutations in patients with Warburg micro syndrome. These findings suggest that RAB3GAP dysregulation may result in a spectrum of phenotypes that range from Warburg micro syndrome to Martsolf syndrome. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, neural coordination, molecular genetics, medical genetics, Martsolf syndrome, endocrine disease, gonads, congenital disease, reproductive system disease, genetic disease, eye disease, etiology, exocytosis, missense mutation, RAB3GAP dysregulation.

Alimuddin, G. Yoshizaki, V. Kiron, S. Satoh, and T. Takeuchi (2005). **Enhancement of EPA and DHA biosynthesis by over-expression of masu salmon delta6-desaturase-like gene in zebrafish.** *Transgenic Research* 14(2): 159-65. ISSN: 0962-8819.
NAL Call Number: QH442.6T74
Abstract: The n - 3 polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have important nutritional benefits in humans. Farmed fish could serve as promising sources of EPA/DHA, but they need these fatty acids or their precursors in their diets. Here we transferred masu salmon delta6-desaturase-like gene in zebrafish to increase its ability for synthesizing EPA and DHA. Expression of this gene in transgenic fish elevated their EPA content by 1.4-fold and DHA by 2.1-fold. On the other hand, the alpha-linolenic acid (ALA) content decreased, it being a substrate of delta6-desaturase, while the total lipid remained constant. This achievement demonstrates that fatty acid metabolic pathway in fish can be modified by the transgenic technique, and perhaps this could be applied to tailor farmed fish as even better sources of valuable human food.
Descriptors: transgenic zebrafish, genetically modified, docosahexaenoic acids, eicosapentaenoic acid biosynthesis and genetics, fatty acid desaturases genetics, salmon genetics, aquaculture , fatty

acid desaturases biosynthesis, gene expression profiling, linoleoyl Co A desaturase, nutritional status, farmed fish application.

Alimuddin, G. Yoshizaki, V. Kiron, S. Satoh, and T. Takeuchi (2007). **Expression of masu salmon delta5-desaturase-like gene elevated EPA and DHA biosynthesis in zebrafish.** *Marine Biotechnology* 9(1): 92-100. ISSN: 1436-2228.
NAL Call Number: TP248.27.M37M37
Abstract: Farmed fish could substitute for marine capture fish as a source of fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) beneficial for human health; however, they require these compounds in their diets. In the present study on a model fish species, we modified the EPA/DHA biosynthesis pathway by overexpression of masu salmon Delta5-desaturase-like gene in zebrafish to increase its ability to synthesize EPA and DHA. Expression of this gene in transgenic fish fed a commercial diet and Artemia helped to improve their EPA content by 1.21-fold and DHA by 1.24-fold. In similar fish that were fed only Artemia the increments were 1.14-fold for EPA and 1.13-fold for DHA, compared with nontransgenic fish. In contrast, eicosatetraenoic acid content decreased, as it is a substrate of Delta5-desaturase, while the total lipid remained constant. The results demonstrated that masu salmon Delta5-desaturase is functional in zebrafish and can modify its fatty acid metabolic pathway. The technique could be applied to farmed fish to generate a nutritionally richer product for human consumption.
Descriptors: transgenic zebrafish, genetically modified physiology, fatty acid desaturases biosynthesis, omega 3 fatty acids biosynthesis, food technology, enzymologic gene expression regulation physiology, actins analysis, animal feed analysis, Artemia chemistry, body weight physiology, DNA primers, docosahexaenoic acids analysis and metabolism, eicosapentaenoic acid analysis and biosynthesis, fatty acid desaturases genetics, omega 3 fatty acids analysis, lipids analysis, *Oncorhynchus* genetics, messenger RNA analysis, masu salmon.

Alioto, T.S. and J. Ngai (2006). **The repertoire of olfactory C family G protein-coupled receptors in zebrafish: candidate chemosensory receptors for amino acids.** *BioMed Central Medical Genomics* 7: 309. ISSN: 1755 8794.
Abstract: BACKGROUND: Vertebrate odorant receptors comprise at least three types of G protein-coupled receptors (GPCRs): the OR, V1R, and V2R/V2R-like receptors, the latter group belonging to the C family of GPCRs. These receptor families are thought to receive chemosensory information from a wide spectrum of odorant and pheromonal cues that influence critical animal behaviors such as feeding, reproduction and other social interactions. RESULTS: Using genome database mining and other informatics approaches, we identified and characterized the repertoire of 54 intact "V2R-like" olfactory C family GPCRs in the zebrafish. Phylogenetic analysis - which also included a set of 34 C family GPCRs from fugu - places the fish olfactory receptors in three major groups, which are related to but clearly distinct from other C family GPCRs, including the calcium sensing receptor, metabotropic glutamate receptors, GABA-B receptor, T1R taste receptors, and the major group of V2R vomeronasal receptor families. Interestingly, an analysis of sequence conservation and selective pressure in the zebrafish receptors revealed the retention of a conserved sequence motif previously shown to be required for ligand binding in other amino acid receptors. CONCLUSION: Based on our findings, we propose that the repertoire of zebrafish olfactory C family GPCRs has evolved to allow the detection and discrimination of a spectrum of amino acid and/or amino acid-based compounds, which are potent olfactory cues in fish. Furthermore, as the major groups of fish receptors and mammalian V2R receptors appear to have diverged significantly from a common ancestral gene(s), these receptors likely mediate chemosensation of different classes of chemical structures by their respective organisms.

Descriptors: zebrafish, receptors, amino acid genetics, odorant receptors genetics, amino acid sequence, chromosome mapping, fishes genetics, genomics methods, mice, molecular models, molecular sequence data, olfactory receptor neurons physiology, phylogeny, tertiary protein structure, receptors, amino acid chemistry, receptors, amino acid classification, G protein Coupled receptors classification, odorant receptors chemistry, odorant receptors classification, sequence alignment, amino acid sequence homology, species specificity, Takifugu genetics, proteins chemistry.

Allende, M.L., M. Manzanares, J.J. Tena, C.G. Feijoo, and J. Gomez Skarmeta (2006). **Cracking the genome's second code: enhancer detection by combined phylogenetic footprinting and transgenic fish and frog embryos**. *Methods* 39(3): 212-219. ISSN: 1046 2023.
NAL Call Number: QP519.7.M47
Abstract: Genes involved in vertebrate development are unusually enriched for highly conserved non-coding sequence elements. These regions are readily detected in silico, by genome-wide sequence comparisons between different vertebrates, from mammals to fish (phylogenetic footprinting). It follows that sequence conservation must be the result of positive selection for an essential physiological role. An obvious possibility is that these conserved sequences possess regulatory or structural functions important for gene expression and, thus, an in vivo assay becomes necessary. We have developed a rapid testing system using zebrafish and *Xenopus laevis* embryos that allows us to assign transcriptional regulatory functions to conserved non-coding sequence elements. The sequences are cloned into a vector containing a minimal promoter and the GFP reporter, and are assayed for their putative cis-regulatory activity in zebrafish or *Xenopus* transgenic experiments. Vectors used include plasmid DNA and the Tol2 transposon system in fish and *X laevis.* We have followed this logic to detect and analyze conserved elements in an intergenic region present in the Iroquois (Irx) gene clusters of zebrafish, *Xenopus tropicalis*, *Fugu rubripes* and mouse. We have assayed similar to 50 of these conserved elements and shown that the majority behave as modular positive regulatory elements (enhancers) that contribute to specific temporal and spatial domains that are part of the endogenous gene expression pattern. Moreover, comparison of the activity of cognate Irx enhancers from different organisms demonstrates that conservation of sequence is accompanied by in vivo functional conservation across species. Finally, for some of the most conserved elements, we have been able to identify a critical core sequence, essential for correct enhancer function. (c) 2006 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: transgenic zebrafish embryos, frog embryos, *Xenopus* species, *Fugu rubripes*, mice, molecular genetics, phylogenetic footprinting, genetic techniques, enhancer detection, genome, cis regulatory activity.

Alonso, M., C.H. Kim, M.C. Johnson, M. Pressley, and J.A. Leong (2004). **The NV gene of snakehead rhabdovirus (SHRV) is not required for pathogenesis, and a heterologous glycoprotein can be incorporated into the SHRV envelope**. *Journal of Virology* 78(11): 5875-5882. ISSN: 0022 538X.
NAL Call Number: QR360.J6
Abstract: Snakehead rhabdovirus (SHRV) affects warm-water fish in Southeast Asia and belongs to the genus Novirhabdovirus by virtue of its "nonvirion" (NV) gene. To examine the function of the NV gene, we used a recently developed reverse genetic system to produce a viable recombinant SHRV carrying an NV gene deletion. The recombinant virus was produced at the same rate and same final concentrations as the wild-type virus in cultured fish cells in spite of the NV gene deletion. The role of the NV protein in fish pathogenesis was also investigated. Zebra fish (*Danio rerio*) were infected with the NV deletion mutant or with a recombinant virus contain-

ing a copy of the SHRV genome, and similar mortality rates as well as final mortalities were recorded, suggesting no apparent role for the NV protein in fish pathogenesis. Interestingly, the unsuccessful rescue of fully viable recombinants with genomes containing deletions in the G/NV gene junction suggested a role for the gene junction in virus transcription and replication. Finally, we demonstrated that the SHRV glycoprotein can be replaced by the glycoprotein of infectious hematopoietic necrosis virus (IHNV) or by a hybrid protein composed of SHRV and IHNV sequences. Copyright © 2008 CABI

Descriptors: zebrafish, deletions, envelope glycoproteins, envelope proteins, genes, genomes, mortality, pathogenesis, recombinant DNA, transcription, viral proteins, viral replication, *Danio rerio*, Rhabdoviridae.

Altmann, S.M., M.T. Mellon, D.L. Distel, and C.H. Kim (2003). **Molecular and functional analysis of an interferon gene from the zebrafish, *Danio rerio*.** *Journal of Virology* 77(3): 1992-2002. ISSN: 0022-538X.

NAL Call Number: QR360.J6

Abstract: The interferon (IFN) family consisting of alpha IFN (IFN-alpha), IFN-beta, IFN-omega, IFN-delta, IFN-kappa, and IFN-tau is a large group of cytokines involved in the innate immune response against various microorganisms. Genes for IFN have been cloned from a variety of mammalian and avian species; however, IFN genes from lower-order vertebrates have not been forthcoming. Here, we report the cloning and characterization of the IFN gene from the zebrafish, *Danio rerio*. Zebrafish IFN (zfIFN) is 185 amino acids in length, with the first 22 amino acids representing a putative signal peptide. Treatment with the known IFN inducer polyinosinic acid-polycytidylic acid (poly[I]-poly[C]) resulted in an increase in zfIFN mRNA transcripts. zfIFN was also able to activate the IFN-inducible Mx promoter when cotransfected with a plasmid containing the zebrafish Mx promoter upstream of a luciferase reporter gene. To demonstrate antiviral activity, zebrafish cells were transfected with zfIFN and challenged with a fish rhabdovirus. A 36% reduction in plaque number was seen in zfIFN-transfected cells, compared to cells transfected with a control vector. Phylogenetic analysis has shown zfIFN to be approximately equally divergent from avian and mammalian IFN, consistent with its origin from an IFN present in the most recent common ancestor of these divergent lineages. A putative IFN from puffer, Fugu rubripes, was also found when zfIFN was used to search the fugu genome database, demonstrating that zfIFN can be used to find additional fish IFN genes. These results demonstrate that zebrafish can be used as an effective model for studying innate immunity and immune response to infectious disease.

Descriptors: zebrafish, interferons genetics, amino acid and base sequence, molecular cloning, interferons chemistry, molecular sequence data, phylogeny, poly I C pharmacology, promoter regions genetics, sequence alignment, immune response model.

Altmann, S.M., M.T. Mellon, M.C. Johnson, B.H. Paw, N.S. Trede, L.I. Zon, and C.H. Kim (2004). **Cloning and characterization of an Mx gene and its corresponding promoter from the zebrafish, *Danio rerio*.** *Developmental and Comparative Immunology* 28(4): 295-306. ISSN: 0145 305X.

NAL Call Number: QR180.D4

Abstract: Type I interferons (IFNs) represent a crucial component of the innate immune response to viruses. An important downstream effector of IFN is the Mx gene, which is activated solely through this pathway. Mx proteins are characterized by a tripartite GTP-binding domain, dynamin family signature, and leucine zipper motif. Mx genes are transcribed upon activation of an interferon-stimulated response element (ISRE) located in the Mx promoter region. In this article, we describe the cloning and analysis of an Mx gene and its corresponding promoter from

the zebrafish (*Danio rerio*). The deduced amino acid sequence of zebrafish Mx contains the conserved GTP-binding domain, dynamin family signature, and leucine zipper motif common to Mx proteins, and shows a 50% identity to human MxA and 69% identity both to rainbow trout and to Atlantic salmon. Zebrafish liver cells produced high levels of Mx mRNA in response to induction by the known IFN-inducer polyinosinic-polycytidylic acid (Poly[I:C]). The zebrafish Mx promoter contains two ISREs homologous to those found in the promoter regions of many IFN-inducible genes, and was able to drive transcription of a luciferase reporter gene when induced by either purified zebrafish IFN or Poly[I:C].
Descriptors: zebrafish, GTP binding amino acid and base sequence, cell line, molecular cloning, complementary DNA genetics, leucine zippers genetics, molecular sequence data, mutation, promoter regions genetics, recombinant amino acid sequence homology, transfection.

Alvarez, M.C., J. Bejar, L. Chen Song, and H. Hong Yun (2007). **Fish embryonic stem cells and applications to biotechnology**. *Marine Biotechnology* 9(2): 117-127. ISSN: 1436 2228.
NAL Call Number: TP248.27.M37M37
Abstract: ES cells provide a promising tool for the generation of transgenic animals with site-directed mutations. When ES cells colonize germ cells in chimeras, transgenic animals with modified phenotypes are generated and used either for functional genomics studies or for improving productivity in commercial settings. Although the ES cell approach has been limited to mice, there is strong interest for developing the technology in fish. We describe the step-by-step procedure for developing ES cells in fish. Key aspects include avoiding cell differentiation, specific in vitro traits of pluripotency, and, most importantly, testing for production of chimeric animals as the main evidence of pluripotency. The entire process focuses on two model species, zebrafish and medaka, in which most work has been done. The achievements attained in these species, as well as their applicability to other commercial fish, are discussed. Because of the difficulties relating to germ line competence, mostly of long-term fish ES cells, alternative cell-based approaches such as primordial germ cells and nuclear transfer need to be considered. Although progress to date has been slow, there are promising achievements in homologous recombination and alternative avenues yet to be explored that can bring ES technology in fish to fruition. Copyright © 2008 CABI
Descriptors: zebrafish embryos, fish breeding, chimaeras, embryonic stem cells, genetic engineering, *Danio rerio*, *Oryzias latipes* , *medaka.*

Amack, J.D. and H.J. Yost (2004). **The T box transcription factor no tail in ciliated cells controls zebrafish left-right asymmetry.** *Current Biology* 14(8): 685-90. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Abstract: The heart, brain, and gut develop essential left-right (LR) asymmetries. Specialized groups of ciliated cells have been implicated in LR patterning in mouse, chick, frog, and zebrafish embryos. In zebrafish, these ciliated cells are found in Kupffer's vesicle (KV) and are progeny of dorsal forerunner cells (DFCs). However, there is no direct evidence in any vertebrate that the genes involved in LR development are specifically required in ciliated cells. By using a novel method in zebrafish, we knocked down the function of no tail (ntl, homologous to mouse brachyury) in DFCs without affecting its expression in other cells in the embryo. We find that the Ntl transcription factor functions cell autonomously in DFCs to regulate KV morphogenesis and LR determination. This is the first evidence that loss-of-gene function exclusively in ciliated cells perturbs vertebrate LR patterning. Our results demonstrate that the ciliated KV, a transient embryonic organ of previously unknown function, is involved in the earliest known step in zebrafish LR development, suggesting that a ciliary-based mechanism establishes the LR axis in all vertebrate embryos.

Descriptors: zebrafish, body patterning physiology, gene expression, T-box domain and genetics, body patterning genetics, cilia physiology, epithelial cells physiology, gene silencing, immunohistochemistry, in situ hybridization, fluorescence microscopy, oligonucleotides.

Amanuma, K., T. Nakamura, and Y. Aoki (2004). **MNNG-induced mutations in the adult gill and hepatopancreas and in embryos of rpsL transgenic zebrafish.** *Mutation Research* 556(1-2): 151-61. ISSN: 0027-5107.

NAL Call Number: QH431.M8

Abstract: To evaluate the feasibility of a mutagenicity assay using adult rpsL transgenic zebrafish, 4- to 8-month-old females were exposed to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (0, 15 or 30 mg/L in a water bath for 2 h). At 2 weeks after exposure, MNNG showed a concentration-dependent significant increase in mutant frequency (MF) of 8 x 10(-5), 18 x 10(-5), and 51 x 10(-5), respectively, in the gill. DNA sequencing revealed that 60-74% of the induced mutations were G:C to A:T transitions, consistent with the known mutagenic effects of MNNG. A marginal but significant increase in MF was observed in the hepatopancreas only in the group exposed to 30 mg/L, with the induction of some G:C to A:T transitions. A time-course of the appearance of mutations was determined in fish treated with 15 mg/L MNNG. In both, the gill and hepatopancreas, a higher MF was observed at 3 weeks than at 2 weeks, suggesting that an expression time of at least 3 weeks is preferable for the assay. When embryos (29 h post-fertilization) were exposed to MNNG (0, 50, and 150 mg/L) for 1 h, MFs increased significantly with an increase in the concentration of MNNG (5 x 10(-5), 40 x 10(-5), and 144 x 10(-5), respectively) at 3 days after exposure. G:C to A:T transitions were the predominant mutations, and these occurred at the same sites in the rpsL gene as in adult tissues. Thus, MNNG induces typical mutations in the gill and hepatopancreas of adult fish, and in embryos, suggesting that the rpsL zebrafish is a useful tool for monitoring genotoxicity caused by water-borne mutagens.

Descriptors: transgenic zebrafish, gills drug effects, hepatopancreas drug effects, methylnitronitrosoguanidine toxicity, mutagens toxicity, genetically modified, drug dose response relationship, gills metabolism, hepatopancreas metabolism.

Amsterdam, A. (2006). **Insertional mutagenesis in zebrafish: genes for development, genes for disease.** *Briefings in Functional Genomics and Proteomics* 5(1): 19-23. ISSN: 1473 9550.

Abstract: In order to rapidly identify a substantial fraction of the genes with a unique and essential role in vertebrate development, the laboratory of Nancy Hopkins at MIT has performed a large insertional mutagenesis screen in zebrafish using a pseudotyped retroviral vector as the mutagen. We have recovered mutations in about one-quarter of the embryonic essential genes in this organism, and have identified the mutated genes in nearly all of these (333). As the ease of gene identification allowed us to clone the mutated genes for nearly all of the mutants rather than prioritizing based upon the initially observed phenotypes, this has provided an unbiased view of the diversity of genes required for vertebrate development as well as a large collection of mutants to be screened for more specific phenotypes. In collaboration with other labs, we have screened the insertional mutant for the development of a variety of organs and cell types, as well as phenotypes that could represent disease models, such as cystic kidney and hepatomegaly. Furthermore, while all of these mutants are embryonic lethal in their homozygous state, we are investigating the heterozygous adults for additional phenotypes, such as cancer predisposition.

Descriptors: zebrafish embryos, hepatomegaly genetics, cystic kidney diseases genetics, liver diseases genetics, insertional mutagenesis developmental gene expression regulation, cystic kidney diseases embryology, liver diseases embryology, retroviridae genetics.

Amsterdam, A. and N. Hopkins (2006). **Mutagenesis strategies in zebrafish for identifying genes involved in development and disease.** *Trends in Genetics* 22(9): 473-8. ISSN: 0168-9525.
NAL Call Number: QH426.T744
Abstract: It has been nearly a decade since the completion of two large-scale chemical mutagenesis screens in zebrafish, and two years since the completion of a large-scale insertional mutagenesis. In this article, we use the accumulated data from these screens to compare the efficiency of each mutagen to isolate mutants and to identify mutated genes, and argue that the two mutagens target the same set of genes. We then review how both forward genetic screens and reverse genetic techniques, such as morpholinos and TILLING, and transgenics are being used to develop models of human disease.
Descriptors: zebrafish, genetic inborn diseases genetics, growth and development genetics, mutagenesis, disease models, insertional mutagenesis model, zebrafish growth and development.

Amsterdam, A. and N. Hopkins (2004). **Retroviral-mediated insertional mutagenesis in zebrafish.** *Methods in Cell Biology* 77: 3-20. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, insertional mutagenesis methods, retroviridae genetics, virus integration genetics, molecular cloning, DNA mutational analysis, metabolism, genetic vectors genetics, proviruses genetics.

Amsterdam, A., R.M. Nissen, Z. Sun, E.C. Swindell, S. Farrington, and N. Hopkins (2004). **Identification of 315 genes essential for early zebrafish development.** *Proceedings of the National Academy of Sciences of the United States of America* 101(35): 12792-7. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, mutation collection, base sequence, *Caenorhabditis elegans* genetics, conserved sequence, insertional mutagenesis, phenotype, *Saccharomyces cerevisiae* genetics, terminology.

Amsterdam, A., K.C. Sadler, K. Lai, S. Farrington, R.T. Bronson, J.A. Lees, and N. Hopkins (2004). **Many ribosomal protein genes are cancer genes in zebrafish.** *Public Library of Science Biology* 2(5): E139. ISSN: 1544-9173.
NAL Call Number: QH301.P56
Abstract: We have generated several hundred lines of zebrafish (*Danio rerio*), each heterozygous for a recessive embryonic lethal mutation. Since many tumor suppressor genes are recessive lethals, we screened our colony for lines that display early mortality and/or gross evidence of tumors. We identified 12 lines with elevated cancer incidence. Fish from these lines develop malignant peripheral nerve sheath tumors, and in some cases also other tumor types, with moderate to very high frequencies. Surprisingly, 11 of the 12 lines were each heterozygous for a mutation in a different ribosomal protein (RP) gene, while one line was heterozygous for a mutation in a zebrafish paralog of the human and mouse tumor suppressor gene, neurofibromatosis type 2. Our findings suggest that many RP genes may act as haploinsufficient tumor suppressors in fish. Many RP genes might also be cancer genes in humans, where their role in tumorigenesis could easily have escaped detection up to now.
Descriptors: zebrafish, gene expression regulation, neoplastic, ribosomal proteins chemistry, ribosomes chemistry, tumor suppressor genes, heterozygote, loss of heterozygosity, mutagenesis, mutation, neurofibromin 2 genetics , phenotype, RNA chemistry and ribosomal chemistry.

Anand, S., W. Wang, D. Powell, S. Bolanowski, J. Zhang, C. Ledje, A. Pawashe, C. Amemiya, and C. Shashikant (2003). **Divergence of hoxc8 early enhancer parallels diverged axial morphologies between mammals and fishes.** *Proceedings of the National Academy of Sciences of the United States of America* 100(26): 15666-15669. ISSN: 0027 8424.
NAL Call Number: 500N21P
Abstract: There is considerable interest in understanding how cis-regulatory modifications drive morphological changes across species. Because developmental regulatory genes, including Hox genes, are remarkably conserved, their noncoding regulatory regions are likely sources for variations. Modifications of Hox cis-regulatory elements have potential to alter Hox gene expression and, hence, axial morphologies. In vertebrates, differences in the axial levels of Hox gene expression correlate with differences in the number and relative position of thoracic vertebrae. Variation in cis-regulatory elements of Hox genes can be identified by comparative sequence and reporter gene analyses in transgenic mouse embryos. Using these approaches, we show a remarkable divergence of the Hoxc8 early enhancers between mammals and fishes representing diverse axial morphologies. Extensive restructuring of the Hoxc8 early enhancer including nucleotide substitutions, inversion, and divergence result in distinct patterns of reporter gene expression along the embryonic axis. Our results provide an evolutionary perspective on how the enhancer elements are engineered and support the hypothesis that remodeling of Hox regulatory elements in different species has played a significant role in generating morphological diversity. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, biochemistry, molecular biophysics, development, genetics, axial morphology, nucleotide sequence.

Anderson, C., S.J. Bartlett, J.M. Gansner, D. Wilson, L. He, J.D. Gitlin, R.N. Kelsh, and J. Dowden (2007). **Chemical genetics suggests a critical role for lysyl oxidase in zebrafish notochord morphogenesis.** *Molecular BioSystems* 3(1): 51-9. ISSN: 1742-206X.
NAL Call Number: QH506.M66426
Abstract: As a result of a chemical genetic screen for modulators of metalloprotease activity, we report that 2-mercaptopyridine-N-oxide induces a conspicuous undulating notochord defect in zebrafish embryos, a phenocopy of the leviathan mutant. The location of the chemically-induced wavy notochord correlated with the timing of application, thus defining a narrow chemical sensitivity window during segmentation stages. Microscopic observations revealed that notochord undulations appeared during the phase of notochord cell vacuolation and notochord elongation. Notochord cells become swollen as well as disorganized, while electron microscopy revealed disrupted organization of collagen fibrils in the surrounding sheath. We demonstrate by assay in zebrafish extracts that 2-mercaptopyridine-N-oxide inhibits lysyl oxidase. Thus, we provide insight into notochord morphogenesis and reveal novel compounds for lysyl oxidase inhibition. Taken together, these data underline the utility of small molecules for elucidating the dynamic mechanisms of early morphogenesis and provide a potential explanation for the recently established role of copper in zebrafish notochord formation.
Descriptors: zebrafish embryos, morphogenesis genetics, notochord embryology, protein lysine 6 oxidase physiology , cell differentiation, abnormalities, enzymology, metals metabolism, molecular structure, notochord abnormalities due to drug effects, notochord enzymology, protein lysine 6 oxidase metabolism, pyridines pharmacology, sulfhydryl compounds pharmacology, thiones pharmacology, time factors.

Anderson, K.V. and P.W. Ingham (2003). **The transformation of the model organism: a decade of developmental genetics**. *Nature Genetics* 33: 285-293. ISSN: 1061-4036.
NAL Call Number: QH426.N37

Descriptors: zebrafish, mice, biological development model, cell cycle, cytology, development, developmental genetics, intercellular signalling, literature reviews, reviews, signal transduction, *Danio rerio*, cell polarity.
Notes: A review.

Ando, H., T. Furuta, and H. Okamoto (2004). **Photo-mediated gene activation by using caged mRNA in zebrafish embryos.** *Methods in Cell Biology* 77: 159-71. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, azo compounds pharmacology and chemistry, coumarins pharmacology, gene expression regulation, messenger RNA chemistry, ultraviolet rays, coumarins chemistry, dimethyl sulfoxide chemistry, drug effects, microinjections instrumentation.

Ando, H. and H. Okamoto (2006). **Efficient transfection strategy for the spatiotemporal control of gene expression in zebrafish.** *Marine Biotechnology* 8(3): 295-303. ISSN: 1436-2228.
NAL Call Number: TP248.27.M37M37
Abstract: Functional analyses of gene function by knockdown and expression approaches strongly enhance the genetic study of development. In vivo application of the introduction of inhibitors of gene expression, mRNA, and expression constructs in the target region make it possible to perform region- and stage-specific regulation of gene function in a simple manner. As a basic tool for the conditional regulation of gene expression in target tissue, we present methods for the efficient introduction of antisense morpholino oligonucleotide (MO), mRNA, and expression plasmid constructs into early and later stage zebrafish embryo and larva. Lipofection of a neuron-specific expression construct plasmid encoding green fluorescent protein (GFP) into optic vesicle resulted in clear GFP expression in the retinotectal pathway in hatched larva. Co-lipofection of MO and GFP mRNA to the presumptive head region resulted in brain-specific knockdown of the gene in mid-stage embryos.
Descriptors: zebrafish brain embryology and metabolism, developmental gene expression regulation, transfection methods, DNA genetics and metabolism, eye embryology and metabolism, larva genetics and metabolism, antisense oligonucleotides genetics and metabolism, messenger RNA genetics and metabolism.

Ando, H. and H. Okamoto (2003). **Practical procedures for ectopic induction of gene expression in zebrafish embryos using Bhc-diazo-caged mRNA.** *Methods in Cell Science* 25(1-2): 25-31. ISSN: 1381-5741.
NAL Call Number: QH573.T52
Abstract: We previously reported mRNA caging technology as a novel and simple technique for photo-mediated temporal and spatial control of gene activation in zebrafish embryos and as an alternative to the 'gene knockdown' approach using antisense morpholino oligonucleotides. The caging reagent used is 6-bromo-4-diazomethyl-7-hydroxycoumarin (Bhc-diazo), which forms a covalent bond with the phosphate moiety of the sugar-phosphate backbone of RNA. Mainly because of the reduced solubility of caged mRNA in aqueous solutions, special care in handling is needed. The Bhc-diazo group binds to the phosphate moieties of RNA and abolishes the translational activity of the latter. The translational activity of Bhc-caged mRNA is restored by photolysis/uncaging when exposed to long-wave UV light (350 approximately 365 nm). In this paper we describe the technique and detailed procedures for spatially and temporally controlled induction of gene expression in zebrafish embryos.
Descriptors: zebrafish embryos, azo compounds chemistry, coumarins chemistry, photolysis radiation effects, messenger RNA metabolism, beta galactosidase metabolism, molecular cloning,

anatomy and histology, microinjections, messenger RNA genetics, ultraviolet rays, beta galactosidase genetics.

Angers, S., C.J. Thorpe, T.L. Biechele, S.J. Goldenberg, N. Zheng, M.J. Maccoss, and R.T. Moon (2006). **The klhl12-cullin-3 ubiquitin ligase negatively regulates the wnt-beta-catenin pathway by targeting dishevelled for degradation**. *Nature Cell Biology* 8(4): 348. ISSN: 1465 7392.
NAL Call Number: QH573
Abstract: Dishevelled is a conserved protein that interprets signals received by Frizzled receptors. Using a tandem-affinity purification strategy and mass spectrometry we have identified proteins associated with Dishevelled, including a Cullin-3 ubiquitin ligase complex containing the Broad Complex, Tramtrack and Bric a Brac (BTB) protein Kelch-like 12 (KLHL12). This E3 ubiquitin ligase complex is recruited to Dishevelled in a Wnt-dependent manner that promotes its polyubiquitination and degradation. Functional analyses demonstrate that regulation of Dishevelled by this ubiquitin ligase antagonizes the Wnt-beta-catenin pathway in cultured cells, as well as in *Xenopus* and zebrafish embryos. Considered with evidence that the distinct Cullin-1 based SCF beta-TrCP complex regulates beta-catenin stability, our data on the stability of Dishevelled demonstrates that two distinct ubiquitin ligase complexes regulate the Wnt-beta-catenin pathway. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, *Xenopus*, development, mass spectrometry, spectrum analysis techniques, tandem affinity purification strategy.

Anken, R., M. Ibsch, U. Kniesel, and H. Rahmann (2004). **No correlation between multilamellar bodies in the inner ear and further organs of mutant (backstroke, bks) and wildtype zebrafish embryos.** *Advances in Space Research* 33(8): 1411-5. ISSN: 0273 1177.
Abstract: The origin of the proteinacious matrix of the inner ear stones (otoliths) of vertebrates has not yet been clarified. Using the backstroke mutant (bks) of the zebrafish *Danio rerio*, which is characterized by a complete lack of otoliths, we searched for possibly missing or aberrant structural components within the macular epithelia of the inner ears of embryos on the ultrastructural level. Numerous multilamellar bodies (MLBs) were found. The MLBs were, however, not restricted to the inner ears of mutants but were also found in wildtype individuals and in further organs such as brain and liver. MLBs have hitherto never been described from the inner ear of fish and are generally estimated to be rare structures. Their occurrence in fish liver can, however, be induced by using particular chemical substances, which seem to effect adaptive compensatory processes on the cellular level. Such a chemical treatment also affects the ultrastructure of further organelles. Since the occurrence of MLBs in the liver of zebrafish was not accompanied by an alteration of the morphology of other organelles, their occurrence seems not to be due to environmental stress. The findings indicate that the MLBs cannot be correlated with bks-inherent features as well as with missing otolith development/growth. Since the occurrence of MLBs was independent from the developmental stage of a specimen and its overall tissue preservation, it can moreover be excluded that these MLBs merely represent fixation artifacts. Their presence more likely indicates cellular remodelling processes of hitherto unknown significance. c2003 COSPAR. Published by Elsevier Ltd. All rights reserved.
Descriptors: zebrafish, acoustic maculae ultrastructure, epithelium ultrastructure, organelles ultrastructure, otolithic membrane ultrastructure, electron transmission microscopy, anatomy and histology, otoliths.

Annilo, T., Z.Q. Chen, S. Shulenin, J. Costantino, L. Thomas, H. Lou, S. Stefanov, and M. Dean (2006). **Evolution of the vertebrate ABC gene family: analysis of gene birth and death**. *Genomics* 88(1): 1-11. ISSN: 0888 7543.

NAL Call Number: QH445.2.G45

Abstract: Vertebrate evolution has been largely driven by the duplication of genes that allow for the acquisition of new functions. The ATP-binding cassette (ABC) proteins constitute a large and functionally diverse family of membrane transporters. The members of this multigene family are found in all cellular organisms, most often engaged in the translocation of a wide variety of substrates across lipid membranes. Because of the diverse function of these genes, their large size, and the large number of orthologs, ABC genes represent an excellent tool to study gene family evolution. We have identified ABC proteins from the sea squirt (*Ciona intestinalis*), zebrafish (*Danio rerio*), and chicken (*Gallus gallus*) and, using phylogenetic analysis, identified those genes with a one-to-one orthologous relationship to human ABC proteins. All ABC protein subfamilies found in Ciona and zebrafish correspond to the human subfamilies, with the exception of a single ABCH subfamily gene found only in zebrafish. Multiple gene duplication and deletion events were identified in different lineages, indicating an ongoing process of gene evolution. As many ABC genes are involved in human genetic diseases, and important drug transport phenotypes, the understanding of ABC gene evolution is important to the development of animal models and functional studies. Copyright © 2008 CABI

Descriptors: zebrafish, humans, fowls, biochemical transporters , evolution, ABC genes evolution, genomes, phylogenetics, poultry, surface proteins, chordata, *Danio rerio.*

Antunes, A. and M.J. Ramos (2005). **Discovery of a large number of previously unrecognized mitochondrial pseudogenes in fish genomes**. *Genomics* 86(6): 708-717. ISSN: 0888 7543.

NAL Call Number: QH445.2.G45

Abstract: Nuclear inserted copies of mitochondrial origin (numts) vary widely among eukaryotes, with human and plant genomes harboring the largest repertoires. Numts were previously thought to be absent from fish species, but the recent release of three fish nuclear genome sequences provides the resource to obtain a more comprehensive insight into the extent of mtDNA transfer in fishes. From the sequence analyses of the genomes of *Fugu rubripes, Tetraodon nigroviridis*, and *Danio rerio*, we have identified 2, 5, and 10 recent numt integrations, respectively, which integrated into those genomes less than 0.6 million years (Myr) ago. Such results contradict the hypothesis of absence or rarity of numts in fishes, as (i) the ratio of numts to the total size of the nuclear genome in *T. nigroviridis* was superior to the ratio observed in several higher vertebrate species (e.g., chicken, mouse, and rat), and only surpassed by humans, and (ii) the mtDNA coverage transferred to the nuclear genome of *D. rerio* is exceeded only by human and mouse, within the whole range of eukaryotic genomes surveyed for numts. Additionally, 335, 336, and 471 old numts (>12.5 Myr) were detected in *F. rubripes, T. nigroviridis, and D. rerio*, respectively. Surprisingly, old numts are inserted preferentially into known or predicted genes, as inferred for recent numts in human. However, because in fish genomes such integrations are old, they are likely to represent evolutionary successes and they may be considered a potential important evolutionary mechanism for the enhancement of genomic coding regions. Copyright © 2008 CABI

Descriptors: zebrafish, fish genomes, mitochondrial genetics , nucleotide sequences, pseudogenes, fishes , Tetraodontidae.

Appelbaum, L., A. Anzulovich, R. Baler, and Y. Gothilf (2005). **Homeobox-clock protein interaction in zebrafish. A shared mechanism for pineal-specific and circadian gene expression.** *Journal of Biological Chemistry* 280(12): 11544-51. ISSN: 0021-9258.

NAL Call Number: 381J824

Abstract: In non-mammalian vertebrates, the pineal gland is photoreceptive and contains

an intrinsic circadian oscillator that drives rhythmic production and secretion of melatonin. These features require an accurate spatiotemporal expression of an array of specific genes in the pineal gland. Among these is the arylalkylamine N-acetyltransferase, a key enzyme in the melatonin production pathway. In zebrafish, pineal specificity of zfaanat2 is determined by a region designated the pineal-restrictive downstream module (PRDM), which contains three photoreceptor conserved elements (PCEs) and an E-box, elements that are generally associated with photoreceptor-specific and rhythmic expression, respectively. Here, by using in vivo and in vitro approaches, it was found that the PCEs and E-box of the PRDM mediate a synergistic effect of the photoreceptor-specific homeobox OTX5 and rhythmically expressed clock protein heterodimer, BMAL/CLOCK, on zfaanat2 expression. Furthermore, the distance between the PCEs and the E-box was found to be critical for PRDM function, suggesting a possible physical feature of this synergistic interaction. OTX5-BMAL/CLOCK may act through this mechanism to simultaneously control pineal-specific and rhythmic expression of zfaanat2 and possibly also other pineal and retinal genes.
Descriptors: zebrafish, arylalkylamine n acetyltransferase genetics, circadian rhythm, gene expression regulation, homeodomain, trans activators physiology, basic helix loop helix transcription factors, DNA binding, dimerization, homeobox genes, OTX transcription factors, pineal gland metabolism, transcription factors physiology.

Appelbaum, L., G. Skariah, P. Mourrain, and E. Mignot (2007). **Comparative expression of p2x receptors and ecto-nucleoside triphosphate diphosphohydrolase 3 in hypocretin and sensory neurons in zebrafish.** *Brain Research* 1174: 66-75. ISSN: 0006-8993.
NAL Call Number: QP376
Abstract: The hypocretin/orexin (HCRT/ORX) excitatory neuropeptides are expressed in a small population of lateral hypothalamic cells in mammals and fish. In humans, loss of these cells causes the sleep disorder narcolepsy. Identification of genes expressed in HCRT-producing cells may be revealing as to the regulation of sleep and the pathophysiology of narcolepsy. In this study, in situ hybridization analyses were performed to characterize the expression pattern of receptors and enzyme, which regulate ATP-mediated transmission in hypocretin cells of zebrafish larvae. The zebrafish cDNA encoding the ecto-nucleoside triphosphate diphosphohydrolase 3 (ENTPD3/NTPDase3) was isolated. This transcript was found to be expressed in zebrafish HCRT cells as previously reported in mammals. It was also expressed in the cranial nerves (gV, gVII, gIV and gX) and in primary sensory neurons (i.e., Rohon-Beard neurons) in the spinal cord. The expression of known zebrafish p2rx purinergic receptor family members was next studied and found to overlap with the entpd3 expression pattern. Specifically, p2rx2, p2rx3.1, p2rx3.2 and p2rx8 were expressed in the trigeminal ganglia and subsets of Rohon-Beard neurons. In contrast to mammals, p2rx2 was not expressed in HCRT cells; rather, p2rx8 was expressed with entpd3 in this hypothalamic region. The conservation of expression of these genes in HCRT cells and sensory neurons across vertebrates suggests an important role for ATP mediated transmission in the regulation of sleep and the processing of sensory inputs.
Descriptors: zebrafish , p2x receptors, ecto-nucleoside triphosphate diphosphohydrolase 3, hypocretin, orexin, sensory neurons HCRT cells, sleep regulation.

Appelbaum, L., R. Toyama, I.B. Dawid, D.C. Klein, R. Baler, and Y. Gothilf (2004). **Zebrafish serotonin-N-acetyltransferase-2 gene regulation: pineal-restrictive downstream module contains a functional E-box and three photoreceptor conserved elements.** *Molecular Endocrinology* 18(5): 1210-21. ISSN: 0888-8809.
NAL Call Number: QP187.3M64M6

Abstract: Pineal function is defined by a set of very narrowly expressed genes that encode proteins required for photoperiodic transduction and rhythmic melatonin secretion. One of these proteins is serotonin N-acetyltransferase (arylalkylamine N-acetyltransferase, AANAT), which controls the daily rhythm in melatonin production. Here, pineal-specific expression of the zebrafish aanat-2 (zfaanat-2) was studied using in vivo transient expression analyses of promoter-reporter constructs; this revealed that specificity is determined by two regions located 12 kb away from each other. One is the 5'-flanking region, and the other is a 257-bp sequence, located 6 kb downstream of the transcribed region. This 3'-sequence, designated pineal-restrictive downstream module (PRDM), has a dual function: enhancement of pineal expression and inhibition of extrapineal expression. The former is an autonomic property of PRDM whereas the later function requires interaction with the upstream regulatory region of zfaanat-2. Functional analyses of the PRDM sequence revealed that three photoreceptor conserved elements (TAATC) and a single perfect E-box (CACGTG) are crucial for the dual function of PRDM. These results indicate that pineal specificity of zfaanat-2 is determined by the dual functionality of the PRDM and the interaction between upstream regulatory region and downstream photoreceptor conserved elements and E-box element.
Descriptors: zebrafish, arylalkylamine n acetyltransferase metabolism, circadian rhythm physiology, melatonin metabolism, pineal gland enzymology, arylalkylamine n acetyltransferase genetics, base sequence, molecular cloning, enzymology, molecular sequence data, photoreceptors metabolism, nucleic acid regulatory sequences genetics.

Appelbaum, L., D. Vallone, A. Anzulovich, L. Ziv, M. Tom, N.S. Foulkes, and Y. Gothilf (2006).
Zebrafish arylalkylamine-N-acetyltransferase genes - targets for regulation of the circadian clock. *Journal of Molecular Endocrinology* 36(2): 337-47. ISSN: 0952-5041.
NAL Call Number: QP187.3.M64J68
Abstract: Daily rhythms of melatonin production are controlled by changes in the activity of arylalkylamine-N-acetyltransferase (AANAT). Zebrafish possess two aanats, aanat1 and aanat2; the former is expressed only in the retina and the latter is expressed in both the retina and the pineal gland. Here, their differential expression and regulation were studied using transcript quantification and transient and stable in vivo and in vitro transfection assays. In the pineal gland, the aanat2 promoter exhibited circadian clock-controlled activity, as indicated by circadian rhythms of Enhanced green fluorescent protein (EGFP) mRNA in AANAT2:EGFP transgenic fish. In vivo transient expression analyses of the aanat2 promoter indicated that E-box and photoreceptor conserved elements (PCE) are required for expression in the pineal gland. In the retina, the expression of both genes was characterized by a robust circadian rhythm of their transcript levels. In constant darkness, the rhythmic expression of retinal aanat2 persisted while the aanat1 rhythm disappeared; indicating that the former is controlled by a circadian clock and the latter is also light driven. In the light-entrainable clock-containing PAC-2 zebrafish cell line, both stably transfected aanat1 and aanat2 promoters exhibited a clock-controlled circadian rhythm, characteristic for an E-box-driven expression. Transient co-transfection experiments in NIH-3T3 cells revealed that the two, E-box- and PCE-containing, promoters are driven by the synergistic action of BMAL/CLOCK and orthehodenticle homeobox 5. This study has revealed a shared mechanism for the regulation of two related genes, yet describes their differential phases and photic responses which may be driven by other gene-specific regulatory mechanisms and tissue-specific transcription factor profiles.
Descriptors: zebrafish, rats, arylalkylamine N acetyltransferase genetics and metabolism, circadian rhythm physiology, basic helix loop helix transcription factors genetics and metabolism, cell line, dimerization, enzymologic gene expression regulation, isoenzymes genetics,

isoenzymes metabolism, otx transcription factors genetics and metabolism, promoter regions genetics , protein binding, messenger RNA genetics, regulatory elements, transcriptional genetics, retina enzymology, trans activators genetics and metabolism, transcription factors metabolism.

Armas, P., S. Cachero, V.A. Lombardo, A. Weiner, M.L. Allende, and N.B. Calcaterra (2004). **Zebrafish cellular nucleic acid-binding protein: gene structure and developmental behaviour.** *Gene* 337: 151-61. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: Here we analyse the structural organisation and expression of the zebrafish cellular nucleic acid-binding protein (zCNBP) gene and protein. The gene is organised in five exons and four introns. A noteworthy feature of the gene is the absence of a predicted promoter region. The coding region encodes a 163-amino acid polypeptide with the highly conserved general structural organisation of seven CCHC Zn knuckle domains and an RGG box between the first and the second Zn knuckles. Although theoretical alternative splicing is possible, only one form of zCNBP is actually detected. This form is able to bind to single-stranded DNA and RNA probes in vitro. The analysis of zCNBP developmental expression shows a high amount of CNBP-mRNA in ovary and during the first developmental stages. CNBP-mRNA levels decrease while early development progresses until the midblastula transition (MBT) stage and increases again thereafter. The protein is localised in the cytoplasm of blastomeres whereas it is mainly nuclear in developmental stages after the MBT. These findings suggest that CNBP is a strikingly conserved single-stranded nucleic acid-binding protein which might interact with maternal mRNA during its storage in the embryo cell cytoplasm. It becomes nuclear once MBT takes place possibly in order to modulate zygotic transcription and/or to associate with newly synthesised transcripts.
Descriptors: zebrafish, RNA binding proteins genetics, alternative splicing, amino acid sequence, molecular cloning, complementary DNA chemistry and genetics, embryonic development metabolism, developmental gene expression regulation, genetics, immunohistochemistry, in situ hybridization, molecular sequence data, ovary embryology, growth, development and metabolism, protein binding, protein isoforms genetics and metabolism, RNA probes genetics and metabolism, messenger RNA genetics and metabolism, RNA binding proteins metabolism, sequence alignment, DNA sequence analysis.

Arnaud, E., K.F. Ferri, J. Thibaut, Z. Haftek Terreau, A. Aouacheria, D. Le Guellec, T. Lorca, and G. Gillet (2006). **The zebrafish bcl-2 homologue Nrz controls development during somitogenesis and gastrulation via apoptosis-dependent and -independent mechanisms.** *Cell Death and Differentiation* 13(7): 1128-37. ISSN: 1350 9047.
NAL Call Number: QH671
Abstract: Although the role of the b-cell lymphoma (Bcl)-2 family of apoptosis inhibitors is well documented in tumor cells and tissue morphogenesis, their role during the early development of vertebrates is unknown. Here, we characterize Nrz, a new Bcl-2-related inhibitor of apoptosis in zebrafish. Nrz is a mitochondrial protein, antagonizing the death-accelerator Bax. The nrz gene is mainly expressed during gastrulation and somitogenesis. The knockdown of nrz with antisense morpholinos leads to alterations of the somites, correlated with an increase in apoptosis. In addition, earlier during development, in the zebrafish gastrula, nrz knockdown results in an increase of snail-1 expression at the margin and frequent gastrulation arrest at the shield stage, independently of apoptosis. Together these data suggest that Nrz, in addition to its effect on apoptosis, contributes to cell movements during gastrulation by negatively regulating the expression of Snail-1, a transcription factor that controls cell

adhesion.

Descriptors: zebrafish gastrulation, apoptosis physiology, gastrula physiology, proto oncogene and genetics, somites physiology, amino acid sequence, Western blotting, COS cells, *Cercopithecus aethiops*, molecular cloning, gastrula cytology and metabolism, developmental gene expression regulation, in situ hybridization, confocal microscopy, molecular sequence data, antisense oligonucleotides genetics, proto oncogene proteins C Bcl-2 genetics, metabolism and physiology, reverse transcriptase PCR, amino acid sequence homology, somites cytology and metabolism, transcription factors genetics, metabolism and physiology, zebrafish protein genetics and metabolism.

Asai, Y., D.K. Chan, C.J. Starr, J.A. Kappler, R. Kollmar, and A.J. Hudspeth (2006). **Mutation of the atrophin2 gene in the zebrafish disrupts signaling by fibroblast growth factor during development of the inner ear.** *Proceedings of the National Academy of Sciences of the United States of America* 103(24): 9069-74. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: The development of the vertebrate inner ear depends on the precise expression of fibroblast growth factors. In a mutagenesis screen for zebrafish with abnormalities of inner-ear development and behavior, we isolated a mutant line, ru622, whose phenotypic characteristics resembled those of null mutants for the gene encoding fibroblast growth factor 8 (Fgf8): an inconsistent startle response, circular swimming, fused otoliths, and abnormal semicircular canals. Positional cloning disclosed that the mutant gene encodes the transcriptional corepressor Atrophin2. Both the Fgf8 protein and zebrafish "similar expression to fgf genes" protein (Sef), an antagonist of fibroblast growth factors induced by Fgf8 itself, were found to be overexpressed in ru622 mutants. We therefore hypothesized that an excess of Sef eliminates Fgf8 signals and produces an fgf8 null phenotype in ru622 mutants. In support of this idea, we could rescue larvae whose atrophin2 expression had been diminished with morpholinos by reducing the expression of Sef as well. We propose that Atrophin2 plays a role in the feedback regulation of Fgf8 signaling. When mutation of the atrophin2 gene results in the overexpression of both Fgf8 and Sef, the excessive Sef inhibits Fgf8 signaling. The resultant imbalance of Fgf8 and Sef signals then underlies the abnormal aural development observed in ru622.

Descriptors: zebrafish, inner ear anatomy, histology, embryology, growth and development, fibroblast growth factor 8 metabolism and genetics, intracellular signaling peptides and proteins genetics and metabolism, nerve tissue proteins genetics and metabolism, repressor proteins genetics and metabolism, signal transduction physiology, behavior physiology, developmental gene expression regulation, in situ hybridization, membrane proteins genetics and metabolism, mutation , phenotype, reflex physiology and metabolism.

Asakawa, K., M.L. Suster, K. Mizusawa, S. Nagayoshi, T. Kotani, A. Urasaki, Y. Kishimoto, M. Hibi, and K. Kawakami (2008). **Genetic dissection of neural circuits by Tol2 transposon-mediated Gal4 gene and enhancer trapping in zebrafish**. *Proceedings of the National Academy of Sciences, USA* 105(4): 1255-1260. ISSN: 0027-8424.

NAL Call Number: 500N21P

Descriptors: transgenic zebrafish, brain, embryos, enhancers, Gal4 gene, gene expression, genetic analysis, light chains, neural networks, neurons, spinal cord, synaptic transmission, tactile stimuli, transcription factors, transposons, trapping, tetanus toxin, *Danio rerio*, *Drosophila*, development and cell cycle.

Ashurst, J.L., C.K. Chen, J.G.R. Gilbert, K. Jekosch, S. Keenan, P. Meidl, S.M. Searle, J. Stalker, R. Storey, S. Trevanion, L. Wilming, and T. Hubbard (2005). **The vertebrate genome annotation (Vega) database**. *Nucleic Acids Research* 33(1): D459-D465. ISSN: 0305-1048.
NAL Call Number: QD341.A2N8
Descriptors: zebrafish, bibliographic information, database browsing, chromosomes, DNA sequencing, data collections, freshwater fish, genomes, nucleic acids, transcription, *Danio rerio*, schema.

Ashworth, R. and C. Brennan (2005). **Use of transgenic zebrafish reporter lines to study calcium signalling in development.** *Briefings in Functional Genomics and Proteomics* 4(2): 186-93. ISSN: 1473 9550.
Abstract: Calcium signals are associated with many of the events common to animal development. Understanding the role of these calcium signals requires the ability to visualise and manipulate calcium levels in the developing embryo. Recent work has led to the development of sensitive protein-based probes that can be used to generate transgenic animals for the analysis of calcium signalling in vivo. This paper focuses on the use of genetically encoded calcium probes to follow calcium signals in zebrafish. It reviews progress and speculates on the potential for use in the future.
Descriptors: zebrafish embryos, genetically modified, calcium metabolism, developmental gene expression regulation, reporter genes, calcium signaling, physiology, embryonic development, fluorescence resonance energy transfer methods, luminescent proteins chemistry, neurons metabolism, signal transduction, time factors.

Babb, S.G., B. Shah, L.N. Bell, Q. Liu, and J.A. Marrs (2004). **R-cadherin (cdh4) is essential for differentiation of the neural retina and normal retinotectal projections**. *Molecular Biology of the Cell* 15(Suppl. S): 182A. ISSN: 1059 1524.
NAL Call Number: QH604.C452
Descriptors: zebrafish embryos, nervous system, neural coordination, molecular biophysics, sense organs, neural retina, sensory reception, immunostaining, histology and cytology techniques, immunologic techniques, two photon microscopy, imaging and microscopy, embryonic development, cell death, retinogenesis.

Badakov, R. and A. Jazwinska (2006). **Efficient transfection of primary zebrafish fibroblasts by nucleofection**. *Cytotechnology* 51(2): 105-110. ISSN: 0920-9069.
NAL Call Number: QH585.C97
Descriptors: zebrafish, cell culture, DNA, embryo fibroblasts, fusion protein, gene transfer, green fluorescent protein, histones, plasmids, transfection, geneticin, *Danio rerio.*

Bae, M.K., K. Kidd, J. Torres-Vazquez, M. Kamei, H. Pine, J. Berk, M. Mcelwain, and B. Weinstein (2004). **An F3 genetic screen for vascular-specific mutants using transgenic zebrafish expressing EGFP in blood vessels**. *Federation of American Societies for Experimental Biology Journal* 18(4-5): Abst. 539.11. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: The zebrafish is a genetically tractable vertebrate with an optically clear embryo that creates an ideal vertebrate model system for functional analysis of embryonic blood vessel development in vivo. These advantages make zebrafish useful for screening to discover novel vascular genes affecting early blood vessel development. We are currently performing an F3 genetic screen to identify novel vascular-specific mutants in ENU-treated zebrafish, *Danio rerio.* To facilitate the screening process, we are using a germline transgenic line gen-

erated previously (Lawson & Weinstein, Dev. Biol. 248, 2002) that expresses EGFP under the control of the vascular-specific promoter, fli1, throughout the vasculature. This robust expression of EGFP in vascular endothelium allows us to screen visually for vascular patterning defects and blood vessel morphogenesis changes. At 3 days post-fertilization, F3 embryos are examined for vascular and circulatory defects under a fluorescent dissecting microscope. Thus far, we have identified 36 mutants with vascular defects related to either increased or decreased vessel number, vessel pattern defects, malformed vessels or loss of circulation. The vascular mutants generated through this screen will provide powerful models for future vascular research endeavors as well as identify novel genes involved in or contributing to vascular development. Copyright©Thomson Reuters 2009

Descriptors: zebrafish F3 embryos, cardiovascular system, transport and circulation, genetic screening, genetic techniques, vascular development, model.

Bae, Y.K., T. Shimizu, O. Muraoka, T. Yabe, T. Hirata, H. Nojima, T. Hirano, and M. Hibi (2004). **Expression of sax1/nkx1.2 and sax2/nkx1.1 in zebrafish.** *Gene Expression Patterns* 4(4): 481-6. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: sax1/nkx1.2 and sax2/nkx1.1 are members of the evolutionally conserved NK-1 homeobox gene family. sax1/nkx1.2 is reported to be expressed in the central nervous system during early and late neurogenesis in the chick and mouse, but the expression of sax2/nkx1.1 has not been reported. We isolated zebrafish cDNAs for sax1/nkx1.2 and sax2/nkx1.1 and examined their expression. In zebrafish, unlike chick and mouse, sax1/nkx1.2 was expressed in the prospective medial floor plate from the mid-gastrula period and was dependent on Nodal signaling. From the early segmentation period, sax1/nkx1.2 was also expressed in the posterior neuroectoderm. sax2/nkx1.1 was expressed in the prospective extraocular muscles, mesencephalic neurons residing along the tract of the posterior commissure, ventral neurons in the hindbrain, and interneurons in the spinal cord.

Descriptors: zebrafish, NK-1 homeobox gene family, central nervous system embryology, developmental gene expression regulation physiology and genetics , homeodomain proteins biosynthesis and genetics, transcription factors biosynthesis, proteins biosynthesis, molecular sequence data, signal transduction physiology, transcription factors genetics, transforming growth factor beta metabolism.

Bagnat, M., I.D. Cheung, K.E. Mostov, and D.Y. Stainier (2007). **Genetic control of single lumen formation in the zebrafish gut.** *Nature Cell Biology* 9(8): 954-60. ISSN: 1465-7392.

NAL Call Number: QH573

Abstract: Most organs consist of networks of interconnected tubes that serve as conduits to transport fluid and cells and act as physiological barriers between compartments. Biological tubes are assembled through very diverse developmental processes that generate structures of different shapes and sizes. Nevertheless, all biological tubes invariably possess one single lumen. The mechanisms responsible for single lumen specification are not known. Here we show that zebrafish mutants for the MODY5 and familial GCKD gene tcf2 (also known as vhnf1) fail to specify a single lumen in their gut tube and instead develop multiple lumens. We show that Tcf2 controls single lumen formation by regulating claudin15 and Na+/K+-ATPase expression. Our in vivo and in vitro results indicate that Claudin15 functions in paracellular ion transport to specify single lumen formation. This work shows that single lumen formation is genetically controlled and appears to be driven by the accumulation of fluid.

Descriptors: zebrafish mutants, hepatocyte nuclear factor 1 beta metabolism, intestines

abnormalities, anatomy, histology and embryology, morphogenesis, genetically modified cultured cells, hepatocyte nuclear factor 1 beta genetics, in situ hybridization, ion channels metabolism, ion transport physiology, membrane proteins, molecular sequence data, Na+ K+ exchanging ATPase metabolism.

Bahadori, R., O. Rinner, H.B. Schonthaler, O. Biehlmaier, Y.V. Makhankov, P. Rao, P. Jagadeeswaran, and S.C. Neuhauss (2006). **The zebrafish fade out mutant: a novel genetic model for Hermansky-Pudlak syndrome.** *Investigative Ophthalmology and Visual Science* 47(10): 4523-31. ISSN: 0146-0404.

Abstract: PURPOSE: To characterize retinal morphology and visual system function in the zebrafish mutant fade out (fad) and to establish the mutant as a lower vertebrate model for Hermansky-Pudlak syndrome (HPS). METHODS: Retinal morphology of fad larvae was examined between 3 and 9 days postfertilization (dpf) by standard histology, transmission electron microscopy, and immunohistochemistry examination. Apoptotic cells were visualized by TdT-mediated dUTP nick-end labeling (TUNEL) staining. Visual system function was probed by electroretinography and behavioral assessment by optokinetic response measurements. Blood clotting was evaluated by time to occlusion testing of blood vessels as an arterial thrombosis assay. The chromosomal location of fad was determined by simple sequence-length polymorphism mapping. Genomic fragments of candidate genes were cloned by standard molecular techniques and mapped to the zebrafish genome by radiation hybrid mapping. RESULTS: Mutant fad larvae are hypopigmented and show structural defects in the outer retina. Melanosomes of these larvae in the retinal pigment epithelium are hypopigmented, generally smaller, and progressively reduced in number compared to nonmutant larvae. Progressive microvilli protrusions into the photoreceptor cell layer are not detectable, and photoreceptor outer segments get shorter and are misaligned. Photoreceptors subsequently undergo apoptosis, with a peak of cell death at 6 dpf. Electrical responses of the retina and visual performance are severely reduced. Blood clotting is prolonged in mutant fad larvae. Genomic mapping of fad reveals distinct genomic positions of the mutant gene from known human HPS genes. CONCLUSIONS: The fad mutant shows syndromic defects in pigmentation, outer retinal structure and function, and blood clotting. This syndrome is characteristic of Hermansky-Pudlak syndrome (HPS), making fad a novel genetic model of HPS. The gene does not cosegregate with the known human HPS genes, suggesting a novel molecular cause of HPS.

Descriptors: zebrafish Hermanski-Pudlak syndrome genetics, hypopigmentation genetics, melanosomes genetics, genetic models, mutation, retinal diseases genetics, apoptosis, electroretinography, fluorescent antibody technique, indirect, recessive genes, glutamate ammonia ligase metabolism, Hermanski-Pudlak syndrome pathology, in situ nick end labeling, linkage genetics, melanosomes pathology, optokinetic nystagmus physiology, vertebrate photoreceptors physiology and ultrastructure, pigment epithelium of eye pathology, radiation hybrid mapping, retinal diseases pathology, synaptosomal associated protein 25 metabolism, whole blood coagulation time.

Bahary, N., A. Davidson, D. Ransom, J. Shepard, H. Stern, N. Trede, Y. Zhou, B. Barut, and L.I. Zon (2004). **The Zon laboratory guide to positional cloning in zebrafish.** *Methods in Cell Biology* 77: 305-29. ISSN: 0091-679X.

NAL Call Number: 442P92

Descriptors: zebrafish, chromosome mapping methods, molecular cloning methods, gene order, human genone, isotope labeling, oligonucleotides chemistry, synteny genetics, *Takifugu* genetics.

Bai, Q., J.A. Garver, N.A. Hukriede, and E.A. Burton (2007). **Generation of a transgenic zebrafish model of Tauopathy using a novel promoter element derived from the zebrafish eno2 gene.** *Nucleic Acids Research* 35(19): 6501-16. ISSN: 0305-1048.

NAL Call Number: QD341.A2N8

Abstract: The aim of this study was to isolate cis-acting regulatory elements for the generation of transgenic zebrafish models of neurodegeneration. Zebrafish enolase-2 (eno2) showed neuronal expression increasing from 24 to 72 h post-fertilization (hpf) and persisting through adulthood. A 12 kb eno2 genomic fragment, extending from 8 kb upstream of exon 1 to exon 2, encompassing intron 1, was sufficient to drive neuronal reporter gene expression in vivo over a similar time course. Five independent lines of stable Tg(eno2 : GFP) zebrafish expressed GFP widely in neurons, including populations with relevance to neurodegeneration, such as cholinergic neurons, dopaminergic neurons and cerebellar Purkinje cells. We replaced the exon 2-GFP fusion gene with a cDNA encoding the 4-repeat isoform of the human microtubule-associated protein Tau. The first intron of eno2 was spliced with high fidelity and efficiency from the chimeric eno2-Tau transcript. Tau was expressed at approximately 8-fold higher levels in Tg(eno2 : Tau) zebrafish brain than normal human brain, and localized to axons, neuropil and ectopic neuronal somatic accumulations resembling neurofibrillary tangles. The 12 kb eno2 promoter drives high-level transgene expression in differentiated neurons throughout the CNS of stable transgenic zebrafish. This regulatory element will be useful for the construction of transgenic zebrafish models of neurodegeneration.

Descriptors: transgenic zebrafish Tauopathy disease models, phosphopyruvate hydratase genetics, promoter regions genetics, Tauopathies genetics, Tau amino acid sequence, genetically modified genetics, genetically modified metabolism, brain metabolism, molecular sequence data, phosphopyruvate hydratase metabolism, repetitive sequences, amino acid, DNA sequence analysis, transcription initiation site, metabolism, Tau proteins chemistry and metabolism.

Bai, Q., S.J. Mullett, J.A. Garver, D.A. Hinkle, and E.A. Burton (2006). **Zebrafish DJ-1 is evolutionarily conserved and expressed in dopaminergic neurons.** *Brain Research* 1113(1): 33-44. ISSN: 0006 8993.

NAL Call Number: QP376

Abstract: Loss-of-function mutations in the human PARK7 gene, encoding DJ-1, are a rare cause of autosomal recessive Parkinson's disease (ARPD). To facilitate generation of a novel vertebrate model, in which to examine the biochemical functions of DJ-1 in vivo, we cloned and characterized the zebrafish orthologue of DJ-1 (zDJ-1). The 0.95 kb zDJ-1 mRNA is expressed in adult zebrafish brain, muscle and gut, and in the embryo from 24 h post-fertilization. The zDJ-1 transcript encodes a 19.8 kDa, 189 amino acid protein, which is 83% identical to human DJ-1. Residues thought to be functionally important sites of post-translational modification in human DJ-1, and critical positions affected by pathogenic missense mutations in ARPD patients, are conserved in zDJ-1. The 14 kb zDJ-1 gene contains six exons and is located on zebrafish chromosome 8; the structure of the gene is highly homologous to human DJ-1, except that there are no alternatively spliced non-coding 5' exons. The single zDJ-1 first exon shows 5' end heterogeneity, reflecting multiple transcription start sites. In the adult zebrafish brain, zDJ-1 immunoreactivity was prominent in the cytoplasm of most neurons, and in the neuropil, but was less evident within white matter tracts, consistent with neuronal somatic and dendritic localization. Dopaminergic neurons in each of the major forebrain and diencephalic TH-positive cell groups expressed zDJ-1. These studies show that zDJ-1 is very similar to human DJ-1 and delineate essential resources, allowing

further examination of the function and regulation of DJ-1, using the zebrafish as a model.
Descriptors: zebrafish embryos, rats, mice, dopamine metabolism, molecular evolution, gene expression physiology, developmental gene expression regulation physiology, intracellular signaling peptides and proteins metabolism, nerve tissue proteins metabolism, oncogene proteins metabolism, Northern blotting and Western blotting methods, human chromosomes pair 8, molecular cloning methods immunohistochemistry methods, in situ hybridization methods, intracellular signaling peptides and nerve tissue neurons metabolism, oncogene messenger RNA metabolism, reverse transcriptase PCR methods, sequence alignment methods, tyrosine 3 monooxygenase metabolism, *Xenopus*.

Bai, S., R. Thummel, A.R. Godwin, H. Nagase, Y. Itoh, L. Li, R. Evans, J. McDermott, M. Seiki, and M.P.J. Sarras (2005). **Matrix metalloproteinase expression and function during fin regeneration in zebrafish: analysis of MT1-MMP, MMP2 and TIMP2.** *Matrix Biology* 24(4): 247-60. ISSN: 0945-053X.
NAL Call Number: QP552.C6
Abstract: Matrix metalloproteinases (MMPs) play key roles in the turnover of extracellular matrix (ECM) and, thereby, function as key regulators of cell-ECM interactions during development. In spite of their importance during developmental processes, relatively little has been reported about the role of these metalloproteinases during limb development and regeneration. To approach the problem of cell-ECM interactions during limb (fin) regeneration, we have utilized zebrafish as an experimental model. Based on previous MMP cloning studies from our laboratory, the current study has focused on the expression of membrane-type 1 metalloproteinase (MT1-MMP), gelatinase A (MMP-2) and endogenous tissue inhibitor 2 of metalloproteinases (TIMP-2) during fin regeneration in adult zebrafish. In situ analysis indicated co-expression of zmt1-mmp, zmmp-2, and ztimp-2 mRNA transcripts in regenerating caudal fins. In situ gelatin-zymography confirmed the presence of active metalloproteinases in regenerating fins. zmt1-mmp, zmmp-2, and ztimp-2 mRNA transcripts were expressed in the blastema and basal epithelium during caudal fin regeneration while expression of type IV collagen [zcol-IV(a5)] transcripts (a basal lamina component) was restricted to the basal epithelium. Fin outgrowth was greatly reduced in the presence of GM6001 (an inhibitor of MMP activity) indicating the importance of these enzymes during fin regeneration. Previous studies by Itoh (EMBO, 2001) indicated that expression of a vertebrate MT1-MMP construct containing only the hemopexin-transmembrane-cytoplasmic domains (MT1HPX) resulted in blockage of MT1-MMP homophilic complex formation and subsequent inhibition of pro-MMP-2 activation. Interference with homophilic complex formation was attributed to expression of the hemopexin domain at the cell surface. Building upon these earlier findings, the current study found that ectopic expression of MT1HPX in fin regenerates inhibited the regeneration process and resulted in a reduction in cell proliferation in the blastema. Taken together, these results indicate that MMPs have an important role during fin regeneration in zebrafish.
Descriptors: zebrafish fin regeneration, enzymologic gene expression regulation, matrix metalloproteinase 2 metabolism, metalloendopeptidases metabolism, regeneration genetics, tissue inhibitor of metalloproteinase 2 metabolism, COS cells, cell proliferation, *Cercopithecus aethiops*, dipeptides pharmacology, enzyme inhibitors pharmacology, epithelium enzymology and metabolism, hemopexin metabolism, matrix metalloproteinase 2 antagonists, inhibitors and genetics, matrix metalloproteinases, membrane associated, metalloendopeptidases antagonists, inhibitors and genetics, messenger RNA genetics and metabolism, tissue inhibitor of metalloproteinase 2 genetics.

Bainy, A.C.D., B.R. Woodin, S. Karchner, M. Hahn, and J.J. Stegernan (2006). **Cloning and expression of two new cytochrome p450 genes (cyp2aa1 and cyp2aa2) in zebrafish (*Danio rerio*)**. *Marine Environmental Research* 62(Suppl. S): S163. ISSN: 0141 1136.
NAL Call Number: QH545.W3M36
Descriptors: zebrafish, toxicology, development, molecular genetics, urinary system, chemical coordination and homeostasis, cloning, laboratory techniques, genetic techniques, immunoblotting, immunologic techniques, semi quantitative reverse transcriptase PCR, genetic techniques, xenobiotic biotransformation.

Baker, M.E. (2004). **Evolutionary analysis of 11 beta -hydroxysteroid dehydrogenase-type 1, -type 2, -type 3 and 17 beta -hydroxysteroid dehydrogenase-type 2 in fish**. *Federation of European Biochemical Societies Letters* 574(1-3): 167-170. ISSN: 0014-5793.
NAL Call Number: QD415.F41
Descriptors: zebrafish, *Oryzias latipes*, amino acid sequence, dehydrogenases, freshwater fish, genomes, hormones, phylogeny, receptors, sex hormones, steroids, Ciona, *Danio rerio* , *Takifugu* , Teleostei, Tetraodon.

Bakkers, J., M. Camacho Carvajal, M. Nowak, C. Kramer, B. Danger, and M. Hammerschmidt (2005). **Destabilization of DeltaNp63alpha by Nedd4-mediated ubiquitination and Ubc9-mediated sumoylation, and its implications on dorsoventral patterning of the zebrafish embryo.** *Cell Cycle* 4(6): 790-800. ISSN: 1538 4101.
Abstract: We have recently identified the p53-related DeltaNp63 gene as a transcriptional target of Bmp signaling that encodes a transcriptional repressor blocking neural development in the zebrafish ectoderm. However, in contrast to Bmps, the neural-repressing effect of forced DeltaNp63alpha expression is restricted to the presumptive forebrain, while posterior regions of the brain are not affected. Here, we show that this is due to instability of DeltaNp63alpha protein on the dorsal side of the embryo. In a yeast-two-hybrid screen, we isolated two DeltaNp63alpha-modifying enzymes, the SUMO-conjugating enzyme Ubc9 and the ubiquitin ligase Nedd4. The proteins bind to distinct sites in the C-terminal region of DeltaNp63alpha, which are absent in the shorter and more stable DeltaNp63gamma isoform. Similarly, mutant versions of DeltaNp63alpha unable to bind Nedd4 or Ubc9 are stabilized. DeltaNp63alpha is sumoylated and ubiquitinated both in HEK293 cells and in zebrafish embryos, and Nedd4 promotes ubiquitination and instability of DeltaNp63alpha protein, with lysine residue 637 serving as a potential alternative sumoylation and ubiquitination site that is crucial for DeltaNp63alpha destabilization. In zebrafish, ubc9.1 and nedd4 show restricted expression on the dorsal side of the embryo, where DeltaNp63alpha instability can be overcome upon blockage of endogenous Nedd4 activity, or upon injection of mutant versions of DeltaNp63alpha that are unable to bind Nedd4 or Ubc9. This results in a more widespread neural repression, affecting the entire Bmp-sensitive neuroectoderm. In sum, our data indicate that DeltaNp63alpha is ubiquitinated in a Nedd4- and sumoylated in a Ubc9-dependent fashion, and that these modifications can regulate DeltaNp63alpha stability in the zebrafish ectoderm.
Descriptors: zebrafish embryos, mice, body patterning, metabolism, protein processing, post translational, ubiquitin conjugating enzymes metabolism, amino acid sequence, cultured cells, ectoderm cytology and metabolism, gastrula cytology and metabolism, gene expression, lysine metabolism, mice, molecular sequence data, mutation genetics, protein binding, messenger RNA genetics, sumo 1 protein metabolism , tissue culture techniques.

Balciunas, D., A.E. Davidson, S. Sivasubbu, S.B. Hermanson, Z. Welle, and S.C. Ekker (2004). **Enhancer trapping in zebrafish using the Sleeping Beauty transposon.** *BioMed Central Medical Genomics* 5(1): 62. ISSN: 1755 8794.

Abstract: BACKGROUND: Among functional elements of a metazoan gene, enhancers are particularly difficult to find and annotate. Pioneering experiments in *Drosophila* have demonstrated the value of enhancer "trapping" using an invertebrate to address this functional genomics problem. RESULTS: We modulated a Sleeping Beauty transposon-based transgenesis cassette to establish an enhancer trapping technique for use in a vertebrate model system, zebrafish *Danio rerio*. We established 9 lines of zebrafish with distinct tissue- or organ-specific GFP expression patterns from 90 founders that produced GFP-expressing progeny. We have molecularly characterized these lines and show that in each line, a specific GFP expression pattern is due to a single transposition event. Many of the insertions are into introns of zebrafish genes predicted in the current genome assembly. We have identified both previously characterized as well as novel expression patterns from this screen. For example, the ET7 line harbors a transposon insertion near the mkp3 locus and expresses GFP in the midbrain-hindbrain boundary, forebrain and the ventricle, matching a subset of the known FGF8-dependent mkp3 expression domain. The ET2 line, in contrast, expresses GFP specifically in caudal primary motoneurons due to an insertion into the poly(ADP-ribose) glycohydrolase (PARG) locus. This surprising expression pattern was confirmed using in situ hybridization techniques for the endogenous PARG mRNA, indicating the enhancer trap has replicated this unexpected and highly localized PARG expression with good fidelity. Finally, we show that it is possible to excise a Sleeping Beauty transposon from a genomic location in the zebrafish germline. CONCLUSIONS: This genomics tool offers the opportunity for large-scale biological approaches combining both expression and genomic-level sequence analysis using as a template an entire vertebrate genome.

Descriptors: zebrafish, DNA transposable elements, enhancer elements genetics, genomics methods, embryonic development genetics, gene transfer techniques, germ cells, glycoside hydrolases genetics, green fluorescent proteins biosynthesis, in situ hybridization, motor neurons metabolism, pilot projects, PCR, promoter regions genetics , DNA sequence analysis methods.

Balter, M. (2005). **Genetics: zebrafish researchers hook gene for human skin color.** *Science* 310(5755): 1754-5. ISSN: 0036-8075.

NAL Call Number: 470Sci2

Descriptors: zebrafish, antiporters genetics, skin pigmentation genetics, African Americans genetics, African continental ancestry group genetics, alanine genetics, alleles, antiporters chemistry, antiporters physiology, Asian continental ancestry group genetics, European continental ancestry group genetics, genes, mutation, selection genetics, threonine genetics, variation genetics, proteins chemistry and physiology.

Baraban, S.C., M.T. Dinday, P.A. Castro, S. Chege, S. Guyenet, and M.R. Taylor (2007). **A large-scale mutagenesis screen to identify seizure-resistant zebrafish.** *Epilepsia* 48(6): 1151-7. ISSN: 0013 9580.

Abstract: PURPOSE: Zebrafish are a vertebrate organism ideally suited to mutagenesis screening strategies. Although a genetic basis for seizure susceptibility and epilepsy is well established, no efforts have been made to study seizure resistance. Here we describe a novel strategy to isolate seizure-resistant zebrafish mutants from a large-scale mutagenesis screen. METHODS: Seizures were induced with pentylenetetrazole (PTZ). Zebrafish were analyzed between 3 and 7 days postfertilization (dpf). Genome mutations were induced in founders

by using N-ethyl-nitrosourea (ENU). Seizure behavior was monitored by using a high-speed camera and quantified by locomotion-tracking software. Electrographic activity was monitored by using a field-recording electrode placed in the optic tectum of agar-immobilized zebrafish. RESULTS: Short-term PTZ exposure elicited a burst-suppression seizure pattern in 3-dpf zebrafish and more complex activity consisting of interictal- and ictal-like discharges at 7 dpf. Prolonged exposure to PTZ induced status epilepticus-like seizure activity and fatality in wild-type zebrafish larvae. With a PTZ survival assay at 6-7 dpf, we identified six zebrafish mutants in a forward-genetic screen covering nearly 2,000 F(2) families. One mutant (s334) also was shown to exhibit reduced behavioral activity on short-term PTZ exposure and an inability to generate long-duration ictal-like discharge. CONCLUSIONS: Zebrafish offers a powerful tool for the identification and study of a genetic basis for seizure resistance.
Descriptors: zebrafish, disease models, genetic screening, mutagenesis genetics, seizures prevention and control, behavior drug effects, behavior physiology, genetic predisposition to disease genetics, larva genetics, larva physiology, locomotion drug effects, locomotion genetics, pentylenetetrazole pharmacology, seizures chemically induced, seizures epidemiology, status epilepticus chemically induced, status epilepticus prevention and control, superior colliculus physiology.

Bardet, P.L., S. Obrecht Pflumio, C. Thisse, V. Laudet, B. Thisse, and J.M. Vanacker (2004). **Cloning and developmental expression of five estrogen-receptor related genes in the zebrafish.** *Development Genes and Evolution* 214(5): 240-9. ISSN: 0949 944X.
NAL Call Number: QL951.R68
Abstract: Estrogen-receptor related receptors (ERRalpha, beta and gamma) are so-called orphan (i.e. for which no natural ligand has been identified to date) nuclear receptors that are closely related to the estrogen receptors. To gain insights into the possible functions of ERRs during early development, we have cloned their homologs in the zebrafish. Five err cDNA types were recovered in a PCR screen. Transient transfection experiments indicated that zebrafish ERRalpha, ERRgamma and ERRdelta display transcriptional activities that are identical to those of their mammalian counterparts. The expression patterns of err genes were determined during zebrafish development. Tissues such as the hindbrain or the pronephric tubes express several errs whereas others, such as the presumptive mesoderm at the level of the margin, specifically express a single err. Our analysis also points to tissues in which err expression is conserved through evolution, such as slow muscles that specifically express erralpha, suggesting important functions in these tissues.
Descriptors: zebrafish, molecular cloning, developmental gene expression regulation, estrogen receptors genetics, amino acid sequence, molecular sequence data, phylogeny, cytoplasmic receptors and nuclear.

Barnes, D.W., C.J. Mattingly, A. Parton, L.M. Dowell, C.J. Bayne, and J.N. Forrest (2004). **Marine organism cell biology and regulatory sequence discovery in comparative functional genomics**. *Cytotechnology* 46(2-3): 123-137. ISSN: 0920-9069.
NAL Call Number: QH585.C97
Descriptors: zebrafish, heart urchins, little skate, piked dogfish, sand dollars, sea urchins, spiny dogfish alternative splicing, bioinformatics, cell culture, computer applications, conserved sequence, gene expression, gene regulation, introns, marine organisms, regulatory sequences, reviews, structure-function relationships, transcription, genomics, molecular evolution, *Ciona intestinalis*, *Danio rerio*, echinoidea, Elasmobranchii, *Fugu rubripes*, *raja erinacea*, *Squalus acanthias*, *Strongylocentrotus purpuratus*, *Tetraodon nigroviridis*.
Notes: A review.

Bartfai, R., C. Balduf, T. Hilton, Y. Rathmann, Y. Hadzhiev, L. Tora, L. Orban, and F. Muller (2004). **TBP2, a vertebrate-specific member of the TBP family, is required in embryonic development of zebrafish.** *Current Biology* 14(7): 593-8. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Abstract: TATA binding protein (TBP) is a key regulator of RNA polymerase transcription. It binds to core promoters, often in large multiprotein complexes, and nucleates RNA polymerase II (Pol II) transcription initiation. In addition to the previously described TBP-like factor present in metazoans (TLF/TRF2/TRP/TLP), we describe a third, vertebrate-specific member of the TBP protein family from zebrafish, called TBP2. Evolutionary conserved TBP2 homologs were also found in human, mouse, frog, and pufferfish. The N-terminal domains of TBP2s are divergent amongst themselves and different from those of TBPs; however, the core domain of TBP2s and TBPs are almost identical. TBP2 binds the TATA box, interacts with TFIIA and TFIIB (similarly to TBP), and can mediate Pol II transcription initiation. However, TBP2 shows contrasting expression patterns in the gonads and during embryonic development in comparison to TBP, suggesting differential function. Knockdown of zebrafish TBP2 results in specific reduction of the protein level, leading to a phenotype, which indicates the requirement of TBP2 for embryonic patterning. The presence of three different TBP family members in vertebrates suggests the existence of developmental stage- and tissue-specific preinitiation complexes with specific requirements for different TBP family members.
Descriptors: zebrafish embryonic development, gene expression profiling, TATA box binding protein genetics and metabolism, base sequence, Western blotting, complementary DNA genetics, gene components, in situ hybridization, molecular sequence data, radiation hybrid mapping, reverse transcriptase PCR, DNA sequence analysis.

Bartfai, R. and L. Orban (2003). **The vasa locus in zebrafish: multiple RGG boxes from duplications**. *DNA and Cell Biology* 22(1): 47-54. ISSN: 1044 5498.
NAL Call Number: QH443.D5
Abstract: Vasa is the most extensively characterized germ cell-specific marker in metazoans. We determined the sequence of the zebrafish vasa locus, which - together with the flanking regions - is 25 kb long. It contains 26+1 exons, with an average length of 83 bp. The 5' end of zebrafish vasa is rich in repeats; it includes a peculiar tandem repeat with three units spanning a total of eight exons, each unit containing several tandemly arranged RNA-binding motifs (RGG boxes). The presence of various, nonmicrosatellite type repeats in vasa seems to be universal among the species studied, and could have functional importance during the evolution of the gene, due to the increase in the number of RGG boxes located in these repeats. Analysis of vasa transcripts in zebrafish identified numerous isoforms resulting from alternative splicing (seven) and polyadenylation (four). Mapping to two radiation hybrid panels strengthened the position of the gene on LG10 of zebrafish. Copyright © 2008 CABI
Descriptors: zebrafish, gene mapping, genes, genetic analysis, markers, LG10 genetics, germ cells, loci, *Danio rerio*.

Bartman, T., E.C. Walsh, K.K. Wen, M. McKane, J. Ren, J. Alexander, P.A. Rubenstein, and D.Y. Stainier (2004). **Early myocardial function affects endocardial cushion development in zebrafish.** *Public Library of Science Biology* 2(5): E129. ISSN: 1544-9173.
NAL Call Number: QH301.P56
Abstract: Function of the heart begins long before its formation is complete. Analyses in mouse and zebrafish have shown that myocardial function is not required for early steps of organogenesis, such as formation of the heart tube or chamber specification. However,

whether myocardial function is required for later steps of cardiac development, such as endocardial cushion (EC) formation, has not been established. Recent technical advances and approaches have provided novel inroads toward the study of organogenesis, allowing us to examine the effects of both genetic and pharmacological perturbations of myocardial function on EC formation in zebrafish. To address whether myocardial function is required for EC formation, we examined silent heart (sih(-/-)) embryos, which lack a heartbeat due to mutation of cardiac troponin T (tnnt2), and observed that atrioventricular (AV) ECs do not form. Likewise, we determined that cushion formation is blocked in cardiofunk (cfk(-/-)) embryos, which exhibit cardiac dilation and no early blood flow. In order to further analyze the heart defects in cfk(-/-) embryos, we positionally cloned cfk and show that it encodes a novel sarcomeric actin expressed in the embryonic myocardium. The Cfk(s11) variant exhibits a change in a universally conserved residue (R177H). We show that in yeast this mutation negatively affects actin polymerization. Because the lack of cushion formation in sih- and cfk-mutant embryos could be due to reduced myocardial function and/or lack of blood flow, we approached this question pharmacologically and provide evidence that reduction in myocardial function is primarily responsible for the defect in cushion development. Our data demonstrate that early myocardial function is required for later steps of organogenesis and suggest that myocardial function, not endothelial shear stress, is the major epigenetic factor controlling late heart development. Based on these observations, we postulate that defects in cardiac morphogenesis may be secondary to mutations affecting early myocardial function, and that, in humans, mutations affecting embryonic myocardial function may be responsible for structural congenital heart disease.

Descriptors: zebrafish, endocardium embryology, developmental gene expression regulation, heart embryology, myocardium pathology, actins chemistry and metabolism, genetically modified, chromosome mapping, molecular cloning, genetic epigenesis, genotype, green fluorescent proteins metabolism, heart diseases pathology, in situ hybridization, genetic models, mutation, oligonucleotides chemistry, phenotype, RNA chemistry, time factors, transgenes.

Bass, T., M. Ebert, M. Hammerschmidt, and M. Frank (2007). **Differential expression of four protocadherin alpha and gamma clusters in the developing and adult zebrafish: DrPcdh2gamma but not DrPcdh1gamma is expressed in neuronal precursor cells, ependymal cells and non-neural epithelia.** *Development Genes and Evolution* 217(5): 337-51. ISSN: 0949 944X.

NAL Call Number: QL951.R68

Abstract: Protocadherins are cadherin-like molecules with adhesive and signaling functions, in particular, during neuronal development. Large protocadherin (Pcdh) gene clusters are present in the genome of vertebrates. In the zebrafish, two Pcdh clusters are found on chromosomes 10 (DrPcdh1) and 14 (DrPcdh2), each divided into subclusters of DrPcdhalpha and DrPcdhgamma family genes. In total, about 100 different DrPcdh molecules are predicted. We have analyzed the expression of the four DrPcdh subclusters and find that DrPcdh transcripts are upregulated in the developing zebrafish nervous system. In the adult fish brain, all four DrPcdh clusters are expressed in differentiated neurons, in particular, in the thalamic nuclei, tectum, and cerebellum. We show that expression patterns grossly overlap for each cluster but with regional differences and variations in strength of expression. Strikingly, the DrPcdh2gamma cluster, distinct from the three other clusters, is also expressed in neuronal precursor cells and ependymal cells of the embryonic and adult nervous system, as well as in specific non-neuronal epithelia. Antibodies to a conserved motif in the constant region of DrPcdh2gamma stain fiber tracts and neuropil of the zebrafish brain and cell-cell junctions in epithelia. Our results indicate that multiple DrPcdhs of the different clusters are

expressed in differentiated zebrafish neurons, suggesting evolutionarily conserved functions of protocadherin clusters in cell adhesion and signaling. In addition, DrPcdh2gamma may exert more specific roles in neuronal precursor and non-neural epithelial cells, which have not yet been described for mammalian Pcdhgamma. Thus, our findings in zebrafish open new perspectives to examine these functions in other vertebrate model organisms.
Descriptors: zebrafish, cadherins genetics, DrPcdh2gamma, ependyma metabolism, epithelium metabolism, developmental gene expression regulation, neurons metabolism, stem cells metabolism, brain cytology and embryology, cadherins metabolism, digestive system metabolism, ependyma cytology, nervous system metabolism, neurons cytology, proliferating cell nuclear antigen metabolism, protein transport, messenger RNA genetics and metabolism, stem cells cytology, synapses metabolism, up regulation genetics, proteins genetics.

Bassett, D.I. (2003). **Identification and developmental expression of a macrophage stimulating 1/ hepatocyte growth factor-like 1 orthologue in the zebrafish.** *Development Genes and Evolution* 213(7): 360-2. ISSN: 0949 944X.
NAL Call Number: QL951.R68
Abstract: The cytokine Macrophage Stimulating 1 (MST1/MSP/Hepatocyte Growth Factor-Like) is a ligand of the Met-related MST1-Receptor (MST1R/RON). Although MST1-deficient mice are viable, MST1R is essential in mice before gastrulation for implantation, and is a known oncogene in man. Here I report the identification, sequence, chromosomal location and embryonic expression of a novel zebrafish orthologue, termed macrophage stimulating 1 (mst1). mst1 shows a striking restriction of expression to the dorsal side of the embryo prior to gastrulation, and as gastrulation and somitogenesis proceed is expressed sequentially in the presumptive neurectoderm, the notochord, the somites, endodermal cells and in the syncytial yolk. This dynamic pattern is largely conserved in tetrapod vertebrates, suggesting that the appearance of MST1, may have played an early role in the evolution of the vertebrate body plan.
Descriptors: zebrafish embryo, hepatocyte growth factor genetics, proto oncogene embryology, evolution, developmental gene expression regulation, hepatocytegrowth factor analysis, morphogenesis, proto oncogene proteins analysis, receptor protein tyrosine kinases genetics.

Bateman, M.J., R. Cornell, C. d'Alencon, and A. Sandra (2004). **Expression of the zebrafish Staufen gene in the embryo and adult.** *Gene Expression Patterns* 5(2): 273-8. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Staufen, a double stranded RNA binding protein, has been shown to be involved in creating and maintaining cellular asymmetry in the *Drosophila* oocyte, neuroblast, and mammalian neuron. Staufen binds to the 3' UTR of specific mRNAs and acts in their localization and anchoring to various subcellular domains. Staufen's molecular interactions during development have been limited to investigations in *Drosophila* melanogaster. Since a vertebrate Staufen has not been studied in a developmental system, the aim of this study was to clone and characterize a staufen orthologue gene in the vertebrate developmental model, zebrafish. The zebrafish staufen-like sequence shows a 64% homology to the human staufen with a 81.2% homology in the highly conserved double stranded RNA binding domain (dsRBDs). Staufen maps on the LN54 radiation hybrid panel to linkage group 6, 16.25 cR from Z265 between fb22h06 and fi16e01. Northern blot and in situ hybridization showed that staufen is expressed both maternally and zygotically. Zygotically expressed staufen is localized to the developing nervous system and at 24 h is highly concentrated in the subventricular zone of the developing brain. Maternally expressed staufen is dispersed in the mature

oocyte and early embryo. In the adult, staufen is expressed in specific brain nuclei, the testis, neurons and Leydig cells.
Descriptors: zebrafish embryos, RNA binding proteins biosynthesis, metabolism, proteins biosynthesis, amino acid sequence, brain embryology, brain growth and development, brain metabolism, *Drosophila*, metabolism, developmental gene expression regulation, in situ hybridization, Leydig cells metabolism, molecular sequence data, RNA binding amino acid sequence homology.

Bates, M.D., J.M. Well, and B.A. Venkatesh (2005). **Comparative genomics of the Hlx homeobox gene and protein: conservation of structure and expression from fish to mammals**. *Gene* 352: 45-56. ISSN: 0378 1119.
NAL Call Number: QH442.A1G4
Abstract: Hlx is a homeobox transcription factor gene that is expressed in intestinal and hepatic mesenchyme of the developing mouse embryo and is essential for normal intestinal and hepatic development. Because of the morphological and molecular similarities in the development of the digestive system across species, we hypothesized that the Hlx gene and protein sequences and expression patterns would be conserved among vertebrates. Comparison of the Hlx gene orthologues of human, chimpanzee, mouse, rat, pufferfish (Fugu) and zebrafish demonstrates that these six genes share an identical organization with four exons and three introns. Comparison of the inferred Hlx protein sequences from these and three additional species (chick, Spanish ribbed newt and rainbow trout) reveals significant sequence identity, with identical homeodomains. The expression of Hlx in the mesenchyme of developing chick embryos is highly similar to that of mouse. Fugu Hlx is expressed in a tissue-specific manner that is similar though not identical to that of mouse, suggesting a conservation of Hlx function between mammals and birds. The mammalian and fish Hlx genes share a putative 5' upstream enhancer as well as an inverted repeat containing CCAAT boxes on opposite strands that we have previously shown to be important for mouse Hlx gene expression. These results suggest that the function of Hlx and the mechanisms regulating its expression are highly conserved in mammals, birds, amphibians and fish. (c) 2005 Elsevier B.V. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, molecular genetics, biochemistry, molecular biophysics, digestive system, ingestion, assimilation, comparative genomics, genetic techniques, expression pattern.

Baxendale, S., C. Davison, C. Muxworthy, C. Wolff, P.W. Ingham, and S. Roy (2004). **The B-cell maturation factor Blimp-1 specifies vertebrate slow-twitch muscle fiber identity in response to Hedgehog signaling**. *Nature Genetics* 36(1): 88-93. ISSN: 1061-4036.
NAL Call Number: QH426.N37
Descriptors: zebrafish embryos, cell fate, developmental genetics, gene expression, genes, marine fish, muscles, mutations, myoblasts, skeletal muscle, *Danio rerio*, B cells, Blimp 1, Hedgehog gene, slow twitch, u boot.

Becker, T., M. Bossenz, B. Tursun, A. Schluter, M.A. Peters, C.G. Becker, H.P. Ostendorff, and I. Bach (2003). **Comparing protein stabilities during zebrafish embryogenesis.** *Methods in Cell Science* 25(1-2): 85-9. ISSN: 1381-5741.
NAL Call Number: QH573.T52
Abstract: The stabilities of many key proteins are regulated, e.g. via ubiquitination and proteasomal degradation, with important biological consequences. We present a convenient method that allows the analysis and comparison of protein stabilities during embryogenesis

using early zebrafish development as a model system. Basically, this method involves ectopic overexpression of epitope-tagged proteins via mRNA injections in one-to-four-cell stage embryos and subsequent protein detection after various time points. Indeed, the protein stability of the ubiquitin ligase RLIM, which is able to autoubiquitinate and target itself for proteasomal degradation, was much shorter when compared to a protein consisting of a Myc epitope-tag and a nuclear localization domain. Thus, this method may be used more widely for the study of developmental protein stability.
Descriptors: zebrafish, developmental gene expression regulation physiology, RNA stability physiology, messenger RNA metabolism, repressor proteins metabolism, molecular cloning, metabolism, microinjections, protein denaturation .

Bedell, V.M., S.Y. Yeo, K.W. Park, J. Chung, P. Seth, V. Shivalingappa, J. Zhao, T. Obara, V.P. Sukhatme, L. Drummond, D.Y. Li, and R. Ramchandran (2005). **Roundabout 4 is essential for angiogenesis in vivo**. *Proceedings of the National Academy of Sciences of the United States of America* 102(18): 6373-6378. ISSN: 0027 8424.
NAL Call Number: 500N21P
Abstract: Stereotypical patterns of vascular and neuronal networks suggest that specific genetic programs tightly control path determination and, consequently, angiogenesis and axon-guidance mechanisms. Our study focuses on one member of the roundabout family of receptors, which traditionally mediate repulsion from the midline. Here, we characterize a fourth member of this family, roundabout4 (robo4), which is the predominant roundabout (robo) that is expressed in embryonic zebrafish vasculature. Gene knockdown and overexpression approaches show that robo4 is essential for coordinated symmetric and directed sprouting of intersomitic vessels and provide mechanistic insights into this process. Also, human robo4 gene functionally compensates for loss of robo4 gene function, suggesting evolutionary conservation. This article reports an endothelial-specific function for a robo gene in vertebrates in vivo. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics, biochemistry, angiogenesis, evolutionary conservation, axon guidance mechanism, predominant roundabout.

Beetz, S., D. Diekhoff, and L.A. Steiner (2007). **Characterization of terminal deoxynucleotidyl transferase and polymerase mu in zebrafish.** *ImmunoGenetics* 59(9): 735-44. ISSN: 0093-7711.
NAL Call Number: QR184.I4
Abstract: Terminal deoxynucleotidyl transferase (TdT) contributes to the junctional diversity of immunoglobulin and T-cell receptors by incorporating nucleotides in a template-independent manner. A closely related enzyme, polymerase mu (polmu), a template-directed polymerase, plays a role in general end-joining double-strand break repair. We cloned zebrafish TdT and polmu and found them to be 43% identical in amino acid sequence. Comparisons with sequences of other species revealed conserved residues typical for TdT in the zebrafish sequence that support the template independence of this enzyme. Some but not all of these features were identified in zebrafish polmu. In adult fish, TdT expression was most prominent in thymus, pro- and mesonephros, the primary lymphoid organs in teleost fish and in spleen, intestine, and the tissue around the intestine. Polmu expression was detected not only in pro- and mesonephros, the major sites for B-lymphocyte development, but also in ovary and testis and in all tissue preparations to a low extent. TdT expression starts at 4 dpf and increases thereafter. Polmu is expressed at all times to a similar extent. In situ studies showed a strong expression of TdT and polmicro in the thymic cortex of 8-week-old fish. The characterization of zebrafish TdT and polmu provide new insights in fish

lymphopoiesis and addresses the importance and evolution of TdT and polmu themselves.
Descriptors: zebrafish , DNA nucleotidylexotransferase metabolism , DNA directed DNA polymerase metabolism, amino acid sequence, DNA nucleotidylexotransferase genetics, DNA directed DNA polymerase genetics, molecular sequence data, organ specificity, phylogeny.

Bellingham, J., S.S. Chaurasia, Z. Melyan, C. Liu, M.A. Cameron, E.E. Tarttelin, P.M. Iuvone, M.W. Hankins, G. Tosini, and R.J. Lucas (2006). **Evolution of melanopsin photoreceptors: discovery and characterization of a new melanopsin in nonmammalian vertebrates**. *Public Library of Science Biology* 4(8): 1334-1343. ISSN: 1544 9173.
Online: http://biology.plosjournals.org/perlserv/?request=get-pdf&file=10.1371_journal.pbio.0040254-S.pdf
NAL Call Number: QH301.P56
Abstract: In mammals, the melanopsin gene (Opn4) encodes a sensory photopigment that underpins newly discovered inner retinal photoreceptors. Since its first discovery in *Xenopus laevis* and subsequent description in humans and mice, melanopsin genes have been described in all vertebrate classes. Until now, all of these sequences have been considered representatives of a single orthologous gene (albeit with duplications in the teleost fish). Here, we describe the discovery and functional characterization of a new melanopsin gene in fish, bird and amphibian genomes, demonstrating that, in fact, the vertebrates have evolved two quite separate melanopsins. On the basis of sequence similarity, chromosomal localization and phylogeny, we identify our new melanopsins as the true orthologues of the melanopsin gene previously described in mammals and term this grouping Opn4m. By contrast, the previously published melanopsin genes in nonmammalian vertebrates represent a separate branch of the melanopsin family which we term Opn4x. Reverse transcription-polymerase chain reaction (RT-PCR) analysis in chicken, zebrafish and *Xenopus* identifies expression of both Opn4m and Opn4x genes in tissues known to be photosensitive (eye, brain and skin). In the day-14 chicken eye, Opn4m messenger RNA is found in a subset of cells in the outer nuclear, inner nuclear and ganglion cell layers, the vast majority of which also express Opn4x. Importantly, we show that a representative of the new melanopsins (chicken Opn4m) encodes a photosensory pigment capable of activating G protein signalling cascades in a light- and retinaldehyde-dependent manner under heterologous expression in Neuro-2a cells. A comprehensive in silico analysis of vertebrate genomes indicates that while most vertebrate species have both Opn4m and Opn4x genes, the latter is absent from eutherian and, possibly, marsupial mammals, lost in the course of their evolution as a result of chromosomal reorganization. Thus, our findings show for the first time that nonmammalian vertebrates retain two quite separate melanopsin genes, while mammals have just one. These data raise important questions regarding the functional differences between Opn4x and Opn4m pigments, the associated adaptive advantages for most vertebrate species in retaining both melanopsins and the implications for mammalian biology of lacking Opn4x. Copyright © 2008 CABI
Descriptors: zebrafish, poultry, brain, evolution, eyes, gene expression, genes, messenger RNA, photoreceptors, pigments, PCR, skin, tissue distribution, *Danio rerio*, *Xenopus laevis.*

Ben Abdelkhalek, H., A. Beckers, K. Schuster-Gossler, M.N. Pavlova, H. Burkhardt, H. Lickert, J. Rossant, R. Reinhardt, L.C. Schalkwyk, I. Mueller, B.G. Herrmann, M. Ceolin, R. Rivera-Pomar, and A. Gossler (2004). **The mouse homeobox gene NOT is required for caudal notochord development and affected by the truncate mutation**. *Genes & Development* 18(14): 1725-1736 . ISSN: 0890-9369.
NAL Call Number: QH426.G46

Descriptors: zebrafish, mice, alleles, biological development, cell fate, developmental genetics, embryonic development, freshwater fish, genes, homeobox, mutations, NOT protein, notochord, pattern formation, phenotypes, phenylalanine, population genetics, stem cells, *Danio rerio*, *Xenopus*, truncate gene, floating head protein.

Ben, J., E.W. Jabs, and S.S. Chong (2005). **Genomic, cDNA and embryonic expression analysis of zebrafish IRF6, the gene mutated in the human oral clefting disorders Van der Woude and popliteal pterygium syndromes.** *Gene Expression Patterns* 5(5): 629-38. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Van der Woude syndrome (VWS) and popliteal pterygium syndrome (PPS) are autosomal dominant clefting disorders recently discovered to be caused by mutations in the IRF6 (Interferon Regulatory Factor 6) gene. The IRF gene family consists of nine members encoding transcription factors that share a highly conserved helix-turn-helix DNA-binding domain and a less conserved protein-binding domain. Most IRFs regulate the expression of interferon-alpha and -beta after viral infection, but the function of IRF6 remains unknown. We have isolated a full-length zebrafish irf6 cDNA, which encodes a 492 amino acid protein that contains a Smad-IRF interaction motif and a DNA-binding domain. The zebrafish irf6 gene consists of eight exons and maps to linkage group 22 closest to marker unp1375. By in situ hybridization analysis of embryo whole-mounts and cryosections, we demonstrate that irf6 is first expressed as a maternal transcript. During gastrulation, irf6 expression was concentrated in the forerunner cells. From the bud stage to the 3-somite stage, irf6 expression was observed in the Kupffer's vesicle. No expression could be detected at the 6-somite and 10-somite stages. At the 14-somite stage, expression was detected in the otic placode. At the 17-somite stage, strong expression was also observed in the cloaca. During the pharyngula, hatch and larva periods up to 5 days post-fertilization, irf6 was expressed in the pharyngeal arches, olfactory and otic placodes, and in the epithelial cells of endoderm derived tissues. The latter tissues include the mouth, pharynx, esophagus, endodermal lining of swim bladder, liver, exocrine pancreas, and associated ducts. Overall, the zebrafish expression data are consistent with the observations of lip pits in VWS patients, as well as more recent reports of alae nasi, otitis media and sensorineural hearing loss documented in some patients.

Descriptors: zebrafish, cleft lip genetics, cleft palate genetics, DNA binding proteins genetics, proteins biosynthesis and genetics, developmental gene expression regulation, transcription factors biosynthesis and genetics, proteins biosynthesis and genetics, amino acid motifs and sequence, base sequence, chromosome mapping, molecular cloning, DNA metabolism, complementary DNA metabolism, epithelial cells cytology and metabolism, gastrula metabolism, in situ hybridization, interferon regulatory factors, linkage genetics, mice, molecular sequence data, mutation , open reading frames, phylogeny, tertiary protein structure, amino acid sequence homology, syndrome, time factors.

Bendig, G., M. Grimmler, I.G. Huttner, G. Wessels, T. Dahme, S. Just, N. Trano, H.A. Katus, M.C. Fishman, and W. Rottbauer (2006). **Integrin-linked kinase, a novel component of the cardiac mechanical stretch sensor, controls contractility in the zebrafish heart.** *Genes and Development* 20(17): 2361-72. ISSN: 0890-9369.

NAL Call Number: QH426.G46

Abstract: The vertebrate heart possesses autoregulatory mechanisms enabling it first to sense and then to adapt its force of contraction to continually changing demands. The molecular components of the cardiac mechanical stretch sensor are mostly unknown but of immense medical importance, since dysfunction of this sensing machinery is suspected to be respon-

sible for a significant proportion of human heart failure. In the hearts of the ethylnitros-urea (ENU)-induced, recessive embryonic lethal zebrafish heart failure mutant main squeeze (msq), we find stretch-responsive genes such as atrial natriuretic factor (anf) and vascular endothelial growth factor (vegf) severely down-regulated. We demonstrate through positional cloning that heart failure in msq mutants is due to a mutation in the integrin-linked kinase (ilk) gene. ILK specifically localizes to costameres and sarcomeric Z-discs. The msq mutation (L308P) reduces ILK kinase activity and disrupts binding of ILK to the Z-disc adaptor protein beta-parvin (Affixin). Accordingly, in msq mutant embryos, heart failure can be suppressed by expression of ILK, and also of a constitutively active form of Protein Kinase B (PKB), and VEGF. Furthermore, antisense-mediated abrogation of zebrafish beta-parvin phenocopies the msq phenotype. Thus, we provide evidence that the heart uses the Integrin-ILK-beta-parvin network to sense mechanical stretch and respond with increased expression of ANF and VEGF, the latter of which was recently shown to augment cardiac force by increasing the heart's calcium transients.

Descriptors: zebrafish, mechanotransduction, cellular physiology, myocardial contraction physiology, myocardium enzymology, protein serine threonine kinases physiology, actinin physiology, amino acid sequence, atrial natriuretic factor antagonists, inhibitors, biosynthesis and genetics, calcium metabolism, down regulation genetics, genetic markers, mechanotransduction, cellular genetics, molecular sequence data, myocardial contraction genetics, protein serine threonine kinases deficiency and genetics, vascular endothelial growth factor A antagonists, inhibitors, biosynthesis and genetics.

Bennett, J.T., H.L. Stickney, W.Y. Choi, B. Ciruna, W.S. Talbot, and A.F. Schier (2007). **Maternal nodal and zebrafish embryogenesis.** *Nature* 450(7167): E1-2- E2-4. ISSN: 0028-0836.

NAL Call Number: 472 N21

Abstract: In fish and amphibians, the dorsal axis is specified by the asymmetric localization of maternally provided components of the Wnt signalling pathway. Gore et al. suggest that the Nodal signal Squint (Sqt) is required as a maternally provided dorsal determinant in zebrafish. Here we test their proposal and show that the maternal activities of sqt and the related Nodal gene cyclops (cyc) are not required for dorsoventral patterning.

Descriptors: zebrafish, body patterning, intracellular signaling peptides and proteins metabolism, proteins metabolism, intracellular signaling peptides and mothers, ovary metabolism, ovum metabolism, RNA splicing, reproducibility of results, metabolism.

Berezikov, E. and R.H. Plasterk (2005). **Camels and zebrafish, viruses and cancer: a microRNA update.** *Human Molecular Genetics* 14(Spec No. 2): R183-90. ISSN: 0964-6906.

NAL Call Number: RB155.5.H87

Abstract: MicroRNAs (miRNAs) form an extensive class of RNA molecules that regulate gene expression at post-transcriptional level. In recent years, much progress has been made in dissection of biogenesis and functions of miRNAs. There are at least several hundred miRNA genes in the human genome, and the emerging evidence suggests that miRNAs are broadly implicated in gene regulation. Here, we review some recent advances, and particularly we discuss how comparative genomics helps to identify novel miRNA genes, how studies in zebrafish reveal roles of miRNAs in morphogenesis, how changes in miRNA expression patterns are connected with cancer and how host-virus coevolution exploits miRNA regulatory pathways.

Descriptors: zebrafish, gene expression regulation, microRNAs genetics, neoplasms etiology, viruses genetics, molecular evolution, genomics, in situ hybridization, microRNAs metabolism, microRNAs physiology, morphogenesis genetics, neoplasms genetics, species specificity.

Berger, J. and P. Currie (2007). **The role of zebrafish in chemical genetics.** *Current Medicinal Chemistry* 14(22): 2413-20. ISSN: 0929 8673.

Abstract: The identification and exploration of new drug candidates to fight diseases is a major imperative for improving human health. The traditional mechanism utilised to identify new compounds with therapeutic potential has been to systematically analyse large libraries of small molecules for lead compounds with a desired bioactivity in protein or cell based assays. Identified lead compounds were subsequently assessed for their potential as lead drugs. In the last few years, small molecule screens were also carried out in vivo, on whole organisms such as the zebrafish. Cost efficient maintenance together with abundant manipulatory techniques and molecular tools have made the zebrafish a preferred system in which to perform large-scale screens. Numerous studies have revealed that zebrafish mutants can accurately model many aspects of human diseases. Therefore, small molecules identified in zebrafish-based screens can be particularly valuable in identifying lead compounds with direct therapeutic relevance to specific human disease states. Here, we review the role of zebrafish-based screens in the emerging field of chemical genetics.

Descriptors: zebrafish, preclinical drug evaluation, gene regulatory networks, signal transduction, chemical genetics.

Berghmans, S., R.D. Murphey, E. Wienholds, D. Neuberg, J.L. Kutok, C.D. Fletcher, J.P. Morris, T.X. Liu, S. Schulte Merker, J.P. Kanki, R. Plasterk, L.I. Zon, and A.T. Look (2005). **Tp53 mutant zebrafish develop malignant peripheral nerve sheath tumors.** *Proceedings of the National Academy of Sciences of the United States of America* 102(2): 407-12. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: TP53 is the most frequently mutated tumor suppressor gene in human cancer, with nearly 50% of all tumors exhibiting a loss-of-function mutation. To further elucidate the genetic pathways involving TP53 and cancer, we have exploited the zebrafish, a powerful vertebrate model system that is amenable to whole-genome forward-genetic analysis and synthetic-lethal screens. Zebrafish lines harboring missense mutations in the tp53 DNA-binding domain were identified by using a target-selected mutagenesis strategy. Homozygous mutant fish from two of these lines were viable and exhibited mutations similar to those found in human cancers (tp53(N168K) and tp53(M214K)). Although homozygous tp53(N168K) mutants were temperature-sensitive and suppressed radiation-induced apoptosis only at 37 degrees C, cells in the tp53(M214K) embryos failed to undergo apoptosis in response to gamma radiation at both 28 and 37 degrees C. Unlike wild-type control embryos, irradiated tp53(M214K) embryos also failed to up-regulate p21 and did not arrest at the G(1)/S checkpoint. Beginning at 8.5 months of age, 28% of tp53(M214K) mutant fish developed malignant peripheral nerve sheath tumors. In addition to providing a model for studying the molecular pathogenic pathways of malignant peripheral nerve sheath tumors, these mutant zebrafish lines provide a unique platform for modifier screens to identify genetic mutations or small molecules that affect tp53-related pathways, including apoptosis, cell-cycle delay, and tumor suppression.

Descriptors: zebrafish, p53 genes physiology, nerve sheath neoplasms etiology, alleles, apoptosis radiation effects, mutation.

Bernardos, R.L. and P.A. Raymond (2006). **GFAP transgenic zebrafish.** *Gene Expression Patterns* 6(8): 1007-13. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: We have generated transgenic zebrafish that express green fluorescent protein

(GFP) in glial cells driven by the zebrafish glial fibrillary acidic protein (GFAP) regulatory elements. Transgenic lines Tg(gfap:GFP) were generated from three founders; the results presented here are from the mi2001 line. GFP expression was first visible in the living embryo at the tail bud-stage, then in the developing brain by the 5-somite-stage (approximately 12 h post-fertilization, hpf) and then spreading posteriorly along the developing spinal cord by the 12-somite stage (approximately 15 hpf). At 24 hpf GFP-expressing cells were in the retina and lens. By 72 hpf GFP expression levels were strong and localized to the glia of the brain, neural retina, spinal cord, and ventral spinal nerves, with moderate expression in the enteric nervous system and weaker levels in the olfactory sensory placode and otic capsule. GFP expression in glia co-localized with anti-GFAP antibodies, but did not co-localize with the neuronal antibodies HuC/D or calretinin in mature neurons.

Descriptors: zebrafish, genetically modified embryology, genetically modified metabolism, glial fibrillary acidic protein genetics, central nervous system embryology and metabolism gene expression, green fluorescent proteins metabolism, neuroglia metabolism, tissue distribution, transgenes.

Bernardos, R.L., M.S. Wolfe, and P.A. Raymond (2004). **Notch-delta signaling is required for mueller glia differentiation in the zebrafish retina**. *Investigative Ophthamology and Visiual Science* 45(Suppl. 2): U633. ISSN: 0146 0404.

Abstract: Purpose: Notch-Delta signaling is involved in cell fate decisions. Notch is a Type I transmembrane receptor that binds to Delta, a transmembrane ligand. Ligand-receptor binding triggers intramembrane proteolysis, releasing the intracellular domains (ICD) of both receptor and ligand, which translocate to the nucleus to alter gene transcription. Two models of Notch-Delta signaling in the nervous system have been proposed: lateral inhibition in which Delta ICD promotes neuron differentiation and Notch ICD promotes continued proliferation, and art instructive mechanism in which Notch ICD promotes gliogenesis. The goal ofthe experiments presented here was to clarify the role of Notch-Delta signaling during retinal neurogenesis and gliogenesis.Methods: We used gamma-secretase inhibitors that prevent intramembrane proteolysis of both Delta and Notch ICD, which were applied to zebrafish embryos beginning at 9 hours post-fertilization (hpf) and ending at different time points.The effects on retinal development were assayed by immunocytochemistry and insitu hybridization.Results: After blocking Notch-Delta signaling from 9 to 65 hpf, neuronal markers for photoreceptor, and ganglion cells were expressed, but retinal lamination was disrupted. In contrast, Mueller glia (MG) were absent from the retina, whereas GFAP-labeled astrocytes were present, but reduced, in the brain. When embryos were removed from the drug at 48 hpf and continued developing in drug-free embryo media until 96 hpf, retinogenesis recovered, and laminated retina, including MG, was generated by retinal progenitors at the periphery. However, in embryos that remained in the drug from 9 to 65 hpf and were then transferred to embryo media to develop to 96 hpf, no recovery was observed and no MG were produced. Mitotic activity was similar in control and drug-treated embryos, with numerous mitotic cells present at 48hpf and a steady decrease in the number of mitotic cell a as development continued. TUNEL-labeling revealed a significant increase in apoptosis in the retina of drug-treated embryos compared tocontrols at 65hpf.Conclusions: Notch-Delta signaling in the zebrafish retina has an instructive role and is essential for MG differentiation but is not required for retinal neurons to differentiate. We further propose that the lack of MG in the drug-treated zebrafish embryos may result in the absence of laminar organization, suggesting that MG are present in the developing retina at an earlier time than generally believed. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, sense organs, sensory reception, apoptosis, cell differentiation, mitosis, retinogenesis, gliogenesis, retinal neurogenesis, retinas, Notch-Delta signaling.

Bertrand, S., S. Pinte, N. Stankovic Valentin, S. Deltour Balerdi, C. Guerardel, A. Begue, V. Laudet, and D. Leprince (2004). **Identification and developmental expression of the zebrafish orthologue of the tumor suppressor gene HIC1.** *Biochimica Et Biophysica Acta* 1678(1): 57-66. ISSN: 0006 3002.

NAL Call Number: 381B522

Abstract: Hypermethylated in Cancer 1 (HIC1) is a human tumor suppressor gene located at chromosome 17p13.3 which is frequently hypermethylated and transcriptionally silent in many types of tumors. In addition, its location in the Miller-Dieker syndrome's (MDS) deletion region, its embryonic expression pattern in mice and the phenotype of the HIC1-deficient mice have provided strong evidence for its implication in this contiguous-gene syndrome. HIC1 encodes a five C2H2-type zinc finger transcriptional repressor belonging to the BTB/POZ family. We have isolated the true zebrafish orthologue of human HIC1 since it has a comparable intron-exon structure and since its predicted gene product, ZfHIC1 displays much higher sequence similarities in its overall sequence (737 residues) with human HIC1 (714 residues) than the 454 residues encoded by the only zebrafish HIC1 sequence (AF111712) described so far, which has been renamed ZfHIC1alpha. Notably, the C-terminal end and one zinc finger in the DNA-binding domain are missing in ZfHIC1alpha. As a consequence, ZfHIC1 proteins bind the human HIC1 consensus DNA-binding sequence in vitro, whereas ZfHIC1alpha cannot. Analyses of the expression pattern of ZfHIC1 and of its paralogue ZfHRG22 (HIC1 related gene on chromosome 22) show that they share expression domains with their respective orthologous vertebrate genes. ZfHRG22 is prominently expressed in the brain and in neural tissues. Interestingly, the predominant expression of ZfHIC1 in the mesenchyme of the head, around the nose and the eye and in the branchial arches is possibly consistent with some of the abnormalities seen in the HIC1-deficient mice and provides another clue for the implication of HIC1 in MDS.

Descriptors: zebrafish, amino acid sequence, molecular cloning, DNA binding proteins genetics , proteins, tumor suppressor genes, HIC1, mesoderm metabolism, molecular sequence data, sequence alignment, transcription factors genetics, tumor suppressor proteins, proteins genetics.

Besser, J., C.P. Bagowski, E. Salas Vidal, M.J. van Hemert, J. Bussmann, and H.P. Spaink (2007). **Expression analysis of the family of 14-3-3 proteins in zebrafish development.** *Gene Expression Patterns* 7(4): 511-20. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: 14-3-3 proteins comprise a family of dimeric multi-functional proteins present in all eukaryotes, that are important in a whelm of ubiquitous biological processes. We have analyzed the genomic structure of all 14-3-3s from zebrafish comprising 11 genes and have analyzed their phylogeny. The gene family was cloned and its expression pattern in zebrafish embryogenesis was analyzed by whole mount in situ hybridization and microarray analysis with gene specific probes. We demonstrate that maternal mRNA of 14-3-3s is expressed evenly at the first cell division. At later stage all genes are expressed in a patterned way with, in most cases, intricate patterns in the developing brain. Our result shows distinct expression patterns of various genes. Microarray results show that differences in expression levels of highly similar 14-3-3 genes also occur in the adult stage.

Descriptors: zebrafish, 14-3-3 gene expression , metabolism, embryonic development, oligonucleotide array sequence analysis, phylogeny.

Bettegowda, A., J. Yao, Y.M. Sik, J.J. Ireland, and G.W. Smith (2006). **The search for vertebrate orthologs of bovine JY-1: a novel oocyte/embryo specific gene.** *Biology of Reproduction*(Special Issue): 146-147. ISSN: 0006 3363.
NAL Call Number: QL876.B5
Descriptors: zebrafish, blood and lymphatics, transport and circulation, development, reproductive system, hybridization, genome, folliculogenesis, oogenesis, NcBI.

Biehlmaier, O., Y. Makhankov, and S.C. Neuhauss (2007). **Impaired retinal differentiation and maintenance in zebrafish laminin mutants.** *Investigative Ophthalmology and Visual Science* 48(6): 2887-94. ISSN: 0146-0404.
Abstract: PURPOSE: To characterize morphologic and physiological alterations in the retina of three laminin mutant zebrafish, bashful (bal, lama1), grumpy (gup, lamb1), and sleepy (sly, lamc1), which were identified in forward genetic screens and were found to be impaired in visual functions. METHODS: Mutant larvae were observed for defects in visual behavior by testing their optokinetic response (OKR). In addition, electroretinograms (ERG) were measured and retinal morphology was examined by standard histology, immunocytochemistry, TUNEL assay, and electron microscopy. RESULTS: Both, gup and sly showed no OKR at any light intensity tested, whereas bal embryos showed some remaining OKR behavior at more than 40% of contrast. Consistent with the OKR result, gup and sly did not show an ERG response at any light intensity tested, whereas bal mutants exhibited small a- and b-waves at high light intensities. All three laminin mutants showed altered ganglion cell layers, optic nerve fasciculations, and lens defects. Again, bal showed the least severe morphologic phenotype with no additional defects. In contrast, both, gup and sly, showed severe photoreceptor outer segment shortening and synapse alteration (floating ribbons) as well as increased cell death. CONCLUSIONS: Lamb1 and lamc1 chains play an important role in the morphogenesis of photoreceptors and their synapses. In contrast, lama1 is not involved in outer retina development.
Descriptors: zebrafish, cell differentiation, pathology, laminin mutant genetics, retina embryology, electroretinography, fluorescent antibody technique, indirect, in situ nick end labeling, crystalline lens embryology and pathology, fluorescence microscopy, morphogenesis, mutation, optokinetic nystagmus, optic nerve embryology, optic nerve pathology, retina pathology, retinal ganglion cells pathology.

Biehlmaier, O., S.C. Neuhauss, and K. Kohler (2003). **Double cone dystrophy and RPE degeneration in the retina of the zebrafish gnn mutant.** *Investigative Ophthalmology and Visual Science* 44(3): 1287-98. ISSN: 0146-0404.
Abstract: PURPOSE: To characterize morphologic alterations in the retina of the visual mutant zebrafish gantenbein (gnn) and to examine whether these alterations correlate with those present in human hereditary eye diseases. METHODS: The gnn mutant was isolated by behavioral and macroscopic screening. Retinas of gnn zebrafish larvae were examined at different developmental stages from 2 to 9 days postfertilization (dpf) by standard histologic staining techniques and by immunocytochemistry. Ultrastructural alterations were examined by electron microscopy. The genetic map position of the induced mutation was identified by mapping with two candidate primer pairs on single larvae. RESULTS: The gnn mutant exhibited shortened outer photoreceptor segments and altered RPE morphology. In the photoreceptor layer of the mutant, the total number of lectin-labeled cones was reduced in all developmental stages from 2 to 7 dpf, whereas the amount of rhodopsin-positive cells remained at the wild-type (WT) level. Labeling with zebrafish opsin antibodies revealed dystrophic red cones at 5 dpf, whereas the morphology of all other cone types was largely

unaffected. Electron microscopy unveiled electron-dense deposits between the discs of the double cone outer segments. In addition, the onset of progressive RPE degeneration was observed at this stage of development. At later stages, all cone types and the RPE became degenerative. The morphology of distinct second-order neurons remained largely unaffected by the mutation. The gnn mutation was located approximately 4.3 cM from the simple sequence length polymorphism (SSLP) marker Z15453 on linkage group 16. CONCLUSIONS: In gnn mutant zebrafish, cones, and especially red cones, are dystrophic in early retinal development. Subsequent to this cone dystrophy, the RPE becomes dysfunctional and starts to degenerate in later stages of development. Thus, the early developmental morphology of gnn exhibits similarities to cone dystrophies most commonly seen in age-related macular degeneration (AMD) among humans, whereas the later stages of degeneration in gnn resemble RPE alterations in retinitis pigmentosa (RP) in humans. The gnn zebrafish mutant may therefore be a useful model for examining the possible interplay and connection between cone dystrophy and RPE degeneration.

Descriptors: zebrafish, retina cones pathology, pigment epithelium of eye pathology, retinal degeneration genetics, retinal degeneration pathology, retina cones growth and development, retina cones metabolism, indirect fluorescent antibody technique, mutagenesis, neurons ultrastructure, opsin metabolism, vertebrate photoreceptors ultrastructure, retinal degeneration metabolism.

Bijlsma, M.F., C.A. Spek, D. Zivkovic, S. VanDeWater, F. Rezaee, and M.P. Peppelenbosch (2006). **Repression of smoothened by patched-dependent (pro-)vitamin D3 secretion**. *Public Library of Science: Biology* 4(8): 1397-1410. ISSN: 1544 9173.

NAL Call Number: QH301.P56

Abstract: The developmentally important hedgehog (Hh) pathway is activated by binding of Hh to patched (Ptch1), releasing smoothened (Smo) and the downstream transcription factor glioma associated (Gli) from inhibition. The mechanism behind Ptch1-dependent Smo inhibition remains unresolved. We now show that by mixing Ptch1-transfected and Ptch1 small interfering RNA-transfected cells with Gli reporter cells, Ptch1 is capable of non-cell autonomous repression of Smo. The magnitude of this non - cell autonomous repression of Smo activity was comparable to the fusion of Ptch1-transfected cell lines and Gli reporter cell lines, suggesting that it is the predominant mode of action. CHOD-PAP analysis of medium conditioned by Ptch1-transfected cells showed an elevated 3 beta-hydroxysteroid content, which we hypothesized to mediate the Smo inhibition. Indeed, the inhibition of 3 beta-hydroxysteroid synthesis impaired Ptch1 action on Smo, whereas adding the 3 beta-hydroxysteroid (pro-)vitamin D3 to the medium effectively inhibited Gli activity. Vitamin D3 bound to Smo with high affinity in a cyclopamine-sensitive manner. Treating zebrafish embryos with vitamin D3 mimicked the smo(-/-) phenotype, confirming the inhibitory action in vivo. Hh activates its signalling cascade by inhibiting Ptch1-dependent secretion of the 3 beta-hydroxysteroid (pro-) vitamin D3. This action not only explains the seemingly contradictory cause of Smith-Lemli-Opitz syndrome (SLOS), but also establishes Hh as a unique morphogen, because binding of Hh on one cell is capable of activating Hh-dependent signalling cascades on other cells. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, metabolism, molecular genetics: biochemistry and molecular biophysics, genetics, Smith Lemli Opitz syndrome, SLOS , congenital disease, genetic and metabolic disease.

Birely, J., V. Schneider, E. Santana, R. Dosch, D.S. Wagner, M.C. Mullins, and M. Granato (2005). **Genetic screens for genes controlling motor nerve-muscle development and interactions**. *Developmental Biology* 280(1): 162-176. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Motor growth cones navigate long and complex trajectories to connect with their muscle targets. Experimental studies have shown that this guidance process critically depends on extrinsic cues. In the zebrafish embryo, a subset of mesodermal cells, the adaxial cells, delineates the prospective path of pioneering motor growth cones. Genetic ablation of adaxial cells causes profound pathfinding defects, suggesting the existence of adaxial cell derived guidance factors. Intriguingly, adaxial cells are themselves migratory, and as growth cones approach they migrate away from the prospective axonal path to the lateral surface of the myotome, where they develop into slow-twitching muscle fibers. Genetic screens in embryos stained with an antibody cocktail identified mutants with specific defects in differentiation and migration of adaxial cells/slow muscle fibers, as well as mutants with specific defects in axonal pathfinding, including exit from the spinal cord and pathway selection. Together, the genes underlying these mutant phenotypes define pathways essential for nerve and muscle development and interactions between these two cell types. (c) 2005 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, nervous system, neural coordination, molecular genetics, muscular system: movement and support, development, genetic screening, genetic techniques.

Bladen, C.L., W.K. Lam, W.S. Dynan, and D.J. Kozlowski (2005). **DNA damage response and Ku80 function in the vertebrate embryo**. *Nucleic Acids Research* 33(9): 3002-3010. ISSN: 0305 1048.

NAL Call Number: QD341.A2N8

Abstract: Cellular responses to DNA damage reflect the dynamic integration of cell cycle control, cell-cell interactions and tissue-specific patterns of gene regulation that occurs in vivo but is not recapitulated in cell culture models. Here we describe use of the zebrafish embryo as a model system to identify determinants of the in vivo response to ionizing radiation-induced DNA damage. To demonstrate the utility of the model we cloned and characterized the embryonic function of the XRCC5 gene, which encodes Ku80, an essential component of the nonhomologous end joining pathway of DNA repair. After the onset of zygotic transcription, Ku80 mRNA accumulates in a tissue-specific pattern, which includes proliferative zones of the retina and central nervous system. In the absence of genotoxic stress, zebrafish embryos with reduced Ku80 function develop normally. However, low dose irradiation of these embryos during gastrulation leads to marked apoptosis throughout the developing central nervous system. Apoptosis is p53 dependent, indicating that it is a downstream consequence of unrepaired DNA damage. Results suggest that nonhomologous end joining components mediate DNA repair to promote survival of irradiated cells during embryogenesis. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, development, molecular genetics, apoptosis, embryogenesis, ionizing radiation DNA damage, XRCC5 gene.

Blasiole, B., N. Kabbani, W. Boehmler, B. Thisse, C. Thisse, V. Canfield, and R. Levenson (2005). **Neuronal calcium sensor-1 gene ncs-1a is essential for semicircular canal formation in zebrafish inner ear.** *Journal of Neurobiology* 64(3): 285-97. ISSN: 0022-3034.

NAL Call Number: QP351.J55

Abstract: We have analyzed the functional role of neuronal calcium sensor-1 (Ncs-1)

in zebrafish development. We identified two orthologs of the mammalian NCS-1 gene. Full-length cDNAs encoding zebrafish Ncs-1a and Ncs-1b polypeptides were cloned and characterized. Whole-mount in situ hybridization revealed that ncs-1a mRNA was expressed beginning at early somitogenesis. As development progressed, ncs-1a mRNA was present throughout the embryo with expression detected in ventral hematopoietic mesoderm, pronephric tubules, CNS nuclei, and otic vesicle. By 4.5 days post fertilization (dpf), ncs-1a expression was detected primarily in the brain. Expression of ncs-1b mRNA was first detected at 36 hours post fertilization (hpf) and was restricted to the olfactory bulb. By 4.5 dpf, ncs-1b was expressed at low levels throughout the brain. Knockdown of ncs-1a mRNA translation with antisense morpholinos blocked formation of semicircular canals. These studies identify a novel function for ncs-1a in inner ear development and suggest that this calcium sensor plays an important role in vestibular function.
Descriptors: zebrafish embryo inner ear development, brain embryology, calcium binding neuropeptides genetics, semicircular canals embryology, amino acid sequence, Western blotting, molecular cloning, developmental gene expression regulation, in situ hybridization, molecular sequence data, neuronal calcium sensor proteins, antisense oligonucleotides, phylogeny, messenger RNA analysis , reverse transcriptase PCR, amino acid sequence homology.

Boehm, T., C.C. Bleul, and M. Schorpp (2003). **Genetic dissection of thymus development in mouse and zebrafish.** *Immunological Reviews* 195: 15-27. ISSN: 0105-2896.
NAL Call Number: QP89.T7
Abstract: Lymphoid organs represent a specialized microenvironment for interaction of stromal and lymphoid cells. In primary lymphoid organs, these interactions are required to establish a self-tolerant repertoire of lymphocytes. While detailed information is available about the genes that control lymphocyte differentiation, little is known about the genes that direct the establishment and differentiation of principal components of such microenvironments. Here, we discuss genetic studies addressing the role of thymic epithelial cells (TECs) during thymopoiesis. We have identified an evolutionarily conserved key regulator of TEC differentiation, Foxn1, that is required for the immigration of prothymocytes into the thymic primordium. Because Foxn1 specifies the prospective endodermal domain that gives rise to thymic epithelial cells, it can be used to identify the evolutionary origins of this specialized cell type. In the course of these studies, we have found that early steps of thymus development in zebrafish are very similar to those in mice. Subsequently, we have used chemical mutagenesis to derive zebrafish lines with aberrant thymus development. Strengths and weaknesses of mouse and zebrafish models are largely complementary such that genetic analysis of mouse and zebrafish mutants may lead to a better understanding of thymus development.
Descriptors: zebrafish, thymus gland cytology and immunology, immunology, biological adaptation, cell differentiation, DNA binding proteins genetics , proteins genetics and proteins metabolism, evolution, forkhead transcription factors, mice, thymus gland abnormalities, thymus gland embryology, transcription factors genetics and metabolism.

Boehmler, W., T. Carr, C. Thisse, B. Thisse, V.A. Canfield, and R. Levenson (2007). **D4 Dopamine receptor genes of zebrafish and effects of the antipsychotic clozapine on larval swimming behaviour.** *Genes, Brain, and Behavior* 6(2): 155-66. ISSN: 1601-1848.
Abstract: Zebrafish, a model developmental genetic organism, is being increasingly used in behavioural studies. We have initiated studies designed to evaluate the response of zebrafish to antipsychotic drugs. This study focuses on characterization of zebrafish D4 dopamine receptors (D4Rs) and the response of larval zebrafish to the atypical antipsychotic clozapine. The D4R is of interest because of its high affinity for clozapine, while interest in clozapine

stems from its effectiveness in reducing symptoms in acutely psychotic, treatment-resistant schizophrenic patients. By mining the zebrafish genomic database, we identified three distinct D4R genes, drd4a, drd4b and drd4c, and generated full-length open reading frames encoding each of the three D4Rs by reverse transcription-polymerase chain reaction. Gene mapping studies showed that each D4R gene mapped to a distinct chromosomal location in the zebrafish genome, and each gene exhibited a unique expression profile during embryogenesis. When administered to larval zebrafish, clozapine produced a rapid and profound effect on locomotor activity. The effect of clozapine was dose-dependent, resulted in hypoactivity and was prevented by the D4-selective agonist ABT-724. Our data suggest that the inhibitory effect of clozapine on the locomotor activity of larval zebrafish may be mediated through D4Rs.

Descriptors: larva zebrafish locomotor activity, clozapine pharmacology, dopamine antagonists pharmacology, motor activity drug effects, dopamine receptors d4 drug effects, proteins drug effects, amino acid sequence, analysis of variance, chromosome mapping, drug dose response relationship, developmental gene expression regulation, gene library, larva drug effects, molecular sequence data, motor activity genetics, dopamine D4 receptors genetics and metabolism, amino acid sequence homology, swimming, .

Boonanuntanasarn, S., G. Yoshizaki, and T. Takeuchi (2003). **Specific gene silencing using small interfering RNAs in fish embryos**. *Biochemical and Biophysical Research Communications* 310(4): 1089-1095. ISSN: 0006-291X.

NAL Call Number: 442.8B5236

Descriptors: zebrafish embryos, aquaculture, developmental stages, embryogenesis, embryonic development, freshwater fish, gene expression, gene silencing, genes, genetic diversity, green fluorescent protein, population genetics, RNA, reporter gene, *Danio rerio*, *Oncorhynchus mykiss*, *Oryzias latipes*, medaka, rainbow trout, siRNA, gene regulation, population genetics.

Bosenko, D.V., N.S. Zinkevich, R.C. Tyler, B.A. Link, and E.V. Semina (2004). **Sequence and expression of zebrafish optineurin gene suggests conserved function in vertebrate eye**. *Investigative Ophthalmology and Visual Science* 45(Suppl. 2): U439. ISSN: 0146 0404.

Abstract: Purpose: The zebrafish represents an excellent model organism for developmental biology and is rapidly expanding to adult phenotypes with resemblance to human disease: We seek to use zebrafish to sin gene interactions important for normal development and maintenance of ocular function and, in particular, as it applies to glaucoma phenotypes. Along this path, we identified and studied zebrafish homologs of genes known to play an important role in mammalian eye development/function. Here we present studies of a zebrafish homolog of the human optineurin (OPTN) gene that was shown to be involved in glaucoma.Methods: Analysis of public nucleotide databases was performed using BLASTN and BLASTX engines. Multiple sequence alignments were carried out using BCM Search Launcher suite. RNA extractions, RT-PCR, in situ hybridization, cloning andsequencing were performed using standard techniques.Results: Examination of public databases identified several zebrafish genomic sequences with homology to human optineurin. Oligonucleotides were designed based on sequences corresponding to the most 5' and 3' ends of the gene and usedin RT-PCR reactions to amplify mRNA. The resultant transcript was similar to 1,800 nt in length, containing the complete coding region sequence. The predicted open reading frame encodes a 522-amino-acid protein with overall 46% identity to the human and 44% to the mouse OPTN proteins; identity between the mouse and human proteins is 77%. The genomic structure of zebrafish optn was foundto be similar to mamma-

lian genes with the coding region divided into thirteenexons. Comparative analysis of OPTN proteins showed conservation of E50 but not M98 or R545 amino acids across species. Based on literature analysis, E50K mutation is strongly associated with familial normal-tension glaucoma. The other mutations appear to likely represent population-specific variants. Expression analysis of the zebrafish optn gene showed a dynamic pattern during embryonic development with transcripts first observed in 3-8 hpf embryos and the moststrong expression in 72 hpf embryos. Zebrafish optineurin mRNA was also detected in adult tissues, with most abundant expression in the eye.Conclusions: We identified a new zebrafish gene with strong homology to human optineurin, which is associated with glaucoma. Expression of zebrafish optn inthe adult eye suggests this gene is important for normal ocular function, which can now be investigated using a multitude of techniques offered by this powerful vertebrate. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, development, molecular genetics, sense organs, glaucoma , eye disease, etiology, reverse transcriptase PCR, embryonic development, genetic conservation, ocular function.

Bovenkamp, D.E., K. Goishi, N. Bahary, A.J. Davidson, Y. Zhou, T. Becker, C.G. Becker, L.I. Zon, and M. Klagsbrun (2004). **Expression and mapping of duplicate neuropilin-1 and neuropilin-2 genes in developing zebrafish.** *Gene Expression Patterns* 4(4): 361-70. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Previously, we described the isolation and characterization of the first zebrafish neuropilin gene, which we now call nrp1a, and found its protein to be a mediator of vascular endothelial growth factor (VEGF)-dependent angiogenesis [Proc. Natl Acad. Sci. USA 99 (2002) 10470]. Subsequently, we have isolated three other full-length neuropilin genes (nrp1b, nrp2a, and nrp2b) and find that they map to independent zebrafish linkage groups. The nrp1s and nrp2s had differential spatio-temporal gene expression profiles with nrp1a being most prominent in the gut, brain, retina, hypochord, motorneurons, fin bud and mandibular cartilage, nrp1b in the brain, dorsal aorta, melanophores, ventral fin, and heart, nrp2a in the brain, retina, heart, and caudal vessels, and nrp2b in the brain, retina, gut, fin bud, melanophores, heart, and caudal vessels. In addition, we have identified an alternatively-spliced transcript of the nrp1b gene (denoted as nrp1b(s)) which is predicted to encode a soluble form of Nrp1b, containing only the a, b, and c extracellular domains. Transcript expression of nrp1b(s) was different from full-length nrp1b transcript, with prominence in the brain, developing mouth, heart, and fin bud. The NRP1s were tested for VEGF-binding ability. Both 125 kDa Nrp1a and 145 kDa Nrp1b bound 125I-labelled VEGFA165. In summary, two nrp1 and two nrp2 genes, with expression patterns similar to higher vertebrates, have been isolated from zebrafish.

Descriptors: zebrafish, developmental gene expression regulation physiology, physiologic neovascularization physiology, neuropilin 1 biosynthesis, neuropilin 2 biosynthesis, alternative splicing genetics and physiology, developmental gene expression regulation genetics , duplicate physiologic neovascularization genetics, neuropilin 1 genetics, neuropilin 2 genetics, organ specificity genetics and physiology, protein binding genetics, protein binding physiology, tertiary protein structure genetics and physiology, vascular endothelial growth factor A physiology.

Boyadjiev, S.A., J.C. Fromme, J.i.n. Ben, S.S. Chong, C. Nauta, D.J. Hur, G. Zhang, S. Hamamoto, R. Schekman, M. Ravazzola, L. Orci, and W. Eyaid (2006). **Cranio-lenticulo-sutural dysplasia is caused by a SEC23A mutation leading to abnormal endoplasmic-reticulum-**

to-Golgi trafficking. *Nature Genetics* 38(10): 1192-1197. ISSN: 1061-4036.
NAL Call Number: QH426.N37
Descriptors: zebrafish, amino acid substitution, budding, cataracts, chromosomes, dysplasia, electron microscopy, embryos, endoplasmic reticulum, fibroblasts, freshwater fish, genetic abnormalities, Golgi apparatus, immunofluorescence, morphogenesis, mutation, mutations, phenotypes, secretory vesicles, symptoms, vesicles, chromosome 14, *Danio rerio*, development and cell cycle, genetics, genetics and evolution.

Bradham, C. and D.R. Mcclay (2006). **P38 MAPK in development and cancer**. *Cell Cycle* 5(8): 824-828. ISSN: 1538 4101.
NAL Call Number: A review.
Abstract: p38 is a MAPK that has been shown to induce a wide variety of biological effects in cell culture in response to a wide range of stimuli. These effects are dependent not only on the stimuli, but also on the cellular context, resulting in a bewildering array of possibilites. For example, p38 was shown to induce apoptosis in some cells, but prevent apoptosis in others. Similarly opposed effects had been observed with respect to cell cycle regulation. The role of p38 in inflammatory disease has been appreciated from the beginning, since it was initially identified as an cytokine inducer. 3 More recently, p38 function has been evaluated in vivo, and through these studies p38 has emerged as an important regulator of both embryonic development and cancer progression. This review will focus on these in vivo studies in an effort to provide perspective on p38 biologically and as a pharmacological target. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics, tumor biology, inflammatory disease, immune system disease, cancer, neoplastic disease, disease progression, cell apoptosis.

Bradley, K.M., J.B. Elmore, J.P. Breyer, B.L. Yaspan, J.R. Jessen, E.W. Knapik, and J.R. Smith (2007). **A major zebrafish polymorphism resource for genetic mapping**. *Genome Biology* 8(4): R55. ISSN: 1465 6906.
NAL Call Number: QH431.G452
Abstract: We have identified 645,088 candidate polymorphisms in zebrafish and observe a single nucleotide polymorphism (SNP) validation rate of 71% to 86%, improving with polymorphism confidence score. Variant sites are non-random, with an excess of specific novel T- and A-rich motifs. We positioned half of the polymorphisms on zebrafish genetic and physical maps as a resource for positional cloning. We further demonstrate bulked segregant analysis using the anchored SNPs as a method for high-throughput genetic mapping in zebrafish. Copyright © 2008 CABI
Descriptors: zebrafish, gene mapping, genetic mapping, genetic polymorphism, linkage, single nucleotide polymorphism, *Danio rerio.*

Brandhorst, B.P. and G.E. Corley Smith (2004). **Production of haploid and diploid androgenetic zebrafish.** *Methods in Molecular Biology* 254: 255-70. ISSN: 1064-3745.
Descriptors: zebrafish, diploidy, haploidy, uniparental disomy, gamma rays, X rays.

Bree, R.T., S. McLoughlin, S.W. Jin, O.M. McMeel, D.Y. Stainier, M. Grealy, and L. Byrnes (2005). **Nanor, a novel zygotic gene, is expressed initially at the midblastula transition in zebrafish.** *Biochemical and Biophysical Research Communications* 333(3): 722-8. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: A novel, developmentally regulated gene, nanor, was identified by suppression

subtractive hybridization. It is first expressed following the midblastula transition (MBT), a critical developmental stage in the early vertebrate embryo when the zygotic genome is activated. The nanor cDNA (626bp) includes a complete open reading frame but neither the gene nor the deduced amino acid sequence shows significant similarity to any known gene or protein. Nanor encodes a 175 amino acid putative protein with a protein kinase C and three casein kinase II phosphorylation sites, an N-myristoylation site and an NFX-type zinc-finger domain, indicating a potential role in transcriptional regulation. Semi-quantitative RT-PCR, Northern blot, and in situ hybridization analysis revealed that nanor expression is developmentally regulated. It is initially expressed after the MBT at the sphere stage and during epiboly it is expressed in the forerunner cells. At 24 h post-fertilization, expression is solely anterior.

Descriptors: zebrafish blastula metabolism, carrier developmental gene expression regulation, zygote metabolism, base sequence, casein kinase II metabolism, DNA primers, complementary DNA, molecular sequence data, phosphorylation, reverse transcriptase PCR.

Brembeck, F.H., T. Schwarz-Romond, J. Bakkers, S. Wilhelm, M. Hammerschmidt, and W. Birchmeier (2004). **Essential role of BCL9-2 in the switch between beta-catenin's adhesive and transcriptional functions**. *Genes and Development* 18(18): 2195-2230. ISSN: 0890 9369.

NAL Call Number: QH426.G46

Abstract: Beta-Catenin controls both cadherin-mediated cell adhesion and activation of Wnt target genes. We demonstrate here that the beta-catenin-binding protein BCL9-2, a homolog of the human proto-oncogene product BCL9, induces epithelial-mesenchymal transitions of nontransformed cells and increases beta-catenin-dependent transcription. RNA interference of BCL9-2 in carcinoma cells induces an epithelial phenotype and translocates beta-catenin from the nucleus to the cell membrane. The switch between beta-catenin's adhesive and transcriptional functions is modulated by phosphorylation of Tyr 142 of beta-catenin, which favors BCL9-2 binding and precludes interaction with alpha-catenin. During zebrafish embryogenesis, BCL9-2 acts in the WNT8-signaling pathway and regulates mesoderm patterning. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, cell biology, development, molecular genetics, cell adhesion, embryogenesis, epithelial phenotype, epithelial mesenchymal transition.

Bricaud, O. and A. Collazo (2003). **The transcription factor Six1 differentially affects neuronal versus sensory cell lineages in The developing zebrafish (*Danio rerio*) inner ear.** *33rd Annual Meeting of the Society of Neuroscience ,November 8, 2003-November 8, 2003, New Orleans, LA, USA,*

Abstract: We cloned a zebrafish Six1 homologue that is expressed in the ventral region of the developing otocyst. From this region arise neurons of the statoacoustic (VIIIth cranial) ganglia and the first sensory organs (consisting of mechanosensory hair cells). Six1 loss and gain of function experiments had different effects on developing statoacoustic neurons and hair cells in an anterior sensory organ (utricular macula). Six1 expression was knocked down by injecting morpholino-modified oligonucleotides specifically directed against Six1. Six1 loss of function resulted in the number of hair cells in the anterior macula being reduced by approximately half and a dramatic increase in statoacoustic neurons. Six1 gain of function (accomplished by over-expressing Six1 mRNA) resulted in an increase in hair cell numbers and almost the complete loss of statoacoustic neurons. How is Six1 acting differently in the lineages leading to these two cell types? Both Six1 knock-down and over-expression lead to an increase in cell death and proliferation. Subsequently we were able to identify the supernumerary dividing cells as neuronal precursors when Six1 is knocked-down and hair cell

precursors when Six1 is over-expressed. These results suggest that Six1 normally promotes cell death of neuronal progenitors and proliferation of hair cell progenitors. Six1 is a transcription factor that acts with other transcription factors and cofactors in the Pax-Eya-Six-Dach regulatory pathway. The Pax-Eya-Six-Dach regulatory pathway could be an important actor in the specification of cell fates in the developing inner ear of the zebrafish embryo. The role of other members of this pathway and their relationship with Six1 is currently being investigated. Copyright©Thomson Reuters 2009
Descriptors: zebrafish Six1 homologue, nervous system, neural coordination, sense organs, transcription, zebrafish embryo.

Bricaud, O. and A. Collazo (2006). **The transcription factor six1 inhibits neuronal and promotes hair cell fate in The developing zebrafish (*Danio rerio*) inner ear.** *Journal of Neuroscience* 26(41): 10438-51. ISSN: 0270-6474.
Abstract: The developmental processes leading to the differentiation of mechanosensory hair cells and statoacoustic ganglion neurons from the early otic epithelium remain unclear. Possible candidates include members of the Pax-Six-Eya-Dach (paired box-sine oculis homeobox-eyes absent-dachshund) gene regulatory network. We cloned zebrafish six1 and studied its function in inner ear development. Gain- and loss-of-function experiments show that six1 has opposing roles in hair cell and neuronal lineages. It promotes hair cell fate and, conversely, inhibits neuronal fate by differentially affecting cell proliferation and cell death in these lineages. By independently targeting hair cells with atoh1a (atonal homolog 1a) knockdown or neurons with neurog1 (neurogenin 1) knockdown, we showed that the remaining cell population, neurons or hair cells, respectively, is still affected by gain or loss of six1 function. six1 interacts with other members of the Pax-Six-Eya-Dach regulatory network, in particular dacha and dachb in the hair cell but not neuronal lineage. Unlike in mouse, six1 does not appear to be dependent on eya1, although it seems to be important for the regulation of eya1 and pax2b expression in the ventral otic epithelium. Furthermore, six1 expression appears to be regulated by pax2b and also by foxi1 (forkhead box I1) as expected for an early inducer of the otic placode. Our results are the first to demonstrate a dual role for a member of the Pax-Six-Eya-Dach regulatory network in inner ear development.
Descriptors: zebrafish embryo, inner ear embryology, inner embryology hair cells, homeodomain proteins biosynthesis, neural inhibition physiology, transcription factors biosynthesis, proteins biosynthesis, inner ear metabolism, developmental gene expression regulation physiology, inner hair cells metabolism, homeodomain neurons metabolism, transcription factors genetics.

Brockerhoff, S.E. (2006). **Measuring the optokinetic response of zebrafish larvae.** *Nature Protocols* 1(5): 2448-51. ISSN: 1754-2189.
Abstract: Our laboratory screens for visual mutants by examining larval eye movements in response to rotating illuminated stripes. This behavior, which is termed an optokinetic response (OKR), is a reflex that appears in zebrafish at the same time as the development of the visual system. The OKR can be accurately measured by 4 d post-fertilization, which is the age when larvae begin foraging for food. The OKR requires approximately 1 min per larva analyzed. After identifying fish with defective eye movements, we conduct secondary screens (such as histological analysis and electroretinography) to identify the subset of fish with disruptions in the function of the outer retina. This paper describes our protocol for the OKR. Our setup is simple to construct and the materials needed are inexpensive. This makes our system especially useful for new undergraduate and graduate students, as well as introductory science lecturers.

Descriptors: zebrafish, eye movement measurements instrumentation, eye movements genetics, genetic screening, mutation.

Bromee, T., J.M. Conlon, and D. Larhammar (2005). **Bradykinin receptors in the zebrafish (*Danio rerio*).** *Annals of the New York Academy of Sciences* 1040: 246-8. ISSN: 0077 8923.
NAL Call Number: 500 N484
Abstract: Ligand interactions of a zebrafish bradykinin (BK) receptor expressed in vitro were characterized by measuring inositol phosphate accumulation. The ligands were analogues of zebrafish BK. Substitutions of Arg1, Gly4, Ser6, Pro7, Leu8, and Arg9 caused greatly reduced potency and maximum response. The Pro3 to Ala analogue had higher potency, but lower maximum response. These and other differences show that the zebrafish BK receptor has a ligand-interaction profile that is distinct from mammalian B1 and B2 receptors and from the previously characterized BK receptor in trout stomach. The results increase our understanding of the evolution of BK receptors and their ligands.
Descriptors: zebrafish, bradykinin receptors chemistry and metabolism, molecular cloning methods, ligands, bradykinin receptors genetics.

Brott, B.K. and S.Y. Sokol (2005). **Frodo proteins: modulators of Wnt signaling in vertebrate development** . *Differentiation* 73(7): 323-329. ISSN: 0301 4681.
NAL Call Number: QH607.D5
Abstract: The Frodo/dapper (Frd) proteins are recently discovered signaling adaptors, which functionally and physically interact with Wnt and Nodal signaling pathways during vertebrate development. The Frd1 and Frd2 genes are expressed in dynamic patterns in early embryos, frequently in cells undergoing epithelial-mesenchymal transition. The Frd proteins function in multiple developmental processes, including mesoderm and neural tissue specification, early morphogenetic cell movements, and organogenesis. Loss-of-function studies using morpholino antisense oligonucleotides demonstrate that the Frd proteins regulate Wnt signal transduction in a context-dependent manner and may be involved in Nodal signaling. The identification of Frd-associated factors and cellular targets of the Frd proteins should shed light on the molecular mechanisms underlying Frd functions in embryonic development and in cancer. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryo Frd functions, development , molecular genetics, signaling pathway, epithelial mesenchymal transition, organogenesis, vertebrate development, morphogenetic cell , neural tissue specification.

Buchner, D.A., F. Su, J.S. Yamaoka, M. Kamei, J.A. Shavit, L.K. Barthel, B. McGee, J.D. Amigo, S. Kim, A.W. Hanosh, P. Jagadeeswaran, D. Goldman, N.D. Lawson, P.A. Raymond, B.M. Weinstein, D. Ginsburg, and S.E. Lyons (2007). **Pak2a mutations cause cerebral hemorrhage in redhead zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 104(35): 13996-4001. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: The zebrafish is a powerful model for studying vascular development, demonstrating remarkable conservation of this process with mammals. Here, we identify a zebrafish mutant, redhead (rhd(mi149)), that exhibits embryonic CNS hemorrhage with intact gross development of the vasculature and normal hemostatic function. We show that the rhd phenotype is caused by a hypomorphic mutation in p21-activated kinase 2a (pak2a). PAK2 is a kinase that acts downstream of the Rho-family GTPases CDC42 and RAC and has been implicated in angiogenesis, regulation of cytoskeletal structure, and endothelial cell migration and contractility among other functions. Correction of the Pak2a-deficient phenotype

by Pak2a overexpression depends on kinase activity, implicating Pak2 signaling in the maintenance of vascular integrity. Rescue by an endothelial-specific transgene further suggests that the hemorrhage seen in Pak2a deficiency is the result of an autonomous endothelial cell defect. Reduced expression of another PAK2 ortholog, pak2b, in Pak2a-deficient embryos results in a more severe hemorrhagic phenotype, consistent with partially overlapping functions for these two orthologs. These data provide in vivo evidence for a critical function of Pak2 in vascular integrity and demonstrate a severe disease phenotype resulting from loss of Pak2 function.

Descriptors: zebrafish redhead mutant, cerebral hemorrhage genetics, mutation, protein serine threonine kinases genetics, alternative splicing, cerebral hemorrhage embryology, cerebrovascular circulation genetics, chromosome mapping recessive genes, polymorphism, restriction fragment length , protein serine threonine kinases deficiency, RNA genetics, reverse transcriptase PCR, genetic transcription, variation genetics.

Buckingham, M. (2006). **Myogenic progenitor cells and skeletal myogenesis in vertebrates**. *Current Opinion in Genetics and Development* 16(5): 525-532. ISSN: 0959 437X.

NAL Call Number: QH426.C88

Abstract: Continuing research on the onset of skeletal myogenesis in the somite is providing new insights into the behaviour of early myogenic progenitor cells and how signalling molecules affect cell fate decisions, in addition to subsequent muscle growth. Genetic manipulations have revealed new regulatory aspects, including the role of Six transcription factors and the CXCR4 cytokine receptor during embryonic myogenesis. An important recent development is the identification of a novel population of somite-derived cells that make a major contribution to muscle growth. These cells, which are characterised by the expression of Pax3 and Pax7, also give rise to the satellite cells of postnatal muscle. The relationship between Pax and Myogenic regulatory factors has been explored. Furthermore, Pax7 is now shown to be required for the maintenance of satellite cells. New approaches that permit the grafting of purified satellite cells demonstrate their capacity for efficient muscle repair and for self-renewal. Regeneration in amphibians is now also shown to involve Pax-positive progenitor cells. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, development, molecular genetics, molecular biophysics, skeletal system, muscular system, phenotype, aymmetric cell division, self renewal, muscle growth, somitogenesis, muscle repair, skeletal myogenesis, factor crosstalk.

Bukanov, N.O., T. Obara, B.L. Roberts, K.W. Klinger, I. Drummond, and O. Ibraghimov-Beskrovnaya (2003). **Proteome analysis of the zebrafish double bubble mutant suggests a role for cathepsins in pronephric cystogenesis.** *Journal of the American Society of Nephrology* 14(Abstracts Issue): 365A. ISSN: 1046 6673.

Descriptors: zebrafish, enzymology, molecular genetics, pronephric cystogenesis, urinary system, pronephric cyst, urologic disease, proteome analysis, genetic techniques.

Burnam, L., A. Novillo, and G.V. Callard (2006). **Development of an endocrine disrupter screen based on gene expression in zebrafish embryos**. *Journal of Experimental Zoology* 305A(2): 114. ISSN: 1552-5007.

NAL Call Number: QL1.J87

Descriptors: zebrafish embryo, endocrine system, chemical coordination and homeostasis, real time PCR, genetic techniques, reporter gene assay, genetic techniques.

Cadieux, B., B.P. Chitramuthu, D. Baranowski, and H.P. Bennett (2005). **The zebrafish progranulin gene family and antisense transcripts.** *BioMed Central Medical Genomics* 6: 156. ISSN: 1755 8794.

Abstract: BACKGROUND: Progranulin is an epithelial tissue growth factor (also known as proepithelin, acrogranin and PC-cell-derived growth factor) that has been implicated in development, wound healing and in the progression of many cancers. The single mammalian progranulin gene encodes a glycoprotein precursor consisting of seven and one half tandemly repeated non-identical copies of the cystine-rich granulin motif. A genome-wide duplication event hypothesized to have occurred at the base of the teleost radiation predicts that mammalian progranulin may be represented by two co-orthologues in zebrafish. RESULTS: The cDNAs encoding two zebrafish granulin precursors, progranulins-A and -B, were characterized and found to contain 10 and 9 copies of the granulin motif respectively. The cDNAs and genes encoding the two forms of granulin, progranulins-1 and -2, were also cloned and sequenced. Both latter peptides were found to be encoded by precursors with a simplified architecture consisting of one and one half copies of the granulin motif. A cDNA encoding a chimeric progranulin which likely arises through the mechanism of trans-splicing between grn1 and grn2 was also characterized. A non-coding RNA gene with antisense complementarity to both grn1 and grn2 was identified which may have functional implications with respect to gene dosage, as well as in restricting the formation of the chimeric form of progranulin. Chromosomal localization of the four progranulin (grn) genes reveals syntenic conservation for grna only, suggesting that it is the true orthologue of mammalian grn. RT-PCR and whole-mount in situ hybridization analysis of zebrafish grns during development reveals that combined expression of grna and grnb, but not grn1 and grn2, recapitulate many of the expression patterns observed for the murine counterpart. This includes maternal deposition, widespread central nervous system distribution and specific localization within the epithelial compartments of various organs. CONCLUSION: In support of the duplication-degeneration-complementation model of duplicate gene retention, partitioning of expression between grna and grnb was observed in the intermediate cell mass and yolk syncytial layer, respectively. Taken together these expression patterns suggest that the function of an ancestral grn gene has been devolved upon four paralogues in zebrafish.

Descriptors: zebrafish, developmental gene expression regulation, intercellular signaling peptides and antisense oligonucleotides genetics, proteins chemistry, amino acid motifs, amino acid sequence, Northern blotting, high pressure liquid chromatography, chromosome mapping, molecular cloning, complementary DNA metabolism, gene dosage, gene library, in situ hybridization, intercellular signaling peptides and proteins biosynthesis, genetic models, molecular sequence data, multigene family, antisense oligonucleotides chemistry, phylogeny, RNA metabolism, messenger RNA metabolism, reverse transcriptase PCR, amino acid sequence homology, tissue distribution, genetic transcription, zebrafish, proteins biosynthesis.

Cameron, D.A., K.L. Gentile, F.A. Middleton, and P. Yurco (2005). **Gene expression profiles of intact and regenerating zebrafish retina.** *Molecular Vision* 11: 775-91. ISSN: 1090-0535.

NAL Call Number: QP475 .M65

Abstract: PURPOSE: Investigate the molecular determinants of retinal regeneration in adult vertebrates by analyzing the gene expression of control and post-lesion retina of adult zebrafish, a system that regenerates following injury. METHODS: Gene expression of zebrafish retina and brain were determined with DNA microarray, RT-PCR, and real-time quantitative PCR analyses. Damaged retinas and their corresponding controls were analyzed 2-5 days post-lesion (acute injury condition) or 14 d post-lesion (cell regeneration condition). RESULTS: Expected similarities and differences in the gene expression profile

of zebrafish retina and brain were observed, confirming the applicability of the gene expression techniques. Mechanical lesion of retina triggered significant, time-dependent changes in retinal gene expression. The induced transcriptional changes were consistent with cellular phenomena known to occur, in a time-dependent manner, subsequent to retinal lesion, including cell cycle progression, axonal regeneration, and regenerative cytogenesis. CONCLUSIONS: The results indicate that retinal regeneration in adult zebrafish involves a complex set of induced, targeted changes in gene transcription, and suggest that these molecular changes underlie the ability of the adult vertebrate retina to regenerate.
Descriptors: zebrafish, gene expression physiology, gene expression profiling, oligonucleotide array sequence analysis, retina physiology, wound healing physiology, brain physiology, DNA primers chemistry, retina injuries, reverse transcriptase PCR.

Camp, E., P. Badhwar, G.J. Mann, and M. Lardelli (2003). **Expression analysis of a tyrosinase promoter sequence in zebrafish.** *Pigment Cell Research* 16(2): 117-26. ISSN: 0893-5785.
Abstract: Sequence comparisons and functional analysis of the 5' upstream regions of tyrosinase genes have revealed the importance of cis-regulatory elements acting to control the spatiotemporal expression of tyrosinase in the melanocytes and retinal pigmented epithelium of developing embryos. To date there are no reports addressing the control of tyrosinase gene transcription in zebrafish, a vertebrate model organism of increasing importance. To exploit the tyrosinase gene as a marker in zebrafish we set out to clone its promoter and analyse its regulation during embryogenesis. Amplification of a zebrafish tyrosinase complementary DNA fragment by reverse transcriptase polymerase chain reaction allowed us to isolate and sequence a 1041 nt genomic DNA fragment that includes a transcription initiation site and 73 nt of the open reading frame. Bioinformatic analysis of this genomic sequence revealed five E-box motifs, including one CATGTG type E-box present in a putative initiation region. These are conserved positive regulatory elements in vertebrate tyrosinase promoters. We show that a region of 814 nt upstream from the translation start site of the zebrafish tyrosinase gene can drive expression in retinal pigmented epithelium in transiently transgenic zebrafish embryos but that its activity is not restricted to melanin-producing cells. This region is unable to drive transcription in human melanoma cell lines. Ectopic expression from this zebrafish tyrosinase promoter fragment is probably due to the absence of positive and negative cis-regulatory elements, such as a tyrosinase distal element, which is known to function as a pigment cell-specific enhancer.
Descriptors: zebrafish, embryology, developmental gene expression regulation genetics and genetics, melanins biosynthesis, monophenol monooxygenase genetics, pigment epithelium of eye embryology, promoter regions genetics, base sequence genetics, e box elements genetics, cytology, enzymology, regulator genes genetics, melanocytes enzymology, melanoma enzymology and genetics, molecular sequence data, pigment epithelium of eye cytology and enzymology, transcription initiation site physiology, tumor cultured cells enzymology.

Camp, E., R. Hope, R.D. Kortschak, T.C. Cox, and M. Lardelli (2003). **Expression of three spalt (sal) gene homologues in zebrafish embryos.** *Development Genes and Evolution* 213(1): 35-43. ISSN: 0949 944X.
NAL Call Number: QL951.R68
Abstract: Three homologues of the *Drosophila* region-specific homeotic gene spalt (sal) have been isolated in zebrafish, sall1a, sall1b and sall3. Phylogenetic analysis of these genes against known salDNA sequences showed zebrafish sall1aand sall1b to be orthologous to other vertebrate sal-1 genes and zebrafish sall3to be orthologous to other vertebrate sal-3 genes, except *Xenopus* sall3. Phylogenetic reconstruction suggests that zebrafish sall1a and sall1bre-

sulted from a gene duplication event occurring prior to the divergence of the ray-finned and lobe-finned fish lineages. Analysis of the expression pattern of the zebrafish sal genes shows that sall1a and sall3 share expression domains with both orthologous and non-orthologous vertebrate sal genes. Both are expressed in various regions of the CNS, including in primary motor neurons. Outside of the CNS, sall1a expression is observed in the otic vesicle (ear), heart and in a discrete region of the pronephric ducts. These analyses indicate that orthologies between zebrafish sal genes and other vertebrate sal genes do not imply equivalence of expression pattern and, therefore, that biological functions are not entirely conserved. However we suggest that, like other vertebrate sal genes, zebrafish sal genes have a role in neural development. Also, expression of zebrafish sall1a in the otic vesicle, heart sac and the pronephric ducts of zebrafish embryos is possibly consistent with some of the abnormalities seen in Sall1-deficient mice and in Townes-Brocks Syndrome, a human disorder which is caused by mutations in the human spalt gene SALL1.
Descriptors: zebrafish embryos, developmental gene expression regulation genetics, homeodomain phylogeny, transcription factors genetics, *Xenopus* proteins, base sequence, molecular evolution, gene expression profiling , Markov chains, molecular sequence data, monte carlo method, sequence alignment, sequence homology.

Candy, J. and C. Collet (2005). **Two tyrosine hydroxylase genes in teleosts**. *Biochimica Et Biophysica Acta, Gene Structure and Expression* 1727(1): 35-44. ISSN: 0167 4781.
NAL Call Number: QH450.B56
Abstract: We report the finding of two non-allelic genes encoding tyrosine hydroxylase (TH) from the diploid teleost barramundi *Lates calcarifer*. Barramundi TH1 is the homologue of the higher vertebrate TH genes and encodes a protein of 489 amino acids that shares 90% sequence identity to the THs of other teleost species. A second non-allelic tyrosine hydroxylase gene (TH2) encodes a protein of 472 amino acids and shares 62% identity with TH1 and the vertebrate THs. TH1 mRNA is found in the brain and kidney of barramundi while TH2 mRNA is found only in brain. The TH2 gene is also present in the genomes of the pufferfish *Takifugu* and zebrafish *Danio*. Estimates of the rates of nucleotide substitution suggest the teleost TH2 genes are selectively constrained although not to the degree seen in the TH1 genes. Differential regulation of the two TH genes is, however, indicated by differences in transcript distribution, the nature of the Ca2+-responsive elements found in the proximal promoter region and the lack of recognised phosphorylation sites in TH2. Preservation of two apparently functional TH genes in phylogenetically distant teleost species is consistent with the notion of partitioning of function between duplicate genes. Copyright © 2008 CABI
Descriptors: zebrafish, amino acid sequences, brain, characterization, diploidy, genes, genomes, isolation, kidneys, messenger RNA, phylogeny, regulatory genes, RNA, tyrosine 3 monooxygenase, Danio rerio, fishes, *Lates calcarifer*, *Takifugu* .

Carmany Rampey, A. and C.B. Moens (2006). **Modern mosaic analysis in the zebrafish.** *Methods* 39(3): 228-38. ISSN: 0882-4010.
NAL Call Number: QP519.7.M47
Abstract: One of the most powerful tools used to gain insight into complex developmental processes is the analysis of mosaic embryos. A mosaic is defined as an organism that contains cells of more than one genotype, usually wild-type and mutant. It is the interplay between wild-type and mutant cells in the mosaic that reveals information about the normal function of the mutated gene. Mosaic analysis has been utilized extensively in *Caenorhabditis elegans*, *Drosophila*, mice, and zebrafish to elucidate when, where, and how a gene acts during

development. In the zebrafish, mosaic analysis has been used to dissect a number of different developmental processes, including gastrulation movements, mesoderm and endoderm specification, neuronal patterning and migration, axon pathfinding, angiogenesis, and cardiac, retinal, and neural crest development. Mosaic analysis is a particularly effective method for understanding gene function in the zebrafish, a model organism particularly suited to forward genetic, molecular, and classical embryological approaches. These attributes, when combined with the accessibility and optical clarity of the zebrafish embryo, facilitate the real time observation of individual cell behaviors and interactions within mosaic embryos.
Descriptors: zebrafish , genetic engineering methods, mosaicism, cell differentiation genetics, cell lineage, cell transplantation methods, chimera, cytology, signal transduction, proteins metabolism.

Carvan, M.J., B.A. Wimpee, G.W. Goetz, and M.L. Rise (2005). **A cross-species environmental genome project for understanding the complexities of chemically-induced birth defects and neurobehavioral deficits**. *Birth Defects Research* 73(5): 318. ISSN: 1542 0752.
NAL Call Number: QL991.T4
Descriptors: zebrafish, behavior, toxicology, nervous system, ecology, molecular genetics, birth defect, congenital disease, chemically induced, genetics, neurobehavioral deficit, nervous system disease, edema, skeletal defect, cDNA microarray, quantitative real time reverse transcriptase PCR, cDNA sequence comparison, environmental genome project.

Castellano, S., S.V. Novoselov, G.V. Kryukov, A. Lescure, E. Blanco, A. Krol, V.N. Gladyshev, and R. Guigo (2004). **Reconsidering the evolution of eukaryotic selenoproteins: a novel non-mammalian family with scattered phylogenetic distribution**. *European Molecular Biology Organization Journal Reports* 5(1): 71-77. ISSN: 1469 221X.
NAL Call Number: QH506 .E463
Abstract: While the genome sequence and gene content are available for an increasing number of organisms, eukaryotic selenoproteins remain poorly characterized. The dual role of the UGA codon confounds the identification of novel selenoprotein genes. Here, we describe a comparative genomics approach that relies on the genome-wide prediction of genes with in-frame TGA codons, and the subsequent comparison of predictions from different genomes, wherein conservation in regions flanking the TGA codon suggests selenocysteine coding function. Application of this method to human and fugu genomes identified a novel selenoprotein family, named SelU, in the puffer fish. The selenocysteine-containing form also occurred in other fish, chicken, sea urchin, green algae and diatoms. In contrast, mammals, worms and land plants contained cysteine homologues. We demonstrated selenium incorporation into chicken SelU and characterized the SelU expression pattern in zebrafish embryos. Our data indicate a scattered evolutionary distribution of selenoproteins in eukaryotes, and suggest that, contrary to the picture emerging from data available so far, other taxa-specific selenoproteins probably exist. Copyright © 2008 CABI
Descriptors: zebrafish embryos, codons, fish, phylogenetics, poultry, proteins, selenium, Bacillariophyta, Echinoidea, fowls, mammals, plants.

Cerda, G.A., J.E. Thomas, M.L. Allende, R.O. Karlstrom, and V. Palma (2006). **Electroporation of DNA, RNA, and morpholinos into zebrafish embryos.** *Methods* 39(3): 207-11. ISSN: 0882-4010.
NAL Call Number: QP519.7.M47
Abstract: The combination of accessible embryology and forward genetic techniques has made zebrafish a powerful model system for the study of vertebrate development. One

limitation of genetic analysis is that the study of gene function is usually limited to the first developmental event affected by a gene. In vivo electroporation has recently matured as a method for studying gene function at different developmental time points and in specific regions of the organism. The focal application of current allows macromolecules to be efficiently introduced into a targeted region at any time in the life cycle. Here we describe a rapid protocol by which DNA, RNA and morpholinos can all be precisely electroporated into zebrafish in a temporally and spatially controlled manner. This versatile technique allows gene function to be determined by both gain and loss of function analyses in specific regions at specific times. This is the first report that describes the electroporation of three different molecules into embryonic and larval zebrafish cells.
Descriptors: zebrafish embryo and larva cells, DNA, electroporation methods, antisense oligonucleotides, RNA, electroporation instrumentation, chemistry, green fluorescent proteins analysis, proteins genetics.

Chakraborty, C., S.S. Nandi, S. Sinha, and V.K. Gera (2006). **Zebrafish caspase-3: molecular cloning, characterization, crystallization and phylogenetic analysis.** *Protein and Peptide Letters* 13(6): 633-40. ISSN: 0929-8665.
Abstract: Apoptosis plays an important role in maintaining the normal function of various tissues and organs in different species. Caspase-3 is a terminal caspases which plays an important role in the execution of apoptosis in all vertebrates. It was cloned from zebra fish embryos and its properties were identified through Western blotting and biological activity. In the cells over-expressing caspase-3, Western blotting with an anti-His-tag antibody confirmed the presence of caspase-3 in the three bands that were proposed to correspond to the precursor form (33 kDa), the mature forms processed at the prodomain alone (29 kDa, large subunit) and small sub unit (13 kD). Fish kidney cells were transiently co-transfected with the beta-galactosidase reporter gene and either vector alone (mock), pZCASP3His (caspase-3) or pZCASP3His mutant (caspase-3 mutant). After 72 h following transfection of fish kidney cells, 35% of cells transfected with the zebra fish caspase-3 construct, pZCASP3His, showed apoptotic morphology when compared with cells transfected with the mock vector or an expression construct (pZCASP3His mutant) encoding the caspase-3 mutant lacking Cys. The fusion proteins were expressed in *Escherichia coli*, isolated from cell lysates by nickel-affinity column chromatography, and cleaved with thrombin. A thrombin cleavage recognition site was positioned at the fusion junction to release the caspase-3 from the fusion protein. Phylogenetic analysis showed that the cloned zebra fish caspase was a member of the caspase-3 subfamily with approximately 60% identity with caspase-3 from *Xenopus*, chicken and mammals. We have obtained structural information by X-ray crystallography. Orthorhombic crystals of the caspase-3 that diffracted to 1.8 A were obtained in a mixture of 0.1 M imidazole (pH 6.0) and 0.4 M NaOAc (pH 7.0 -7.5), containing 30% glycerol. The space group is C222 with cell dimensions of a = 36.07 A, b = 38.80 A, c = 135.20 A.
Descriptors: zebrafish, caspases chemistry and genetics, phylogeny, amino acid sequence, caspase 3, cell line, molecular cloning , crystallization, crystallography, X-ray, DNA, complementary, kidney cytology, molecular sequence data, recombinant proteins chemistry, recombinant proteins genetics.

Chakraborty, C., G. Saha, B. Sarkar, S. Pal, T.K. Chatterjee, and A.K. Sadhu (2006). **Caspase-3 induced apoptosis in transgenic zebrafish.** *Biotechnology Letters* 28(3): 189-96. ISSN: 0141 5492.
NAL Call Number: QR53.B56
Abstract: Zebrafish is an attractive model organism for studying apoptosis development

because of its genetic accessibility. Here we describe the induction of clonally derived apoptosis in transgenic zebrafish expressing mouse caspase-3 (CASP3) under control of the zebrafish beta-actin promoter (betap). Visualization of apoptotic cells, expressing a chimeric transgene encoding CASP3 fused to green fluorescent protein (GFP) gene, revealed that apoptosis arose in the thymus, spread locally into gill arches and retro-orbital soft tissue, and then disseminated into abdominal organs like testis, kidney. This transgenic model provides a platform for over-expression of caspase-3 induced extensive apoptosis in embryos and adult.
Descriptors: zebrafish embryos and adults, apoptosis, caspases metabolism, actins genetics, genetically modified, caspase 3, caspases genetics, abnormalities, anatomy and histology, green fluorescent proteins genetics and metabolism, mice, organ specificity, promoter regions genetics, recombinant fusion proteins genetics and metabolism, zebrafish abnormalities.

Challa, M., G.R. Chapa, S. Govindaraju, M. Gonzalez Garcia, and R.P. Ballestero (2006). **Characterization of the domains of zRICH, a protein induced during optic nerve regeneration in zebrafish.** *Brain Research* 1100(1): 42-54. ISSN: 0006 8993.
NAL Call Number: QP376
Abstract: Teleost fish show a remarkable capability of nerve regeneration in their CNS, while injuries to axon fibers in the CNS of mammals result in degeneration and loss of function. Understanding this difference has biomedical consequences to humans. Both extrinsic factors from the neuronal environment and intrinsic neuronal factors seem to play a role in successful nerve regeneration. Among the intrinsic factors, a number of proteins termed axonal growth associated proteins (GAPs) are strongly induced during axon regeneration. RICH proteins are axonal GAPs that show homology to mammalian myelin marker proteins termed CNPases. Sequence analysis distinguishes three domains in these proteins. In this report, mutant versions of zebrafish RICH proteins were generated to study the roles of the domains of the protein at biochemical and cellular levels. The central CNPase homology domain was sufficient for catalytic activity. The amino terminal acidic domain causes the anomalous electrophoretic migration observed for RICH proteins. The small C-terminal domain bears an isoprenylation motif and is necessary for the interaction of zRICH with cellular membranes. At the cellular level, expression of wild-type zRICH protein in PC12 cells did not induce neurite generation. Additionally, neither the expression of wild-type zRICH nor the expression of mutant versions of the protein interfered with the levels of differentiation of PC12 cells induced by nerve growth factor, suggesting that, at least in this model of neuronal differentiation, zRICH proteins do not participate in the process of generation of neurites.
Descriptors: zebrafish, nerve growth factors genetics , nerve regeneration physiology, optic nerve physiology, Western blotting, catalysis, cell differentiation genetics, cultured cells, cytosol metabolism, polyacrylamide gel electrophoresis, immunohistochemistry, kinetics, mutation physiology, nerve growth factors physiology, pc12 cells, plasmids genetics, rats, recombinant proteins chemistry, recombinant proteins isolation and purification, transfection, .

Chan, K.M., L.L. Ku, P.C. Chan, and W.K. Cheuk (2006). **Metallothionein gene expression in zebrafish embryo-larvae and ZFL cell-line exposed to heavy metal ions.** *Marine Environmental Research* 62(Suppl): S83-7. ISSN: 0141-1136.
NAL Call Number: QH545.W3M36
Abstract: The aim of this study is to investigate the induction of zebrafish metallothionein (zMT) gene expression following the administration of different metal ions using in vivo and in vitro models. The zebrafish embryo-larvae were used for the in vivo study, and MT gene

expression was studied during the development from fertilization (8hpf) to embryo-larval stage using real-time PCR. The LC50 values and zMT mRNA levels were also measured in embryo-larvae exposed to various metal ions. The general trend of 24 h LC50 values as determined is Cu2+ < Hg2+ < Cd2+ << Zn2+. However, Hg2+ was found to be the most potent metal inducer with the highest level of zMT mRNA induction (40-50 folds) in 8hpf embryo-larvae, followed by Cd2+ (approximately 20 folds); Cu2+ and Zn2+ only gave approximately 5 fold of induction. In the in vitro study of ZFL cell-line, Cd2+ is the most potent inducer of zMT mRNA (up to 250 folds), Cu2+ and Zn2+ gave similar potency of approximately 50-100 folds, and Hg2+ gave approximately 40-50 folds of zMT mRNA levels over the control group.
Descriptors: zebrafish, in vitro, ZFL cell-line, Cd2+, zMT mRNA, Cu2+, Zn2+, Hg2+, metallothionein gene.

Chang, C.P., J.R. Neilson, J.H. Bayle, J.E. Gestwicki, A. Kuo, K. Stankunas, I.A. Graef, and G.R. Crabtree (2004). **A field of myocardial-endocardial NFAT signaling underlies heart valve morphogenesis**. *Cell* 118(5): 649-663. ISSN: 0092 8674.
NAL Call Number: QH573.C42
Abstract: The delicate leaflets that make up vertebrate heart valves are essential for our moment-to-moment existence. Abnormalities of valve formation are the most common serious human congenital defect. Despite their importance, relatively little is known about valve development. We show that the initiation of heart valve morphogenesis in mice requires calcineurin/NFAT to repress VEGF expression in the myocardium underlying the site of prospective valve formation. This repression of VEGF at E9 is essential for endocardial cells to transform into mesenchymal cells. Later, at Ell, a second wave of calcineurin/NFAT signaling is required in the endocardium, adjacent to the earlier myocardial site of NFAT action, to direct valvular elongation and refinement. Thus, NFAT signaling functions sequentially from myocardium to endocardium within a valvular morphogenetic field to initiate and perpetuate embryonic valve formation. This mechanism also operates in zebrafish, indicating a conserved role for calcineurin/NFAT signaling in vertebrate heart valve morphogenesis. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, cardiovascular system, transport and circulation, development, calcineurin, NFAT, heart valve development model.

Chang, H.T. and S.J. Lee (2005). **Expression of Cofilin iosforms in zebrafish embryos**. *Journal of the Fisheries Society of Taiwan* 32(1): 13-14. ISSN: 0379-4180.
NAL Call Number: SH135.T3
Descriptors: zebrafish embryos, actin, bioassays, embryonic development, freshwater fish, gene expression, proteins, transcription, *Danio rerio*, reproduction and development, Taiwan.

Chang, L., B. Khoo, L. Wong, and V. Tropepe (2006). **Genomic sequence and spatiotemporal expression comparison of zebrafish mbx1 and its paralog, mbx2.** *Development Genes and Evolution* 216(10): 647-54. ISSN: 0949 944X.
NAL Call Number: QL951.R68
Abstract: The expression of midbrain homeobox-1 (mbx1) defines a discrete region in the vertebrate neural plate that will give rise to the mesencephalon, as well as subregions of the diencephalon and retinal field. Here, we report on the identification and cloning of a second Mbx gene in zebrafish, termed mbx2. Genomic sequence comparison suggests that mbx1 and mbx2 are derived from the duplication of a single putative ancestral gene that is conserved in other vertebrates as a single copy gene. Furthermore, phylogenetic analyses indicate that

the mbx genes belong to a novel subgroup of paired-like homeobox genes. Finally, quantitative reverse transcriptase-PCR and whole mount in situ hybridization experiments revealed a pattern of partial spatiotemporal expression divergence between the mbx paralogs that correlates with sequence divergence in noncoding regulatory domains. Our data support a subfunctionalization model that may explain the retention of duplicate mbx genes in teleosts.
Descriptors: zebrafish, developmental gene expression regulation, genome, homeodomain amino acid and base sequence, DNA primers, homeodomain proteins chemistry, molecular sequence data, phylogeny, reverse transcriptase PCR , amino acid sequence homology, zebrafish, proteins chemistry.

Chang, M.H., C.M. Chou, Y.C. Hsieh, I.C. Lu, M.K. Devi, J.P. Chang, T.F. Kuo, and C.J. Huang (2004). **Identification of 5'-upstream region of pufferfish ribosomal protein L29 gene as a strong constitutive promoter to drive GFP expression in zebrafish.** *Biochemical and Biophysical Research Communications* 314(1): 249-58. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: The genomic structure of Tetraodon fluviatilis L29 gene was determined and its promoter activity was analyzed in COS-1 cells and zebrafish embryos. The TfL29 gene comprises four exons and three introns, spanning approximately 1.7kb. The 5'-upstream 2.2-kb of the first exon contains 10 E-boxes and many putative binding motifs for transcription factors GATA-1, AML-1a, c-Myb, Oct-1, CdxA, and NRF-2. Promoter activity assay showed that the distal 2.2-kb fragment not only had high luciferase activity in COS-1 cells, but also strong and ubiquitous GFP expression in a variety of tissues in zebrafish embryos. On the other hand, there are no TATA or CAAT boxes within a 300-bp region upstream from the transcription initiation site. Although this region has high luciferase activity in COS-1 cells, it is not sufficient to drive GFP expression in zebrafish embryos. In this proximal 300-bp region, there are two E-boxes, two CdxA sites, and one NRF-2 site that is immediately downstream of the transcription start site.
Descriptors: zebrafish embryos, gene expression regulation, luminescent luminescent proteins metabolism, promoter regions genetics, ribosomal proteins chemistry, ribosomal protein sequence analysis, amino acid and base sequence, COS cells, *Caenorhabditis elegans*, *Drosophila*, green fluorescent proteins, molecular sequence data, recombinant fusion proteins chemistry, genetics and metabolism, ribosomal proteins metabolism, sequence alignment, amino acid sequence homology, species specificity, structure activity relationship, tetraodontiformes, trans activation genetics.

Chang, M.X., P. Nie, H.X. Xie, G.L. Wang, and Y. Gao (2006). **Characterization and expression analysis of TNF-related apoptosis inducing ligand (TRAIL) in grass carp *Ctenopharyngodon idella*.** *Veterinary Immunology and Immunopathology* 110(1/2): 51-63. ISSN: 0165 2427.
NAL Call Number: SF757.2.V38
Abstract: TRAIL (Apo2 ligand) described as a type II transmembrane protein belonging to the TNF superfamily can induce apoptotic cell death in a variety of cell types. In the present study, a putative cDNA sequence encoding the 299 amino acids of TRAIL (GC-TRAIL) and its genomic organization were identified in grass carp *Ctenopharyngodon idella*. The predicted GC-TRAIL sequence showed 44 and 41% identities to chicken and human TRAILs, respectively. In a domain search, a tumour necrosis factor homology domain (THD) was identified in the C-terminal portion of TRAILs. The GC-TRAIL gene consists of five exons, with four intervening introns, spaced over approximately 4 kb of genomic sequence. Analysis of GC-TRAIL promoter region revealed the presence of a number of putative transcription factor

binding sites, such as Sp1, NF-kappaB, AP-1, GATA, NFAT, HNF, STAT, P53 and IRF1 sequences which are important for the expression of other TNF family members. Phylogenetic analysis placed GC-TRAIL and the putative zebrafish (*Danio rerio*) TRAIL obtained from searching the zebrafish database into one separate cluster near mammalian TRAIL genes, but apart from the reported zebrafish TRAIL-like protein, indicating that the GC-TRAIL is an authentic fish TRAIL. Expression analysis revealed that GC-TRAIL is expressed in many tissues, such as in gills, liver, trunk kidney, head kidney, intestine and spleen. Copyright © 2008 CABI

Descriptors: zebrafish, apoptosis, binding sites, complementary DNA, exons, gene expression, genes, genome analysis, genomes, ligands, nucleotide sequences, phylogeny, promoters, transcription factors, tumor necrosis factor, *Ctenopharyngodon idella*, *carp.*

Chang, W.N., J.N. Tsai, B.H. Chen, and T.F. Fu (2006). **Cloning, expression, purification, and characterization of zebrafish cytosolic serine hydroxymethyltransferase.** *Protein Expression and Purification* 46(2): 212-20. ISSN: 1046-5928.

NAL Call Number: QP551.P695818

Abstract: A cDNA which encodes for zebrafish serine hydroxymethyltransferase (SHMT) has been cloned into a pET43.1a vector as a NdeI-EcoRI insert and transformed into HMS174(DE3) cells. After induction with isopropyl thiogalactoside, the enzyme was purified with a three-step purification protocol and about 15 mg of pure enzyme was obtained per liter of culture. Spectral and structural characteristics of the recombinant zebrafish SHMT are similar to the rabbit and human cytosolic SHMT. Kinetic constants for the natural substrates l-serine and tetrahydrofolate are also comparable to the values obtained previously for the rabbit and human cytosolic enzyme.

Descriptors: zebrafish, glycine hydroxymethyltransferase biosynthesis, isolation and purification, recombinant proteins biosynthesis, isolation and purification, proteins biosynthesis, isolation and purification, cytoplasm enzymology and genetics, *Escherichia coli* genetics, growth and development, gene expression, glycine hydroxymethyltransferase chemistry and genetics, rabbits, recombinant proteins chemistry, recombinant amino acid sequence homology, proteins chemistry and genetics.

Chang, Y.T. (2007). **Small-molecule switch for zebrafish gene expression.** *Nature Chemical Biology* 3(3): 135-6. ISSN: 1552-4450.

Descriptors: zebrafish, gene expression genetics, physiology, regulation and physiology, genetic models.

Cheesman, S.E. and J.S. Eisen (2004). **Gsh1 demarcates hypothalamus and intermediate spinal cord in zebrafish.** *Gene Expression Patterns* 5(1): 107-12. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: We have cloned and characterized the expression of zebrafish genetic screen homeobox 1 (gsh1). Early expression is confined to hindbrain rhombomeres; by mid-somitogenesis gsh1 is expressed in precise domains within the mesencephalon and diencephalon, as well as in intermediate spinal cord. Double-label experiments revealed that the diencephalic domain is coincident with hypothalamus and that spinal cord expression is in a region that generates interneurons. These data suggest gsh1 may play a role in patterning cell types generated in these domains.

Descriptors: zebrafish, homeodomain proteins genetics and metabolism, hypothalamus embryology and metabolism, spinal cord embryology, amino acid sequence, molecular sequence data, spinal cord metabolism.

Chen, C. and M.M. Shen (2004). **Two modes by which lefty proteins inhibit nodal signaling**. *Current Biology* 14(7): 618-624. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: During vertebrate embryogenesis, members of the Lefty subclass of Transforming Growth Factor-beta (TGFbeta) proteins act as extracellular antagonists of the signaling pathway for Nodal, a TGFbeta-related ligand essential for mesendoderm formation and left-right patterning (1-3). Genetic and biochemical analyses have shown that Nodal signaling is mediated by activin receptors but also requires EGF-CFC coreceptors, such as mammalian Cripto or Cryptic (4-8). Misexpression experiments in zebrafish and frogs have suggested that Lefty proteins can act as long-range inhibitors for Nodal, possibly through competition for binding to activin receptors (9-13). Here we demonstrate two distinct and unexpected mechanisms by which Lefty proteins can antagonize Nodal activity. In particular, using a novel assay for Lefty activity in mammalian cell culture, we find that Lefty can inhibit signaling by Nodal but not by Activin or TGFbeta1, which are EGF-CFC independent. We show that Lefty can interact with Nodal in solution and thereby block Nodal from binding to activin receptors. Furthermore, Lefty can also interact with EGF-CFC proteins and prevent their ability to form part of a Nodal receptor complex. Our results provide mechanistic insights into how Lefty proteins can achieve efficient and stringent regulation of a potent signaling factor. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, cell biology, development, molecular genetics, biochemistry and molecular biophysics, biochemical analysis, laboratory techniques, cell culture, culturing techniques, laboratory techniques for genetic analysis, genetic techniques, embryogenesis, left right patterning.

Chen, E., P.B. Hackett and S.C. Ekker (2004). **Gene 'knockdown' approaches using unconventional antisense oligonucleotides**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd.: Singapore, Singapore , p. 454-475. ISBN: 9812388214.

NAL Call Number: QL638.C94 F57

Abstract: Derived from therapeutic tools developed for the clinic, unconventional antisense technology has emerged as a new and broadly applicable RNA-based gene inhibition approach. Its targeting mechanisms of action are both RNaseH-independent and distinct from other sequence-based tools such as small inhibitory RNAs. Among the novel classes of antisense oligonucleotides, morpholino phosphorodiamidate oligonucleotides (morpholinos) have emerged as the preferred effector molecules of targeted gene "knockdown" strategies. Morpholinos have been shown to be extremely effective, specific and convenient for elucidating gene functions in a variety of model systems. This chapter provides an overview on technical aspects of morpholino usage and some examples of the many potential biological applications of this technology ranging from human disease modeling to functional genomics. Copyright © 2008 CABI

Descriptors: zebrafish, DNA, exons, genes, genetic models, genomes, messenger RNA, nucleotide sequences, oligonucleotides, RNA, translation, *Danio rerio*.

Chen, G.D., C.M. Chou, S.P. Hwang, F.F. Wang, Y.C. Chen, C.C. Hung, J.Y. Chen, and C.J. Huang (2006). **Requirement of nuclear localization and transcriptional activity of p53 for its targeting to the yolk syncytial layer (YSL) nuclei in zebrafish embryo and its use for apoptosis assay.** *Biochemical and Biophysical Research Communications* 344(1): 272-82. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: We expressed zebrafish p53 protein fused to GFP by a neuron-specific HuC promoter in zebrafish embryos. Instead of displaying neuronal expression patterns, p53-GFP was targeted to zebrafish YSL nuclei. This YSL targeting is p53 sequence-specific because GFP fusion proteins of p63 and p73 displayed neuronal-specific patterns. To dissect the underlying mechanisms, various constructs encoding a series of p53 mutant proteins under the control of different promoters were generated. Our results showed that expression of p53, in early zebrafish embryo, is preferentially targeted to the nuclei of YSL, which is mediated by importin. Similarly, this targeting is abrogated when p53 nuclear localization signal is disrupted. In addition, the transcriptional activity of p53 is required for this targeting. We further showed that fusion of pro-apoptotic BAD protein to p53-GFP led to apoptosis of YSL cells, and subsequent imperfect microtubule formation and abnormal blastomere movements.

Descriptors: zebrafish, apoptosis, cell nucleus metabolism, genetic transcription, yolk sac metabolism, biological assay, cell nucleus chemistry, cytosol chemistry and metabolism, cytology, metabolism, green fluorescent proteins analysis, genetics and metabolism, Hu paraneoplastic encephalomyelitis antigens genetics, karyopherins metabolism, mutation, neurons chemistry and metabolism, nuclear localization signals genetics and metabolism, recombinant fusion proteins analysis, genetics and metabolism, tumor suppressor protein p53 analysis, genetics and metabolism, yolk sac chemistry and cytology, proteins analysis, genetics and metabolism, BCl associated death protein analysis, genetics and metabolism.

Chen, H., T. Leung, K.E. Giger, A.M. Stauffer, J.E. Humbert, S. Sinha, E.J. Horstick, C.A. Hansen, and J.D. Robishaw (2007). **Expression of the G protein gammaT1 subunit during zebrafish development.** *Gene Expression Patterns* 7(5): 574-83. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Here, we report the identification and expression analysis of the zebrafish G protein gammaT1 subunit gene (gngT1) during development. Similar to its human and mouse homologs, we confirm zebrafish gngT1 is expressed in the developing retina, where its transcription overlaps with the photoreceptor cell-specific marker, rhodopsin (rho). Surprisingly, we also show zebrafish gngT1 is expressed in the dorsal diencephalon, where its transcription overlaps with the pineal specific markers, arylalkylamine N-acetyltransferase-2 (annat-2) and extra-ocular rhodopsin (exorh). Analysis of the proximal promoter sequence of the zebrafish gngT1 gene identifies several conserved binding sites for the cone-rod homeobox/orthodenticle (Crx/Otx) homeodomain family of transcription factors. Using a morpholino anti-sense approach in zebrafish, we show that targeted knockdown of otx5 potently suppresses gngT1 expression in the pineal gland, whereas knockdown of crx markedly reduces gngT1 expression in the retina. Taken together, these data indicate that pineal- and retinal-specific expression of the gngT1 gene are controlled by different transcription factors and exogenous signals.

Descriptors: zebrafish , GTP binding protein gamma subunits metabolism genetics, developmental gene expression regulation, proteins metabolism, amino acid and base sequence, molecular cloning, homeodomain proteins genetics and metabolism, in situ hybridization, molecular sequence data, antisense oligonucleotides pharmacology, otx transcription factors genetics and metabolism, photoreceptors metabolism, pineal gland cytology and metabolism, RNA probes, retina cytology and metabolism, rhodopsin metabolism, amino acid sequence homology, trans activators genetics and metabolism, zebrafish growth, development and metabolism.

Chen, J., C. Jette, J.P. Kanki, J.C. Aster, A.T. Look, and J.D. Griffin (2007). **NOTCH1-induced T-cell leukemia in transgenic zebrafish.** *Leukemia* 21(3): 462-71. ISSN: 0887-6924.

Abstract: Activating mutations in the NOTCH1 gene have been found in about 60% of patients with T-cell acute lymphoblastic leukemia (T-ALL). In order to study the molecular mechanisms by which altered Notch signaling induces leukemia, a zebrafish model of human NOTCH1-induced T-cell leukemia was generated. Seven of sixteen mosaic fish developed a T-cell lymphoproliferative disease at about 5 months. These neoplastic cells extensively invaded tissues throughout the fish and caused an aggressive and lethal leukemia when transplanted into irradiated recipient fish. However, stable transgenic fish exhibited a longer latency for leukemia onset. When the stable transgenic line was crossed with another line overexpressing the zebrafish bcl2 gene, the leukemia onset was dramatically accelerated, indicating synergy between the Notch pathway and the bcl2-mediated antiapoptotic pathway. Reverse transcription-polymerase chain reaction analysis showed that Notch target genes such as her6 and her9 were highly expressed in NOTCH1-induced leukemias. The ability of this model to detect a strong interaction between NOTCH1 and bcl2 suggests that genetic modifier screens have a high likelihood of revealing other genes that can cooperate with NOTCH1 to induce T-ALL.

Descriptors: zebrafish, neoplastic cell transformation genetics, acute T cell leukemia etiology and genetics, proto oncogene proteins C Bcl 2 physiology, Notch1 receptor physiology and genetics, genetically modified, apoptosis, basic helix loop helix transcription factors physiology, gamma rays, gene expression profiling and regulation, leukemic, gene rearrangement, alpha chain T cell antigen receptor, genes, Bcl 2, mosaicism, neoplasm proteins biosynthesis, genetics, physiology and transplantation, oncogenes, radiation chimera, radiation tolerance, recombinant fusion, signal transduction, time factors, .

Chen, J., H. Ruan, S.M. Ng, C. Gao, H.M. Soo, W. Wu, Z. Zhang, Z. Wen, D.P. Lane, and J. Peng (2005). **Loss of function of def selectively up-regulates Delta113p53 expression to arrest expansion growth of digestive organs in zebrafish.** *Genes and Development* 19(23): 2900-11. ISSN: 0890-9369.

NAL Call Number: QH426.G46

Abstract: Transcription factor p53 forms a network with associated factors to regulate the cell cycle and apoptosis in response to environmental stresses. However, there is currently no direct genetic evidence to show if or how the p53 pathway functions during organogenesis. Here we present evidence to show that the zebrafish def (digestive-organ expansion factor) gene encodes a novel pan-endoderm-specific factor. A loss-of-function mutation in def confers hypoplastic digestive organs and selectively up-regulates the expression of Delta113p53, counterpart to a newly identified isoform of p53 produced by an alternative internal promoter in intron 4 of the p53 gene in human. The increased Delta113p53 expression is limited to within the mutant digestive organs, and this increase selectively induces the expression of p53-responsive genes to trigger the arrest of the cell cycle but not apoptosis, resulting in compromised organ growth in the mutant. Our data demonstrate that, while induction of expression of p53 and/or its isoforms is crucial to suppress abnormal cell growth, Delta113p53 is tightly regulated by an organ/tissue-specific factor Def, especially during organogenesis, to prevent adverse inhibition of organ/tissue growth.

Descriptors: zebrafish, organogenesis genetics, tumor suppressor protein p53 genetics, up regulation, cell cycle, cell proliferation, digestive system growth and development endoderm, promoter regions genetics, protein isoforms, .

Chen, J.Y., J.C. Chen, W.T. Huang, C.W. Liu, C.F. Hui, T.T. Chen, and J.L. Wu (2004). **Molecular cloning and tissue-specific, developmental-stage-specific, and hormonal regulation of IGFBP3 gene in zebrafish.** *Marine Biotechnology* 6(1): 1-7. ISSN: 1436-2228.
NAL Call Number: TP248.27.M37M37
Abstract: The biological functions of insulin-like growth factor (IGF) I and II are modulated by a family of IGF-binding proteins (IGFBPs) in complex IGF-dependent and IGF-independent pathways. For further understanding of the actions of IGFs, some of these binding proteins have been cloned and characterized. We report the molecular cloning of IGFBP-3 cDNA for zebrafish. The tissue-specific and developmental stage-specific expression of IGFBP-3 and the hormonal regulation of its expression have also been determined by comparative reverse transcription polymerase chain reaction. Zebrafish IGFBP-3 cDNA contains an open reading frame of 879 bp, encoding a polypeptide of 293 amino acid residues. Results of this analysis revealed high levels of IGFBP-3 messenger RNA in ovary and fin tissue. Expression of IGFBP-3 mRNA was throughout the entire embryonic development, with the highest level of expression observed at 36 hours after the onset of development. Elevated levels of expression of IGFBP-3 were observed 24 hours after injection with IGF-I and 48 hours with IGF-II or insulin. These results suggest that the expression of IGFBP3 gene might be modulated by IGF-I, IGF-II, and insulin.
Descriptors: zebrafish, developmental gene expression regulation physiology, insulin like growth factor binding protein 3 genetics, amino acid sequence, DNA primers, complementary DNA genetics, food deprivation physiology, developmental gene expression regulation drug effects, insulin pharmacology, insulin like growth factor binding protein 3 metabolism, molecular sequence data, ovary metabolism, messenger RNA genetics, reverse transcriptase PCR, sequence alignment, DNA sequence analysis, somatomedins pharmacology.

Chen, J.Y., M.J. Chou, H.Y. Gong, T.C. Huang, J.L. Wu, and C.M. Kuo (2005). **Cloning and biological analysis of the zebrafish *(Danio rerio*) insulin-like growth factor binding protein-2 proximal promoter region.** *DNA and Cell Biology* 24(3): 199-208. ISSN: 1044 5498.
NAL Call Number: QH443.D5
Abstract: Insulin-like growth factor binding protein 2 (IGFBP-2) plays an important role in the regulation of IGF's action and endocrinology in fish. To understand the molecular mechanism which controls transcription of the IGFBP-2 gene, we cloned and sequenced the IGFBP-2 proximal promoter region of the zebrafish IGFBP-2 gene and characterized its activity by firefly luciferase transient transfection expression assays. Different fragments of the zebrafish IGFBP-2 5'-flanking region were transfected into Hela and ZFL cells. In these cell lines, maximum promoter activity was located in the 900 base pairs (bp) of the zebrafish IGFBP-2 5' flanking region in the ZFL cell line and 318 bp of the zebrafish IGFBP-2 5' flanking region in the Hela cell line. The in vivo actions of the IGFBP-2 promoter on developmental stage expression were further investigated in transgenic zebrafish in which an IGFBP-2 (900-bp) promoter-driven green fluorescent protein encoding the GFP cDNA transgene was microinjected into zebrafish embryos. Morphological and RT-PCR studies of transgenic zebrafish indicated that the IGFBP-2 promoter-driven GFP transcripts appeared for the first time in the 32-cell stage. These results indicate that the IGFBP-2 promoter is active in a development-specific manner. These results suggest that the IGFBP-2 promoter plays an important role in teleost embryo growth.
Descriptors: zebrafish embryos, insulin like growth factor binding protein 2 genetics, base sequence, molecular cloning, fireflies genetics, developmental gene expression regulation, molecular sequence data, promoter regions genetics, transfection.

Chen, J.Y., C.C. Wei, M.J. Chiou, H.Y. Su, and C.M. Kuo (2007). **Cloning and expression analysis of a protein kinase C gene, PKCmu, and its regulation of the promoter region in zebrafish.** *DNA and Cell Biology* 26(6): 415-24. ISSN: 1044 5498.

NAL Call Number: QH443.D5

Abstract: The cDNA and genomic DNA of zebrafish (*Danio rerio*) protein kinase Cmu (PKCmu), with its promoter region, were obtained. The 508-amino acid zebrafish PKCmu has 86.17% similarity to human PKCmu. Real-time reverse-transcription polymerase chain reaction analysis with starvation and hormonal treatment found significant differences between the control group and the experimental group after 14 days of starvation. After injecting insulin-like growth factor II (IGF-II), growth hormone (GH), insulin, or human chorionic gonadotropin, significant differences were observed between the control and experimental groups 24 h after treatment. After injecting the gonadotropin-releasing hormone or luteotropin-releasing hormone, significant differences were seen between the control and experimental groups 15 h after treatment. These results suggest that in vivo PKCmu expression is regulated by the insulin family or by the GH, but other sex hormones produced a significant expression level more quickly than the insulin family and GH. The zebrafish PKCmu gene is located on zebrafish chromosome 17 and consists of 16 exons. A 2.6 kilobase pair on the 5' flanking region displayed maximal promoter activity in the zebrafish liver (ZFL) cell line after treatment with IGF-I, IGF-II, and GH. However, a 1.6 kilobase pair on the 5' flanking region displayed maximal promoter activity in the HeLa cell line after treatment with IGF-I, IGF-II, and GH. Finally, PKCmu may have important nuclear effects on cell growth and may involve nuclear localization. By transiently transfecting ZFL cells with various zebrafish PKCmu segments, we identified a nuclear localization signal: the amino acid sequence between amino acids 206 and 209 was able to predominantly direct enhanced green fluorescence protein (EGFP) into the nucleus, whereas a deletion of this motif abrogated the nuclear localization property.

Descriptors: zebrafish, protein kinase C genetics, 5' untranslated regions, amino acid and base sequence, molecular cloning, DNA primers genetics, complementary DNA genetics, enzymologic gene expression regulation drug effects, reporter genes , green fluorescent proteins genetics and metabolism, hormones pharmacology, molecular sequence data, nuclear localization signals chemistry and genetics, promoter regions genetics, protein kinase C chemistry, recombinant proteins genetics and metabolism, amino acid sequence homology, starvation enzymology and genetics.

Chen, J.Y., Y.K. You, J.C. Chen, T.C. Huang, and C.M. Kuo (2005). **Organization and promoter analysis of the zebrafish (*Danio rerio*) interferon gene.** *DNA and Cell Biology* 24(10): 641-50. ISSN: 1044 5498.

NAL Call Number: QH443.D5

Abstract: Interferon plays important roles in confronting viral infections as the first line of defense. For the purpose of understanding the molecular mechanism which controls transcription of the interferon gene, we cloned and sequenced the interferon promoter region of the zebrafish interferon gene and characterized its activity using firefly luciferase transient transfection expression assays. Different fragments of the zebrafish interferon 5'-flanking region were transfected into ZFL cells. In these cell lines, maximum promoter activity was located in 2.2 kb of the zebrafish interferon 5' flanking region of the ZFL cell line. In this study, we investigated whether the viral replicative intermediate double-stranded RNA (herein we used synthetic polyinosinic-polycytidylic acid [poly(I):poly(C)] modifies the effects of interferon on gene expression. For this purpose, all zebrafish interferon promoter fragments were treated with either 1, 10, or 100 microg/ml poly(I):poly(C). The results

showed that after treatment with 10 microg/ml poly(I):poly(C), high promoter activity was observed in the -2.2-kb interferon promoter fragment. Several putative transcription factors were shown in the promoter region, including IRF-1, C/EBP, NFkappaB, and GATA-1. Further study of the in vivo expression of the interferon promoter during development was carried out in transgenic zebrafish expressing an interferon promoter-driven green fluorescent protein (GFP) encoding the GFP cDNA transgene. Morphological studies of transgenic zebrafish indicated that the interferon promoter-driven GFP transcripts appeared in the yolk, head, and lymphoid organs. These results indicate that the interferon promoter is active in a tissue-specific manner, and suggest that the interferon promoter plays an important role in virus resistance during teleost growth.

Descriptors: zebrafish, developmental gene expression regulation, interferons genetics, promoter regions genetics, transcription factors metabolism, amino acid sequence, genetically modified, base sequence, cultured cells, complementary DNA genetics, physiology, green fluorescent proteins genetics and metabolism, interferons metabolism, mice, molecular sequence data, NIH 3t3 cells, poly C genetics, poly I genetics, double stranded RNA genetics, genetic transcription, zebrafish growth and development.

Chen, J.Y., J.C. Chen, S.H. Lin, C.Y. Pan, and C.M. Kuo (2006). **Organization and promoter analysis of a tiger shrimp *Penaeus monodon* single wap domain-containing protein gene**. *Fisheries Science (Tokyo)* 72(5): 1086-1095. ISSN: 0919 9268.

NAL Call Number: SH1.F8195

Abstract: The main function of the single whey acidic protein domain (SWD)-containing protein in shrimp is unknown. To elucidate the function of the SWD-containing protein in vivo, the SWD-containing protein gene was isolated and characterized. A 9.3-kb shrimp SWD-containing protein gene fragment was sequenced, including a 0.9-kb 5'-flanking region, the 4.5-kb SWD-containing protein gene, and a 3.9-kb 3'-flanking region. The shrimp SWD-containing protein gene contained three exons and two introns. Different fragments of the shrimp SWD-containing protein 5'-flanking region were transfected into HeLa cells. The promoter activities were assayed by basal human chorionic gonadotropin (HCG), luteinizing hormone-releasing hormone (LRH), and gonadotropin-releasing hormone (GnRH) treatments. The in vitro actions of the SWD-containing protein promoter expression pattern were studied by transfection of an SWD-containing protein promoter (1 kb)-driven green fluorescent protein (GFP) encoding the GFP cDNA transgene into the HeLa cell line, which was then microinjected into zebrafish *Danio rerio* embryos. These results indicate that the shrimp SWD-containing protein promoter might play an important role in gene regulation of sex hormones in mammalian cell lines and in gene regulation of developmental stages in zebrafish. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, molecular genetics, protein organization, gene promoter analysis, acidic protein domain, gonadotropin releasing hormone.

Chen, J.-Y., J.-C. Chen, and J.-L. Wu (2004). **Functional analysis of zebrafish *(Danio rerio)* IGFBP-1 and IGFBP-2 and biological activities of fish recombinant protein in Inhibition of tumor cell Differentiation**. *Journal of the Fisheries Society of Taiwan* 31(4): 295-310. ISSN: 0379-4180.

NAL Call Number: SH135.T3

Descriptors: zebrafish, DNA, differentiation, endocrinology, functional analysis, gene expression, growth factors, growth hormone, heart, hormones, insulin, insulin-like growth factor-binding protein 1 and 2, liver, mitochondria, muscles, nucleotide sequence, ovaries, PCR, polypeptides, proteins, tumor cells, mRNA, reductase, *Danio rerio.*

Chen, K., X. Huang, Y. Chai, and H.Y. Gaisano (2007). **Characterization of SNAP-25 gene from marine teleostean, *Lateolabrax japonicus*.** *Chinese Journal of Oceanology and Limnology* 25(4): 378-385. ISSN: 0254-4059.

Descriptors: zebrafish, bioinformatics, exocytosis, gene expression, limnology, nerves, plasma membranes, PCR, SNAp receptors, SNAp-25 protein, vesicle fusion, Western blotting, *Carassius auratus* , *Danio rerio*, *Lateolabrax japonicus.*

Chen, L., J.H. Yao, S.H. Zhang, L. Wang, H.D. Song, and J.L. Xue (2005). **Slit-like 2, a novel zebrafish slit homologue that might involve in zebrafish central neural and vascular morphogenesis.** *Biochemical and Biophysical Research Communications* 336(1): 364-71. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: Nervous and vascular systems grow as parallel networks, indicating common cues in distal targets. We have identified a novel zebrafish gene slit-like 2 (slitl2) that might involve in zebrafish central neural and vascular morphogenesis. Whole-mount in situ hybridization of zebrafish embryo detected distinct signals of slitl2 transcripts in zebrafish midline structure of central nervous system similar to that of slits. Strong expression is also observed in zebrafish vasculature. Zebrafish slitl2 shares amino acid sequence identity of 41% with *Homo sapiens* slitl2 (vasorin) and Mus musculus slitl2, and 35%, 33% with *Danio rerio* slit3, slit2. Analysis of zebrafish slitl2 cripto growth factor domain, extracellular matrix protein slit domain, and putative signal peptide confirms that as a secreted and cell-surface protein slitl2 may be essential in axon guidance, vessel development, and axis patterning. These results provide evidence that slitl2 may play important roles in zebrafish central nervous system and vascular morphogenesis.

Descriptors: zebrafish, blood vessels embryology, central nervous system embryology, amino acid and base sequence, complementary DNA, molecular sequence data, morphogenesis, amino acid sequence homology, proteins chemistry.

Chen, P.Y., H. Manninga, K. Slanchev, M. Chien, J.J. Russo, J. Ju, R. Sheridan, B. John, D.S. Marks, D. Gaidatzis, C. Sander, M. Zavolan, and T. Tuschl (2005). **The developmental miRNA profiles of zebrafish as determined by small RNA cloning.** *Genes and Development* 19(11): 1288-93. ISSN: 0890-9369.

NAL Call Number: QH426.G46

Abstract: MicroRNAs (miRNAs) represent a family of small, regulatory, noncoding RNAs that are found in plants and animals. Here, we describe the miRNA profile of the zebrafish *Danio rerio* resolved in a developmental and cell-type-specific manner. The profiles were obtained from larger-scale sequencing of small RNA libraries prepared from developmentally staged zebrafish, and two adult fibroblast cell lines derived from the caudal fin (ZFL) and the liver epithelium (SJD). We identified a total of 154 distinct miRNAs expressed from 343 miRNA genes. Other experimental/computational sources support an additional 10 miRNAs encoded by 19 genes. The miRNAs can be classified into 87 distinct families. Cross-species comparison indicates that 81 families are conserved in mammals, 17 of which also have at least one member conserved in an invertebrate. Our analysis reveals that the zygotes are essentially devoid of miRNAs and that their expression begins during the blastula period with a zebrafish-specific family of miRNAs encoded by closely spaced multicopy genes. Computational predictions of zebrafish miRNA targets are provided that take into account the depth of evolutionary conservation. Besides miRNAs, we identified a prominent class of repeat-associated small interfering RNAs (rasiRNAs).

Descriptors: zebrafish, microRNAs genetics, base sequence, cell line, molecular cloning, microRNAs chemistry, rasiRNAs.

Chen WeiJen, G. Orti, and A. Meyer (2004). **Novel evolutionary relationship among four fish model systems**. *Trends in Genetics* 20(9): 424-431. ISSN: 0168 9525.
NAL Call Number: QH426.T744
Abstract: Knowledge of the correct phylogenetic relationships among animals is crucial for the valid interpretation of evolutionary trends in biology. Zebrafish, medaka, pufferfish and cichilds are fish models for development, genomics and comparative genetics studies, although their phylogenetic relationships have not been tested rigorously. The results of phylogenomic analysis based on 20 nuclear protein-coding genes confirmed the basal placement of zebrafish in the fish phylogeny but revealed an unexpected relationship among the other three species, contrary to traditionally held systematic views based on morphology. Our analyses show that medaka (Beloniformes) and cichlids (Perciformes) appear to be more closely related to each other than either of them is to pufferfish (Tetraodontiformes), suggesting that a re-interpretation of some findings in comparative biology might be required. In addition, phylogenomic analyses show that fish typically have more copies of nuclear genes than land vertebrates, supporting the fish-specific genome duplication hypothesis. Copyright © 2008 CABI
Descriptors: zebrafish, evolution, models, phylogenetics, reviews, cichlidae, *Danio rerio*, *Oryzias latipes*, Tetraodontidae, comparative biology data.
Notes: A review.

Chen, X., Y. Masuda, T. Yoshida, and M. Mishina (2006). **Molecular cloning and expression patterns of zebrafish receptor protein tyrosine phosphatase sigma**. *Journal of Pharmacological Sciences* 100(Suppl. 1): 245P. ISSN: 1347 8613.
Descriptors: zebrafish, enzymology, muscular system, nervous system, cloning, genetic techniques, reverse transcriptase PCR, in situ hybridization, histology and cytology.

Chen, Y.G., P. Song, D.Y. Lu, W. Zhou, and J.F. Gui (2005). **Study on the expression of zebrafish (*Danio rerio*) nanos1 during gametogenesis by in situ hybridization**. *Yi Chuan; Hereditas/ Zhongguo Yi Chuan Xue Hui Bian Ji* 27(4): 589-94. ISSN: 0253-9772.
Abstract: Using in situ hybridization on tissue sections with DIG antisense RNA probe, the distribution of zebrafish (*Danio rerio*) nanos1 mRNA during oogenesis and spermatogenesis was detected, and the expression and function of nanos 1 during zebrafish gametogenesis was preliminarily studied. The results were as follows: nanos 1 mRNA uniformly dispersed throughout the cytoplasm of oocytes at all stages. Strong expression of nanos1 mRNA was observed in oogonia and stage I, II oocytes, but the signal became weaker in later stage oocytes. Strong expression of nanos 1 mRNA was detected in spermatogonia and relatively weaker signal was found in primary spermatocytes but no nanos 1 mRNA expression was observed in spermatids. The results suggested that nanos 1 may play an important role in maintenance and functioning of the germline stem cell-oogonia and spermatogonia.
Descriptors: zebrafish, DIG antisense, RNA probe, zebrafish, nanos1, mRNA, oogenesis, oogonia, oocytes, spermatogenesis.
Language of Text: Chinese.

Chen, Y.H., F.L. Huang, Y.C. Cheng, C.J. Wu, C.N. Yang, and H.J. Tsay (2008). **Knockdown of zebrafish Na sub(v)1.6 sodium channel impairs embryonic locomotor activities**. *Journal of Biomedical Science* 15(1): 69-78. ISSN: 1021-7770.

NAL Call Number: R850.A1
Descriptors: zebrafish, Na sub(v)1.6, sodium, channel, locomotor movement, antisense, morpholino, knockdown, MO1, MO2, RNA splicing.

Cheng, L., M. Chong, W. Fan, X. Guo, W. Zhang, X. Yang, F. Liu, Y. Gui, and D. Lu (2007). **Molecular cloning, characterization, and developmental expression of foxp1 in zebrafish**. *Development Genes and Evolution* 217(10): 699-707. ISSN: 0949-944X.
NAL Call Number: QL951.R68
Descriptors: zebrafish, forkhead transcription factor, gene, development, foxp1, cDNA.

Cheung, C.Y., S.E. Webb, A. Meng, and A.L. Miller (2006). **Transient expression of apoaequorin in zebrafish embryos: extending the ability to image calcium transients during later stages of development.** *International Journal of Developmental Biology, The* 50(6): 561-9. ISSN: 0214-6282.
Abstract: When aequorin is microinjected into cleavage-stage zebrafish embryos, it is largely used up by ~24 hours. Thus, it is currently not possible to image Ca(2+) signals from later stages of zebrafish development using this approach. We have, therefore, developed protocols to express apoaequorin, i.e., the protein component of aequorin, transiently in zebrafish embryos and then reconstitute intact aequorin in vivo by loading the coelenterazine co-factor into the embryos separately. Two types of apoaequorin mRNA, aeq-mRNA and aeq::EGFP-mRNA, the latter containing the enhanced green fluorescent protein (EGFP) sequence, were in vitro transcribed and when these were microinjected into embryos, they successfully translated apoaequorin and a fusion protein of apoaequorin and EGFP (apoaequorin-EGFP), respectively. We show that aeq::EGFP -mRNA was more toxic to embryos than equivalent amounts of aeq-mRNA. In addition, in an in vitro reconstitution assay, apoaequorin-EGFP produced less luminescence than apoaequorin, after reconstitution with coelenterazine and with the addition of Ca(2+). Furthermore, when imaging intact coelenterazine-loaded embryos that expressed apoaequorin, Ca(2+)signals from ~2.5 to 48 hpf were observed, with the spatio-temporal pattern of these signals up to 24 hpf, being comparable to that observed with aequorin. This transient aequorin expression approach using aeq-mRNA provides a valuable tool for monitoring Ca(2+)signaling during the 2448 hpf period of zebrafish development. Thus, it effectively extends the aequorin-based Ca(2+) imaging window by an additional 24 hours.
Descriptors: zebrafish embryos, aequorin genetics, apocalcium signaling physiology, aequorin biosynthesis, apoproteins biosynthesis, imidazoles, fluorescence microscopy, pyrazines, recombinant proteins biosynthesis and genetics, transfection.

Chien, C.B. (2005). **Using genetics and imaging to understand retinal axon guidance**. *Federation of American Societies for Experimental Biology* 19(4, Suppl. S, Part 1): A786. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: watch neural connections develop in an intact vertebrate embryo. Not only can we visualize growing axons in fixed and living tissue with single-cell resolution, but we can examine the mechanisms that guide these axons using molecular,genetic, and embryological perturbations. The highly stereotyped development of the wildtype visual projection allows us to see errors (and their correction), as well as allowing us to detect mutant phenotypes with great sensitivity.The large set of available mutants show specific defects in pathfinding from the eye to the tectum (astray/robo); in topographic sorting of axons in the optic tract between the eye and the tectum (boier/extl3, dackel/ext2 and pinscher); and in topographic

targeting along the dorsoventral axis of the tectum (nevermind and who-cares). We have systematically cloned the affected genes and analyzing their function. Complementing this forward-genetic approach, we are using a reverse-genetic approach to analyze the functions of candidate retinal axon guidance genes, by injecting morpholino antisense oligos to analyze loss-of-function phenotypes.The combination of genetic analysis with in vivo imaging should eventually lead to a truly comprehensive understanding of the developmental mechanisms of this axonal projection. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, molecular genetics, biochemistry , nervous system, neural coordination, sense organs, sensory reception, retinal axon guidance genes.

Chinen, A., T. Hamaoka, Y. Yamada, and S. Kawamura (2003). **Gene duplication and spectral diversification of cone visual pigments of zebrafish.** *Genetics* 163(2): 663-75. ISSN: 0016-6731.
NAL Call Number: 442.8G28
Abstract: Zebrafish is becoming a powerful animal model for the study of vision but the genomic organization and variation of its visual opsins have not been fully characterized. We show here that zebrafish has two red (LWS-1 and LWS-2), four green (RH2-1, RH2-2, RH2-3, and RH2-4), and single blue (SWS2) and ultraviolet (SWS1) opsin genes in the genome, among which LWS-2, RH2-2, and RH2-3 are novel. SWS2, LWS-1, and LWS-2 are located in tandem and RH2-1, RH2-2, RH2-3, and RH2-4 form another tandem gene cluster. The peak absorption spectra (lambdamax) of the reconstituted photopigments from the opsin cDNAs differed markedly among them: 558 nm (LWS-1), 548 nm (LWS-2), 467 nm (RH2-1), 476 nm (RH2-2), 488 nm (RH2-3), 505 nm (RH2-4), 355 nm (SWS1), 416 nm (SWS2), and 501 nm (RH1, rod opsin). The quantitative RT-PCR revealed a considerable difference among the opsin genes in the expression level in the retina. The expression of the two red opsin genes and of three green opsin genes, RH2-1, RH2-3, and RH2-4, is significantly lower than that of RH2-2, SWS1, and SWS2. These findings must contribute to our comprehensive understanding of visual capabilities of zebrafish and the evolution of the fish visual system and should become a basis of further studies on expression and developmental regulation of the opsin genes.
Descriptors: zebrafish, gene duplication, opsin genetics, amino acid sequence, Southern blotting, molecular sequence data, opsin chemistry, phylogeny, physical chromosome mapping, sequence alignment, spectrophotometry.

Chinen, A., Y. Matsumoto, and S. Kawamura (2005). **Reconstitution of ancestral green visual pigments of zebrafish and molecular mechanism of their spectral differentiation.** *Molecular Biology and Evolution* 22(4): 1001-10. ISSN: 0707-4038.
NAL Call Number: QH506.M642
Abstract: We previously reported that zebrafish have four tandemly duplicated green (RH2) opsin genes (RH2-1, RH2-2, RH2-3, and RH2-4). Absorption spectra vary widely among the four photopigments reconstituted with 11-cis retinal, with their peak absorption spectra (lambda(max)) being 467, 476, 488, and 505 nm, respectively. In this study, we inferred the ancestral amino acid (aa) sequences of the zebrafish RH2 opsins by likelihood-based Bayesian statistics and reconstituted the ancestral opsins by site-directed mutagenesis. The ancestral pigment (A1) to the four zebrafish RH2 pigments and that (A3) to RH2-3 and RH2-4 showed lambda(max) at 506 nm, while that (A2) to RH2-1 and RH2-2 showed a lambda(max) at 474 nm, indicating that a spectral shift had occurred toward the shorter wavelength on the evolutionary lineages A1 to A2 by 32 nm, A2 to RH2-1 by 7 nm, and A3 to RH2-3 by 18 nm. Pigment chimeras and site-directed mutagenesis revealed a large con-

tribution (approximately 15 nm) of glutamic acid to glutamine substitution at residue 122 (E122Q) to the A1 to A2 and A3 to RH2-3 spectral shifts. However, the remaining spectral differences appeared to result from complex interactive effects of a number of aa replacements, each of which has only a minor spectral contribution (1-3 nm). The four zebrafish RH2 pigments cover nearly an entire range of lambda(max) distribution among vertebrate RH2 pigments and provide an excellent model to study spectral tuning mechanisms of RH2 in vertebrates.

Descriptors: zebrafish, retinal pigments chemistry, amino acid sequence, Bayes theorem, molecular sequence data, mutation, retinal pigments genetics, amino acid sequence homology, spectrum analysis.

Chiou, M.J., Y.D. Wang, C.M. Kuo, J.C. Chen, and J.Y. Chen (2007). **Functional analysis of Mitogen-Activated Protein Kinase-3 (MAPK3) and Its regulation of the promoter region in zebrafish.** *DNA and Cell Biology* 26(11): 781-90. ISSN: 1044 5498.

NAL Call Number: QH443.D5

Abstract: Mitogen-activated protein kinase (MAPK) plays a pivotal role in intracellular actions in response to a variety of extracellular stimuli. Real-time reverse-transcription polymerase chain reaction analysis of MAPK3 tissue distribution in zebrafish showed significant differences in the fin and liver compared with muscle. A 1.2-kilobase (kb) pair and a 2.3-kb fragment of the 5'-flanking region displayed minimal promoter activity in the zebrafish liver (ZFL) and HeLa cell lines after treatment with insulin-like growth factors (IGF-I and IGF-II). Targeted knockdown of the MAPK3 gene by two antisense morpholino oligonucleotides revealed that although the zebrafish MAPK3 MO 1-targeted sequence was located at 5' untranslated region and the zebrafish MAPK3 MO 2-targeted sequence was located in the mature peptide region, similar results were shown in zebrafish for disruption of notochord development, with the whole body exhibiting distortion. From a comparative point of view, this study of the MAPK3 gene in zebrafish might not correlate well with previously published studies on mice. These molecular results suggest that MAPK3 plays an important role in whole-body development and is required for general embryonic development. Finally, MAPK3 may play important roles in fish cell growth.

Descriptors: zebrafish, functional analysis, Mitogen-Activated Protein kinase-3, MAPK3, regulation, notochord development, promoter region.

Chiu, C., K. Dewar, G.P. Wagner, K. Takahashi, F. Ruddle, C. Ledje, P. Bartsch, J.L. Scemama, E. Stellwag, C. Fried, S.J. Prohaska, P.F. Stadler, and C.T. Amemiya (2004). **Bichir HoxA cluster sequence reveals surprising trends in ray-finned fish genomic evolution**. *Genome Research* 14(1): 11-17. ISSN: 1054-9803.

Descriptors: zebrafish, developmental genetics, evolution, evolutionary genetics, fluorescence in situ hybridization, freshwater fish, gene clusters, gene families, genes, genetic diversity, genomes, homeobox, marine fish, nucleotide sequence, phylogeny, population genetics, actinopterygii, *Danio rerio*, *Morone saxatilis*, *Polypterus senegalus*, *Takifugu rubripes*, Teleostei, Hoxa gene, human, ray finned fishes, rockfish, spiny rayed fishes, gene duplication, shark, striped bass, developmental genetics, genetics and evolution.

Cho, S. and J. Zhang (2007). **Zebrafish ribonucleases are bactericidal: implications for the origin of the vertebrate RNase A superfamily.** *Molecular Biology and Evolution* 24(5): 1259-68 . ISSN: 0707-4038.

NAL Call Number: QH506.M642

Abstract: Understanding the evolutionary origin of the ribonuclease (RNase) A superfam-

ily is of great interest because the superfamily is the sole vertebrate-specific enzyme family known to date. Although mammalian RNases have a diverse array of biochemical and physiological functions, the original function of the superfamily at its birth is enigmatic. Such information may be obtained by studying basal lineages of the vertebrate phylogeny and is necessary for discerning how and why this superfamily originated. Here, we clone and characterize 3 RNase genes from the zebrafish, the most basal vertebrate examined for RNases. We report 1) that all the 3 zebrafish RNases are ribonucleolytically active, with one of them having an RNase activity comparable to that of bovine RNase A, the prototype of the superfamily; 2) that 2 zebrafish RNases have prominent expressions in adult liver and gut, whereas the 3rd is expressed in adult eye and heart; and 3) that all 3 RNases have antibacterial activities in vitro. These results, together with the presence of antibacterial and/or antiviral activities in multiple distantly related mammalian RNases, strongly suggest that the superfamily started as a host-defense mechanism in vertebrate evolution.

Descriptors: zebrafish, anti-bacterial agents metabolism, molecular evolution, ribonuclease, pancreatic genetics, ribonucleases physiology, metabolism, amino acid sequence, molecular cloning, fishes genetics, fishes metabolism, gene expression, genome, liver enzymology, microbial sensitivity tests, molecular sequence data, transfer RNAmetabolism, ribonucleases classification, ribonucleases genetics, tissue distribution, vertebrates, zebrafish microbiology.

Chou, C.M., Y.C. Chen, M.T. Lee, G.D. Chen, I.C. Lu, S.T. Chen, and C.J. Huang (2006). **Expression and characterization of a brain-specific protein kinase BSK146 from zebrafish.** *Biochemical and Biophysical Research Communications* 340(3): 767-75. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: We have previously identified a novel protein kinase, pk146, in the brain of Tetraodon. In the present study, we cloned the homologous protein kinase gene encoding a protein of 385 amino acid residues from zebrafish. The overall amino acid sequence and the kinase domain of zebrafish BSK146 shows 48% and 69% identity to that of rat sbk, a SH3-containing serine/threonine protein kinase. By whole-mount in situ hybridization and RT-PCR, the expression of bsk146 mRNA was mainly in the brain. To explore the in vivo function of BSK146 during zebrafish development, we used morpholino knockdown approach and found that BSK146 morphants displayed enlarged hindbrain ventricle and smaller eyes. Whole-mount in situ hybridization was further performed to analyze the brain defects in BSK146-MO-injected embryos. The expression of brain-specific markers, such as otx2, pax2.1, and krox20, was found normal in morphant embryos at 24hpf, while expression of pax2.1 exerted changes in midbrain-hindbrain boundary and hindbrain in morphant embryos at 48hpf. These data suggest that BSK146 may play an important role in later ventricle expansion in zebrafish brain development. Although the recombinant BSK146 protein produced in insect cells was active and could phosphorylate both histone H1 and histone 2B, the endogenous substrate of BSK146 in the embryonic brain of zebrafish is not clear at the present time and needs further investigation.

Descriptors: zebrafish, developmental gene expression regulation, enzymologic gene expression regulation, protein kinases biosynthesis, protein serine threonine kinases physiology, amino acid sequence, Western blotting, brain embryology, brain metabolism, molecular cloning, DNA primers chemistry, complementary DNA metabolism, early growth response protein 2 biosynthesis, exons, gene expression regulation, gene library, genome, histones metabolism, in situ hybridization, insects, genetic models, molecular sequence data, mutation, neoplasm proteins metabolism, nerve tissue proteins metabolism, OTX transcription factors biosynthesis, pax2 transcription factor biosynthesis, phosphorylation, tertiary protein structure, protein serine threonine kinases biosynthesis, RNA metabolism, messenger RNA

metabolism, rats, reverse transcriptase PCR, DNA sequence analysis, amino acid sequence homology, time factors, tissue distribution.

Christie, T.L., R. Mui, T.W. White, and G. Valdimarsson (2004). **Molecular cloning, functional analysis, and RNA expression analysis of connexin45.6: a zebrafish cardiovascular connexin.** *American Journal of Physiology. Heart and Circulatory Physiology* 286(5): H1623-32. ISSN: 0363 6135.

Abstract: In the vertebrate cardiovascular system, gap junctions function in intercellular communication essential for both the coordinated propagation of the heartbeat and the control of vasomotor responses in the vascular system. Connexins, the protein subunits of gap junctions, are coded by a multigene family. In this study, a connexin gene (zfCx45.6), which exhibits 53% amino acid identity to chick Cx42, was cloned from zebrafish genomic DNA. With the use of the LN54 radiation hybrid panel, zfCx45.6 was mapped to zebrafish linkage group 9. Northern blots and RT-PCR revealed the presence of zfCx45.6 mRNA in the embryo before 2 h postfertilization (hpf) and then again beginning at about 12 hpf, after which time no major changes in relative expression levels were detected. In the adult, zfCx45.6 mRNA continued to be detected in the heart, as well as the brain, liver, and ovary, but not the lens. Whole mount in situ hybridization revealed zfCx45.6 mRNA was expressed at high levels in the major vessels of the entire embryo and in both the atrium and ventricle of the adult heart. Expression of zfCx45.6 channels in paired Xenopus oocytes produced high levels of intercellular coupling that was voltage sensitive. With the previous isolation of zebrafish Cx43 and Cx43.4, zebrafish orthologues have now been isolated for three of the four connexins expressed in the mammalian cardiovascular system.

Descriptors: zebrafish embryos, cardiovascular system metabolism, molecular cloning, connexins genetics and metabolism, RNA, amino acid sequence, Northern blotting, connexin45.6, cardiovascular system embryology, electric conductivity, in situ hybridization, ion channels physiology, molecular sequence data, oocytes, reverse transcriptase PCR, *Xenopus laevis*.

Christie, T.L., O. Starovic Subota, and S. Childs (2006). **Zebrafish collapsin response mediator protein (CRMP)-2 is expressed in developing neurons.** *Gene Expression Patterns* 6(2): 193-200. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: The collapsin response mediator proteins (CRMPs) are highly expressed in the vertebrate nervous system. CRMP2 has been shown to function in Semaphorin and lysophosphatidic acid induced growth cone collapse. Correspondingly, the highest levels of CRMP2 protein are found in the distal portion of growing axons. To understand the role of CRMP2 during embryonic development we have documented its expression pattern in zebrafish embryos at multiple stages. We find that CRMP2 is expressed in the major neural clusters of the embryonic brain during the primary stages of neurogenesis. From 20 somites through 30 hpf CRMP2 is expressed in the dorsal rostral cluster of the telencephalon, the ventral rostral cluster of the diencephalon, the ventral caudal cluster of the mesencephalon, and the hindbrain clusters. CRMP2 is also expressed in the trigeminal sensory ganglia and the Rohon Beard cells of the neural tube from 15 somites. By 48 hpf, we find expression of CRMP2 throughout the developing brain, trigeminal sensory ganglia, and Rohon Beard cells. CRMP2 is also detected in the retinal ganglion cell layer of the eye, and in the otic vesicle. Finally, we have compared the expression of CRMP2 to PlexinA4, a Semaphorin receptor expressed in sensory neurons, and find that their expression partially overlaps.

Descriptors: zebrafish, central nervous system embryology, nerve tissue amino acid and base

sequence, central nervous system metabolism, molecular cloning, DNA genetics, developmental gene expression regulation, in situ hybridization, intercellular signaling peptides and proteins, molecular sequence data, neurons metabolism, phylogeny, cell surface receptors genetics.

Christoffels, A., R. Bartfai, H. Srinivasan, H. Komen, and L. Orban (2006). **Comparative genomics in cyprinids: common carp ESTs help the annotation of the zebrafish genome.** *BioMed Central Bioinformatics* 7(Suppl 5): S2. ISSN: 1471 2105.

NAL Call Number: QH324.2.B53

Abstract: BACKGROUND: Automatic annotation of sequenced eukaryotic genomes integrates a combination of methodologies such as ab-initio methods and alignment of homologous genes and/or proteins. For example, annotation of the zebrafish genome within Ensembl relies heavily on available cDNA and protein sequences from two distantly related fish species and other vertebrates that have diverged several hundred million years ago. The scarcity of genomic information from other cyprinids provides the impetus to leverage EST collections to understand gene structures in this diverse teleost group. RESULTS: We have generated 6,050 ESTs from the differentiating testis of common carp (*Cyprinus carpio*) and clustered them with 9,303 non-gonadal ESTs from CarpBase as well as 1,317 ESTs and 652 common carp mRNAs from GenBank. Over 28% of the resulting 8,663 unique transcripts are exclusively testis-derived ESTs. Moreover, 974 of these transcripts did not match any sequence in the zebrafish or fathead minnow EST collection.A total of 1,843 unique common carp sequences could be stringently mapped to the zebrafish genome (version 5), of which 1,752 matched coding sequences of zebrafish genes with or without potential splice variants. We show that 91 common carp transcripts map to intergenic and intronic regions on the zebrafish genome assembly and regions annotated with non-teleost sequences. Interestingly, an additional 42 common carp transcripts indicate the potential presence of new splicing variants not found in zebrafish databases so far. The fact that common carp transcripts help the identification or confirmation of these coding regions in zebrafish exemplifies the usefulness of sequences from closely related species for the annotation of model genomes. We also demonstrate that 5' UTR sequences of common carp and zebrafish orthologs share a significant level of similarity based on preservation of motif arrangements for as many as 10 ab-initio motifs. CONCLUSION: Our data show that there is sufficient homology between the transcribed sequences of common carp and zebrafish to warrant an even deeper cyprinid transcriptome comparison. On the other hand, the comparative analysis illustrates the value in utilizing partially sequenced transcriptomes to understand gene structure in this diverse teleost group. We highlight the need for integrated resources to leverage the wealth of fragmented genomic data.

Descriptors: zebrafish, carps genetics, Cyprinidae, *Cyprinus carpio* genetics, expressed sequence tags, genomics methods, algorithms, chromosome mapping methods, nucleic acid databases and sequence homology, gene library, genome, testis metabolism, untranslated regions genetics.

Chu, C.Y., C.H. Cheng, G.D. Chen, Y.C. Chen, C.C. Hung, K.Y. Huang, and C.J. Huang (2007). **The zebrafish erythropoietin: functional identification and biochemical characterization.** *Federation of European Biochemical Societies Journal* 581(22): 4265-71. ISSN: 1742 464X.

NAL Call Number: QP501.E8

Abstract: In the present study, the zebrafish epo cDNA was cloned. The encoded protein displays 90%, 55% and 32% identity to the Epo from carp, fugu and human, respectively.

Through RT-PCR, the expression of zepo mRNA was mainly in the heart and liver. In the COS-1 cell transfection experiments, the recombinant zEpo-HA protein was efficiently secreted into the culture medium as a glycoprotein and the carbohydrate moiety can be cleaved by the treatment of peptide-N-glycosidase F (PNGase F). Using the morpholino approach, we showed that zepo morphants displayed severe anemia leading to high mortality during development. Such an effect can be significantly rescued by zepo RNA. Furthermore, in the absence of functional zEpo, the expression of specific markers for adult globin genes, such as alphaA1- and betaA1-globin, but not the embryonic betae1-globin, was affected.
Descriptors: zebrafish, erythropoietin genetics and metabolism, amino acid sequence, COS cells, *Cercopithecus aethiops*, molecular cloning, cytology, metabolism, erythroid cells metabolism, erythropoietin chemistry and secretion, gene expression profiling, developmental gene expression regulation, hemoglobins biosynthesis, molecular sequence data, organ specificity, protein transport, messenger RNA genetics and metabolism, sequence alignment, proteins chemistry, proteins secretion.

Cinquin, O. (2003). **Is the somitogenesis clock really cell-autonomous? A coupled-oscillator model of segmentation**. *Journal of Theoretical Biology* 224(4): 459-468. ISSN: 0022-5193.
NAL Call Number: 442.8J8223
Descriptors: zebrafish, chronometers, embryonic development, freshwater fish, gene expression, modelling, morphogenesis, *Danio rerio*, genetics and evolution.

Clark, K.J., A.M. Geurts, J.B. Bell, and P.B. Hackett (2004). **Transposon vectors for gene-trap insertional mutagenesis in vertebrates**. *Genesis* 39(4): 225-233. ISSN: 1526 954X.
NAL Call Number: QH426.D32
Abstract: The function of most vertebrate genes remains unknown or uncertain. Insertional mutagenesis offers one approach to identify and understand the function of these genes. Transposons have been used successfully in lower organisms and plants for insertional mutagenesis, but until activation of the Sleeping Beauty (SB) transposon system, there was no indication of active DNA-based transposons in vertebrates. Investigator-driven insertional mutagenesis in vertebrates has relied on retroviral insertions or selection of low-frequency integration of naked DNA in ES cell lines. We have combined the highly active SB transposon with gene-trapping technology to demonstrate that transposon traps can be used for insertional mutagenesis screens in vertebrates. In our studies about one-fourth of the trap insertions appear to be in transcriptional units, a rate that is commensurate with random integration. We show that gene-traps coupled to a fluorescent protein reporter gene can be used to detect insertions into genes active in specific cells of living zebrafish embryos, supporting use of our transposon traps for high-throughput functional genomic screens in vertebrates. Copyright © 2008 CABI
Descriptors: zebrafish, embryos, gene transfer, genes, genetic engineering, genetic vectors, insertional mutagenesis, mutagenesis, mutants, reporter genes, techniques, transcription, transposable elements, *Danio rerio*, vertebrates.

Clark, M.S., Y.J.K. Edwards, D. Peterson, S.W. Clifton, A.J. Thompson, M. Sasaki, Y. Suzuki, K. Kikuchi, S. Watabe, K. Kawakami, S. Sugano, G. Elgar, and S.L. Johnson (2003). **Fugu expressed sequence tags: new resources for transcription analysis and genome annotation**. *Genome Research* 13(12): 2747-2753. ISSN: 1054-9803.
Descriptors: zebrafish, freshwater fish, genes, genetic analysis, genetic diversity, genomes, population genetics, expressed, tags, *Danio rerio*, *Fugu rubripes*, expressed sequence tags, zebra fish, structure and sequence, genetics and evolution.

Clelland, E., G. Kohli, R.K. Campbell, S. Sharma, S. Shimasaki, and C. Peng (2006). **Bone morphogenetic protein-15 in the zebrafish ovary: complementary deoxyribonucleic acid cloning, genomic organization, tissue distribution, and role in oocyte maturation.** *Endocrinology* 147(1): 201-9. ISSN: 0013 7227.
NAL Call Number: 448.8EN2
Abstract: Bone morphogenetic protein-15 (BMP-15) is a member of the TGFbeta family known to regulate ovarian functions in mammals. The structure and function of BMP-15 in lower vertebrates are less known. In this study, we cloned the zebrafish BMP-15 (zfBMP-15) cDNA and depicted its genomic organization. The zfBMP-15 cDNA encodes a protein of 384 amino acids. The mature protein has 46-51% sequence identities to fugu, chicken, and mammalian BMP-15. It also shares 38-46% homology with growth and differentiation factor-9 in fishes, chicken, and mammals. Phylogenetic analysis further confirms that the zfBMP-15 is most closely related to BMP-15 from other species, whereas the growth and differentiation factor-9 peptides from fish to mammals form a distinct branch. Comparison of zfBMP-15 cDNA with zebrafish genome database revealed that zfBMP-15 is encoded by a gene with two exons and one intron, located on chromosome 6. BMP-15 mRNA is expressed in the ovary and testis and, to a lesser extent, brain, liver, gut, heart, and muscle. Real-time PCR revealed that BMP-15 is expressed in follicles at all stages of development with no significant changes over the course of folliculogenesis. Using in situ hybridization and immunocytochemistry, we detected BMP-15 in both oocytes and follicular cells. Incubation of follicles with antiserum against zfBMP15 increased oocyte maturation, whereas incubation with recombinant human BMP-15 suppressed human chorionic gonadotropin-induced oocyte maturation. These findings suggest that BMP-15 plays a role in regulating gonadal functions in fish, in particular oocyte maturation.
Descriptors: zebrafish, complementary DNA genetics, intercellular signaling peptides and oocytes physiology, amino acid and base sequence, chickens, molecular cloning, DNA primers, mammals, molecular sequence data, organ specificity, phylogeny, PCR, zebrafish classification, proteins genetics.

Cohen, A.J. and R.K. Pope (2003). **Cloning and analysis of zebrafish supervillin**. *Integrative and Comparative Biology* 43(6): 961. ISSN: 1540 7063.
NAL Call Number: QL1.I67
Descriptors: zebrafish, development, molecular genetics, population genetics, population studies, cell biology, complementary DNA cloning, genetic techniques, embryonic development, tissue differentiation.

Cohen, S.M., J. Brennecke, and A. Stark (2006). **Denoising feedback loops by thresholding - a new role for microRNAs**. *Genes and Development* 20(20): 2769-2772. ISSN: 0890 9369.
NAL Call Number: QH426.G46
Descriptors: zebrafish, development, molecular genetics, nervous system, sense organs, phenotype, thresholding , denoising feedback loop.

Colombo, A., G. Reig, M. Mione, and M.L. Concha (2006). **Zebrafish BarH-like genes define discrete neural domains in the early embryo.** *Gene Expression Patterns* 6(4): 347-52. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: BarH (Barhl) genes encode for highly conserved homeodomain-containing transcription factors involved in critical functions during development, including cell fate specification, migration and survival. Here, we report the dynamic and restricted expres-

sion of three zebrafish barhl within the developing central nervous system. barhl2 becomes expressed in the late gastrula as a transverse diencephalic domain located immediately caudal to the prospective eyes. At early somitogenesis, barhl1.1 and barhl1.2 are expressed in the diencephalon in domains that partially overlap with the ventral and dorsal aspects of barhl2 expression, respectively. At later stages, expression of all zebrafish barhl shows large extent of overlap in the pretectum, tectum and dorsal hindbrain. The presence of a unique territory of barhl2 expression in the dorsal telencephalon and the high levels of expression in the retina are both consistent with expression reports of other Barhl2 orthologues, and support the subdivision of vertebrate Barhl into two paralogue groups based on the phylogenetic analysis of nucleotide and amino acid sequences.

Descriptors: zebrafish embryos, central nervous system embryology, homeobox genes, homeodomain proteins genetics, amino acid sequence, genetically modified, molecular cloning gastrula, developmental gene expression regulation, immunohistochemistry, in situ hybridization, molecular sequence data, phylogeny, tertiary protein structure, amino acid sequence homology, transgenes, proteins chemistry.

Connolly, M.H. and B.K. Hall (2006). **More than skin deep: embryonic heat shock increases pigment and skeletal variation in zebrafish, *Danio rerio*.** *Federation of American Societies for Experimental Biology Journal* 20(5, Part 2): A869-A870. ISSN: 0892 6638.

NAL Call Number: QH301.F3

Abstract: There is increasing evidence that more genetic variation is present among metazoans than normally expressed by the phenotype, due in part to the canalization of development. Among teleosts this genetic variation is often expressed as phenotypic change in response to environmental cues. For example, some fish species demonstrate meristic variation in caudal fin ray number and vertebrae counts as a result of seasonal or experimental temperature change. Using embryonic zebrafish (*Danio rerio*), this intrinsic relationship is being explored to examine the influence of environmental disturbance on development form. Following a 37 degrees C heat shock during early somitogenesis, phenotypic variability was noted in the lower trunk and tail bud regions, where somite development was reduced or ceased prematurely. Phenotypic variation was examined among 400 mature individuals to further elucidate the degree of change. Here, bone-specific staining indicates increased variation in the shape and number of skeletal elements among fish heat-shocked during a narrow developmental window (9-16 somite stages), with an increase in the number of caudal vertebrae and decrease in the number of caudal fin rays. Similarly, variation among zebrafish pigment patterns is accentuated with heat shock treatment. Exposure of such phenotypic variation creates a basis for selection and sheds light on the underlying mechanism for genetic assimilation. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, skeletal system, development, genetics, phenotypic variability, heat shock, somitogenesis, pigment pattern, meristic variation.

Conway, G., M. Torrejon, S. Lin, and S. Reinsch (2006). **Fluorescent tagged analysis of neural gene function using mosaics in zebrafish and *Xenopus laevis*.** *Brain Research* 1070(1): 150-9. ISSN: 0006 8993.

NAL Call Number: QP376

Abstract: An important question in the neurosciences is the role of specific gene expression in the control of neural morphology and connectivity. To address this question, methods are needed for expression of exogenous genes in a subset of neurons. This limited and mosaic expression allows the assessment of gene expression in a cell autonomous fashion without environmental contributions from neighboring expressing cells. These methods must also

label neurons so that detailed morphology and neural connections can be evaluated. The labeling method should label only a subset of neurons so that neuronal morphology can be viewed upon a non-stained background, in a Golgi staining fashion. Here, we report methods using plasmids called pTAGUM (tagged analysis of genes using mosaics) that accomplish these goals. These methods should prove useful for the analysis of neural gene function in two important model organisms, the zebrafish and *Xenopus laevis.*
Descriptors: zebrafish, green fluorescent mosaicism, nervous system embryology, *Xenopus laevis* embryology and genetics, genetically modified, blastomeres, DNA metabolism, circular DNA physiology, Type II deoxyribonucleases site specific pharmacology, embryo metabolism and physiology, embryonic development, fluorescent dyes, gene expression, injections, neurons classification, metabolism and physiology, osmolar concentration, time factors.

Cooke, J.E., H.A. Kemp, and C.B. Moens (2005). **EphA4 is required for cell adhesion and rhombomere-boundary formation in the zebrafish.** *Current Biology* 15(6): 536-42. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Abstract: The formation of boundaries between or within tissues is a fundamental aspect of animal development. In the developing vertebrate hindbrain, boundaries separate molecularly and neuroanatomically distinct segments called rhombomeres. Transplantation studies have suggested that rhombomere boundaries form by the local sorting out of cells with different segmental identities. This sorting-out process has been shown to involve repulsive interactions between cells expressing an Eph receptor tyrosine kinase, EphA4, and cells expressing its ephrinB ligands. Although a model for rhombomere-boundary formation based on repulsive Eph-ephrin signaling is well established in the literature, the predictions of this model have not been tested in loss-of-function experiments. Here, we eliminate EphA4 and ephrinB2a proteins in zebrafish with antisense morpholinos (MO) and find that rhombomere boundaries are disrupted in EphA4MO embryos, consistent with a requirement for Eph-ephrin signaling in boundary formation. However, in mosaic embryos, we observe that EphA4MO cells and EphA4-expressing cells sort from one another, an observation that is not predicted by the Eph-ephrin repulsion model but instead suggests that EphA4 promotes cell adhesion within the rhombomeres in which it is expressed. Differential cell adhesion is known to be an effective mechanism for cell sorting. We therefore propose that the well-known EphA4-dependent repulsion between rhombomeres operates in parallel with the EphA4-dependent adhesion within rhombomeres described here to drive the cell sorting that underlies rhombomere-boundary formation.
Descriptors: zebrafish, cell adhesion physiology, cell differentiation physiology, developmental gene expression regulation, epha4 receptor physiology, rhombencephalon embryology and metabolism, signal transduction, ephrin B2 metabolism, immunohistochemistry, confocal microscopy, morphogenesis, oligonucleotides.
Notes: Comment In:Curr. Biol. 2005 Mar 29;15(6):R210-2
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Cooper, B.J., B. Key, A. Carter, N.Z. Angel, D.N. Hart, and M. Kato (2006). **Suppression and overexpression of adenosylhomocysteine hydrolase-like protein 1 (AHCYL1) influences zebrafish embryo development: a possible role for AHCYL1 in inositol phospholipid signaling.** *Journal of Biological Chemistry* 281(32): 22471-84. ISSN: 0021-9258.
NAL Call Number: 381J824
Abstract: Adenosylhomocysteine hydrolase-like protein 1 (AHCYL1) is a novel intracel-

lular protein with approximately 50% protein identity to adenosylhomocysteine hydrolase (AHCY), an important enzyme for metabolizing S-adenosyl-l-homocysteine, the by-product of S-adenosyl-l-homomethionine-dependent methylation. AHCYL1 binds to the inositol 1,4,5-trisphosphate receptor, suggesting that AHCYL1 is involved in intracellular calcium release. We identified two zebrafish AHCYL1 orthologs (zAHCYL1A and -B) by bioinformatics and reverse transcription-PCR. Unlike the ubiquitously present AHCY genes, AHCYL1 genes were only detected in segmented animals, and AHCYL1 proteins were highly conserved among species. Phylogenic analysis suggested that the AHCYL1 gene diverged early from AHCY and evolved independently. Quantitative reverse transcription-PCR showed that zAHCYL1A and -B mRNA expression was regulated differently from the other AHCY-like protein zAHCYL2 and zAHCY during zebrafish embryogenesis. Injection of morpholino antisense oligonucleotides against zAHCYL1A and -B into zebrafish embryos inhibited zAHCYL1A and -B mRNA translation specifically and induced ventralized morphologies. Conversely, human and zebrafish AHCYL1A mRNA injection into zebrafish embryos induced dorsalized morphologies that were similar to those obtained by depleting intracellular calcium with thapsigargin. Human AHCY mRNA injection showed little effect on the embryos. These data suggest that AHCYL1 has a different function from AHCY and plays an important role in embryogenesis by modulating inositol 1,4,5-trisphosphate receptor function for the intracellular calcium release.

Descriptors: zebrafish embryogenesis, adenosylhomocysteinase chemistry, developmental gene expression regulation, intracellular signaling peptides and proteins genetics and physiology, phosphatidylinositols metabolism, phospholipids metabolismand physiology, alternative splicing, amino acid and base sequence, calcium channels metabolism, molecular cloning, inositol 1,4,5 trisphosphate receptors, molecular sequence data, phylogeny, cytoplasmic receptors and nuclear metabolism, amino acid sequence homology.

Cooper, M.S., G. Sommers Herivel, C.T. Poage, M.B. McCarthy, B.D. Crawford, and C. Phillips (2004). **The zebrafish DVD Exchange Project: a bioinformatics initiative.** *Methods in Cell Biology* 77: 439-57. ISSN: 0091-679X.

NAL Call Number: 442P92

Abstract: Scientists who study zebrafish currently have an acute need to increase the rate of visual data exchange within their international community. Although the Internet has provided a revolutionary transformation of information exchange, the Internet is at present unable to serve as a vehicle for the efficient exchange of massive amounts of visual information. Much like an overburdened public water system, the Internet has inherent limits to the services it can provide. It is possible, however, for zebrafishologists to develop and use virtual intranets (such as the approach we outlined in this chapter) to adapt to the growing informatics need of our expanding research community. We need to assess qualitatively the economics of visual bioinformatics in our research community and evaluate the benefit:investment ratio of our collective information-sharing activities. The development of the World Wide Web started in the early 1990s by particle physicists who needed to rapidly exchange visual information within their collaborations. However, because of current limitations in information bandwidth, the World Wide Web cannot be used to easily exchange gigabytes of visual information. The Zebrafish DVD Exchange Project is aimed at by-passing these limitations. Scientists are curiosity-driven tool makers as well as curiosity-driven tool users. We have the capacity to assimilate new tools, as well as to develop new innovations, to serve our collective research needs. As a proactive research community, we need to create new data transfer methodologies (e.g., the Zebrafish DVD Exchange Project) to stay ahead of our bioinformatics needs.

Descriptors: zebrafish, computational biology methods, computer communication networks, videodisc recording, Zebrafish DVD Exchange Project, image, visual bioimformatics.

Corbit, K.C., P. Aanstad, V. Singla, A.R. Norman, D.Y.R. Stainier, and J.F. Reiter (2005). **Vertebrate smoothened functions at the primary cilium**. *Nature* 437(7061): 1018-1021,1012. ISSN: 0028-0836.
NAL Call Number: 472 N21
Abstract: The unanticipated involvement of several intraflagellar transport proteins in the mammalian Hedgehog (Hh) pathway has hinted at a functional connection between cilia and Hh signal transduction(1,2). Here we show that mammalian Smoothened (Smo), a seven-transmembrane protein essential for Hh signalling(3), is expressed on the primary cilium. This ciliary expression is regulated by Hh pathway activity; Sonic hedgehog or activating mutations in Smo promote ciliary localization, whereas the Smo antagonist cyclopamine inhibits ciliary localization. The translocation of Smo to primary cilia depends upon a conserved hydrophobic and basic residue sequence homologous to a domain previously shown to be required for the ciliary localization of seven-transmembrane proteins in *Caenorhabditis elegans*(4). Mutation of this domain not only prevents ciliary localization but also eliminates Smo activity both in cultured cells and in zebrafish embryos. Thus, Hh-dependent translocation to cilia is essential for Smo activity, suggesting that Smo acts at the primary cilium. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, cell culture, culturing techniques, signal transduction, Hedgehog (Hh) pathway, Smoothened (Smo), ciliary expressions.

Corey, D.P., J. Garcia-Anoveros, J.R. Holt, K. Kwan, S.-Y. Lin, M.A. Vollrath, A. Amalfitano, E.L.-M. Cheung, B.H. Derfler, A. Duggan, G.S. Geleoc, P.A. Gray, M.P. Hoffman, H.L. Rehm, D. Tamasauskas, and D.-S. Zhang (2004). **TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells**. *Nature* 432(7018): 723-730. ISSN: 0028-0836.
NAL Call Number: 472 N21
Descriptors: zebrafish, audition, auditory organs, hearing, auditory system, biochemical analysis, gene expression, hair cells, inner ear, mechanoreceptors, mechanotransduction, nervous system, transduction, transient receptor potential proteins, *Danio rerio*, ANKTM1 protein, hair cells, mechanotransduction, TRPA1 protein, auditory and vestibular systems (including echolocation), physiology, biochemistry, biophysics.

Corley Smith, G.E., H.T. Su, J.L. Wang Buhler, H.P. Tseng, C.H. Hu, T. Hoang, W.G. Chung, and D.R. Buhler (2006). **CYP3C1, the first member of a new cytochrome P450 subfamily found in zebrafish (*Danio rerio*).** *Biochemical and Biophysical Research Communications* 340(4): 1039-46. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: We report a new cytochrome P450 (CYP) subfamily CYP3C and the cloning through PCR from zebrafish *(Danio rerio*) of the first member, CYP3C1. The CYP3C1 gene is on Chromosome 3 with 13 ORF exons encoding a 505 amino acid protein which has 44-54% identities with mammalian and teleost CYP3A and CYP3B forms. As evidenced by spectral analysis, the CYP3C1 protein heterologously expressed in yeast is functional. In silico analysis identified, on the same region of the chromosome, three more genes encoding CYP3C1-like proteins that formed a clade with CYP3C1 in a phylogenetic tree. Using RT-PCR, the CYP3C1 mRNA was detected in 1-6dpf embryo/larvae and in adult fish liver and seven extrahepatic tissues. Whole-mount in situ hybridization using a riboprobe dem-

onstrated expression in the brain during 12-120 hpf. At the 120 hpf larval stage, CYP3C1 mRNA was also detected in the pharynx and gastrointestinal tract. TCDD, dexamethasone, and rifampicin, which up-regulated CYP3A65 mRNA in zebrafish larvae, did not alter the CYP3C1 transcript levels suggesting regulatory differences between CYP3A and CYP3C enzymes in this species.

Descriptors: zebrafish larva, cytochrome P450 enzyme system chemistry, genetics and metabolism, cytochromes genetics and metabolism, amino acid and base sequence, cytochromes chemistry, molecular evolution and sequence data, organ specificity, phylogeny, amino acid sequence homology, tissue distribution.

Correa, R.G., T. Matsui, V. Tergaonkar, C. Rodriguez Esteban, J.C. Izpisua Belmonte, and I.M. Verma (2005). **Zebrafish IkappaB kinase 1 negatively regulates NF-kappaB activity.** *Current Biology* 15(14): 1291-5. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: The IkappaB kinase (IKK) activity is critical for processing IkappaB inhibitory proteins and activating the NF-kappaB signaling, which is involved in a series of physiological and developmental steps in vertebrates. The IKK activity resides in two catalytic subunits, IKK1 and IKK2, and two regulatory subunits, NEMO and ELKS. IKK2 is the major cytokine-responsive IkappaB kinase because depletion of IKK1 does not interfere with the IKK activity. In fact, IKK1-/- mice display morphological abnormalities that are independent of its kinase activity and NF-kappaB activation. Hence, using zebrafish (*Danio rerio*) as a model, we examined the evolutionary role of IKK1 in modulating NF-kappaB. Ikk1-/- zebrafish embryos present head and tail malformations and, surprisingly, show upregulation of NF-kappaB-responsive genes and increased NF-kappaB-dependent apoptosis. Overexpression of ikk1 leads to midline structure defects that resemble NF-kappaB blockage in vivo. Zebrafish Ikk1 forms complexes with NEMO that represses NF-kappaB in vertebrate cells. Indeed, truncation of its NEMO binding domain (NBD) restores NF-kappaB-dependent transcriptional activity and, consequently, the ikk1-overexpressing phenotype. Here, we report that Ikk1 negatively regulates NF-kappaB by sequestering NEMO from active IKK complexes, indicating that IKK1 can function as a repressor of NF-kappaB.

Descriptors: zebrafish, down regulation, NF-kappaB metabolism, protein serine threonine kinases metabolism, signal transduction physiology, metabolism, Western blotting, cell line, chromosome mapping, molecular cloning, computational biology, DNA primers, gene components, I kappa B kinase, immunohistochemistry, in situ hybridization, protein serine threonine kinases genetics, reverse transcriptase PCR.

Correa, R.G., V. Tergaonkar, J.K. Ng, I. Dubova, J.C. Izpisua Belmonte, and I.M. Verma (2004). **Characterization of NF-kappa B/I kappa B proteins in zebra fish and their involvement in notochord development.** *Molecular and Cellular Biology* 24(12): 5257-68. ISSN: 0270-7306.

NAL Call Number: QH506.M664

Abstract: Although largely involved in innate and adaptive immunity, NF-kappa B plays an important role in vertebrate development. In chicks, the inactivation of the NF-kappa B pathway induces functional alterations of the apical ectodermal ridge, which mediates limb outgrowth. In mice, the complete absence of NF-kappa B activity leads to prenatal death and neural tube defects. Here, we report the cloning and characterization of NF-kappa B/I kappa B proteins in zebra fish. Despite being ubiquitously expressed among the embryonic tissues, NF-kappa B/I kappa B members present distinct patterns of gene expression during the early zebra fish development. Biochemical assays indicate that zebra fish NF-kappa B proteins are

able to bind consensus DNA-binding (kappa B) sites and inhibitory I kappa B alpha proteins from mammals. We show that zebra fish I kappa B alphas are degraded in a time-dependent manner after induction of transduced murine embryo fibroblasts (MEFs) and that these proteins are able to rescue NF-kappa B activity in I kappa B alpha(-/-) MEFs. Expression of a dominant-negative form of the murine I kappa B alpha (mI kappa B alpha M), which is able to block NF-kappa B in zebra fish cells, interferes with the notochord differentiation, generating no tail (ntl)-like embryos. This phenotype can be rescued by coinjection of the T-box gene ntl (Brachyury homologue), which is typically required for the formation of posterior mesoderm and axial development, suggesting that ntl lies downstream of NF-kappa B . We further show that ntl and Brachyury promoter regions contain functional kappa B sites and NF-kappa B can directly modulate ntl expression. Our study illustrates the conservation and compatibility of NF-kappa B/I kappa B proteins among vertebrates and the importance of NF-kappa B pathway in mesoderm formation during early embryogenesis.
Descriptors: zebrafish, I kappa B proteins metabolism and genetics, NF kappa B metabolism, notochord embryology, metabolism, cultured cells, complementary DNA genetics, developmental gene expression regulation, knockout mice, NF-kappaB genetics, notochord metabolism, phenotype, recombinant proteins genetics and metabolism, species specificity, T-box domain proteins genetics and metabolism, proteins metabolism.

Cortemeglia, C. and T.L. Beitinger (2006). **Projected U.S. distributions of transgenic and wild-type zebra danios, *Danio rerio*, based on temperature tolerance data**. *Journal of Thermal Biology* 31(5): 422-428. ISSN: 0306 4565.
NAL Call Number: QP82.2.T4J6
Abstract: A genetically modified version of the south Asian, zebra danio, *Danio rerio*, a common aquarium fish, has become the first transgenic pet sold in the USA. Mean chronic lethal maxima of wildtype (39.8 degrees C, n=16) and transgenic (39.3 degrees C, n=10) zebra danios initially acclimated to 30 degrees C were statistically (but not dramatically) different as were mean chronic lethal minima of wildtype (5.3 degrees C, n=16) and transgenic (5.6 degrees C, n=20) zebra danios initially acclimated to 20 degrees C. These temperature tolerance values were used to estimate potential geographic distributions of the two varieties in the USA. Distributions of these D. rerio varieties in the USA should not be limited by their upper temperature tolerances, and low-temperature tolerance data suggest that both varieties are capable of overwintering in some southern and western US waters. Copyright © 2008 CABI
Descriptors: transgenic zebrafish, aquarium fishes, cold tolerance, environmental temperature, geographical distribution, heat tolerance, *Danio rerio*.

Cortemeglia, C. and T.L. Beitinger (2005). **Temperature tolerances of wild-type and red transgenic zebra danios**. *Transactions of the American Fisheries Society* 134(6): 1431-1437. ISSN: 0002 8487.
NAL Call Number: 414.9 Am3
Abstract: Seventy-nine transgenic red zebra danios *Danio rerio* (also known as zebrafish; transgenics also known as glofish) and 78 wild-type zebra danios acclimated to 20 degrees C and 30 degrees C had critical thermal maxima (+or-SD) of 38.8+or-0.54 degrees C and 41.3+or-0.50 degrees C (glofish) and 39.2+or-0.34 degrees C and 41.7+or-0.35 degrees C (wild-type), respectively. The critical thermal minima (+or-SD) were 7.2+or-0.48 degrees C and 10.0+or-0.50 degrees C (glofish) and 6.2+or-0.28 degrees C and 10.6+or-0.53 degrees C (wild-type), respectively. At both 20 degrees C and 30 degrees C acclimation temperatures, critical thermal maxima (CTMax) and critical thermal minima (CTMin) were significantly

different between transgenic and wild zebrafish; however, differences were 1 degrees C and less. The temperature tolerances would classify both varieties of zebra danios as eurythermal and place this species in the relatively rare category of fishes with a CTMax above 40.0 degrees C. Compared with similar temperature tolerance data reported for other tropical fishes that have successfully been introduced into U.S. waters, the present data suggest both wild-type zebra danios and red glofish are capable of overwintering in some southern U.S. waters. Copyright © 2008 CABI

Descriptors: transgenic zebrafish, red glofish, overwintering southern U.S. waters, temperature, tolerance, *Danio rerio*, .

Costagli, A., B. Felice, A. Guffanti, S.W. Wilson, and M. Mione (2006). **Identification of alternatively spliced dab1 isoforms in zebrafish.** *Development Genes and Evolution* 216(6): 291-9. ISSN: 0949 944X.

NAL Call Number: QL951.R68

Abstract: We have investigated the genomic organization, the occurrence of alternative splicing and the differential expression of the zebrafish disabled1 (dab1) gene. Dab1 is a key effector of the Reelin pathway, which regulates neuronal migration during brain development in vertebrates. The coding region of the zebrafish dab1 gene spans over 600 kb of genomic DNA and is composed of 15 exons. Alternative splicing in a region enriched for tyrosine residues generates at least three different isoforms. These isoforms are developmentally regulated and show differential tissue expression. Comparison with mouse and human data shows an overall conservation of the genomic organization with different alternative splicing events generating species-specific isoforms. Because these alternative splicing events give rise to isoforms with different numbers of phosphorylateable tyrosines, we speculate that alternative splicing of the dab1 gene in zebrafish and in other vertebrates regulates the nature of the cellular response to the Reelin signal.

Descriptors: zebrafish, alternative splicing, nerve tissue proteins genetics and metabolism, signal transducing adaptor proteins, genetically modified, base sequence, initiator codon, terminator codon, complementary DNA, exons, gene expression regulation, in situ hybridization, mice, molecular sequence data, neurons physiology, phylogeny, protein isoforms genetics and metabolism, species specificity, tyrosine genetics.

Cotto, E., M. Andre, J. Forgue, H.J. Fleury, and P.J. Babin (2005). **Molecular characterization, phylogenetic relationships, and developmental expression patterns of prion genes in zebrafish (*Danio rerio*).** *Federation of European Biochemical Societies Journal* 272(2): 500-513. ISSN: 1742 464X.

NAL Call Number: QP501.E8

Abstract: Prion diseases are characterized by the accumulation of a pathogenic misfolded form of a prion protein (PrP) encoded by the Prnp gene in humans. In the present study in zebrafish (*D. rerio*), two transcripts and the corresponding genes encoding prion proteins, PrP1 and PrP2, related to human PrP have been characterized with a relatively divergent deduced amino acid sequence, but a well preserved overall organization of structural prion protein motifs. Whole-mount in situ hybridization analysis performed during embryonic and larval development showed a high level of PrP1 messenger RNA spatially restricted to the anterior floor-plate of the central nervous system and in ganglia. Transcripts of prp2 were detected in embryonic cells from the mid-blastula transition to the end of the segmentation period. From 24 h postfertilization up to larval stages, prp2 transcripts were localized in distinct anatomical structures, including a major expression in the brain, eye, kidney, lateral line neuromasts, liver, heart, pectoral fins and posterior intestine. The observed differential

developmental expression patterns of the two long PrP forms, prp1 and prp2, and the short PrP form prp3, a more divergent prion-related gene previously identified in zebrafish, should contribute to understanding of the phylogenetic and functional relationships of duplicated prion gene forms in the fish genome. Together, the complex history of prion-related genes, reflected in the deduced structural features, conserved amino acid sequence and repeat motifs of the corresponding proteins, and the presence of differential developmental expression patterns suggest possible acquisition or loss of prion protein functions during vertebrate evolution. Sequence data for PrP1 and PrP2 mRNAs have been deposited with the EMBL/ GenBank Data Libraries under Accession Numbers AJ850286 and AJ620614, respectively. Copyright © 2008 CABI
Descriptors: zebrafish larva, amino acid sequences, embryonic development, embryos, gene expression, genes, genome analysis, genomes, larvae, messenger RNA, molecular genetics, nucleotide sequences, phylogenetics, prion proteins, *Danio rerio.*
Notes: Sequence data in GenBank Data Libraries.

Covassin, L.D., J.A. Villefranc, M.C. Kacergis, B.M. Weinstein, and N.D. Lawson (2006). **Distinct genetic interactions between multiple Vegf receptors are required for development of different blood vessel types in zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 103(17): 6554-9. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: Recent evidence indicates a specific role for vascular endothelial growth factor a (Vegfa) during artery development in both zebrafish and mouse embryos, whereas less is known about signals that govern vein formation. In zebrafish, loss of vegfa blocks segmental artery formation and reduces artery-specific gene expression, whereas veins are largely unaffected. Here, we describe a mutation in the zebrafish vegf receptor-2 homolog, kdra, which eliminates its kinase activity and leads to specific defects in artery development. We further find that Flt4, a receptor for Vegfc, cooperates with Kdr during artery morphogenesis, but not differentiation. We also identify an additional zebrafish vegfr-2 ortholog, referred to as kdrb, which can partially compensate for loss of kdra but is dispensable for vascular development in wild-type embryos. Interestingly, we find that these Vegf receptors are also required for formation of veins but in distinct genetic interactions that differ from those required for artery development. Taken together, our results indicate that formation of arteries and veins in the embryo is governed in part by different Vegf receptor combinations and suggest a genetic mechanism for generating blood vessel diversity during vertebrate development.
Descriptors: zebrafish, blood vessels embryology and metabolism, receptors, vascular endothelial growth factor genetics and metabolism, base sequence, complementary DNA genetics, molecular sequence data, mutation, phenotype, phylogeny, vascular endothelial growth factor C metabolism, vascular endothelial growth factor receptor 2 genetics and metabolism, vascular endothelial growth factor receptor 3 genetics and metabolism.

Coverdale, L.E., C.J. Martyniuk, V.L. Trudeau, and C.C. Martin (2004). **Differential expression of the methyl-cytosine binding protein 2 gene in embryonic and adult brain of zebrafish.** *Brain Research* 153(2): 281-7. ISSN: 0006 8993.
NAL Call Number: QP376
Abstract: Epigenetic gene repression occurs as the result of the interactions between DNA and a number of proteins, including methyl-cytosine binding protein 2 (MeCP2). We have isolated a 1680 bps MeCP2 cDNA from zebrafish that shows deduced amino acid identity with *Xenopus* and mammalian MeCP2alpha protein sequences. The zebrafish MeCP2 gene was mapped to linkage group 8 using the LN54 radiation hybrid cell panel. The genomic

organizations of the zebrafish MeCP2 and mammalian MeCP2alpha are highly similar. Relatively high levels of expression of MeCP2 mRNA were found in embryos at 1 to 4 h postfertilization (hpf), after 24 hpf, and in adult brain and eyes. Whole mount in situ hybridization was performed on embryos and revealed ubiquitous MeCP2 expression during early zebrafish development. At 24 and 48 hpf, the highest levels of expression are found in the epiphysis, midbrain, ventricular zone, and the otic vesicles. In adult zebrafish, MeCP2-expressing cells were found throughout the brain. Regions that are enriched in MeCP2 mRNA include the pallium layer of the telencephalon, the preoptic area, the periventricular grey zone, lobus caudalis, and the vagus lobes. In the cerebellum, high expression is found in the Purkinje and Golgi type 2 cells.

Descriptors: zebrafish, brain growth and development, brain chemistry genetics, chromosomal proteins, non histone biosynthesis and genetics, DNA binding proteins genetics, proteins biosynthesis and genetics, developmental gene expression regulation physiology, repressor proteins biosynthesis and genetics, amino acid sequence, brain embryology, complementary DNA biosynthesis, chemistry and genetics, in situ hybridization, introns genetics, linkage genetics, methyl CPG binding protein 2, molecular sequence data, phenotype, messenger RNA biosynthesis and genetics, reverse transcriptase PCR, species specificity.

Cox, J.A., S.C. Kucenas, and M.M. Voigt (2003). **NMDA receptors in zebrafish: developmental expression and genomic localization.** *33rd Annual Meeting of the Society of Neuroscience,November 8, 2003-November 8, 2003, New Orleans, LA, USA,* Society for Neuroscience : p. Abstract No.467.4.

Abstract: Glutamate is the primary excitatory neurotransmitter in the vertebrate nervous system. One subclass of glutamate-gated ion channels, the N-methyl-D-aspartate receptor (NMDAR), has been implicated in many neurophysiological functions due to their high calcium permeability and unique voltage-dependence. However, their role in the developing nervous system is poorly understood. In order to elucidate the possible roles these receptors might play in maturation of the nervous system, we have turned to the use of the zebrafish. The zebrafish has proven a useful model for studying neuronal development for several reasons, including the ability to perform large-scale forward genetic screens and the optical clarity of the embryos and larvae. In our initial studies, we have cloned 10 cDNAs encoding the zebrafish family of NMDA receptors from adult and embryonic mRNA: two NMDAR1 receptor genes (NMDAR1.1 and NMDAR1.2) and eight NMDAR2 receptor genes (NMDAR2A.1-D.1 and NMDAR2A.2-D.2). Each receptor subtype has two zebrafish paralogs, only one gene from each pair corresponding to the mammalian ortholog. These paired genes arose from genomic duplication events that occurred during teleost evolution. The 10 identified genes have been placed on the physical map of the genome, using the T54 Zebrafish Radiation Hybrid. The in situ hybridization pattern of these receptor subunits have been examined in 12, 24 and 48 hour post fertilization (hpf) zebrafish embryos using the whole mount technique. The NMDAR1.1 gene is expressed in the spinal cord at 24 hours, whereas the NMDAR1.2 gene shows general brain expression at 72 hours. NMDAR2A.1, 2C.1 and 2D.1 are expressed in the embryo. NMDA2B.1 is not. None of second NMDAR2 paralogs is expressed in the embryo. In order to exploit the utility of the zebrafish system, we are currently in the process of making NMDAR1 transgenic fish using a BAC (Bacterial Artificial Chromosome) modification protocol.This work was supported by NIH Grants NS35534 (M. M. V.). Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, molecular genetics, nervous system, in situ hybridization, genetic techniques, histology and cytology techniques, N-methyl-D-aspartate receptor (NMDAR), T54 Zebrafish Radiation Hybrid.

Crawford, B.D., C.A. Henry, T.A. Clason, A.L. Becker, and M.B. Hille (2003). **Activity and distribution of paxillin, focal adhesion kinase, and cadherin indicate cooperative roles during zebrafish morphogenesis.** *Molecular Biology of the Cell* 14(8): 3065-81. ISSN: 1059-1524.
NAL Call Number: QH604.C452
Abstract: We investigated the focal adhesion proteins paxillin and Fak, and the cell-cell adhesion protein cadherin in developing zebrafish (*Danio rerio*) embryos. Cadherins are expressed in presomitic mesoderm where they delineate cells. The initiation of somite formation coincides with an increase in the phosphorylation of Fak, and the accumulation of Fak, phosphorylated Fak, paxillin, and fibronectin at nascent somite boundaries. In the notochord, cadherins are expressed on cells during intercalation, and phosphorylated Fak accumulates in circumferential rings where the notochord cells contact laminin in the perichordal sheath. Subsequently, changes in the orientations of collagen fibers in the sheath suggest that Fak-mediated adhesion allows longitudinal expansion of the notochord, but not lateral expansion, resulting in notochord elongation. Novel observations showed that focal adhesion kinase and paxillin concentrate at sites of cell-cell adhesion in the epithelial enveloping layer and may associate with actin cytoskeleton at epithelial junctions containing cadherins. Fak is phosphorylated at these epithelial junctions but is not phosphorylated on Tyr397, implicating a noncanonical mechanism of regulation. These data suggest that Fak and paxillin may function in the integration of cadherin-based and integrin-based cell adhesion during the morphogenesis of the early zebrafish embryo.
Descriptors: zebrafish, cadherins metabolism, cytoskeletal proteins metabolism, phosphoproteins metabolism, protein tyrosine kinases metabolism , amino acid sequence, cell adhesion physiology, molecular cloning, cytoskeletal embryology, metabolism, fibronectins metabolism, focal adhesion protein tyrosine kinases, laminin metabolism, molecular sequence data, morphogenesis physiology, notochord embryology and metabolism, paxillin, phosphophosphorylation, protein tyrosine kinases genetics, somites cytology and metabolism.

Criscitiello, M.F., N.E. Wermenstam, L. Pilstrom , and E.C. McKinney (2004). **Allelic polymorphism of T-cell receptor constant domains is widespread in fishes**. *ImmunoGenetics* 55(12): 818-824. ISSN: 0093 7711.
NAL Call Number: QR184.I4
Abstract: T-cell receptor chains contain membrane-proximal constant domains of the immunoglobulin superfamily that are relatively invariant in mammalian species. In contrast, recent studies in the bicolor damselfish have demonstrated surprising allelic polymorphism in the TCR alpha (A) and TCR beta (B) "constant" (C) domain genes. This report extends these initial observations beyond Perciformes to two other orders of teleost fishes. Studies in both the Atlantic cod and zebrafish show high levels of polymorphism in the TCRA constant genes. Levels of 13% and 15% amino acid nonidentity were found within cod and zebrafish, respectively. Evolutionary analysis of codon usage suggests that positive selection maintains the high number of TCRAC alleles in these fish populations. Additionally, investigation of a TCRB constant gene from the Beau Gregory, a sister species of the bicolor damselfish, shows no evidence of transpecies maintenance of constant region alleles. These data argue that the T-cell receptor constant domain is being employed by many vertebrates in a manner inconsistent with our current understanding, and may indicate unheralded complexity in signal transduction through the TCR/CD3 complex. Copyright © 2008 CABI
Descriptors: zebrafish, bicolor damselfish, Atlantic cod, alleles, evolution, genes, genetic polymorphism, immunology, TCR, CD3 complex, T lymphocytes.

Crollius, H.R. and J. Weissenbach (2005). **Fish genomics and biology**. *Genome Research* 15(12): 1675-1682. ISSN: 1088 9051.

NAL Call Number: QP606.D46P34

Abstract: The impact of the genome sequence for fish species for which it is already available is reviewed. How the combination of genomics with more traditional disciplines may pave the way for a wider impact on biology is examined. The use of fish genomic sequence as a model of other vertebrate genomes is discussed. Insights into vertebrate genome evolution are given. Results of sequencing the *Takifugu rubripes* and *Tetraodon* genomes using the whole-genome shotgun method are presented. Two other biological models used, zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*), are discussed. The future of fish genomics is discussed. Copyright © 2008 CABI

Descriptors: zebrafish, biology, chromosomes, DNA sequencing, evolution, genes, genetic models, genome analysis, genomes, mutations, nucleotide sequences, phylogeny, *Danio rerio*, *Oryzias latipes*, *Tetraodontidae*, *Takifugu rubripes.*

Notes: A review.

Crotwell, P.L., A.R. Sommervold, and P.M. Mabee (2004). **Expression of bmp2a and bmp2b in late-stage zebrafish median fin development.** *Gene Expression Patterns* 5(2): 291-6. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Zebrafish bmp2a and bmp2b mRNA expression in the developing median fins (caudal, anal, and dorsal) of late-stage larvae (>5 days post-fertilization) was analyzed by reverse transcriptase-PCR (RT-PCR) and in situ hybridization. bmp2a is expressed in developing fin rays, while bmp2b is expressed in developing fin rays, hypertrophic chondrocytes, and in the zone of segmentation (ZS) in developing anal and dorsal fin radials. This latter pattern of bmp2b expression in the ZS mirrors tetrapod bmp2 expression in developing joints. Additionally, both genes are expressed in neural and hemal arches and spines. bmp2a is strongly expressed in the lens; lens bmp2b expression is detected only weakly via RT-PCR.

Descriptors: zebrafish, bone morphogenetic proteins metabolism, proteins biosynthesis, chondrocytes metabolism, extremities growth and development, developmental gene expression regulation, larva growth , development and metabolism, messenger RNA biosynthesis, proteins metabolism.

Crow, K.D., P.F. Stadler, V.J. Lynch, C. Amemiya, and G.P. Wagner (2006). **The "fish-specific" hox cluster duplication is coincident with the origin of teleosts**. *Molecular Biology and Evolution* 23(1): 121-136. ISSN: 0737-4038.

NAL Call Number: QH506.M642

Descriptors: zebrafish, evolution, evolutionary genetics, freshwater fish, HOX gene, marine fish, molecular biology, nucleotide sequence, phylogenetics, gene duplication, genealogy, *Acipenser*, *Danio rerio*, *Fugu*, *Oryzias latipes*, Teleostei, medaka, spoonfishes, sturgeons, genetics and evolution, developmental genetics.

Crump, J.G., M.E. Swartz, and C.B. Kimmel (2004). **An integrin-dependent role of pouch endoderm in hyoid cartilage development**. *Public Library of Science: Biology* 2(9) ISSN: 1544-9173.

NAL Call Number: QH301.P56

Descriptors: zebrafish, cartilage, cell fate, cell survival, compaction, defects, development, developmental genetics, endoderm, evolution, freshwater fish, head, integrins, muscles, musculoskeletal system, nerves, neural crest, pattern formation, pharynx, craniofacial growth,

Danio rerio, developmental genetics, cartilage and cartilage diseases, fishery charts, grounds and water areas.

Cui, W.W., S.E. Low, H. Hirata, L. Saint Amant, R. Geisler, R.I. Hume, and J.Y. Kuwada (2005). **The zebrafish shocked gene encodes a glycine transporter and is essential for The function of early neural circuits in The CNS.** *Journal of Neuroscience* 25(28): 6610-20. ISSN: 0270-6474.

Abstract: shocked (sho) is a zebrafish mutation that causes motor deficits attributable to CNS defects during the first2dof development. Mutant embryos display reduced spontaneous coiling of the trunk, diminished escape responses when touched, and an absence of swimming. A missense mutation in the slc6a9 gene that encodes a glycine transporter (GlyT1) was identified as the cause of the sho phenotype. Antisense knock-down of GlyT1 in wild-type embryos phenocopies sho, and injection of wild-type GlyT1 mRNA into mutants rescues them. A comparison of glycine-evoked inward currents in *Xenopus* oocytes expressing either the wild-type or mutant protein found that the missense mutation results in a nonfunctional transporter. glyt1 and the related glyt2 mRNAs are expressed in the hindbrain and spinal cord in nonoverlapping patterns. The fact that these regions are known to be required for generation of early locomotory behaviors suggests that the regulation of extracellular glycine levels in the CNS is important for proper function of neural networks. Furthermore, physiological analysis after manipulation of glycinergic activity in wild-type and sho embryos suggests that the mutant phenotype is attributable to elevated extracellular glycine within the CNS.

Descriptors: wild type and shocked mutant zebrafish, central nervous system embryology, glycine metabolism, glycine plasma membrane transport and genetics, nerve tissue, drug effects, pathology, physiopathology, extracellular fluid metabolism, developmental gene expression regulation, gene targeting, glycine plasma membrane transport proteins antagonists and inhibitors and proteins deficiency, muscles embryology and physiology, missense mutation, nerve tissue proteins antagonists and inhibitors deficiency and genetics , oocytes, phenotype, physical stimulation, messenger RNA biosynthesis, messenger RNA genetics, messenger RNApharmacology, recombinant fusion proteins metabolism, rhombencephalon embryology and metabolism, sarcosine analogs and derivatives pharmacology, spinal cord embryology and metabolism, swimming , *Xenopus*, proteins antagonists, inhibitors, deficiency and genetics.

Cui, Z., Y. Yang, C.D. Kaufman, D. Agalliu, and P.B. Hackett (2003). **RecA-mediated, targeted mutagenesis in zebrafish.** *Marine Biotechnology* 5(2): 174-84. ISSN: 1436-2228.

NAL Call Number: TP248.27.M37M37

Abstract: We have evaluated the efficacy of RecA, a prokaryotic protein involved with homologous recombination, to direct site-specific mutagenesis in zebrafish embryos. For this we coinjected a vector containing a mutated enhanced green fluorescent protein (EGFP) gene plus 236-nucleotide corrective single-stranded DNAs coated with RecA into 1-cell zebrafish embryos. Twenty-hours after fertilization, about 5% to 20% of injected embryos showed EGFP expression in 1 or more cells when RecA-coated corrective DNAs were used, but not when RecA was omitted. Mutated EGFP genes with 1-bp insertions or deletions were inefficiently activated, whereas those with 7-bp insertions were activated about 4-fold more efficiently. RecA-coated template strand had a higher efficiency than its complementary strand in activation of EGFP expression. Prior irradiation of the embryos with UV light enhanced RecA-mediated restoration of gene activity, suggesting that the effects we observed were augmented by one or more factors of zebrafish DNA repair systems.

Descriptors: zebrafish embryos, DNA metabolism, gene expression, luminescent site directed mutagenesis Rec A recombinases metabolism, genetically modified, molecular cloning, DNA genetics, DNA primers genetics , DNA probes, recombinant DNA genetics, genetic engineering methods, genetic vectors, green fluorescent proteins.

D'Amico, L., I.C. Scott, B. Jungblut, and D.Y. Stainier (2007). **A mutation in zebrafish hmgcr1b reveals a role for isoprenoids in vertebrate heart-tube formation.** *Current Biology* 17(3): 252-9. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: In vertebrates, the morphogenetic assembly of the primitive heart tube requires the medial migration and midline fusion of the bilateral myocardial epithelia. Several mutations that result in abnormal heart-tube formation have been studied; however, an understanding of the underlying molecular and cellular mechanisms of the migration and fusion of these epithelial sheets is far from complete. In a forward genetic screen to identify genes regulating early zebrafish heart development, we identified a mutation in the 3-hydroxy-3-methylglutaryl-Coenzyme A reductase 1b (hmgcr1b) gene that affects myocardial migration to the midline and subsequent heart-tube morphogenesis. The mutant phenotype can be rescued with injections of mevalonate, the direct product of HMGCR activity. Furthermore, treatment of embryos with pharmacological inhibitors of isoprenoid synthesis, which occurs downstream of mevalonate production, resulted in defective heart-tube formation. Interestingly, in hmgcr1b mutant embryos and embryos treated with HMGCR inhibitors, both RasCT20-eGFP and RhoaCT32-eGFP fusion proteins were mislocalized away from the plasma membrane in embryonic myocardial cells. We conclude that protein prenylation, acting downstream of Hmgcr1b and possibly through Ras and, or, Rho signaling, is required for the morphogenesis of the myocardial sheets for formation of the primitive heart tube.

Descriptors: mutant zebrafish, developmental gene expression regulation, heart embryology, hydroxymethylglutaryl coa reductases metabolism, morphogenesis, protein isoprenylation , proteins metabolism, amino acid sequence, amino acid substitution, genetically modified, cell movement, metabolism, epithelial cells cytology, hydroxymethylglutaryl coa reductases chemistry and genetics, mevalonic acid, molecular sequence data, myocardium cytology, cardiac myocytes cytology, phenotype, point mutation, sequence alignment.

Daggett, D.F., C.A. Boyd, P. Gautier, R.J. Bryson Richardson, C. Thisse, B. Thisse, S.L. Amacher, and P.D. Currie (2004). **Developmentally restricted actin-regulatory molecules control morphogenetic cell movements in the zebrafish gastrula.** *Current Biology* 14(18): 1632-8. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: Although our understanding of the regulation of cellular actin and its control during the development of invertebrates is increasing, the question as to how such actin dynamics are regulated differentially across the vertebrate embryo to effect its relatively complex morphogenetic cell movements remains poorly understood. Intercellular signaling that provides spatial and temporal cues to modulate the subcellular localization and activity of actin regulatory molecules represents one important mechanism. Here we explore whether the localized gene expression of specific actin regulatory molecules represents another developmental mechanism. We have identified a cap1 homolog and a novel guanine nucleotide exchange factor (GEF), quattro (quo), that share a restricted gene expression domain in the anterior mesendoderm of the zebrafish gastrula. Each gene is required for specific cellular behaviors during the anterior migration of this tissue; furthermore, cap1 regulates cortical actin distribution specifically in these cells. Finally, although cap1 and quo are autonomously

required for the normal behaviors of these cells, they are also nonautonomously required for convergence and extension movements of posterior tissues. Our results provide direct evidence for the deployment of developmentally restricted actin-regulatory molecules in the control of morphogenetic cell movements during vertebrate development.
Descriptors: zebrafish, actins metabolism, morphogenetic cell movement physiology, developmental gene expression regulation, guanine nucleotide exchange factors genetics and metabolism, transcription factors metabolism, proteins genetics and metabolism, actins physiology, amino acid and base sequence, basic leucine zipper transcription factors, complementary DNA genetics, genetic databases, fungal proteins, gastrula physiology, immunohistochemistry, in situ hybridization, molecular sequence data, morphogenesis, sequence alignment, DNA sequence analysis, transcription factors genetics.

Daiyasu, H., T. Ishikawa, K. Kuma, S. Iwai, T. Todo, and H. Toh (2004). **Identification of cryptochrome DASH from vertebrates**. *Genes to Cells* 9(5): 479-495. ISSN: 1356-9597.
NAL Call Number: QH441.5
Descriptors: zebrafish, amino acid sequence, amphibiotic species, bacteria, DNA, databases, folic acid, freshwater fish, fungi, new records, nucleotide sequence, phylogenetics, cDNA, cryptochromes, DNA photolyase, flavin, amphibia, *Arabidopsis thaliana*, *Danio rerio*, *Neurospora crassa*, Pisces, Synechocystis, *Vibrio cholerae*, *Xenopus laevis*, African clawed frog, amphibians, palaeontology.

Dalkic, E., C. Kuscu, C. Sucularli, I.T. Aydin, K.C. Akcali, and O. Konu (2006). **Alternatively spliced Robo2 isoforms in zebrafish and rat.** *Development Genes and Evolution* 216(9): 555-63. ISSN: 0949 944X.
NAL Call Number: QL951.R68
Abstract: Robo2, a member of the robo gene family, functions as a repulsive axon guidance receptor as well as a regulator of cell migration and tissue morphogenesis in different taxa. In this study, a novel isoform of the zebrafish robo2 (robo2_tv2), which included an otherwise alternatively spliced exon (CAE), has been characterized. Robo2_tv2 is expressed differentially in most non-neuronal tissues of adult zebrafish whereas robo2_tv1 expression to a great extent is restricted to the brain and eye. In zebrafish, robo2_tv2 exhibits a very-low-level basal expression starting from 1 day post fertilization until the mid-larval stages, at which time its expression increases dramatically and could be detected throughout adulthood. Our findings demonstrate that the amino acid sequence coded by CAE of the robo2 gene is highly conserved between zebrafish and mammals, and also contains conserved motifs shared with robo1 and robo4 but not with robo3. Furthermore, we provide an account of differential transcription of the CAE homolog in various tissues of the adult rat. These results suggest that the alternatively spliced robo2 isoforms may exhibit tissue specificity.
Descriptors: zebrafish, alternative splicing, immunologic receptors and protein metabolism, amino acid sequence, conserved sequence, exons genetics, gene expression, molecular sequence data, protein isoforms chemistry, genetics and metabolism, messenger RNA analysis and metabolism, Sprague Dawley rats, immunologic receptors chemistry, immunologic receptors genetics, sequence alignment, metabolism, proteins chemistry and genetics.

Dalkic, E., C. Kuscu, C. Sucularli, and O. Konu (2006). **Expression profiling of candidate TOR signalling components in zebrafish**. *Federation of European Biochemical Societies Journal* 273(Suppl. 1): 104. ISSN: 1742 464X.
NAL Call Number: QP501.E8

Descriptors: zebrafish embryo, development, molecular genetics, biochemistry and molecular biophysics, TOR signalling, embryogenesis, cell migration.

Danilova, N., A. Visel, C.E. Willett, and L.A. Steiner (2004). **Expression of the winged helix/forkhead gene, foxn4, during zebrafish development.** *Brain Research* 153(1): 115-9. ISSN: 0006 8993.
NAL Call Number: QP376
Abstract: The winged-helix/forkhead transcription factor gene, foxn4, is expressed in the nervous system of developing and adult zebrafish. Prominent expression sites include the olfactory placode, the basal layer of the olfactory epithelium, the neuroepithelium of the developing retina, the germinal zone of the differentiated eye, regions of motoneuron development in the neural tube and periventricular regions of the brain. The adult thymus is the only major site of foxn4 expression outside of the nervous system.
Descriptors: zebrafish, brain embryology, eye proteins biosynthesis, transcription factors biosynthesis, amino acid sequence, eye developmental gene expression regulation, in situ hybridization, foxn4 gene, molecular sequence data, retina embryology, skin embryology, thymus gland embryology, transcription factors genetics.

David, N.B., P. Mourrain and F.M. Rosa (2004). **Molecular Aspects of fish and marine Biology: Endoderm formation in zebrafish**. G. Zhiyuan and V. Korzh *Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd.: Singapore, Singapore: p. 424-453. ISBN: 9812388214.
NAL Call Number: QL638.C94 F57
Descriptors: zebrafish embryos, activins, biochemical pathways, biochemical receptors, blastomere, cell cycle, cell differentiation, cellular biology, embryonic development, epidermal growth factor, epistasis, epithelium, gene expression, genes, genetic models, involution , ligands, molecular biology, mutants, phenotypes, signal transduction, transcription factors, transforming growth factor, yolk sac, *Danio rerio.*

Davidson, A.J., P. Ernst, Y. Wang, M.P.S. Dekens, P.D. Kingsley, J. Palis, S.J. Korsmeyer, G.Q. Daley, and L.I. Zon (2003). **Cdx4 mutants fail to specify blood progenitors and can be rescued by multiple hox genes**. *Nature* 425(6955): 7300-306. ISSN: 0028-0836.
NAL Call Number: 472 N21
Descriptors: zebrafish, blood progenitors, embryonic development , gene expression, hemopoiesis, mesoderm, organogenesis, cdx4 gene, gene regulation, hox genes, hoxa9a gene, hoxb7a gene, hoxb8a gene, scl gene, developmental genetics, evolution.

Davison, J.M., S.D. Leach, and M.J. Parsons (2006). **Identifying genes that regulate organogenesis: a gene-trap approach in zebrafish**. *Laboratory Investigation* 86(Suppl. 1): 289A-290A. ISSN: 0023 6837.
NAL Call Number: 448.8L11
Descriptors: zebrafish, molecular genetics, biochemistry, molecular biophysics, development, gene trap screen, genetic techniques, organogenesis.

De Jong, J.L. and L.I. Zon (2005). **Use of the zebrafish system to study primitive and definitive hematopoiesis.** *Annual Review of Genetics* 39: 481-501. ISSN: 0066 4197.
NAL Call Number: QH431.A1A5
Abstract: The zebrafish (*Danio rerio*) has emerged as an ideal organism for the study of hematopoiesis, the process by which all the cellular elements of the blood are formed. These

elements, including erythrocytes, granulocytes, monocytes, lymphocytes, and thrombocytes, are formed through complex genetic signaling pathways that are highly conserved throughout phylogeny. Large-scale forward genetic screens have identified numerous blood mutants in zebrafish, helping to elucidate specific signaling pathways important for hematopoietic stem cells (HSCs) and the various committed blood cell lineages. Here we review both primitive and definitive hematopoiesis in zebrafish, discuss various genetic methods available in the zebrafish model for studying hematopoiesis, and describe some of the zebrafish blood mutants identified to date, many of which have known human disease counterparts.
Descriptors: zebrafish, hematopoiesis genetics, blood mutants, hematopoiesis physiology, hematopoietic stem cells (HSCs), disease models, hematologic diseases and mutation genetics.

De Souza, F.S., V.F. Bumaschny, M.J. Low, and M. Rubinstein (2005). **Subfunctionalization of expression and peptide domains following the ancient duplication of the proopiomelanocortin gene in Teleost fishes**. *Molecular Biology and Evolution* 22(12): 2417-2427. ISSN: 0737-4038.
NAL Call Number: QH506.M642
Descriptors: zebrafish, brain, enhancers, evolution, evolutionary genetics, gene expression, gene mapping, genomes, hormones, molecular biology, opioids, peptides, phylogeny , pituitary, preoptic area, proopiomelanocortin, regulatory sequences, gene duplication, opioid peptides, *Danio rerio*, Fugu, *Takifugu rubripes*, Teleostei, *Tetraodon*, *Tetraodon nigroviridis*.

Deak, K.L., M.E. Dickerson, E. Linney, D.S. Enterline, T.M. George, E.C. Melvin, F.L. Graham, D.G. Siegel, P. Hammock, L. Mehltretter, A.G. Bassuk, J.A. Kessler, J.R. Gilbert, and M.C. Speer (2005). **Analysis of ALDH1A2, CYP26A1, CYP26B1, CRABP1, and CRABP2 in human neural tube defects suggests a possible association with alleles in ALDH1A2**. *Birth Defects Research* 73(11): 868-875. ISSN: 1542 0752.
NAL Call Number: QL991.T4
Abstract: BACKGROUND: Vitamin A (retinol), in the form of retinoic acid (RA), is essential for normal development of the human embryo. Studies in the mouse and zebrafish have shown that retinol is metabolized in the developing spinal cord and must be maintained in a precise balance along the anteroposterior axis. Both excess and deficiency of RA can affect morphogenesis, including failures of neural tube closure.METHODS: We chose to investigate 5 genes involved in the metabolism or synthesis of RA, ALDH1A2, CYP26A1, CYP26B1, CRABP1, and CRABP2, for their role in the development of human neural tube defects, such as spina bifida.RESULTS: An association analysis using both allelic and genotypic single-locus tests revealed a significant association between the risk for spina bifida and 3 polymorphisms in the gene ALDH1A2; however, we found no evidence of a significant multilocus association.CONCLUSIONS: These results may suggest that polymorphisms in ALDH1A2 may influence the risk for lumbosacral myelomeningocele in humans. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics and biophysics, polymorphisms in ALDH1A2, neurology, development, medical genetics, neural tube defect, nervous system disease, congenital disease, spina bifida model, genetic disease.

DeBruyne, J., M.W. Hurd, L. Gutierrez, M. Kaneko, Y. Tan, D.E. Wells, and G.M. Cahill (2004). **Isolation and phenogenetics of a novel circadian rhythm mutant in zebrafish.** *Journal of NeuroGenetics* 18(2): 403-28. ISSN: 0167-7063.
Abstract: Widespread use of zebrafish *(Danio rerio*) in genetic analysis of embryonic devel-

opment has led to rapid advances in the technology required to generate, map and clone mutated genes. To identify genes involved in the generation and regulation of vertebrate circadian rhythmicity, we screened for dominant mutations that affect the circadian periodicity of larval zebrafish locomotor behavior. In a screen of 6,500 genomes, we recovered 8 homozygous viable, semi-dominant mutants, and describe one of them here. The circadian period of the lager and lime (lag(dg2)) mutant is shortened by 0.7 h in heterozygotes,and 1.3 h in homozygotes. This mutation also shortens the period of the melatonin production rhythm measured from cultured pineal glands, indicating that the mutant gene product affects circadian rhythmicity at the tissue level, as well as at the behavioral level. This mutation also alters the sensitivity of pineal circadian period to temperature, but does not affect phase shifting responses to light. Linkage mapping with microsatellite markers indicates that the lag mutation is on chromosome 7. A zebrafish homolog of period1(per1) is the only known clock gene homolog that maps near the lag locus. However, all sequence variants found in per1 cDNA from lag(dg2) mutants are also present in wild type lines, and we were unable to detect any defect in per1 mRNA splicing, so this mutation may identify a novel clock gene.

Descriptors: zebrafish, circadian rhythm mutant genetics, novel clock gene, DNA mutational analysis, eye proteins genetics, chromosome mapping, homozygote , locomotion genetics, melatonin secretion, organ culture techniques, pineal gland secretion.

DeLaCalle-Mustienes, E., C.G. Feijoo, M. Manzanares, J.J. Tena, E. Rodriguez-Seguel, A. Letizia, M.L. Allende, and J.L. Gomez-Skarmeta (2005). **A functional survey of the enhancer activity of conserved non-coding sequences from vertebrate Iroquois cluster gene deserts**. *Genome Research* 15(8): 1061-1072. ISSN: 1088 9051.

NAL Call Number: QP606.D46P34

Abstract: Recent Studies of the genome architecture of vertebrates have uncovered two unforeseen aspects of its organization. First, large regions of the genome, called gene deserts, are devoid of protein-coding sequences and have no obvious biological role. Second, comparative genomics has highlighted the existence of an array of highly conserved non-coding regions [HCNR]s ill all vertebrates. Most Surprisingly, these structural features are strongly associated with genes that have essential functions during development. Among these, the vertebrate Iroquois (Irx) genes stand Out on both fronts. Mammalian Irx genes are organized in two clusters (IrxA and IrxB) that span >1 Mb each with no other genes interspersed. Additionally, a large number of HCNRs exist within IrX Clusters. We have systematically examined the enhancer activity of HCNRs from the IrxB Cluster using transgenic *Xenopus* and zebrafish embryos. Most of these HCNRs are active in subdomains of endogenous Irx expression, and some are candidates to contain shared enhancers of neighboring genes, which could explain the evolutionary conservation of Irx Clusters. furthermore, HCNRs present in tetrapod IrxB but not in fish may be responsible for novel Irx expression domains that appeared after their divergence. Finally, we have performed a more detailed analysis oil two IrxB Ultraconserved non-coding regions (UCRs) duplicated in IrxA Clusters ill similar relative positions. These four regions share a core re.-ion highly conserved among all of them and drive expression ill similar domains. However, inter-species conserved sequences Surrounding the core, specific for each of these UCRs, are able to modulate their expression.
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Descriptors: zebrafish, molecular genetics, comparative genomics, genetic techniques, genome, gene desert, highly conserved on-coding regions, Iroquois cluster gene deserts.

Delaunay, F., C. Thisse, B. Thisse, and V. Laudet (2003). **Differential regulation of Period 2 and Period 3 expression during development of the zebrafish circadian clock.** *Gene Expression Patterns* 3(3): 319-24. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Circadian (approximately 24h) clocks are endogenous time-keeping systems that drive the daily biological rhythms observed in most living organisms. The oscillation is generated by a transcriptional/translational autoregulatory feedback loop that is reset by external time cues such as the light/dark cycle and which in turn controls rhythms in physiology and behavior through downstream clock-controlled genes (Nature 417 (2002) 329). Genetic and biochemical analysis of *Drosophila* and mammalian clock genes has provided a comprehensive model for the molecular oscillator that generates these rhythms, but the ontogeny of this oscillator remains poorly understood. A circadian oscillator involving the clock genes Per3 and Rev-erb alpha was identified during early development in zebrafish (Science 289 (2000) 297). Here, we report the isolation of zebrafish Per2 and show the presence of a Per2 maternal mRNA in early embryos as for Per3. However, Per2 rhythmic expression occurs late during embryogenesis as compared to that of Per3. Furthermore, our data indicate that Per2 is not required during embryogenesis for the rhythmicity of physiological outputs such as melatonin synthesis. In addition, Per2 but not Per3 is constitutively expressed in the developing olfactory bulb and pituitary. This differential spatio-temporal expression patterns suggest specific roles for Per2 and Per3 in the establishment of the embryonic circadian system.

Descriptors: zebrafish embryos, circadian rhythm genetics, eye proteins genetics and biosynthesis, developmental gene expression regulation physiology, nuclear proteins genetics, amino acid sequence, circadian rhythm physiology, conserved sequence, gene expression profiling, molecular sequence data, nuclear proteins biosynthesis, phylogeny, transcription factors, proteins, Period 2 and Period 3.

Demas, J. and H.T. Cline (2007). **The blu Blur: mutation of a vesicular glutamate transporter reduces the resolution of zebrafish vision.** *Neuron* 53(1): 4-6. ISSN: 0896-6273.

Abstract: Vesicular transporters mediate the packaging of neurotransmitters into synaptic vesicles and can therefore control the amount of neurotransmitter released into the synaptic cleft. In this issue of *Neuron*, Smear et al. demonstrate that mutation of a vesicular glutamate transporter (Vglut) found in the retinal ganglion cells (RGCs) of zebrafish alters both the synaptic transmission and connectivity between RGCs and their targets, limiting the transfer of visually evoked activity from RGCs and degrading behaviors that depend on high-acuity vision.

Descriptors: zebrafish embryos, vision mutation genetics, retinal ganglion cells metabolism, synaptic transmission genetics, vesicular glutamate transport proteins genetics, metabolism, glutamic acid metabolism, neural pathways cytology, neural pathways metabolism, neural pathways physiopathology, retinal ganglion cells ultrastructure, vision genetics, vision disorders genetics, metabolism disorders and physiopathology, zebrafish anatomy and histology.

Den Hertog, J. (2005). **Chemical genetics: Drug screens in zebrafish.** *Bioscience Reports* 25(5-6): 289-97. ISSN: 0144 8463.

NAL Call Number: QH506.B557

Abstract: High throughput chemical genetic screens for compounds with specific biological activity in a whole organism are feasible using zebrafish embryos. At least two medium to large scale drug screens have been carried out to date, leading to the identification of compounds that disturb zebrafish development. Chemical genetics using zebrafish embryos may become an important step in the discovery of drugs and their targets.

Descriptors: zebrafish, preclinical drug evaluation methods, pharmacogenetics methods, metabolism, genetically modified, biological and genetic models, mutagenesis, toxicity tests.

Deniziak, M., C. Thisse, M. Rederstorff, C. Hindelang, B. Thisse, and A. Lescure (2007). **Loss of selenoprotein N function causes disruption of muscle architecture in the zebrafish embryo.** *Experimental Cell Research* 313(1): 156-67. ISSN: 0014 4827.
NAL Call Number: 442.8EX7
Abstract: Mutations in the gene coding for selenoprotein N (SelN), a selenium containing protein of unknown function, cause different forms of congenital muscular dystrophy in humans. These muscular diseases are characterized by early onset of hypotonia which predominantly affect in axial muscles. We used zebrafish as a model system to understand the function of SelN in muscle formation during embryogenesis. Zebrafish SelN is highly homologous to its human counterpart and amino acids corresponding to the mutated positions in human muscle diseases are conserved in the zebrafish protein. The sepn1 gene is highly expressed in the somites and notochord during early development. Inhibition of the sepn1 gene by injection of antisense morpholinos does not alter the fate of the muscular tissue, but causes muscle architecture disorganization and greatly reduced motility. Ultrastructural analysis of the myotomes reveals defects in muscle sarcomeric organization and in myofibers attachment, as well as altered myoseptum integrity. These studies demonstrate the important role of SelN for muscle organization during early development. Moreover, alteration of myofibrils architecture and tendon-like structure in embryo deficient for SelN function provide new insights into the pathological mechanism of SelN-related myopathy.
Descriptors: zebrafish embryos, muscles embryology and metabolism, selenoproteins deficiency , proteins deficiency, amino acid and base sequence, complementary DNA genetics, gene expression, molecular sequence data, nucleic acid conformation, RNA chemistry and genetics, selenoproteins genetics.

Detrich, H.W.3. and D.A. Yergeau (2004). **Comparative genomics in erythropoietic gene discovery: synergisms between the Antarctic icefishes and the zebrafish.** *Methods in Cell Biology* 77: 475-503. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, Antarctic icefishes, erythropoiesis genetics, genomics methods, nucleic acid hybridization methods, perciformes genetics, Antarctic regions, blood cells chemistry, complementary DNA isolation and purification, osmeriformes genetics, messenger RNA analysis and metabolism.

Devine, C.A. and B. Key (2003). **Identifying axon guidance defects in the embryonic zebrafish brain.** *Methods in Cell Science* 25(1-2): 33-7. ISSN: 1381-5741.
NAL Call Number: QH573.T52
Abstract: The method described here outlines a simple protocol to investigate the in vivo function of axon guidance molecules during the development of the embryonic zebrafish brain. By 24 hours postfertilization, a simple scaffold of axon tracts and commissures can be visualised in the brain using acetylated alpha-tubulin, a panaxonal marker that stains all axons. The highly stereotypical trajectory of axons in the embryonic zebrafish brain provides an ideal system in which to study the molecular mechanisms of axon guidance, as defects in the axon scaffold can be clearly visualised. We describe here our approach to identify defects in the trajectory of axons that establish the initial template of tracts in the embryonic fore- and mid-brain. By combining immunohistochemical techniques and confocal microscopy on dissected wholemounts of embryonic brains we are able to observe at high resolution the

complete scaffold of axon tracts. This approach provides a rapid and simple means of assessing axon guidance defects in the developing brain. Given the advantages of the zebrafish as a model system, and the range of molecular perturbation methods now available, this technique provides a valuable tool for assessing the phenotypic effects of gene perturbations in a biologically relevant context.

Descriptors: zebrafish embryo brain, axons physiology, embryonic induction physiology, developmental gene expression regulation physiology, neurons cytology, brain anatomy and histology, brain physiology, cell movement physiology, anatomy and histology, neurons physiology.

Dickmeis, T., C. Plessy, S. Rastegar, P. Aanstad, R. Herwig, F. Chalmel, N. Fischer, and U. Strahle (2004). **Expression profiling and comparative genomics identify a conserved regulatory region controlling midline expression in the zebrafish embryo.** *Genome Research* 14(2): 228-38. ISSN: 1088-9051.

NAL Call Number: QP606.D46P34

Abstract: Differential gene transcription is a fundamental regulatory mechanism of biological systems during development, body homeostasis, and disease. Comparative genomics is believed to be a rapid means for the identification of regulatory sequences in genomes. We tested this approach to identify regulatory sequences that control expression in the midline of the zebrafish embryo. We first isolated a set of genes that are coexpressed in the midline of the zebrafish embryo during somitogenesis stages by gene array analysis and subsequent rescreens by in situ hybridization. We subjected 45 of these genes to Compare and DotPlot analysis to detect conserved sequences in noncoding regions of orthologous loci in the zebrafish and *Takifugu* genomes. The regions of homology that were scored in nonconserved regions were inserted into expression vectors and tested for their regulatory activity by transient transgenesis in the zebrafish embryo. We identified one conserved region from the connective tissue growth factor gene (ctgf), which was able to drive expression in the midline of the embryo. This region shares sequence similarity with other floor plate/notochord-specific regulatory regions. Our results demonstrate that an unbiased comparative approach is a relevant method for the identification of tissue-specific cis-regulatory sequences in the zebrafish embryo.

Descriptors: zebrafish embryos, conserved sequence genetics, gene expression profiling methods, genomics methods, promoter regions genetics, genetically modified, base sequence, DNA probes genetics, chemistry, cytology, metabolism, enhancer elements genetics, developmental gene expression regulation genetics, immediate early in situ hybridization methods, intercellular signaling peptides and molecular sequence data, notochord chemistry, cytology and metabolism, DNA sequence analysis methods, nucleic acid sequence homology.

Diekmann, H., M. Klinger, T. Oertle, D. Heinz, H.-M. Pogoda, M.E. Schwab, and C.A. Stuermer (2005). **Analysis of the reticulon gene family demonstrates the absence of the neurite growth inhibitor Nogo-A in fish**. *Molecular Biology and Evolution* 22(8): 1635-1648. ISSN: 0737-4038.

NAL Call Number: QH506.M642

Descriptors: zebrafish, amino acid sequence, axons, central nervous system, evolution, evolutionary genetics, freshwater fish, gene families, genomes, homology, myelin, N-Terminus, Nogo protein, *Oligodendrocytes*, phylogenetics, phylogeny, regeneration, synteny, *Danio rerio.*

Diks, S.H., R.J. Bink, S. VanDeWater, J. Joore, C. VanRooijen, F.J. Verbeek, J. DenHertog, M.P. Peppelenbosch, and D. Zivkovic (2006). **The novel gene asb11: a regulator of The size of The neural progenitor compartment**. *Journal of Cell Biology* 174(4): 581-592. ISSN: 0021 9525.

NAL Call Number: 442.8J828

Abstract: From a differential display designed to isolate genes that are down-regulated upon differentiation of the central nervous system in *Danio rerio* embryos, we isolated d-asb11 (ankyrin repeat and suppressor of cytokine signaling box-containing protein 11). Knockdown of the d-Asb11 protein altered the expression of neural precursor genes sox2 and sox3 and resulted in an initial relative increase in proneural cell numbers. This was reflected by neurogenin1 expansion followed by premature neuronal differentiation, as demonstrated by HuC labeling and resulting in reduced size of the definitive neuronal compartment. Forced misexpression of d-asb11 was capable of ectopically inducing sox2 while it diminished or entirely abolished neurogenesis. Overexpression of d-Asb11 in both a pluripotent and a neural-committed progenitor cell line resulted in the stimulus-induced inhibition of terminal neuronal differentiation and enhanced proliferation. We conclude that d-Asb11 is a novel regulator of the neuronal progenitor compartment size by maintaining the neural precursors in the proliferating undifferentiated state possibly through the control of SoxB1 transcription factors. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, d-Asb11 protein development, molecular biophysics, sox2 and sox3 genes, nervous system, neuronal differentiation, central nervous system.

Dildrop, R. and U. Ruether (2004). **Organization of Iroquois genes in fish**. *Development Genes and Evolution* 214(6): 267-276. ISSN: 0949-944X.

NAL Call Number: QL951.R68

Descriptors: zebrafish, animal models, chromosomes, comparative studies, developmental genetics, divergence, evolution, freshwater fish, gene clusters, gene families, Iroquois protein, organizations, phylogeny, *Danio rerio*, *Fugu rubripes*, *Tetraodon nigroviridis*, evolutionary divergence, developmental genetics, genetics and evolution.

Dodd, A., S.P. Chambers, and D.R. Love (2004). **Short interfering RNA-mediated gene targeting in the zebrafish.** *Federation of European Biochemical Societies Journal* 561(1-3): 89-93. ISSN: 1742 464X.

NAL Call Number: QP501.E8

Abstract: Short interfering RNAs (siRNAs) have proved to be a useful tool in studying gene function in plants, invertebrates and mammalian systems. Here we report the use of siRNAs for targeting the zebrafish dystrophin gene. This study demonstrates the efficacy of siRNA-based gene silencing in this vertebrate model species, and illustrates the potential of this approach for determining the roles of multiple protein products expressed by a single gene during the early stages of development. In addition this study illustrates the usefulness of zebrafish as a model for muscle disease, and highlights the potential of siRNA-based gene targeting for disease analysis in this model organism.

Descriptors: zebrafish , dystrophin genetics, gene silencing drug effects, small interfering RNA pharmacology, embryonic and fetal development drug effects, histocytochemistry, methods, models, muscle fibers drug effects, small interfering RNA chemical synthesis.

Dong, J. and G.W. Stuart (2004). **Transgene manipulation in zebrafish by using recombinases.** *Methods in Cell Biology* 77: 363-79. ISSN: 0091-679X.

NAL Call Number: 442P92

Abstract: Although much remains to be done, our results to date suggest that efficient and precise genome engineering in zebrafish will be possible in the future by using Cre recombinase and SB transposase in combination with their respective target sites. In this study, we provide the first evidence that Cre recombinase can mediate effective site-specific deletion of transgenes in zebrafish. We found that the efficiency of target site utilization could approach 100%, independent of whether the target site was provided transiently by injection or stably within an integrated transgene. Microinjection of Cre mRNA appeared to be slightly more effective for this purpose than microinjection of Cre-expressing plasmid DNA. Our work has not yet progressed to the point where SB-mediated mobilization of our transgene constructs would be observed. However, a recent report has demonstrated that SB can enhance transgenesis rates sixfold over conventional methods by efficiently mediating multiple single-copy insertion of transgenes into the zebrafish genome (Davidson et al., 2003). Therefore, it seems likely that a combined system should eventually allow both SB-mediated transgene mobilization and Cre-mediated transgene modification. Our goal is to validate methods for the precise reengineering of the zebrafish genome by using a combination of Cre-loxP and SB transposon systems. These methods can be used to delete, replace, or mobilize large pieces of DNA or to modify the genome only when and where required by the investigator. For example, it should be possible to deliver particular RNAi genes to well-expressed chromosomal loci and then exchange them easily with alternative RNAi genes for the specific suppression of alternative targets. As a nonviral vector for gene therapy, the transposon component allows for the possibility of highly efficient integration, whereas the Cre-loxP component can target the integration and/or exchange of foreign DNA into specific sites within the genome. The specificity and efficiency of this system also make it ideal for applications in which precise genome modifications are required (e.g., stock improvement). Future work should establish whether alternative recombination systems (e.g., phiC31 integrase) can improve the utility of this system. After the fish system is fully established, it would be interesting to explore its application to genome engineering in other organisms.
Descriptors: zebrafish, genetically modified genetics, gene targeting methods, gene transfer techniques, insertional mutagenesis methods, recombinases metabolism, chemistry, green fluorescent proteins analysis and genetics, integrases genetics and metabolism, recombinases genetics, genetic recombination genetics, sequence deletion genetics, trans viral proteins genetics and metabolism.

Dong, P.D.S., C.A. Munson, W. Norton, C. Crosnier, X. Pan, Z. Gong, C.J. Neumann, and D.R. Stainier (2007). **Fgf10 regulates hepatopancreatic ductal system patterning and differentiation**. *Nature Genetics* 39(3): 397-402. ISSN: 1061-4036.
NAL Call Number: QH426.N37
Descriptors: zebrafish, differentiation, endoderm, epithelial cells, epithelium, fibroblast growth factor 10, fish physiology, foregut, freshwater fish, gallbladder, hepatopancreas, intestine, intestines, liver, mesenchyme, organogenesis, pancreas, signal transduction, *Danio rerio*.

Douglas, S.E. (2006). **Microarray Studies of Gene Expression in fish**. *Omics: a Journal of Integrative Biology* 10(4): 474-489. ISSN: 1536-2310.
NAL Call Number: QH447.G466
Descriptors: zebrafish, DNA microarrays, gene expression, models, *Danio rerio*, Salmonidae, salmonids.

Down, M., M. Power, S.I. Smith, K. Ralston, M. Spanevello, G.F. Burns, and A.W. Boyd (2005). **Cloning and expression of the large zebrafish protocadherin gene, Fat.** *Gene Expression Patterns* 5(4): 483-90. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: The cadherin superfamily members play an important role in mediating cell-cell contact and adhesion (Takeichi, M., 1991. Cadherin cell adhesion receptors as a morphogenetic regulator. Science 251, 1451-1455). A distinct subfamily, neither belonging to the classical or protocadherins includes Fat, the largest member of the cadherin super-family. Fat was originally identified in *Drosophila*. Subsequently, orthologues of Fat have been described in man (Dunne, J., Hanby, A. M., Poulsom, R., Jones, T. A., Sheer, D., Chin, W. G., Da, S. M., Zhao, Q., Beverley, P. C., Owen, M. J., 1995. Molecular cloning and tissue expression of FAT, the human homologue of the *Drosophila* fat gene that is located on chromosome 4q34-q35 and encodes a putative adhesion molecule. Genomics 30, 207-223), rat (Ponassi, M., Jacques, T. S., Ciani, L., ffrench, C. C., 1999. Expression of the rat homologue of the *Drosophila* fat tumour suppressor gene. Mech. Dev. 80, 207-212) and mouse (Cox, B., Hadjantonakis, A. K., Collins, J. E., Magee, A. I., 2000. Cloning and expression throughout mouse development of mfat1, a homologue of the *Drosophila* tumour suppressor gene fat [In Process Citation]. Dev. Dyn. 217, 233-240). In *Drosophila*, Fat has been shown to play an important role in both planar cell polarity and cell boundary formation during development. In this study we describe the characterization of zebrafish Fat, the first non-mammalian, vertebrate Fat homologue to be identified. The Fat protein has 64% amino acid identity and 80% similarity to human FAT and an identical domain structure to other vertebrate Fat proteins. During embryogenesis fat mRNA is expressed in the developing brain, specialised epithelial surfaces the notochord, ears, eyes and digestive tract, a pattern similar but distinct to that found in mammals.

Descriptors: zebrafish, cadherins genetics, amino acid and base sequence, brain embryology, brain physiology, molecular cloning , complementary DNA genetics, digestive system embryology, *Drosophila* gene library, Fat homologue, mammals, molecular sequence data, morphogenesis, phylogeny, sequence alignment, amino acid sequence homology, zebrafish classification.

Draper, B.W., C.M. McCallum, J.L. Stout, A.J. Slade, and C.B. Moens (2004). **A high-throughput method for identifying N-ethyl-N-nitrosourea (ENU)-induced point mutations in zebrafish.** *Methods in Cell Biology* 77: 91-112. ISSN: 0091-679X.

NAL Call Number: 442P92

Descriptors: zebrafish, DNA mutational analysis methods, endonucleases metabolism, ethylnitrosourea pharmacology, mutagenesis, point mutation genetics, base pair mismatch genetics, cryopreservation, DNA analysis, endonucleases chemistry, gene frequency and library, spermatozoa chemistry.

Du Pasquier, D. (2006). **Survivin increased vascular development during *Xenopus* ontogenesis**. *Differentiation* 74(5): 244-253. ISSN: 0301-4681.

NAL Call Number: QH607.D5

Descriptors: zebrafish, amphibiotic species, biological development, blood vessels, DNA, freshwater fish, juveniles, ontogeny, *Danio rerio*, *Xenopus*, clawed frogs, rainbow trout.

Du, S.J. (2004). **Molecular regulation of fish muscle development and growth**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd. Singapore,

Singapore , p. 339-391. ISBN: 9812388214.
NAL Call Number: QL638.C94 F57
Abstract: The formation of skeletal muscles in vertebrate embryos involves a series of events including induction, specification and differentiation. The multi-potential mesoderm cells in gastrula stage embryos are first induced to become paraxial mesoderm that subsequently form segmented somites. Somitic cells are specified into osteoblasts, myoblasts and dermal mesenchyme that ultimately differentiate into axial skeleton, skeletal muscles, and dermis of the skin, respectively. Somite formation and subsequent specification and differentiation of myoblasts are regulated by many extracellular signaling molecules and intracellular transcription factors. Signaling molecules of the Notch and FGF families are involved in somitogenesis, whereas Hedgehog (Hh), Wnt and TGF- beta families play critical roles in myoblast specification and differentiation. These signaling molecules bind to their receptors and activate or repress intracellular transcription factors, such as members of the MyoD family and the paired transcription factors Pax3 and Pax7 that directly regulate muscle-specific gene expression, and muscle cell differentiation. The challenging task at present is to understand how these signaling cascades coordinate with each other and how they control the myogenic transcription network to allow for precise changes in gene expression during myoblast specification and differentiation. Copyright © 2008 CABI
Descriptors: zebrafish, embryonic development, embryos, gene expression, genes, growth, heat shock proteins, muscle physiology, regulatory genes, skeletal muscle, transcription factors, *Danio rerio* , fishes.

Duffy, K.T., M.F. McAleer, W.R. Davidson, L. Kari, C. Kari, C.G. Liu, S.A. Farber, K.C. Cheng, J.R. Mest, E. Wickstrom, A.P. Dicker, and U. Rodeck (2005). **Coordinate control of cell cycle regulatory genes in zebrafish development tested by cyclin D1 knockdown with morpholino phosphorodiamidates and hydroxyprolyl-phosphono peptide nucleic acids.** *Nucleic Acids Research* 33(15): 4914-21. ISSN: 0305-1048.
NAL Call Number: QD341.A2N8
Abstract: During early zebrafish (*Danio rerio*) development zygotic transcription does not begin until the mid-blastula transition (MBT) 3 h after fertilization. MBT demarcates transition from synchronous short cell cycles of S and M phases exclusively to full cycles encompassing G1 and G2 phases. Transcriptional profiling and RT-PCR analyses during these phases enabled us to determine that this shift corresponds to decreased transcript levels of S/M phase cell cycle control genes (e.g. ccna2, ccnb1, ccnb2 and ccne) and increased transcript levels of ccnd1, encoding cyclin D1, and orthologs of p21 (p21-like) and retinoblastoma (Rb-like 1). To investigate the regulation of this process further, the translation of ccnd1 mRNA, a G1/S checkpoint control element, was impaired by microinjection of ccnd1-specific morpholino phosphorodiamidate (MO) 20mer or hydroxyprolyl-phosphono peptide nucleic acid (HypNA-pPNA) 16mer antisense oligonucleotides. The resulting downregulation of cyclin D1 protein resulted in microophthalmia and microcephaly, but not lethality. The phenotypes were not seen with 3-mismatch MO 20mers or 1-mismatch HypNA-pPNA 16mers, and were rescued by an exogenous ccnd1 mRNA construct with five mismatches. Collectively, these results indicate that transcription of key molecular determinants of asynchronous cell cycle control in zebrafish embryos commences at MBT and that the reduction of cyclin D1 expression compromises zebrafish eye and head development.
Descriptors: zebrafish, cyclin D1 genetics, CDC genes, oligodeoxyribonucleotides pharmacology, antisense oligonucleotides pharmacology, cyclin D1 antagonists and inhibitors, cyclin D1 physiology, down regulation, eye embryology , eye metabolism, developmental gene

expression regulation, oligodeoxyribonucleotides chemistry, antisense oligonucleotides chemistry, peptide nucleic acids chemistry, genetic transcription, metabolism.

Duffy, K.T. and E. Wickstrom (2007). **Zebrafish tp53 knockdown extends the survival of irradiated zebrafish embryos more effectively than the p53 inhibitor pifithrin-alpha.** *Cancer Biology and Therapy* 6(5): 675-8. ISSN: 1538 4047.

Abstract: Cancer treatment strategies using radiation therapy have significantly improved outcomes in patients. Although advances in physics and electronics have enabled physicians to plan and deliver radiotherapy regimens precisely and efficiently, studies of the underlying molecular mechanisms of the radiation response could enable more effective and less toxic treatment strategies, as well as protection against radiation damage following unplanned exposure. Using the developing zebrafish embryo as a model system, we hypothesized that knockdown of tp53 mRNA or small molecule inhibition of p53 could alter the efficacy of radiotherapy against developing zebrafish embryos as a model for tumors. We found that interfering with p53 expression and function using hydroxylprolyl-phosphono peptide nucleic acid oligomers or pifithrin-alpha protected developing zebrafish embryos against radiation damage, consistent with our hypothesis.

Descriptors: zebrafish embryos, cancer, treatment, radiation damage, tp53, survival.

Dufourcq, P., S. Rastegar, U. Strahle, and P. Blader (2004). **Parapineal specific expression of gfi1 in the zebrafish epithalamus.** *Gene Expression Patterns* 4(1): 53-7. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: We describe the isolation of zebrafish growth factor independent 1 (gfi1) and present an analysis of its pattern of expression during early development. As with its murine homologue, gfi1 expression is detected in the ganglion cells of the neural retina and in developing hair cells of the ear. In keeping with a role in the development of sensory hair cells, gfi1 is also expressed in neuromasts of the anterior and posterior lateral line system. Finally, gfi1 is expressed in the developing epithalamus in the dorsal diencephalon where its transcription is restricted to the parapineal.

Descriptors: zebrafish, DNA binding proteins genetics, epithalamus metabolism, developmental gene expression regulation, pineal gland metabolism, amino acid sequence, metabolism, embryonic development, epithalamus embryology, in situ hybridization, molecular sequence data, pineal gland embryology , messenger RNA genetics and metabolism, reverse transcriptase PCR, amino acid sequence homology.

Dufourcq, P. and S. Vriz (2006). **The chemokine SDF-1 regulates blastema formation during zebrafish fin regeneration.** *Development Genes and Evolution* 216(10): 635-9. ISSN: 0949 944X.

NAL Call Number: QL951.R68

Abstract: The work presented in this study focuses on blastema formation in epimorphic regeneration. We describe the expression pattern of Sdf1a and Sdf1b (the chemokines stromal-cell-derived factor-1a and 1b) and their two receptors Cxcr4a and Cxcr4b during zebrafish fin regeneration. We demonstrate that Sdf1a/Cxcr4a plays a critical role in fin regeneration and more precisely in epidermal cell proliferation, an important process for blastema formation. In mammals, a single cxcr4 gene is involved both in chemotaxis and cell proliferation and survival; we discuss in this study a possible functional division of the two cxcr4 zebrafish genes.

Descriptors: zebrafish, Cxc chemokines physiology, regeneration, in situ hybridization.

Dunham, R.A. and Z.J. Liu (2006). **Transgenic fish - where we are and where do we go?** *Israeli Journal of Aquaculture Bamidgeh* 58(4): 297-319. ISSN: 0792 156X.
NAL Call Number: SH117.I75B36
Abstract: Transgenic fish have been developed that have improved growth, color, disease resistance, survival in cold, and body composition, and produce pharmaceutical proteins. Transgenes elicit pleiotropic effects, some positive and some negative. In general, transgenic fish appear to have lower fitness than controls and pose little environmental risk, but this research is not fully conclusive. Transgenic zebrafish with altered coloration have been commercialized and growth hormone transgenic salmon, carp, and tilapia are near commercialization. To enhance commercialization and minimize environmental risk, additional technologies such as transgenic sterilization need to be developed. Genomic research has produced an abundance of molecular genetic information including many genes for consideration for gene transfer, highly regulated gene promoters, and knowledge about their expression and function. Functional genomics analysis should be applied in the future to enhance the capacity and versatility of transgenic technology. Copyright © 2008 CABI
Descriptors: transgenic zebrafish, animal breeding, body composition, breeding methods, cold tolerance, color, disease resistance, genetic engineering, genetics, growth, growth rate, performance traits, pleiotropy, reporter genes, reviews, sterilization, environmental release risk.

Dunzinger, U., T. Haaf, and U. Zechner (2007). **Conserved synteny of mammalian imprinted genes in chicken, frog, and fish genomes**. *Cytogenetic and Genome Research* 117(1/4): 78-85. ISSN: 1424 8581.
NAL Call Number: QH431.C95
Abstract: Conservation of synteny of mammalian imprinted genes between chicken and human suggested that highly conserved gene clusters were selected long before these genes were recruited for genomic imprinting in mammals. Here we have applied in silico mapping of orthologous genes in pipid frog, zebrafish, spotted green and Japanese pufferfish to show considerable conservation of synteny in lower vertebrates. More than 400 million years ago in a common ancestor of teleost fish and tetrapods, 'preimprinted' chromosome regions homologous to human 6q25, 7q21, 7q32, 11p15, and 15q11->q12 already contained most present-day mammalian imprinted genes. Interestingly, some imprinted gene orthologues which are isolated from imprinted clusters in mouse and human could be linked to preimprinted regions in lower vertebrates, indicating that separation occurred during mammalian evolution. On the contrary, newly arisen genes by segmental duplication in the mammalian lineage, i.e. SNRPN and FRAT3, were transposed or translocated to imprinted clusters and recruited for parent-specific activity. By analysis of currently available sequences of non-mammalian vertebrates, the imprinted gene clusters homologous to human chromosomes 14q32 and 19q12 are only poorly conserved in chicken, frog, and fish and, therefore, may not have evolved from ancestral preimprinted gene arrays. Evidently, evolution of imprinted gene clusters is an ongoing and dynamic process in mammals. In general, imprinted gene orthologues do not show a higher degree of synteny conservation in vertebrates than non-imprinted genes interspersed with or adjacent to an imprinted cluster. Copyright © 2008 CABI
Descriptors: zebrafish, evolution, gene mapping, genes, synteny conservation, genomes, genomic imprinting, poultry, fishes, fowls, frogs.

Durr, K., J. Holzschuh, A. Filippi, A.K. Ettl, S. Ryu, I.T. Shepherd, and W. Driever (2006). **Differential roles of transcriptional mediator complex subunits Crsp34/Med27, Crsp150/Med14 and Trap100/Med24 during zebrafish retinal development.** *Genetics* 174(2): 693-

705. ISSN: 0016-6731.
NAL Call Number: 442.8G28
Abstract: The transcriptional mediator complex has emerged as an important component of transcriptional regulation, yet it is largely unknown whether its subunits have differential functions in development. We demonstrate that the zebrafish mutation m885 disrupts a subunit of the mediator complex, Crsp34/Med27. To explore the role of the mediator in the control of retinal differentiation, we employed two additional mutations disrupting the mediator subunits Trap100/Med24 and Crsp150/Med14. Our analysis shows that loss of Crsp34/Med27 decreases amacrine cell number, but increases the number of rod photoreceptor cells. In contrast, loss of Trap100/Med24 decreases rod photoreceptor cells. Loss of Crsp150/Med14, on the other hand, only slightly reduces dopaminergic amacrine cells, which are absent from both crsp34(m885) and trap100(lessen) mutant embryos. Our data provide evidence for differential requirements for Crsp34/Med27 in developmental processes. In addition, our data point to divergent functions of the mediator subunits Crsp34/Med27, Trap100/Med24, and Crsp150/Med14 and, thus, suggest that subunit composition of the mediator contributes to the control of differentiation in the vertebrate CNS.
Descriptors: zebrafish embryos, protein subunits physiology, retina embryology, trans activators physiology, alleles, amacrine cells cytology, cell differentiation genetics, developmental gene expression regulation, phenotype, protein subunits biosynthesis and genetics, retina metabolism, trans activators biosynthesis, trans activators genetics, zebrafish, proteins biosynthesis.

Eames, B.F. and C.B. Kimmel (2007). **Molecular genetics of zebrafish endochondral ossification**. *Developmental Biology* 306(1): 296. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Descriptors: zebrafish, freshwater fish, phylogenetics, *Danio rerio*, genetics and evolution.

Eaton, J.L. and E. Glasgow (2007). **Zebrafish orthopedia (otp) is required for isotocin cell development.** *Development Genes and Evolution* 217(2): 149-58. ISSN: 0949 944X.
NAL Call Number: QL951.R68
Abstract: Several behavioral and physiological processes such as social, sexual, and maternal behaviors, learning and memory, and parturition are influenced by the neurohypophysial peptide oxytocin. Studies in knockout mice have identified four transcriptional regulatory genes that are required for oxytocin neuronal development in the hypothalamus. These are the basic helix-loop-helix PAS genes Single-minded 1 (Sim1) and Arylhydrocarbon receptor nuclear translocator 2 (Arnt2), the POU homeobox gene Pou3f2, and the paired homeobox gene Orthopedia (Otp). Overall, however, the molecular control of oxytocin cell development is poorly understood. Studies in zebrafish provide a complementary view to mouse knockout experiments and facilitate understanding of neuroendocrine cell development. Isotocin, which is orthologous to oxytocin, is expressed early in the developing zebrafish brain. In this paper we show that zebrafish otp mRNA expression in the embryonic forebrain is dynamic and complex, and that it overlaps with isotocin expression in the dorsal preoptic area. Additionally, these studies demonstrate that otp is required for isotocin cell development. Evidence is also provided that otp and sim1 function in parallel to direct the differentiation of isotocin cells, and that otp is unlikely to affect brain patterning. Overall, these studies support the hypothesis that the role of otp in zebrafish neuroendocrine cell development is evolutionarily conserved with that of mammals.
Descriptors: zebrafish, oxytocin analogs and derivatives, transcription factors physiology, base sequence, body patterning genetics and physiology, developmental gene expression

regulation, homeobox genes, homeodomain proteins genetics and physiology, in situ hybridization, mice, nerve tissue proteins deficiency, nerve tissue proteins genetics and physiology, neurosecretory systems cytology, embryology and physiology, oligodeoxyribonucleotides, antisense genetics, oxytocin physiology, preoptic area embryology and metabolism, prosencephalon embryology and metabolism, messenger RNA genetics and metabolism, transcription factors antagonists and inhibitors and genetics, proteins antagonists, inhibitors and genetics.

Eberhart, J.K., X. He, M.E. Swartz, Y.-L. Yan, H.a.o. Song, T.C. Boling, A.K. Kunerth, M.B. Walker, C.B. Kimmel, and J.H. Postlethwait (2008). **MicroRNA Mirn140 modulates Pdgf signaling during palatogenesis**. *Nature Genetics* 40(3): 290-298. ISSN: 1061-4036.
NAL Call Number: QH426.N37
Descriptors: zebrafish embryos, cartilage, cleft lip, palate, ectoderm, gene expression, Hedgehog protein, mouth, neural crest, platelet-derived growth factor, signal transduction, skull, miRNA, optic stalk, *Danio rerio*, development and cell cycle.

Ebert, A.M., C.A. McAnelly, A. Srinivasan, J.L. Linker, W.A. Horne, and D.M. Garrity (2008). **Ca super(2+) channel-independent requirement for MAGUK family CACNB4 genes in initiation of zebrafish epiboly**. *Proceedings of the National Academy of Sciences, USA* 105(1): 198-203. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, blastoderm, calcium, calcium channels (voltage-gated), channel gating, cytoskeleton, dispersal, embryos, guanylate kinase, microtubules, Nocodazole, nuclei, yolk, *Danio rerio* , Teleostei, development and cell cycle.

Eckfeldt, C.E., E.M. Mendenhall, C.M. Flynn, T.F. Wang, M.A. Pickart, S.M. Grindle, S.C. Ekker, and C.M. Verfaillie (2005). **Functional analysis of human hematopoietic stem cell gene expression using zebrafish.** *Public Library of Science Biology* 3(8): E254. ISSN: 1544-9173.
NAL Call Number: QH301.P56
Abstract: Although several reports have characterized the hematopoietic stem cell (HSC) transcriptome, the roles of HSC-specific genes in hematopoiesis remain elusive. To identify candidate regulators of HSC fate decisions, we compared the transcriptome of human umbilical cord blood and bone marrow (CD34+)(CD33-)(CD38-)Rho(lo)(c-kit+) cells, enriched for hematopoietic stem/progenitor cells with (CD34+)(CD33-)(CD38-)Rho(hi) cells, enriched in committed progenitors. We identified 277 differentially expressed transcripts conserved in these ontogenically distinct cell sources. We next performed a morpholino antisense oligonucleotide (MO)-based functional screen in zebrafish to determine the hematopoietic function of 61 genes that had no previously known function in HSC biology and for which a likely zebrafish ortholog could be identified. MO knock down of 14/61 (23%) of the differentially expressed transcripts resulted in hematopoietic defects in developing zebrafish embryos, as demonstrated by altered levels of circulating blood cells at 30 and 48 h postfertilization and subsequently confirmed by quantitative RT-PCR for erythroid-specific hbae1 and myeloid-specific lcp1 transcripts. Recapitulating the knockdown phenotype using a second MO of independent sequence, absence of the phenotype using a mismatched MO sequence, and rescue of the phenotype by cDNA-based overexpression of the targeted transcript for zebrafish spry4 confirmed the specificity of MO targeting in this system. Further characterization of the spry4-deficient zebrafish embryos demonstrated that hematopoietic defects were not due to more widespread defects in the mesodermal development, and therefore represented primary defects in HSC specification, proliferation, and/or

differentiation. Overall, this high-throughput screen for the functional validation of differentially expressed genes using a zebrafish model of hematopoiesis represents a major step toward obtaining meaningful information from global gene profiling of HSCs.
Descriptors: zebrafish, gene expression profiling methods, genomics methods, hematopoietic stem cells metabolism, CD antigens analysis, CD34 antigens analysis, CD38 antigens analysis, antigens, differentiation, myelomonocytic analysis, cell differentiation, cell lineage, cell proliferation, fetal blood cytology, flow cytometry, hematopoietic stem cells cytology, membrane proteins genetics and physiology, multigene family physiology, nerve tissue nerve tissue, oligonucleotide array sequence analysis, phosphophospho, messenger RNA metabolism, rhodamine 123 analysis, proteins genetics.

Eisinger, A.L., L.D. Nadauld, D.N. Shelton, P.W. Peterson, R.A. Phelps, S. Chidester, D.M. Staffo-rini, S.M. Prescott, and D.A. Jones (2006). **The adenomatous polyposis coli tumor suppressor gene regulates expression of cyclooxygenase-2 by a mechanism that involves retinoic acid**. *Journal of Biological Chemistry* 281(29): 20474-20482. ISSN: 0021-9258.
NAL Call Number: 381J824
Descriptors: zebrafish, adenomatous polyposis coli protein, biosynthesis, carcinogenesis, carcinoma, cell proliferation, colon cancer, colorectal cancer, cyclooxygenase-2, epithelial cells , freshwater fish, gene expression, intestine, intestines, mutation, mutations, population genetics, prostaglandin E2, retinoic acid, supplementation, suppressors, transcription factors, tumor cell lines, tumor suppressor genes, tumorigenesis, tumors, Wnt protein, adenomatous polyposis coli, siRNA, *Danio rerio*, tumor suppressors, genetics and evolution.

Ekker, S.C. (2004). **Nonconventional antisense in zebrafish for functional genomics applications.** *Methods in Cell Biology* 77: 121-36. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, antisense elements genetics pharmacology, genomics methods, antisense elements genetics chemistry, mutagenesis, protein biosynthesis drug effects, protein biosynthesis genetics.

Ekker, S.C., A. Nasevicius, H. Kim, and S. Sumanas (Inventors) **Inhibition of gene expression using polynucleotide analogues**. 2005
Descriptors: zebrafish, gene expression, nucleotide sequence, patents, nucleic acids, polynucleotides, *Danio rerio* .

Elicker, K.S. and L.D. Hutson (2007). **Genome-wide analysis and expression profiling of the small heat shock proteins in zebrafish.** *Gene* 403(1-2): 60-9. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: Small Heat Shock Proteins (sHSPs) have important roles in preventing disease and promoting resistance to environmental stressors. Mutations in any one of a number of sHSPs, including HSP27 (HSPB1), HSP22 (HSPB8), alphaA-crystallin (HSPB4), or alphaB-crystallin (HSPB5) can result in neuronal degeneration, myopathy, and/or cataract in humans. Ten sHSPs are known in humans, and thirteen have been identified in teleost fish. Here we report the identification of thirteen zebrafish sHSPs. Using a combination of phylogenetic analysis and analysis of synteny, we have determined that ten are likely orthologs of human sHSPs. We have used quantitative RT-PCR to determine the relative expression levels of all thirteen sHSPs during development and in response to heat shock. Our findings indicate that most of the zebrafish sHSPs are expressed during development, and five of these genes are transcriptionally upregulated by heat shock at one or more stages of development.

Descriptors: zebrafish, gene expression profiling methods, developmental gene expression regulation, genome, heat shock proteins genetics, amino acid sequence, conserved sequence, genetic databases molecular evolution, expressed sequence tags, molecular sequence data, phylogeny, reverse transcriptase PCR, protein sequence analysis, amino acid sequence homology, synteny, alpha crystallins genetics.

Elizondo, M.R., B.L. Arduini, J. Paulsen, E.L. MacDonald, J.L. Sabel, P.D. Henion, R.A. Cornell, and D.M. Parichy (2005). **Defective skeletogenesis with kidney stone formation in dwarf zebrafish mutant for trpm7.** *Current Biology* 15(7): 667-71. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Abstract: Development of the adult form requires coordinated growth and patterning of multiple traits in response to local gene activity as well as to global endocrine and physiological effectors. An excellent example of such coordination is the skeleton. Skeletal development depends on the differentiation and morphogenesis of multiple cell types to generate elements with distinct forms and functions throughout the body. We show that zebrafish touchtone/nutria mutants exhibit severe growth retardation and gross alterations in skeletal development in addition to embryonic melanophore and touch-response defects. These alterations include accelerated endochondral ossification but delayed intramembranous ossification, as well as skeletal deformities. We show that the touchtone/nutria phenotype results from mutations in trpm7, which encodes a transient receptor potential (TRP) family member that functions as both a cation channel and kinase. We find trpm7 expression in the mesonephric kidney and show that mutants develop kidney stones, indicating renal dysfunction. These results identify a requirement for trpm7 in growth and skeletogenesis and highlight the potential of forward genetic approaches to uncover physiological mechanisms contributing to the development of adult form.
Descriptors: zebrafish drawf mutant, ion channels genetics, kidney calculi veterinary, osteogenesis genetics, protein kinases genetics, multiple abnormalities genetics, multiple abnormalities metabolism, base sequence, bone and bones anatomy and histology, chromosome mapping, complementary DNA genetics, histological techniques, in situ hybridization, ion channels metabolism, kidney metabolism, kidney calculi genetics, larva metabolism, molecular sequence data, protein kinases metabolism, DNA sequence analysis, TRPMcation channels.

Ellestad, K.K. and B.G. Magor (2005). **Evolution of transcriptional enhancers in the immunoglobulin heavy-chain gene: functional characteristics of the zebrafish Emu3' enhancer.** *ImmunoGenetics* 57(1-2): 129-39. ISSN: 0093-7711.
NAL Call Number: QR184.I4
Abstract: Past studies of the channel catfish immunoglobulin heavy-chain (IgH) locus indicates that it lacks an Emu enhancer in the J(H)-Cmu1 intron but does have an enhancer, termed Emu3', in the mu-delta intergenic region. The positioning of the catfish enhancer downstream of the mu-chain exons is predicted to be unfavorable for antibody-affinity maturation in catfish, and would also have been an impediment to the evolution of class switch recombination, had it existed in early tetrapods. To determine if this downstream enhancer is a general feature of teleost fish, we have identified the location of the transcriptional enhancer in the zebrafish IgH locus. We find that zebrafish, like catfish, only have an Emu3'-like enhancer that has cross-species activity, but which is B-cell-specific in its activity. A 300-bp region of the zebrafish enhancer shares sequence homology with the core of the catfish Emu3', although there has been loss and gain of specific octamer enhancer motifs. Mutagenesis studies demonstrate that the zebrafish IgH enhancer depends on a pair of E-box motifs

that are found in the enhancer core. Similarly spaced E-box motifs appear to exist in the Emu3' enhancer regions of other teleost fish, suggesting this is a common feature among fish IgH enhancers. We discuss how this distal positioning of the enhancer may influence affinity maturation in extant teleosts as well as the evolution of this process in the early tetrapods.
Descriptors: zebrafish, enhancer elements genetics, molecular evolution, genes, immunoglobulin, immunoglobulin heavy chains genetics, amino acid motifs, base sequence, catfishes, molecular sequence data, trans activation genetics, zebrafish immunology.

Elsalini, O.A. and K.B. Rohr (2003). **Phenylthiourea disrupts thyroid function in developing zebrafish.** *Development Genes and Evolution* 212(12): 593-8. ISSN: 0949 944X.
NAL Call Number: QL951.R68
Abstract: Thyroid hormone (T4) can be detected in thyroid follicles in wild-type zebrafish larvae from 3 days of development, when the thyroid has differentiated. In contrast, embryos or larvae treated with goitrogens (substances such as methimazole, potassium percholorate, and 6-n-propyl-2-thiouracil) are devoid of thyroid hormone immunoreactivity.Phenythiourea (PTurea; also commonly known as PTU) is widely used in zebrafish research to suppress pigmentation in developing embryos/fry. PTurea contains a thiocarbamide group that is responsible for goitrogenic activity in methimazole and 6-n-propyl-2-thiouracil. In the present study, we show that commonly used doses of 0.003% PTurea abolish T4 immunoreactivity of the thyroid follicles of zebrafish larvae. As development of the thyroid gland is not affected, these data suggest that PTurea blocks thyroid hormone production. Like other goitrogens, PTurea causes delayed hatching, retardation and malformation of embryos or larvae with increasing doses. At doses of 0.003% PTurea, however, toxic side effects seem to be at a minimum, and the maternal contribution of the hormone might compensate for compromised thyroid function during the first days of development.
Descriptors: zebrafish embryos and larvae, antithyroid agents pharmacology, phenylthiourea pharmacology, thyroid gland drug effects and metabolism, biological metamorphosis, methimazole pharmacology, mutation, perchloric acid pharmacology, potassium compounds pharmacology, propylthiouracil pharmacology, thyroid gland embryology, thyroid hormones pharmacology, zebrafish growth and development.

Emery, G. and J. Knoblich (2006). **Endosome dynamics during development**. *Current Opinion in Cell Biology* 18(4): 407-415. ISSN: 0955 0674.
NAL Call Number: QH573.C87
Abstract: Endocytosis has traditionally been studied in isolated cells. More recently, however, the analysis of protein trafficking in whole organisms has revealed that it plays exciting roles during development. Endocytic trafficking of cell adhesion molecules regulates epithelial polarity and cell migration. Developmental signaling pathways are regulated by the trafficking of receptors and their ligands through the endocytic pathway. Finally, impairment of the endocytic machinery can affect proliferation control and contribute to tumor development. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics, tumor biology, enzymology, cell biology, development, neoplastic disease, etiology, cell migration, developmental signaling pathway, endosome dynamics.

Emran, F., J. Rihel, A.R. Adolph, K.Y. Wong, S. Kraves, and J.E. Dowling (2007). **OFF ganglion cells cannot drive the optokinetic reflex in zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 104(48): 19126-31. ISSN: 0027-8424.
NAL Call Number: 500N21P

Abstract: Whereas the zebrafish retina has long been an important model system for developmental and genetic studies, little is known about the responses of the inner retinal neurons. Here we report single-unit ganglion cell recordings from 5- to 6-day-old zebrafish larvae. In wild-type larvae we identify at least five subtypes of ganglion cell responses to full-field illumination, with ON-OFF and ON-type cells predominating. In the nrc mutant retina, in which the photoreceptor terminals develop abnormally, we observe normal OFF responses but abnormal ON-OFF responses and no ON responses. Previously characterized as blind, these mutants lack an optokinetic reflex (OKR), but in another behavioral assay nrc mutant fish have near-normal responses to the offset of light and slow and sluggish responses to the onset of light. Pharmacological block of the ON pathway mimics most of the nrc visual defects. We conclude that the abnormal photoreceptor terminals in nrc mutants predominantly perturb the ON pathway and that the ON pathway is necessary to drive the OKR in larval zebrafish.
Descriptors: zebrafish larvae, retina, genetic studies, optokinetic reflex, ganglion cells.

Engels, B., S. van 't Padje, L. Blonden, L.A. Severijnen, B.A. Oostra, and R. Willemsen (2004). **Characterization of Fxr1 in *Danio rerio*; a simple vertebrate model to study costamere development.** *Journal of Experimental Biology* 207(Pt 19): 3329-38. ISSN: 0022-0949.
NAL Call Number: 442.8B77
Abstract: The X-linked FMR1 gene, which is involved in the fragile X syndrome, forms a small gene family with its two autosomal homologs, FXR1 and FXR2. Mouse models for the FXR genes have been generated and proved to be valuable in elucidating the function of these genes, particularly in adult mice. Unfortunately, Fxr1 knockout mice die shortly after birth, necessitating an animal model that allows the study of the role of Fxr1p, the gene product of Fxr1, in early embryonic development. For gene function studies during early embryonic development the use of zebrafish as a model organism is highly advantageous. In this paper the suitability of the zebrafish as a model organism to study Fxr1p function during early development is explored. As a first step, we present here the initial characterization of Fxr1p in zebrafish. Fxr1p is present in all the cells from zebrafish embryos from the 2/4-cell stage onward; however, during late development a more tissue-specific distribution is found, with the highest expression in developing muscle. In adult zebrafish, Fxr1p is localized at the myoseptum and in costamere-like granules in skeletal muscle. In the testis, Fxr1p is localized in immature spermatogenic cells and in brain tissue Fxr1p displays a predominantly nuclear staining in neurons throughout the brain. Finally, the different tissue-specific isoforms of Fxr1p are characterized. Since the functional domains and the expression pattern of Fxr1p in zebrafish are comparable to those in higher vertebrates such as mouse and human, we conclude that the zebrafish is a highly suitable model for functional studies of Fxr1p.
Descriptors: zebrafish, gene expression profiling, Fxr1p models, amino acid and base sequence, Western blotting, cryoultramicrotomy, DNA primers, immunohistochemistry, molecular sequence data, RNA binding proteins metabolism, sequence alignment, DNA sequence analysis.

Epting, D., S. Vorwerk, A. Hageman, and D. Meyer (2007). **Expression of rasgef1b in zebrafish.** *Gene Expression Patterns* 7(4): 389-95. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: The rasgef genes encode a subgroup of highly conserved Ras guanine nucleotide exchange factors. While EST projects revealed the presence of rasgef genes in organisms that range from nematodes to humans, their functions remain to be elucidated. In zebrafish two rasgef genes, rasgef and rasgef1b, have been identified and high throughput analysis revealed

tissue specific embryonic expression for rasgef1b. Here, we show that three rasgef1b-transcripts are generated from two transcriptional start sites and by alternative splicing. Detailed expression analyses show that rasgef1b is expressed in a subset of adaxial cells, in the anterior part of somites, in the rostral part of the mid-hindbrain boundary and in the rhombomere boundaries. In the larva, rasgef1b is further expressed in the pallium and the inner nuclear layer of the retina. We also find that rasgef1b is expressed maternally and that the ubiquitous distribution of maternal transcripts disappears shortly after mid-blastula transition. At early epiboly stages, rasgef1b expression is restricted to the margin with low levels of expression on the ventral and high levels of expression on the dorsal side. Finally, we show that early zygotic expression is regulated by Nodal and FGF signals and that these signals have different activities in regulating the level and distribution of early zygotic rasgef1b mRNA expression.
Descriptors: zebrafish, developmental gene expression regulation, ras guanine nucleotide exchange factors genetics, alternative splicing, amino acid sequence, brain embryology and metabolism, Fgf metabolism, molecular sequence data, organ specificity, phylogeny, protein isoforms, signal transduction, transcription initiation site, transforming growth factor beta metabolism.

Ernest, S., S. Guadagnini, M.C. Prevost, and N. Soussi Yanicostas (2007). **Localization of anosmin-1a and anosmin-1b in the inner ear and neuromasts of zebrafish.** *Gene Expression Patterns* 7(3): 274-81. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Anosmin-1, encoded by the KAL-1 gene, is the protein defective in the X-linked form of Kallmann syndrome. This human developmental disorder is characterized by defects in cell migration and axon target selection. Anosmin-1 is an extracellular matrix protein that plays a role, in vitro, in processes such as cell adhesion, neurite outgrowth, axon guidance, and axon branching. The zebrafish possesses two orthologues of the KAL-1 gene: kal1a and kal1b, which encode anosmin-1a and anosmin-1b, respectively. Previous in situ hybridization studies have shown that kal1a and kal1b mRNAs are expressed in undetermined cells of the inner ear but not in neuromast cells. Using specific antibodies against anosmin-1a and anosmin-1b, we report here that both proteins are expressed in sensory hair cells of the inner ear cristae ampullaris and the lateral line neuromasts. Accumulation of these proteins was observed mainly at the level of the hair bundle and also at the cell membrane. In neuromast hair cells, immunogold scanning electronmicroscopy demonstrated that anosmin-1a and anosmin-1b were present at the surface of the stereociliary bundle. In addition, anosmin-1a, but not anosmin-1b, was detected on the track of the ampullary nerve. This is the first report of anosmin-1 expression in sensory hair cells of the inner ear and lateral line, and along the ampullary nerve track.
Descriptors: zebrafish, inner ear chemistry, extracellular matrix proteins analysis, hair cells chemistry, lateral line system chemistry, nerve tissue proteins analysis, sensory receptors chemistry, metabolism, inner ear anatomy and histology and metabolism, extracellular matrix fluorescent antibody technique, hair cells metabolism and ultrastructure, immunohistochemistry, lateral line system cytology and metabolism, confocal microscopy, scanning electron microscopy, immunoelectron microscopy, nerve tissue sensory receptors metabolism.

Eroshkin, F.M., G.V. Ermakova, A.V. Bayramov, and A.G. Zaraisky (2006). **Multiple noggins in vertebrate genome: cloning and expression of noggin2 and noggin4 in *Xenopus laevis*.** *Gene Expression Patterns* 6(2): 180-186. ISSN: 1567 133X.
NAL Call Number: QH450.B73
Abstract: Noggin is a neural inducer secreted by cells of the Spemann organizer. A single

noggin gene was identified until very recently in all tested vertebrates. The only exception was zebrafish, in which two close homologs of noggin, named noggin1 and noggin3, and one gene more diverged from them, noggin2, were cloned. Nevertheless, finding of three zebrafish noggins was attributed exclusively to specific genomic duplications in the fish evolutionary branch. However, very recently it was shown that *Xenopus tropicalis* have additional noggin homolog, called noggin2 [Fletcher, R.B., Watson, A.L., Harland, R.M. (2004). Expression of *Xenopus tropicalis* noggin1 and noggin2 in early development: two noggin genes in a tetrapod. Gene Expr. Patterns 5, 225-230], which indicates at least two independent noggin genes in vertebrate phylum. Now we report identification of two novel noggin homologs in each of so evolutionary distant species as *Xenopus laevis*, chicken and fugu. One of these noggins is ortholog of the *X tropicalis* and zebrafish noggin2, whereas another, named noggin4, was not known previously. In the *X. laevis* embryos, the expression of noggin2 very resembles that of its counterpart in *X. tropicalis*: it begins with neurulation at the anterior margin of the neural plate and, afterward, continues mainly in the forebrain and dorsal hindbrain. At the same time, noggin4 is expressed starting from the beginning of gastrulation, throughout the ectoderm, with a local expression maximum in the prospective anterior neurectoderm. Later, it is widely expressed on the dorsal side of embryo, including neural tube, eyes, otic vesicles, cranial placodes, branchial arches, and somites. The data presented here demonstrate that the vertebrate phylum contains at least three distinct noggin genes. (C) 2005 Elsevier B.V. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, nervous system, molecular genetics, biochemistry, noggin2, noggin4, sense organs, Spemann organizer, sensory reception, respiratory system, cloning, genetic techniques, *Xenpus laevis.*

Esengil, H., V. Chang, J.K. Mich, and J.K. Chen (2007). **Small-molecule regulation of zebrafish gene expression.** *Nature Chemical Biology* 3(3): 154-5. ISSN: 1552-4450.
Abstract: The zebrafish has emerged as a versatile model organism for biomedical research, yet its potential has been limited by a lack of conditional reverse-genetic tools. Here we report a chemically inducible gene expression technology that has orthogonality to vertebrate signaling processes, high induction levels, and rapid kinetics. Coupled with tissue-specific promoters, this system provides multidimensional control of gene expression and will enable new models of human disorders and diseases.
Descriptors: zebrafish, gene expression regulation physiology, genomics methods, cell line herpes simplex virus protein vmw65 biosynthesis, herpes simplex virus protein vmw65 genetics, insects, steroid receptors genetics, steroid receptors physiology, trans activators genetics.

Evans, B.R., S.I. Karchner, D.G. Franks, and M.E. Hahn (2005). **Duplicate aryl hydrocarbon receptor repressor genes (ahrr1 and ahrr2) in the zebrafish *Danio rerio*: structure, function, evolution, and AHR-dependent regulation in vivo.** *Archives of Biochemistry and Biophysics* 441(2): 151-67. ISSN: 0003 9861.
NAL Call Number: 381 Ar2
Abstract: The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The recently identified AHR repressor (AHRR) forms a negative feedback loop with the AHR. We investigated AHRR structure, function, evolution, and regulation in zebrafish, a powerful model in developmental biology and toxicology. We identified and cloned two distinct AHRR cDNAs that encode predicted proteins of 550 (AHRR1) and 573 (AHRR2) amino acids. The ahrr1 and ahrr2 genes map to zebrafish chromosomes 24 and 2, respectively, both of which share conserved synteny with human chromosome 5, the location of human AHRR. Mapping

and phylogenetic analysis show that AHRR1 and AHRR2 are co-orthologs of the mammalian AHRR. In transient transfection assays, AHRR1 and AHRR2 repressed constitutive and TCDD-inducible transactivation by AHR2. Expression of both AHRR mRNAs was induced in ZF-L cells by AHR agonists but not by non-agonists. TCDD induced AHRR1 and AHRR2 expression in a dose-dependent manner in ZF-L cells, with EC50 values similar to those for induction of CYP1A. Both AHRRs were expressed and induced by TCDD in zebrafish embryos. Thus, zebrafish possess duplicate AHR-regulated AHRR paralogs that act in a negative feedback loop to repress the AHR signaling pathway.
Descriptors: zebrafish, molecular evolution, duplicate aryl hydrocarbon receptors metabolism, repressor proteins chemistry and metabolism, proteins chemistry and metabolism, amino acid and base sequence, molecular sequence data, aryl hydrocarbon receptors genetics, repressor proteins analysis and genetics, amino acid sequence homology, structure activity relationship, proteins analysis.

Evans, T.G., Y. Yamamoto, W.R. Jeffery, and P.H. Krone (2005). **Zebrafish Hsp70 is required for embryonic lens formation.** *Cell Stress and Chaperones* 10(1): 66-78. ISSN: 1355 8145.
NAL Call Number: QH573
Abstract: Heat shock proteins (Hsps) were originally identified as proteins expressed after exposure of cells to environmental stress. Several Hsps were subsequently shown to play roles as molecular chaperones in normal intracellular protein folding and targeting events and to be expressed during discrete periods in the development of several embryonic tissues. However, only recently have studies begun to address the specific developmental consequences of inhibiting Hsp expression to determine whether these molecular chaperones are required for specific developmental events. We have previously shown that the heat-inducible zebrafish hsp70 gene is expressed during a distinct temporal window of embryonic lens formation at normal growth temperatures. In addition, a 1.5-kb fragment of the zebrafish hsp70 gene promoter is sufficient to direct expression of a gfp reporter gene to the lens, suggesting that the hsp70 gene is expressed as part of the normal lens development program. Here, we used microinjection of morpholino-modified antisense oligonucleotides (MOs) to reduce Hsp70 levels during zebrafish development and to show that Hsp70 is required for normal lens formation. Hsp70-MO-injected embryos exhibited a small-eye phenotype relative to wild-type and control-injected animals, with the phenotype discernable during the second day of development. Histological and immunological analysis revealed a small, underdeveloped lens. Numerous terminal deoxynucleotidyl transferase-mediated dUTP-fluoroscein nick-end labeling (TUNEL)-positive nuclei appeared in the lens of small-eye embryos after 48 hours postfertilization (hpf), whereas they were no longer apparent in untreated embryos by this age. Lenses transplanted from hsp70-MO-injected embryos into wild-type hosts failed to recover and retained the immature morphology characteristic of the small-eye phenotype, indicating that the lens phenotype is lens autonomous. Our data suggest that the lens defect in hsp70-MO-injected embryos is predominantly at the level of postmitotic lens fiber differentiation, a result supported by the appearance of mature lens organization in these embryos by 5 days postfertilization, once morpholino degradation or dilution has occurred.
Descriptors: zebrafish, embryonic development, HSP70 heat shock proteins genetics and metabolism, crystalline lens embryology, apoptosis , Western blotting, cell nucleus metabolism and pathology, immunohistochemistry, crystalline lens transplantation, microinjections , antisense oligonucleotides pharmacology, temperature, time factors, homologous transplantation.

Evans, T.R. and A. Heicklen-Klein (2004). **Tbx-20 regulates gata-4 and the integrity of the precardiac epithelium**. *Circulation* 110(17, Suppl. S): 60. ISSN: 0009 7322.
NAL Call Number: RC681.A1C8
Abstract: GATA and T-box binding transcription factors regulate multiple stages of heart development, and mutations in their genes are associated with congenital cardiomyopathies. To investigate regulatory mechanisms for the GATA-4 gene, we generated transgenic zebrafish that express GFP throughout the heart and valves under the control of GATA-4 genomic sequences. These fish allow us to visualize cardiac morphogenesis in living animals. Using a reverse-genetics approach, we defined a conserved GATA-4 enhancer region with a cluster of consensus binding sites for T-box transcription factors. Mutation of these sequences significantly reduces reporter gene expression in the heart. Thus, T-box transcription factors directly regulate the GATA 4 gene. We next investigated the specificity among various T-box factors expressed in the developing heart. Embryos injected with a morpholino that targets the knockdown of Tbx-20 express GATA-4 and MLC2 in the fused heart tube at 19 hpf. However, GATA-4 positive cells are surprisingly found adjacent to but no longer associated with the myocardial epithelium. By 24 hpf, the linear heart tube has formed but jogs right in approximately 20 percent of the knockdown embryos, Furthermore, additional cells that are GATA-4 positive lie on the caudal portion of the yolk, along with a few scarce MLC2 positive cells. By 48 hpf numerous GATA-4 positive cells are scattered along the ventral aspect of the yolk sac. In these embryos, the thin heart continues to express MLC2 but has little or no GATA-4 expression. In Tbx-5 mutant embryos, there are no obvious differences in GATA-4 or MLC2 expression levels, despite the similarity in the resulting cardiac phenotype. While both Tbx-20 and Tbx-5 are required for regulating normal myocardial thickening and contractility, Tbx-20 but not Tbx-5 regulates GATA-4 expression. The Tbx-20 gene appears to be required for retaining GATA-4 positive cells in association with the precardiac epithelium. GATA-4 and/or Tbx-20 may regulate genes that maintain normal epithelial sheet structure during heart tube morphogenesis. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, molecular genetics, cardiovascular system, transport and circulation, reverse genetics, genetic techniques.

Fadool, J.M., A. Davis, A.C. Decarvalho, M. Merritt, and A.C. Morris (2004). **Genetic analysis of development of the photoreceptor mosaic in zebrafish**. *Investigative Ophthalmology and Visual Science* 45(Suppl. 2): U629. ISSN: 0146 0404.
Abstract: Purpose: The well characterized, laminar organization of the vertebrate retinais complemented by the non-random, mosaic organization of the neuronal populations within each of the layers. This is most obvious in the highly ordered, crystalline-like arrangement of the photo receptors in the teleost. The necessity of the regular distribution of cells for uniform light gathering and parallel processing is self-evident. However, developmental mechanisms leading to mosaic formation remain largely unknown. In this study, the role of Notch signaling in the formation of the photoreceptor mosaic in zebrafish (*Danio rerio*) was tested.Methods, A GAL4/UAS system controlled by a heat-shock promoter was used to drive ubiquitous but transient expression of a constitutively active form of zebrafish Notch1a (the notch intracellular domain: NICD). Trans-heterozygous embryos ranging in age from 32 hpf to several days were incubated at 38 degrees C for 30 minutes. Alterations in the mosaic were determined by whole-mount immunolabeling and in situ hybridization.Results: Previously, we described the development and arrangement of the red photoreceptor cell mosaic. Using transgenic zebrafish demonstrating rod-specific expression of eGFP, the spatial and temporal pattern of rod differentiation was observed. In embryo chimeras of transgenic and non-transgenic zebrafish, the regular arrangement of the eGFP expressing rods of the donor

clones suggested a role for local signaling mechanisms in mosaic formation. The origin of the cell-cell signaling was, tested genetically, by blocking neurogenesis at various stages of development by overexpression of the NICD. Induction of NICD expression at early stages of retinal neurogenesis dramatically decreased retinal lamination and blocked the differentiation of neurons, including photoreceptors. However, induction of NICD at later stages of development, i.e. during photoreceptor cell genesis, had little affect upon the inner retina but resultedin dramatic changes in the pattern and arrangement of the cone photoreceptors, a reduction in the number of rods and alterations in their distribution. Surprisingly, in embryos allowed to survive for several days after heat shock, the photoreceptor cell mosaic recovered in the newly generated cells at the retinal margin.Conclusions: These data provide the first genetic evidence that the photoreceptor cell mosaic is derived by local cell-cell interactions between differentiating neuroblasts of the outer retina and suggest an autonomous property, of the neuroepithelial cells in forming neuronal mosaics. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, molecular genetics, sense organs, sensory reception, photoreceptor cell mosaic, Notch intracellular domain, cell-cell signaling and interaction.

Fairbrother, W.G., G.W. Yeo, R. Yeh, P. Goldstein, M. Mawson, P.A. Sharp, and C.B. Burge (2004). **RESCUE-ESE identifies candidate exonic splicing enhancers in vertebrate exons**. *Nucleic Acids Research* 32(2): 187-190. ISSN: 0305-1048.

NAL Call Number: QD341.A2N8

Descriptors: zebrafish, amino acid sequence, bioinformatics, computer applications, DNA, exon skipping, freshwater fish, function, gene expression, mutations, nucleotide sequence, phenotypes, point mutation, prediction, transcription, World Wide Web, mRNA processing, *Danio rerio*, Tetraodontidae, blowfishes, exon skipping, globefishes, puffers, RESCUE-ESE method, rabbitfishes, swellfishes, toadfishes, mRNA processing, genetics and evolution.

Notes: A review.

Fan, L., A. Alestrom, P. Alestrom, and P. Collodi (2004). **Production of zebrafish germline chimeras from cultured cells.** *Methods in Molecular Biology* 254: 289-300. ISSN: 1064-3745.

Descriptors: zebrafish, genetically modified, chimera genetics and metabolism, electroporation methods, luminescent proteins genetics and metabolism, plasmids genetics and metabolism.

Fan, L., J. Crodian, and P. Collodi (2004). **Culture of embryonic stem cell lines from zebrafish.** *Methods in Cell Biology* 76: 151-60. ISSN: 0091-679X.

NAL Call Number: 442P92

Descriptors: zebrafish, cytology, stem cells cytology, blastula cytology, cell culture techniques methods, coculture techniques methods, electroporation methods, gastrula cytology, genetic vectors genetics, transfection methods.

Fan, L., J. Crodian, and P. Collodi (2004). **Production of zebrafish germline chimeras by using cultured embryonic stem (ES) cells.** *Methods in Cell Biology* 77: 113-9. ISSN: 0091-679X.

NAL Call Number: 442P92

Descriptors: zebrafish, cell culture techniques, chimera genetics, stem cells cytology, cytology, germ cells cytology, stem cell transplantation.

Fan, L., J. Moon, J. Crodian, and P. Collodi (2006). **Homologous recombination in zebrafish ES cells.** *Transgenic Research* 15(1): 21-30. ISSN: 0962-8819.
NAL Call Number: QH442.6T74
Abstract: Targeted insertion of a plasmid by homologous recombination was demonstrated in zebrafish ES cell cultures. Two selection strategies were used to isolate ES cell colonies that contained targeted plasmid insertions in either the no tail or myostatin I gene. One selection strategy involved the manual isolation of targeted cell colonies that were identified by the loss of fluorescent protein gene expression. A second strategy used the diphtheria toxin A-chain gene in a positive-negative selection approach. Homologous recombination was confirmed by PCR, sequence and Southern blot analysis and colonies isolated using both selection methods were expanded and maintained for multiple passages. The results demonstrate that zebrafish ES cells have potential for use in a cell-mediated gene targeting approach.
Descriptors: zebrafish, plasmid, ES cell cultures, myostatin I gene, diphtheria toxin A-chain gene.

Fan, X. and S.T. Dougan (2007). **The evolutionary origin of nodal-related genes in teleosts**. *Development Genes and Evolution* 217(11-12): 807-813. ISSN: 0949-944X.
NAL Call Number: QL951.R68
Descriptors: zebrafish, teleost, genes, tetrapod genome, triplicate genes, Nodal-related genes, mesoderm, endoderm, left-right axis, ndr1, squint, ndr2, cyclops, ndr3, southpaw, noda.

Fang, F. (2003). **Phylogenetic analysis of the Asian cyprinid genus *Danio* (Teleostei, Cyprinidae)** . *Copeia* 2003(4): 714-728. ISSN: 0045-8511.
NAL Call Number: QL1
Descriptors: zebrafish, animal morphology, cladistics, freshwater fish, phylogeny, CHeLa, Cyprinidae, *Danio dangila*, *Danio nigrofasciatus*, *Danio rerio*, Devario, Esomus, Inlecypris, carps, minnows, taxonomy and morphology, Asia.

Faraco, J.H., L. Appelbaum, W. Marin, S.E. Gaus, P. Mourrain, and E. Mignot (2006). **Regulation of hypocretin (orexin) expression in embryonic zebrafish.** *Journal of Biological Chemistry* 281(40): 29753-61. ISSN: 0021-9258.
NAL Call Number: 381J824
Abstract: Hypocretins/orexins are neuropeptides involved in the regulation of sleep and energy balance in mammals. Conservation of gene sequence, hypothalamic localization of cell bodies, and projection patterns in adult zebrafish suggest that the architecture and function of the hypocretin system are conserved in fish. We report on the complete genomic structure of the zebrafish and Tetraodon hypocretin genes and the complete predicted hypocretin protein sequences from five teleosts. Using whole mount in situ hybridization, we have traced the development of hypocretin cells in zebrafish from onset of expression at 22 h post-fertilization through the first week of development. Promoter elements of similar size from zebrafish and Tetraodon were capable of driving efficient and specific expression of enhanced green fluorescent protein in developing zebrafish embryos, thus defining a minimal promoter region able to accurately mimic the native hypocretin pattern. This enhanced green fluorescent protein expression also revealed a complex pattern of projections within the hypothalamus, to the midbrain, and to the spinal cord. To further analyze the promoter, a series of deletion and substitution constructs were injected into embryos, and resulting promoter activity was monitored in the first week of development. A critical region of 250 base pairs was identified containing a core 13-base pair element essential for hypocretin expression.

Descriptors: zebrafish, developmental gene expression regulation physiology, intracellular signaling peptides and neuropeptides biosynthesis, neuropeptides genetics, amino acid sequence, molecular sequence data, promoter regions genetics.

Farber, S.A., R.A. De Rose, E.S. Olson, and M.E. Halpern (2003). **The zebrafish annexin gene family.** *Genome Research* 13(6a): 1082-96. ISSN: 1088-9051.
NAL Call Number: QP606.D46P34
Abstract: The Annexins (ANXs) are a family of calcium- and phospholipid-binding proteins that have been implicated in many cellular processes, including channel formation, membrane fusion, vesicle transport, and regulation of phospholipase A2 activity. As a first step toward understanding in vivo function, we have cloned 11 zebrafish anx genes. Four genes (anx1a, anx2a, anx5,and anx11a) were identified by screening a zebrafish cDNA library with a *Xenopus* anx2 fragment. For these genes, full-length cDNA sequences were used to cluster 212 EST sequences generated by the Zebrafish Genome Resources Project. The EST analysis revealed seven additional anx genes that were subsequently cloned. The genetic map positions of all 11 genes were determined by using a zebrafish radiation hybrid panel. Sequence and syntenic relationships between zebrafish and human genes indicate that the 11 genes represent orthologs of human anx1,2,4,5,6,11,13,and suggest that several zebrafish anx genes resulted from duplications that arose after divergence of the zebrafish and mammalian genomes. Zebrafish anx genes are expressed in a wide range of tissues during embryonic and larval stages. Analysis of the expression patterns of duplicated genes revealed both redundancy and divergence, with the most similar genes having almost identical tissue-specific patterns of expression and with less similar duplicates showing no overlap. The differences in gene expression of recently duplicated anx genes could explain why highly related paralogs were maintained in the genome and did not rapidly become pseudogenes.
Descriptors: zebrafish, annexins genetics, multigene family genetics, amino acid sequence, expressed sequence tags , fish gene expression profiling methods and regulation, mice, molecular sequence data, *Oryzias* genetics, phylogeny, sequence alignment methods, synteny, *Takifugu* genetics.

Fausett, B.V. and D. Goldman (2006). **A role for alpha1 tubulin-expressing Muller glia in regeneration of the injured zebrafish retina.** *Journal of Neuroscience* 26(23): 6303-13. ISSN: 0270-6474.
Abstract: Alpha1 tubulin (alpha1T) is a neuron-specific microtubule protein whose expression is induced in the developing and regenerating CNS. In the adult CNS, alpha1T expression remains high in neural progenitors. Transgenic zebrafish harboring a 1.7 kb alpha1T promoter fragment along with the first exon and intron express the transgene in a manner that recapitulates expression of the endogenous gene. We recently showed that this promoter mediates gene induction in retinal ganglion cells during optic nerve regeneration and in a subset of Muller glia that proliferate after retinal injury (Senut et al., 2004). To further characterize these Muller glia, we generated transgenic fish harboring an alpha1T promoter fragment that is specifically induced in these cells after retinal damage. Transgene expression, bromodeoxyuridine (BrdU) labeling, and stem cell marker expression suggested that alpha1T-expressing Muller glia dedifferentiate and become multipotent in response to injury. In addition, green fluorescent protein and BrdU-mediated lineage tracing combined with retinal gene expression analysis indicated that alpha1T-expressing Muller glia were capable of generating retinal neurons and glia. These data strongly suggest alpha1T-expressing Muller glia dedifferentiate and mediate regeneration of the injured zebrafish retina.
Descriptors: zebrafish, neuroglia metabolism, regeneration, retina injuries and physiopa-

thology , tubulin metabolism, genetically modified, cell differentiation and proliferation, gene expression, multipotent stem cells pathology, neuroglia pathology, neurons pathology, promoter regions genetics, retina pathology, transgenes, tubulin genetics, wounds and injuries physiopathology.

Feeney, G.P., D. Zheng, P. Kille, and C. Hogstrand (2005). **The phylogeny of teleost ZIP and ZnT zinc transporters and Their tissue specific expression and response to zinc in zebrafish.** *Biochimica Et Biophysica Acta* 1732(1-3): 88-95. ISSN: 0006 3002.
NAL Call Number: 381B522
Abstract: In contrast to mammals, zinc transporter genes remain largely uncharacterised in teleosts. Teleost zinc transporter genes were data-mined and phylogenetically assigned to mammalian orthologues. For the first time in animals, the tissue-distribution and mRNA expression response to zinc for most zinc transporter genes was tested in zebrafish.
Descriptors: zebrafish , carrier proteins genetics and metabolism , gene expression profiling, phylogeny, zinc metabolism and pharmacology, organ specificity, messenger RNA genetics and metabolism.

Feijoo, C.G., M. Manzanares, E. de la Calle Mustienes, J.L. Gomez Skarmeta, and M.L. Allende (2004). **The Irx gene family in zebrafish: genomic structure, evolution and initial characterization of irx5b.** *Development Genes and Evolution* 214(6): 277-84. ISSN: 0949 944X.
NAL Call Number: QL951.R68
Abstract: Genes of the iroquois (Iro/Irx) family are highly conserved from *Drosophila* to mammals and they have been implicated in a number of developmental processes. In flies, the Iro genes participate in patterning events in the early larva and in imaginal disk specification. In vertebrates, the Irx genes regulate developmental events during gastrulation, nervous system regionalization, activation of proneural genes and organ patterning. The Iro genes in *Drosophila* and the Irx genes of mammals show a clustered organization in the genome. Flies have a single cluster comprising three genes while mammals have two clusters also having three genes each. Moreover, experimental evidence in flies shows that transcriptional regulatory elements are shared among genes within the Iro cluster, suggesting that the same may be true in vertebrates. To date, the genomic organization of the Irx genes in non-mammalian species has not been studied. In this work, we have isolated the irx5b gene from zebrafish, *Danio rerio*, and have characterized its expression pattern. Furthermore, we have identified the complete set of Irx genes in two fish species, the zebrafish and pufferfish, *Takifugu rubripes*, and have determined the genomic organization of these genes. Our analysis indicates that early in fish evolutionary history, the Irx gene clusters have been duplicated and that subsequent events have maintained the clustered organization for some of the genes, while others have been lost. In total there are 11 existing Irx genes in zebrafish and 10 in pufferfish. We propose a new nomenclature for the zebrafish Irx genes based on the analysis of their sequences and their genomic relationships.
Descriptors: zebrafish, molecular evolution, homeodomain multigene family, transcription factors genetics, gene expression, homeodomain proteins metabolism, phylogeny, sequence analysis , *Takifugu* genetics, transcription factors metabolism, proteins.

Fein, A.J., L.S. Meadows, C. Chen, E.A. Slat, and L.L. Isom (2007). **Cloning and expression of a zebrafish SCN1B ortholog and identification of a species-specific splice variant.** *BioMed Central Medical Genomics* 8: 226. ISSN: 1755 8794.
Abstract: BACKGROUND: Voltage-gated Na+ channel beta1 (Scn1b) subunits are multifunctional proteins that play roles in current modulation, channel cell surface expression,

cell adhesion, cell migration, and neurite outgrowth. We have shown previously that beta1 modulates electrical excitability in vivo using a mouse model. Scn1b null mice exhibit spontaneous seizures and ataxia, slowed action potential conduction, decreased numbers of nodes of Ranvier in myelinated axons, alterations in nodal architecture, and differences in Na+ channel alpha subunit localization. The early death of these mice at postnatal day 19, however, make them a challenging model system to study. As a first step toward development of an alternative model to investigate the physiological roles of beta1 subunits in vivo we cloned two beta1-like subunit cDNAs from *D. rerio*. RESULTS: Two beta1-like subunit mRNAs from zebrafish, scn1ba_tv1 and scn1ba_tv2, arise from alternative splicing of scn1ba. The deduced amino acid sequences of Scn1ba_tv1 and Scn1ba_tv2 are identical except for their C-terminal domains. The C-terminus of Scn1ba_tv1 contains a tyrosine residue similar to that found to be critical for ankyrin association and Na+ channel modulation in mammalian beta1. In contrast, Scn1ba_tv2 contains a unique, species-specific C-terminal domain that does not contain a tyrosine. Immunohistochemical analysis shows that, while the expression patterns of Scn1ba_tv1 and Scn1ba_tv2 overlap in some areas of the brain, retina, spinal cord, and skeletal muscle, only Scn1ba_tv1 is expressed in optic nerve where its staining pattern suggests nodal expression. Both scn1ba splice forms modulate Na+ currents expressed by zebrafish scn8aa, resulting in shifts in channel gating mode, increased current amplitude, negative shifts in the voltage dependence of current activation and inactivation, and increases in the rate of recovery from inactivation, similar to the function of mammalian beta1 subunits. In contrast to mammalian beta1, however, neither zebrafish subunit produces a complete shift to the fast gating mode and neither subunit produces complete channel inactivation or recovery from inactivation. CONCLUSION: These data add to our understanding of structure-function relationships in Na+ channel beta1 subunits and establish zebrafish as an ideal system in which to determine the contribution of scn1ba to electrical excitability in vivo.

Descriptors: zebrafish, developmental gene expression regulation, sodium channels genetics, alternative splicing, amino acid sequence, antibodies chemistry and pharmacology, base sequence, cultured cells, molecular cloning, Cricetinae, *Cricetulus*, electrophysiology molecular models, molecular sequence data, protein isoforms genetics, rats, amino acid sequence homology, sodium channels immunology, metabolism and physiology, species specificity, tissue distribution, *Xenopus laevis* , proteins immunology and metabolism.

Feitsma, H., M.C. Leal, P.B. Moens, E. Cuppen, and R.W. Schulz (2007). **Mlh1 deficiency in zebrafish results in male sterility and aneuploid as well as triploid progeny in females.** *Genetics* 175(4): 1561-9. ISSN: 0016-6731.

NAL Call Number: 442.8G28

Abstract: In most eukaryotes, recombination of homologous chromosomes during meiosis is necessary for proper chromosome pairing and subsequent segregation. The molecular mechanisms of meiosis are still relatively unknown, but numerous genes are known to be involved, among which are many mismatch repair genes. One of them, mlh1, colocalizes with presumptive sites of crossing over, but its exact action remains unclear. We studied meiotic processes in a knockout line for mlh1 in zebrafish. Male mlh1 mutants are sterile and display an arrest in spermatogenesis at metaphase I, resulting in increased testis weight due to accumulation of prophase I spermatocytes. In contrast, females are fully fertile, but their progeny shows high rates of dysmorphology and mortality within the first days of development. SNP-based chromosome analysis shows that this is caused by aneuploidy, resulting from meiosis I chromosomal missegregation. Surprisingly, the small percentage of progeny that develops normally has a complete triploid genome, consisting of both sets of maternal and one set of

paternal chromosomes. As adults, these triploid fish are infertile males with wild-type appearance. The frequency of triploid progeny of mlh1 mutant females is much higher than could be expected for random chromosome segregation. Together, these results show that multiple solutions exist for meiotic crossover/segregation problems.

Descriptors: zebrafish, DNA repair enzymes deficiency, male infertility genetics, male infertility metabolism, proteins deficiency, aneuploidy, base sequence , crossing over, genetic genetics, DNA mismatch repair, DNA primers genetics, DNA repair enzymes genetics, male infertility pathology, meiosis genetics, mutation, phenotype , polyploidy, seminiferous tubules pathology.

Ferg, M., R. Sanges, J. Gehrig, J. Kiss, M. Bauer, A. Lovas, M. Szabo, L. Yang, U. Straehle, M.J. Pankratz, F. Olasz, E. Stupka, and F. Muller (2007). **The TATA-binding protein regulates maternal mRNA degradation and differential zygotic transcription in zebrafish.** *European Molecular Biology Organization Journal Journal* 26(17): 3945-56. ISSN: 0261 4189.

NAL Call Number: QH506.E46

Abstract: Early steps of embryo development are directed by maternal gene products and trace levels of zygotic gene activity in vertebrates. A major activation of zygotic transcription occurs together with degradation of maternal mRNAs during the midblastula transition in several vertebrate systems. How these processes are regulated in preparation for the onset of differentiation in the vertebrate embryo is mostly unknown. Here, we studied the function of TATA-binding protein (TBP) by knock down and DNA microarray analysis of gene expression in early embryo development. We show that a subset of polymerase II-transcribed genes with ontogenic stage-dependent regulation requires TBP for their zygotic activation. TBP is also required for limiting the activation of genes during development. We reveal that TBP plays an important role in the degradation of a specific subset of maternal mRNAs during late blastulation/early gastrulation, which involves targets of the miR-430 pathway. Hence, TBP acts as a specific regulator of the key processes underlying the transition from maternal to zygotic regulation of embryogenesis. These results implicate core promoter recognition as an additional level of differential gene regulation during development.

Descriptors: zebrafish , messenger RNA, TATA box binding protein physiology, metabolism, RNA stability, signal transduction, proteins genetics.

Ferguson, J.E.I.I., R.W. Kelley, and C. Patterson (2005). **Mechanisms of endothelial differentiation in embryonic vasculogenesis**. *Arteriosclerosis Thrombosis and Vascular Biology* 25(11): 2246-2254. ISSN: 1079 5642.

Abstract: The formation of new blood vessels in the adult organism not only contributes to the progression of diseases such as cancer and diabetic retinopathy but also can be promoted in therapeutic approaches to various ischemic pathologies. Because many of the signals important to blood vessel development during embryogenesis are recapitulated during adult blood vessel formation, much work has been performed to better-understand the molecular control of endothelial differentiation in the developing embryo. In this review, we describe the current understanding of where endothelial differentiation from pluripotent progenitor cells occurs during development, how this process is controlled at the molecular level, and what model systems can be used to investigate the earliest steps of blood vessel formation. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, blood and lymphatics, transport and circulation, molecular genetics, molecular biophysics, development , vasculogenesis.

Feschotte, C. (2004). **Merlin, a New Superfamily of DNA Transposons Identified in Diverse Animal genomes and Related to Bacterial IS1016 Insertion Sequences**. *Molecular Biology and Evolution* 21(9): 1769-1780. ISSN: 0737-4038.
NAL Call Number: QH506.M642
Descriptors: zebrafish, aquatic animals and insects, bacteria, DNA, endoparasites, evolution, freshwater fish, genomes, insertion sequences, molecular biology, neurofibromin 2, nucleotide sequence, parasites, transposons, transposase, bacteria, Culicidae, *Danio rerio*, Nemata, Nematoda, Platyhelminthes, *Schistosoma mansoni*, Tunicata, DNA transposons, flatworms, Merlin, mosquitoes, sea squirts, tunicates, transposons, insertion sequences, genetics and evolution, public health, medicines, dangerous organisms, species interactions, parasites and diseases.

Fetcho, J.R. and S. Higashijima (2004). **Optical and Genetic Approaches Toward Understanding Neuronal Circuits in zebrafish**. *Integrative and Comparative Biology* 44(1): 57-70. ISSN: 1540-7063.
NAL Call Number: QL.I67
Descriptors: zebrafish, ablation, animal models, brain architecture, circuits, freshwater fish, neural networks, neurons, *Danio rerio*, methodologies.
Notes: Review article.

Figueiredo, M.d.a., C.F.C. Lanes, D.V. Almeida, and et al. (2007). **Improving the production of transgenic fish germlines: in vivo evaluation of mosaicism in zebrafish (*Danio rerio*) using a green fluorescent protein (GFP) and growth hormone cDNA transgene co-injection strategy**. *Genetics and Molecular Biology* 30(1): 31-36. ISSN: 1415-4757.
NAL Call Number: QH426.R48
Descriptors: zebrafish, actin, chromosomes, gene transfer, germ cells, green fluorescent protein, growth hormone, integration, microinjection, mosaicism, promoters, reporter gene, genomics, transgenic fish, *Danio rerio.*

Filippi, A., N. Tiso, G. Deflorian, E. Zecchin, M. Bortolussi, and F. Argenton (2005). **The basic helix-loop-helix olig3 establishes the neural plate boundary of the trunk and is necessary for development of the dorsal spinal cord**. *Proceedings of the National Academy of Sciences of the United States of America* 102(12): 4377-4382. ISSN: 0027 8424.
NAL Call Number: 500N21P
Abstract: olig genes encode a previously unrecognized group of vertebrate-specific basic helix-loop-helix transcription factors. As shown in mice, chickens, and zebrafish, two members of this group, olig1 and olig2, are involved in the differentiation of motoneurons and oligodendrocytes, but nothing is known about the role of the third member, olig3. Here, we show that olig3 plays an essential role in the establishment of the neural crest-lateral neural plate boundary. In zebrafish embryos, morpholino-induced olig,3 inactivation dramatically increases the number of neural crest cells, but lateral neural plate fates (interneurons and astrocytes) are missing. Zebrafish swirl mutants that have impaired bone morphogenetic protein signaling and lack neural crest cells display an expanded olig,3 expression domain. Moreover, olig3 is up-regulated in mind-bomb mutants lacking the neural crest because of an impaired notch signaling, and olig3 repression in such mutants rescues the neural crest. In addition, olig,3 regulates ngn1 and deltaA expression in interneuron precursors. Our results indicate that olig3 has an essential proneural activity in the dorsal spinal cord and cooperates with the Delta/Notch regulatory loop to establish the boundary between the neural crest and the lateral neural plate. Thus, a proper regulation of the olig gene family is essential for the

formation of three cell types (oligodendrocytes, astrocytes, and neural crest) that are unique to vertebrates. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, nervous system, molecular genetics, marine ecology and environmental sciences.

Fisher, S., E.A. Grice, R.M. Vinton, S.L. Bessling, A. Urasaki, K. Kawakami, and A.S. McCallion (2006). **Evaluating the biological relevance of putative enhancers using Tol2 transposon-mediated transgenesis in zebrafish.** *Nature Protocols* 1(3): 1297-305. ISSN: 1754 2189.
Abstract: Evaluating the biological relevance of the myriad putative regulatory noncoding sequences in vertebrate genomes represents a huge challenge. Functional analyses in vivo have typically relied on costly and labor-intensive transgenic strategies in mice. Transgenesis has also been applied in nonrodent vertebrates, such as zebrafish, but until recently these efforts have been hampered by significant mosaicism and poor rates of germline transmission. We have developed a transgenic strategy in zebrafish based on the Tol2 transposon, a mobile element that was recently identified in another teleost, Medaka. This method takes advantage of the increased efficiency of genome integration that is afforded by this intact DNA transposon, activity that is mediated by the corresponding transposase protein. The approach described in this protocol uses a universal vector system that permits rapid incorporation of DNA that is tagged with sequence targets for site-specific recombination. To evaluate the regulatory potential of a candidate sequence, the desired interval is PCR-amplified using sequence-specific primers that are flanked by the requisite target sites for cloning, and recombined into a universal expression plasmid (pGW_cfosEGFP). Purified recombinant DNAs are then injected into 1-2-cell zebrafish embryos and the resulting reporter expression patterns are analyzed at desired timepoints during development. This system is amenable to large-scale application, facilitating rapid functional analysis of noncoding sequences from both mammalian and teleost species.
Descriptors: zebrafish, enhancer elements genetics, gene transfer techniques, genomics methods, DNA primers, DNA transposable elements genetics, PCR methods.

Fisher, S. and P.M. Mabee (2004). **Skeletogenesis in zebrafish *Danio rerio*: evolutionary and developmental aspects**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd.: Singapore, Singapore: p. 392-423. ISBN: 9812388214.
NAL Call Number: QL638.C94 F57
Abstract: The zebrafish, a species within the family Cyprinidae (minnows and carps), has emerged as an important vertebrate model for the study of development, including skeletal development, due to the availability of embryological, molecular, and genetic tools. Ichthyology has a long history, extending from Aristotle through present, fueled by a large number of species relative to most major vertebrate groups and by the considerable fossil record consisting of fish skeletons. The comparative adult osteology of fishes is a traditional area of study within the field, and many of the differences in skeletal anatomy closely track the paths of evolution within various clades. Developmental studies in zebrafish have the potential to clarify historically significant evolutionary questions pertaining to the evolution of the skeleton. We focus on the study of skeletogenesis in zebrafish, although our emphasis is on the extent to which findings can be generalized to other vertebrates. After a review of descriptive studies, we discuss the availability of molecular markers, and the mutational analysis of skeletal development in zebrafish. Finally, we conclude with a discussion of prospects for additional, targeted mutant screens, and studies to examine lineage relationships among skeletogenic cells in the embryo and the adult. Copyright © 2008 CABI

Descriptors: zebrafish embryos, bone formation, embryonic development, evolution, mutants, phylogeny, skeletal development, *Danio rerio*.

Flores, M.V., C. Hall, A. Jury, K. Crosier, and P. Crosier (2007). **The zebrafish retinoid-related orphan receptor (ror) gene family.** *Gene Expression Patterns* 7(5): 535-43. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: The retinoid-related orphan receptors Rora, b and c are highly conserved transcription factors belonging to the steroid hormone receptor superfamily. Mammalian ROR proteins perform key regulatory roles in a number of processes during embryonic development and in the adult including neurogenesis, bone metabolism and modulation of circadian rhythms. A more recent area of interest has been their roles in the development and function of the immune system. In particular, RORA has been implicated in the regulation of inflammatory cytokine production, and RORC has been shown to be essential in the development of the T lymphocyte repertoire and of secondary lymphoid organs. We cloned the zebrafish orthologs for the Ror gene family. Assignment of orthologies was supported by analysis of the phylogenetic relationships between zebrafish and other vertebrate Ror genes based on sequence similarities, and conserved syntenies with the human Ror gene loci.
Descriptors: zebrafish, developmental gene expression regulation, cytoplasmic receptors and nuclear genetics, amino acid sequence, molecular cloning, in situ hybridization, molecular sequence data, RNA probes, messenger RNA genetics and metabolism, cytoplasmic receptors and nuclear metabolism, reverse transcriptase PCR, amino acid sequence homology.

Flores, M.V., V.W. Tsang, W. Hu, M. Kalev Zylinska, J. Postlethwait, P. Crosier, K. Crosier, and S. Fisher (2004). **Duplicate zebrafish runx2 orthologues are expressed in developing skeletal elements.** *Gene Expression Patterns* 4(5): 573-81. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: The differentiation of cells in the vertebrate skeleton is controlled by a precise genetic program. One crucial regulatory gene in the pathway encodes the transcription factor Runx2, which in mouse is required for differentiation of all osteoblasts and the proper development of a subset of hypertrophic chondrocytes. To explore the differentiation of skeletogenic cells in the model organism zebrafish (*Danio rerio*), we have identified two orthologues of the mammalian gene, runx2a and runx2b. Both genes share sequence homology and gene structure with the mammalian genes, and map to regions of the zebrafish genome displaying conserved synteny with the region where the human gene is localized. While both genes are expressed in developing skeletal elements, they show evidence of partial divergence in expression pattern, possibly explaining why both orthologues have been retained through teleost evolution.
Descriptors: zebrafish, bone and bones embryology and metabolism, developmental gene expression regulation, neoplasm proteins metabolism, transcription factors metabolism, amino acid and base sequence, cluster analysis, core bindingfactor alpha 1 subunit, in situ hybridization, molecular sequence data, phylogeny, polymorphism, single stranded conformational, radiation hybrid mapping, reverse transcriptase PCR, sequence alignment, DNA sequence analysis, sequence homology.

Flynt, A.S., N. Li, E.J. Thatcher, L. Solnica Krezel, and J.G. Patton (2007). **Zebrafish miR-214 modulates Hedgehog signaling to specify muscle cell fate.** *Nature Genetics* 39(2): 259-63. ISSN: 1061-4036.
NAL Call Number: QH426.N37

Abstract: Numerous microRNAs (miRNAs) have been discovered in the genomes of higher eukaryotes, and functional studies indicate that they are important during development. However, little is known concerning the function of individual miRNAs. We approached this problem in zebrafish by combining identification of miRNA expression, functional analyses and experimental validation of potential targets. We show that miR-214 is expressed during early segmentation stages in somites and that varying its expression alters the expression of genes regulated by Hedgehog signaling. Inhibition of miR-214 results in a reduction or loss of slow-muscle cell types. We show that su(fu) mRNA, encoding a negative regulator of Hedgehog signaling, is targeted by miR-214. Through regulation of su(fu), miR-214 enables precise specification of muscle cell types by sharpening cellular responses to Hedgehog.
Descriptors: zebrafish, developmental gene expression regulation, Hedgehog proteins metabolism, microRNAs physiology, muscles metabolism and physiology, repressor proteins metabolism, signal transduction, somites metabolism and physiology, cell differentiation morphogenesis.

Fradinger, E.A., J.A. Tello, J.E. Rivier, and N.M. Sherwood (2005). **Characterization of four receptor cDNAs: PAC1, VPAC1, a novel PAC1 and a partial GHRH in zebrafish.** *Molecular and Cellular Endocrinology* 231(1-2): 49-63. ISSN: 0303-7207.
NAL Call Number: QP187.A1M6
Abstract: To understand the role of growth hormone-releasing hormone (GHRH) and pituitary adenylate cyclase-activating polypeptide (PACAP) and to examine the functional significance of the co-expression of GHRH and PACAP in fish, their receptors were characterized in zebrafish. Three cDNAs encoding the PAC(1) receptor, the VPAC(1) receptor, and the partial GHRH receptor were identified from zebrafish. Functional expression of the PAC(1) and VPAC(1) receptors revealed that both are potently coupled to the adenylyl cyclase pathway, but only the PAC(1) receptor is coupled to the phospholipase C pathway. Transcripts for all three receptors were widely distributed, often in an overlapping pattern in the adult zebrafish. Also, one splice variant of the partial GHRH receptor and three splice variants of the PAC(1) receptor were identified from adult zebrafish. The long GHRH receptor transcript contained a 27 amino acid insert in transmembrane domain 5 encoding a premature stop codon leading to a truncated receptor protein. For the PAC(1) receptor, two of the splice variants corresponded to the hop1 and hop2 variants characterized in mammals. The third splice variant identified from the gill encoded a novel 107 bp insert containing a premature stop codon. Therefore, PACAP and GHRH have widespread, overlapping target sites suggesting a coordinated role for these hormones in evolution.
Descriptors: zebrafish, GHRH genetics, cell surface receptors genetics, vasoactive intestinal peptide receptors genetics, adenylate cyclase metabolism, base sequence, nonsense codon, complementary DNA, growth hormone releasing hormone physiology, molecular sequence data, phospholipase C metabolism, RNA splicing, messenger RNA analysis, cell surface receptors physiology, receptors, pituitary adenylate cyclase activating polypeptide, receptors, pituitary adenylate cyclase activating polypeptide, Type I vasoactive intestinal peptide receptors physiology and sequence alighnment, tissue distribution.

Fredriksson, R., E. Larson, Y.L. Yan, J.H. Postlethwait, and D. Larhammar (2004). **Novel neuropeptide Y Y2-like receptor subtype in zebrafish and frogs supports early vertebrate chromosome duplications.** *Journal of Molecular Evolution* 58(1): 106-114. ISSN: 0022 2844.
NAL Call Number: QH359.J6
Abstract: The Y receptors comprise a family of G-protein coupled receptors with neuro-

peptide Y-family peptides as endogenous ligands. The Y receptor family has five members in mammals and evolutionary data suggest that it diversified in the two genome duplications proposed to have occurred early in vertebrate evolution. If this theory holds true, it allows for additional family members to be present. We describe here the cloning, pharmacological characterization, tissue distribution, and chromosomal localization of a novel subtype of the Y-receptor family, named Y7, from the zebrafish. We also present Y7 sequences from rainbow trout and two amphibians. The new receptor is most similar to Y2, with 51-54% identity. As Y2 has also been cloned from some of these species, there clearly are two separate Y2-subfamily genes. Chromosomal mapping in zebrafish supports origin of Y7 as a duplicate of Y2 by chromosome duplication in an early vertebrate. Y7 has probably been lost in the lineage leading to mammals. The pharmacological profile of the zebrafish Y7 receptor is different from mammalian Y2, as it does not bind short fragments of NPY with a high affinity. The Y7 receptor supports the theory of early vertebrate genome duplications and suggests that the Y family of receptors is a result of these early genome duplications.
Descriptors: zebrafish, vertebrata , neurotransmitter neuropeptide Y receptor receptor family, gene sequences, phylogenetic implications, hormones, molecular genetics, neuropeptide Y receptor family gene sequences, homology , phylogeny, neuropeptide Y receptor family gene sequence homology implications.

Fredriksson, R., P. Sjodin, E.T. Larson, J.M. Conlon, and D. Larhammar (2006). **Cloning and characterization of a zebrafish Y2 receptor.** *Regulatory Peptides* 133(1-3): 32-40. ISSN: 0167-0115.
NAL Call Number: QP552.P4
Abstract: The NPY receptors belong to the superfamily of G-protein coupled receptors and in mammals this family has five members, named Y1, Y2, Y4, Y5, and Y6. In bony fish, four receptors have been identified, named Ya, Yb, Yc and Y7. Yb and Y7 arose prior to the split between ray-fined fishes and tetrapods and have been lost in mammals. Yc appeared as a copy of Yb in teleost fishes. Ya may be an ortholog of Y4, but surprisingly no unambiguous receptor ortholog to any of the mammalian subtypes has yet been identified in bony fishes. Here we present the cloning and pharmacological characterization of a Y2 receptor in zebrafish, *Danio rerio*. To date, this is the first Y2 receptor outside mammals and birds that has been characterized pharmacologically. Phylogenetic analysis and synteny confirmed that this receptor is orthologous to mammalian Y2. We show that the receptor is pharmacologically most similar to chicken Y2 which leads to the conclusion that Y2 has acquired several novel characteristics in mammals. Y2 from zebrafish binds very poorly to the Y2-specific antagonist BIIE0246. Our pharmacological characterization supports our previous conclusions regarding the binding pocket of BIIE0246 in the human Y2 receptor.
Descriptors: zebrafish, protein isoforms genetics, neuropeptide Y receptor chemistry, amino acid sequence, amino acid substitution genetics, carrier proteins drug effects, carrier cultured cells, chickens genetics, molecular cloning, conserved sequence, drug dose response relationship, ligands, molecular sequence data, phylogeny, protein isoforms chemistry, neuropeptide Y receptor genetics, sequence alignment, sequence homology, transfection.

Freeman, J.L., A. Adeniyi, R. Banerjee, S. Dallaire, S.F. Maguire, J. Chi, B.L. Ng, C. Zepeda, C.E. Scott, S. Humphray, J. Rogers, Y. Zhou, L.I. Zon, N.P. Carter, F. Yang, and C. Lee (2007). **Definition of the zebrafish genome using flow cytometry and cytogenetic mapping.** *BioMed Central Medical Genomics* 8: 195. ISSN: 1755 8794.
Abstract: BACKGROUND: The zebrafish *(Danio rerio)* is an important vertebrate model organism system for biomedical research. The syntenic conservation between the zebrafish

and human genome allows one to investigate the function of human genes using the zebrafish model. To facilitate analysis of the zebrafish genome, genetic maps have been constructed and sequence annotation of a reference zebrafish genome is ongoing. However, the duplicative nature of teleost genomes, including the zebrafish, complicates accurate assembly and annotation of a representative genome sequence. Cytogenetic approaches provide "anchors" that can be integrated with accumulating genomic data. RESULTS: Here, we cytogenetically define the zebrafish genome by first estimating the size of each linkage group (LG) chromosome using flow cytometry, followed by the cytogenetic mapping of 575 bacterial artificial chromosome (BAC) clones onto metaphase chromosomes. Of the 575 BAC clones, 544 clones localized to apparently unique chromosomal locations. 93.8% of these clones were assigned to a specific LG chromosome location using fluorescence in situ hybridization (FISH) and compared to the LG chromosome assignment reported in the zebrafish genome databases. Thirty-one BAC clones localized to multiple chromosomal locations in several different hybridization patterns. From these data, a refined second generation probe panel for each LG chromosome was also constructed. CONCLUSION: The chromosomal mapping of the 575 large-insert DNA clones allows for these clones to be integrated into existing zebrafish mapping data. An accurately annotated zebrafish reference genome serves as a valuable resource for investigating the molecular basis of human diseases using zebrafish mutant models.

Descriptors: zebrafish, cytogenetics methods, flow cytometry methods, genome, chromosome mapping, artificial bacterial chromosomes, genetic techniques, genomic library, genomics, in situ hybridization, fluorescence, fluorescence microscopy, genetic models, telomere ultrastructure.

Friedberg, F. (2005). **Parvalbumin isoforms in zebrafish**. *Molecular Biology Reports* 32(3): 167-175. ISSN: 0301 4851.

NAL Call Number: QH506.M684

Abstract: By using an analysis of existing genomic information it is concluded that in zebrafish nine genes encode parvalbumin (PV). These genes possess introns that differ in size and show nucleotide variability but they contain the same number of exons, and for each corresponding exon, the number of nucleotides therein are identical in all the paralogs. This rule also applies to the multiple PV genes of other species e.g. mammals. Each of these genes displays, however, characteristic 5' and 3' UTRs which appear highly conserved between closely related species (so that orthologs among these species can be readily identified) but which show larger numbers of mutations between species that are more distant in evolution. A tree is presented which suggests that the traditional classification of PVs as alpha or beta (based mainly on charge of the protein molecule) is not sustainable. Numbers 1-9 are assigned to the various isoforms to facilitate their identification in future studies. A bifurcation of isoforms into 1 and 4; 2 and 3; 6 and 7; 8 and 9 appears to have occurred simultaneously in more recent time, i.e. perhaps approximately 60 mys ago when primates and rodents branched. Copyright © 2008 CABI

Descriptors: zebrafish, evolution, encode parvalbumin, exons, introns, genes, genomes, nucleotides, *Danio rerio.*

Friedberg, F. and L. Taliaferro (2005). **Calmodulin genes in zebrafish (revisited).** *Molecular Biology Reports* 32(1): 55-60. ISSN: 0301-4851.

NAL Call Number: QH506.M684

Abstract: Calmodulin (CaM), a ubiquitous protein, ancestral in early eukaryotes, regulates a large number of physiologically important functions by activating other proteins, some

of them enzymes, usually in response to changes in the local concentration of calcium ions. Invertebrates possess one gene that codes for CaM. Among vertebrates, mammals display three genes that code for a 100% identical CaM molecule, while for zebra fishes etc., a non-mammalian vertebrate, we reported earlier the existence of four such genes. The number of multiple genes coding for a 100% identical CaM molecule present in the zebra fish genome, however, is corrected here, from the four, as previously suggested, to six (alpha, alpha2, beta, beta2, gamma and gamma 2). Identification of each of these genes is readily achieved upon examination of the characteristic 5' and 3' UTRs within their respective mRNAs even though we do not know at present what role these UTRs might play. A scanning of the 3' UTRs for short homology elements among the six genes (and a comparison with the human type I, II, and III CaM 3' UTRs) also suggests that duplication processes for three genes resulted in the formation of six such genes. As they become available, the promoter regions for these six genes should be scanned for possible identification of putative regulatory elements if we are to understand the need for the uniquely rigid evolutionary maintenance of these six genes. A comparison of the promoter regions for the beta and beta 2 genes is presented in this paper. A few common short homologous elements appear to be retained in these generally highly variant two regions, but conclusions about differential expression controls must be delayed until the promoter regions for all the other CaM genes have been examined.

Descriptors: zebrafish, calmodulin genetics, base sequence, molecular evolution, genes, molecular sequence data, sequence alignment.

Friederike, L., G. Heiko, K. Soerge, and D. Frank (2004). **Evolution of sialic acid-binding proteins: molecular cloning and expression of fish siglec-4**. *Glycobiology* 14(11): 959. ISSN: 0959-6658.

NAL Call Number: QP552.G59G593

Descriptors: zebrafish, alternative splicing, cell adhesion molecules, evolution, evolutionary genetics, genomes, immune system, lectins, nervous system, sialic acids, signal transduction, protein families, sialic acid-binding protein, *Danio rerio*, Fugu.

Fuchs, C., V. Heib, L. Kiger, M. Haberkamp, A. Roesner, M. Schmidt, D. Hamdane, M.C. Marden, T. Hankeln, and T. Burmester (2004). **Zebrafish reveals different and conserved features of vertebrate neuroglobin gene structure, expression pattern, and ligand binding.** *Journal of Biological Chemistry* 279(23): 24116-22. ISSN: 0021-9258.

NAL Call Number: 381J824

Abstract: Neuroglobin has been identified as a respiratory protein that is primarily expressed in the mammalian nervous system. Here we present the first detailed analysis of neuroglobin from a non-mammalian vertebrate, the zebrafish *Danio rerio*. The zebrafish neuroglobin gene reveals a mammalian-type exon-intron pattern in the coding region (B12.2, E11.0, and G7.0), plus an additional 5'-non-coding exon. Similar to the mammalian neuroglobin, the zebrafish protein displays a hexacoordinate deoxy-binding scheme. Flash photolysis kinetics show the competitive binding on the millisecond timescale of external ligands and the distal histidine, resulting in an oxygen affinity of 1 torr. Western blotting, immune staining, and mRNA in situ hybridization demonstrate neuroglobin expression in the fish central nervous system and the retina but also in the gills. Neurons containing neuroglobin have a widespread distribution in the brain but are also present in the olfactory system. In the fish retina, neuroglobin is mainly present in the inner segments of the photoreceptor cells. In the gills, the chloride cells were identified to express neuroglobin. Neuroglobin appears to be associated with mitochondria-rich cell types and thus oxygen consumption rates, suggesting a

myoglobin-like function of this protein in facilitated oxygen diffusion.
Descriptors: zebrafish , globins genetics, nerve tissue binding, competitive, Western blotting, chlorides chemistry, molecular cloning, conserved sequence, complementary DNA metabolism, diffusion, exons, introns, gene expression regulation, gills metabolism, histidine chemistry, in situ hybridization, kinetics, ligands, fluorescence microscopy, mitochondria metabolism, genetic models, olfactory pathways metabolism, oxygen metabolism, messenger RNA metabolism, recombinant proteins chemistry, retina metabolism, spectrophotometry.

Fuchs, C., A. Luckhardt, F. Gerlach, T. Burmester, and T. Hankeln (2005). **Duplicated cytoglobin genes in teleost fishes**. *Biochemical and Biophysical Research Communications* 337(1): 216-223. ISSN: 0006-291X.
NAL Call Number: 442.8B5236
Descriptors: zebrafish, cations, DNA, evolution, evolutionary genetics, exons, freshwater fish, genes, introns, phylogenetics, phylogeny, synteny, gene duplication, *Danio rerio*, *Oryzias latipes*, *Takifugu rubripes*, Teleostei, Tetraodon nigroviridis, medaka.

Fujimori, K., T. Inui, N. Uodome, K. Kadoyama, K. Aritake, and Y. Urade (2006). **Zebrafish and chicken lipocalin-type prostaglandin D synthase homologues: Conservation of mammalian gene structure and binding ability for lipophilic molecules, and difference in expression profile and enzyme activity.** *Gene* 375: 14-25. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: Lipocalin-type prostaglandin (PG) D synthase (L-PGDS) is a bifunctional protein possessing both the ability to synthesize PGD(2) and to serve as a carrier protein for lipophilic molecules. L-PGDS has been extensively studied in mammalian species, whereas little is known about non-mammalian forms. Here, we identified and characterized the L-PGDS homologues from non-mammals such as zebrafish and chicken. Phylogenetic analysis revealed that L-PGDSs of mammalian and non-mammalian organisms form a "L-PGDS sub-family" that has been evolutionally separated from other lipocalin gene family proteins. The genes for zebrafish and chicken L-PGDS homologues consisted of 6 exons, and all of the exon/intron boundaries were completely identical to those of mammalian L-PGDS genes. Zebrafish and chicken L-PGDS genes were clustered with several lipocalin genes in the chromosome, as in the case of mouse and human genes. Gene expression profiles were different among chicken, mouse, human, except for conservation of abundant expression in the brain and heart. The chicken L-PGDS homologue carried weak PGDS activity, whereas the zebrafish protein did not show any of the activity. However, when the amino-terminal region of the zebrafish L-PGDS homologue was exchanged for that of mouse L-PGDS carrying the Cys residue essential for PGDS activity, this chimeric protein showed weak PGDS activity. Both zebrafish and chicken L-PGDS homologues bound thyroxine and all-trans retinoic acid, like mammalian L-PGDSs and other lipocalin gene family proteins. These results indicate that non-mammalian and mammalian L-PGDS genes evolved from the same ancestral gene and that the non-mammalian L-PGDS homologue was the primordial form of L-PGDS but whose major function was and is to serve as a carrier protein for lipophilic molecules. During molecular evolution, the mammalian L-PGDS protein might have acquired effective PGDS activity through substitution of several amino acid residues, especially in the amino-terminal region including the Cys residue, which is essential for PGDS activity.
Descriptors: zebrafish, chickens genetics, gene expression profiling, intramolecular oxidoreductases genetics, amino acid and base sequence, chromosome mapping, molecular cloning, DNA primers, complementary DNA, intramolecular oxidoreductases chemistry

and metabolism, molecular sequence data, reverse transcriptase PCR, amino acid sequence homology.

Fuller, C.L., R. Villanueva, and C.A. Byrd (2005). **Changes in glutamate receptor subunit 4 expression in the deafferented olfactory bulb of zebrafish.** *Brain Research* 1044(2): 251-61. ISSN: 0006 8993.
NAL Call Number: QP376
Abstract: The distribution of ionotropic glutamate receptor subunit 4 (iGluR4) was examined in both normal and deafferented olfactory bulbs of adult zebrafish, *Danio rerio*. With the exception of the olfactory nerve layer, there was extensive labeling with antibodies to iGluR4 in the olfactory bulbs, specifically in juxtaglomerular cell bodies and their processes. These results are consistent with previous work, which has suggested differential distribution of glutamate receptors in the vertebrate olfactory system. Analysis of bulbs following olfactory organ removal revealed a significant loss of iGluR4 immunoreactivity by 24 h post-deafferentation. At 48 h after denervation, iGluR4 labeling had returned to normal levels and was retained through 3 weeks post-surgery. Thus, afferent input plays a role in reduced labeling of this protein immediately following injury, but return of immunoreactivity can occur even without sensory innervation.
Descriptors: zebrafish, gene expression regulation physiology, neurons metabolism, olfactory bulb cytology, AMPA receptors metabolism, Western blotting, cell count, denervation adverse effects, immunohistochemistry methods, olfactory bulb physiology, time factors, tyrosine 3 monooxygenase metabolism.

Gahtan, E. and H. Baier (2004). **Of lasers, mutants, and see-through brains: functional neuroanatomy in zebrafish.** *Journal of Neurobiology* 59(1): 147-61. ISSN: 0022-3034.
NAL Call Number: QP351.J55
Descriptors: zebrafish, brain anatomy and histology, neuroanatomy methods, neurons physiology, vision physiology, behavior, brain physiology, escape reaction physiology, genetic techniques, lasers, mutation, nerve net cytology, nerve net physiology, visual pathways cytology, visual pathways physiology.

Ganapathy, V., Z. Chen, L.A. Zhuang, S. Navarre, and D.J. Kozlowski (2004). **Taurine transporter in zebrafish: structure, function, and expression pattern**. *Federation of American Societies for Experimental Biology Journal* 18(4-5): Abst. 841.2. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: We have cloned the taurine transporter from zebrafish (zfTauT), characterized its transport function, analyzed its expression pattern in zebrafish, and assessed the consequences of morpholino-induced knockdown of its function. The zfTauT cDNA encodes a protein of 625 amino acids. In a mammalian cell expression system, the cDNA induces the expression of Na+/Cl- -coupled transport of taurine. Whole mount in situ hybridization with a zfTauT-specific riboprobe reveals robust expression of TauT mRNA in the developing eye and somites at 18 hours post fertilization (hpf). At 24 hpf, expression is detectable in the brain and in the developing heart tube. Expression in somites continues and expression in the eye becomes restricted to the dorsal retina. At 30 hpf, expression is maintained in the heart and retina, with additional expression detectable in the optic nerve. Somites no longer show evidence of expression. At 48 hpf, tissue-specific expression of TauT mRNA includes eye, heart, epiphysis, and posterior tectum. Microinjection of antisense morpholino oligonucleotides specific for TauT mRNA caused massive apoptotic cell death. At 24 hpf, TUNEL-positive cells were detectable in the eye, brain, and along the dorsal trunk. At 48- and 72 hpf, mor-

pholino-injected live embryos had reduced blood circulation, suggesting decreased heart rate and/or decreased volume of heart chambers. We conclude that TauT plays a critical role in the development of retina, heart, and brain in zebrafish. Copyright©Thomson Reuters 2009
Descriptors: zebrafish , molecular biophysics, cloning, genetic techniques, whole mount in situ hybridization, blood circulation, apoptosis, cell death, heart rate.

Gao, S., T. Zhang, X. Zhou, Y. Zhao, Q. Li, Y. Guo, H. Cheng, and R. Zhou (2005). **Molecular cloning, expression of Sox5 and its down-regulation of Dmrt1 transcription in zebrafish.** *Journal of Experimental Zoology. Part B. Molecular and Developmental Evolution* 304(5): 476-83. ISSN: 1552-5007.
NAL Call Number: QL1.J87
Abstract: The doublesex and mab-3-related transcription factor 1 (Dmrt1) is the founding member of a family of DM domain genes, involved in gonadogenesis. Here we report the cloning of zebrafish Sox5 and use real time PCR to show that its expression increases while expression of Dmrt1 decreases during embryogenesis. Characterization of the proximal promoter of zebrafish Dmrt1 revealed two positive and two negative regulatory regions and a Sox5-binding site. Co-transfection of dmrt1 (with or without the Sox-binding site), driving an EGFP reporter and Sox5 showed further that Sox5 bound the Dmrt1 promoter and inhibited Dmrt1 expression. This antagonistic partnership between Dmrt1 and Sox5 suggests a potential transcriptional regulatory mechanism for Dmrt1 in early embryogenesis.
Descriptors: zebrafish, repressor transcription factors biosynthesis , amino acid sequence, molecular cloning, down regulation flow cytometry, developmental gene expression regulation, reporter genes, molecular sequence data, promoter regions genetics, repressor proteins biosynthesis, reverse transcriptase PCR, amino acid sequence homology, sex differentiation genetics, proteins biosynthesis.

Garcia, D.M., H. Bauer, T. Dietz, T. Schubert, J. Markl, and M. Schaffeld (2005). **Identification of keratins and analysis of their expression in carp and goldfish: comparison with the zebrafish and trout keratin catalog.** *Cell and Tissue Research* 322(2): 245-56. ISSN: 0302 766X.
NAL Call Number: QH581.A1Z4
Abstract: With more than 50 genes in human, keratins make up a large gene family, but the evolutionary pressure leading to their diversity remains largely unclear. Nevertheless, this diversity offers a means to examine the evolutionary relationships among organisms that express keratins. Here, we report the analysis of keratins expressed in two cyprinid fishes, goldfish and carp, by two-dimensional polyacrylamide gel electrophoresis, complementary keratin blot binding assay, and immunoblotting. We further explore the expression of keratins by immunofluorescence microscopy. Comparison is made with the keratin expression and catalogs of zebrafish and rainbow trout. The keratins among these fishes exhibit a similar range of molecular weights and isoelectric points, with a similar overall pattern on two-dimensional gels. In addition, immunofluorescence microscopy studies of goldfish and carp tissues have revealed the expression of keratins in both epithelial and mesenchymally derived tissues, as reported previously for zebrafish and trout. We conclude that keratin expression is qualitatively similar among these fishes, with goldfish and carp patterns being more similar to each other than to zebrafish, and the cyprinid fishes being more similar to each other than to the salmonid trout. Because of the detected similarity of keratin expression among the cyprinid fishes, we propose that, for certain experiments, they are interchangeable. Although the zebrafish distinguishes itself as being a developmental and genetic/genomic model organism, we have found that the goldfish, in particular, is a more suitable model for both

biochemical and histological studies of the cytoskeleton, especially since goldfish cytoskeletal preparations seem to be more resistant to degradation than those from carp or zebrafish.
Descriptors: zebrafish, carps, goldfish, keratins metabolism, protein isoforms metabolism, trout, zebrafish, amino acid and base sequence, two dimensional gel electrophoresis, gene expression, immunohistochemistry, keratins genetics, mass spectrometry, molecular sequence data, protein isoforms genetics , tissue distribution.

Garg, R., C.D. Geng, J.L. Miller, S. Callens, X. Tang, B. Appel, and B. Xu (2004). **Molecular cloning and characterization of the catalytic domain of zebrafish homologue of the ataxia-telangiectasia mutated gene.** *Molecular Cancer Research* 2(6): 348-53. ISSN: 1541-7786.
Abstract: Inherited biallelic mutations of the ATM (ataxia-telangiectasia mutated) gene in humans cause ataxia-telangiectasia, a rare autosomal recessive disorder associated with progressive neuro-degeneration, cancer predisposition, immunodeficiency, and hypersensitivity to ionizing radiation. The ATM gene is highly conserved across a wide range of species. In an attempt to establish a zebrafish (*Danio rerio*) model of ataxia-telangiectasia, we cloned the coding sequence of the catalytic domain of the zebrafish homologue of ATM and found it to contain an open reading frame encoding 907 amino acids at the carboxyl terminus of the zebrafish ATM (zATM). The catalytic domain of zATM shares 67% and 66% homology with human ATM (hATM) and mouse ATM (mATM), respectively. The full-length mRNA encoding zATM is found to be approximately 11 kb by Northern hybridization, and the expression of zATM is observed in different adult and embryonic tissues. Overexpression of a kinase-inactive zATM domain in human cells has a dominant-negative effect against hATM function. Expression of the altered zATM in ZF4 cells leads to an A-T-like phenotype in response to ionizing radiation. These results taken together indicate that zATM is the homologue of hATM. Furthermore, using the kinase-inactive form of zATM should allow manipulation of zATM function in fish cells.
Descriptors: zebrafish, catalytic domain, protein serine threonine kinases chemistry and genetics, proteins chemistry and genetics, amino acid sequence, cell cycle proteins, cell line, molecular cloning, DNA binding proteins genetics, proteins, metabolism, dominant molecular sequence data, mutation genetics, protein serine threonine kinases antagonists and inhibitors, protein serine threonine kinases metabolism, messenger RNA genetics and metabolism, S phase radiation effects, amino acid sequence homology, transfection, tumor suppressor proteins, proteins antagonists and inhibitors.

Gasperini, R. and L. Foa (2004). **Homer 1b/c expression correlates with zebrafish olfactory system development.** *Journal of Neurocytology* 33(6): 671-80. ISSN: 0030-4864.
Abstract: The zebrafish, (*Danio rerio*) is an important model organism for the analysis of molecular mechanisms that govern neuronal circuit development. The neuronal circuitry that mediates olfaction is crucial for the development and survival of all teleost fishes. In concert with other sensory systems, olfaction is functional at early stages in zebrafish development and mediates important behavioral and survival strategies in the developing larva. Odorant cues are transduced by an array of signaling molecules from receptors in olfactory sensory neurons. The scaffolding protein family known as Homer is well placed to orchestrate this signaling cascade by interacting with and coupling membrane bound receptors to cytosolic signaling partners. To date, Homer has not been demonstrated in the zebrafish. Here we report that the Homer 1b/c isoform was prominent in the olfactory system from the earliest stages of differentiation. We describe the spatial and temporal distribution of Homer in the zebrafish olfactory system. At 24 hours post fertilization (hpf), Homer expression delineated

the boundary of the presumptive olfactory placode. Subsequent expression steadily increased throughout the developing olfactory placode, with a prominent localization to the dendritic knobs of the olfactory sensory neurons. Homer expression in the developing olfactory bulb was punctate and prominent in the glomeruli, displaying an apparent synaptic localization. This work supports the hypothesis that Homer is an important molecule in neuronal circuit development, necessary for crucial behaviors required for development and survival.
Descriptors: zebrafish, carrier proteins metabolism, olfactory bulb embryology, olfactory mucosa embryology, olfactory receptor neurons embryology, carrier cell differentiation physiology, dendrites metabolism and ultrastructure, developmental gene expression regulation physiology, neuropil metabolism and ultrastructure, olfactory bulb growth, development and metabolism, olfactory mucosa growth, development and metabolism, olfactory receptor neurons growth, development and metabolism, protein isoforms genetics and metabolism, synapses metabolism and ultrastructure, zebrafish growth, development and metabolism.

Gavaia, P.J., D.C. Simes, J.B. Ortiz Delgado, C.S. Viegas, J.P. Pinto, R.N. Kelsh, M.C. Sarasquete, and M.L. Cancela (2006). **Osteocalcin and matrix Gla protein in zebrafish (*Danio rerio*) and Senegal sole (*Solea senegalensis*): comparative gene and protein expression during larval development through adulthood.** *Gene Expression Patterns* 6(6): 637-52. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Bone Gla protein (Bgp or osteocalcin) and matrix Gla protein (Mgp) are important in calcium metabolism and skeletal development, but their precise roles at the molecular level remain poorly understood. Here, we compare the tissue distribution and accumulation of Bgp and Mgp during larval development and in adult tissues of zebrafish (*Danio rerio*) and throughout metamorphosis in Senegal sole (*Solea senegalensis*), two fish species with contrasting environmental calcium levels and degrees of skeletal reorganization at metamorphosis. Mineral deposition was investigated in parallel using a modified Alizarin red/Alcian blue protocol allowing sensitive simultaneous detection of bone and cartilage. In zebrafish, bgp and mgp mRNAs were localized in all mineralized tissues during and after calcification including bone and calcified cartilage of branchial arches. Through immunohistochemistry we demonstrated that these proteins accumulate mainly in the matrix of skeletal structures already calcified or under calcification, confirming in situ hybridization results. Interestingly, some accumulation of Bgp was also observed in kidney, possibly due to the presence of a related protein, nephrocalcin. Chromosomal localization of bgp and mgp using a zebrafish radiation hybrid panel indicated that both genes are located on the same chromosome, in contrast to mammals where they map to different chromosomes, albeit in regions showing synteny with the zebrafish location. Results in Senegal sole further indicate that, during metamorphosis, there is an increase in expression of both bgp and mgp, paralleling calcification of axial skeleton structures. In contrast with results obtained for previously studied marine fishes, in zebrafish and Senegal sole Mgp accumulates in both calcified tissues and non-mieralized vessel walls of the vascular system. These results suggest different patterns of Mgp accumulation between fish and mammals.
Descriptors: zebrafish larvae, Senagal sole, calcium binding proteins genetics and metabolism, extracellular matrix proteins genetics and metabolism, flatfishes growth and development, osteocalcin genetics and metabolism, bone and bones metabolism and resorption, chromosome mapping, fish proteins analysis, flatfishes genetics and metabolism, fresh water, gene expression profiling, immunohistochemistry, larva growth, development and metabolism, biological metamorphosis, osteogenesis, messenger RNA metabolism, seawater, time factors.

Geisler, R., G.J. Rauch, S. Geiger Rudolph, A. Albrecht, F.v. Bebber, A. Berger, E. Busch Nentwich, R. Dahm, M.P. Dekens, C. Dooley, A.F. Elli, I. Gehring, H. Geiger, M. Geisler, S. Glaser, S. Holley, M. Huber, A. Kerr, A. Kirn, M. Knirsch, M. Konantz, A.M. Kuchler, F. Maderspacher, S.C. Neuhauss, T. Nicolson, and E.A.e.a. Ober (2007). **Large-scale mapping of mutations affecting zebrafish development**. *Bio Med Central Genomics* 8(11) ISSN: 1471 2164.

Online: http://www.biomedcentral.com/content/pdf/1471-2164-8-11.pdf

NAL Call Number: QH447.B63

Abstract: Background: Large-scale mutagenesis screens in the zebrafish employing the mutagen ENU have isolated several hundred mutant loci that represent putative developmental control genes. In order to realize the potential of such screens, systematic genetic mapping of the mutations is necessary. Here we report on a large-scale effort to map the mutations generated in mutagenesis screening at the Max Planck Institute for Developmental Biology by genome scanning with microsatellite markers. Results: We have selected a set of microsatellite markers and developed methods and scoring criteria suitable for efficient, high-throughput genome scanning. We have used these methods to successfully obtain a rough map position for 319 mutant loci from the Tubingen I mutagenesis screen and subsequent screening of the mutant collection. For 277 of these the corresponding gene is not yet identified. Mapping was successful for 80% of the tested loci. By comparing 21 mutation and gene positions of cloned mutations we have validated the correctness of our linkage group assignments and estimated the standard error of our map positions to be approximately 6 cM. Conclusion: By obtaining rough map positions for over 300 zebrafish loci with developmental phenotypes, we have generated a dataset that will be useful not only for cloning of the affected genes, but also to suggest allelism of mutations with similar phenotypes that will be identified in future screens. Furthermore this work validates the usefulness of our methodology for rapid, systematic and inexpensive microsatellite mapping of zebrafish mutations. Copyright © 2008 CABI

Descriptors: zebrafish, embryos, gene mapping, developmental genes, genetic mapping and markers, genome analysis, genotypes, microsatellites, mutagenesis, mutations, phenotypes, techniques, *Danio rerio*, fishes.

Geraudie, J. and V. Borday Birraux (2003). **Posterior hoxa genes expression during zebrafish bony fin ray development and regeneration suggests their involvement in scleroblast differentiation.** *Development Genes and Evolution* 213(4): 182-6. ISSN: 0949 944X.

NAL Call Number: QL951.R68

Abstract: Expression of two zebrafish developmental posterior hoxa genes, hoxa11b and hoxa13b, was studied by in situ hybridization during pectoral and caudal fin development and regeneration. Expression was restricted to cells of the bony rays region. During fin development, molecular cytological analysis revealed that a subpopulation of mesenchymal cells expressed these two hoxa genes during their early differentiation in the subapical region of the developing ray. These cells were identified as differentiating dermal bone making cells (scleroblasts). During fin regeneration, hoxa11b and hoxa13b genes are both induced in undifferentiated cells of the distalmost blastema region (DMB) and the proliferating zone (PZ) and later in differentiating bone-forming cells. In addition, the transient regionalization of the hoxa13b expression pattern in differentiated bone-forming cells along the proximodistal axis of the regenerating ray suggests that hoxa13b could participate in ray patterning. This study is the first to establish a correlation between hoxa gene expression and dermal bone cell differentiation.

Descriptors: zebrafish larvae, cell differentiation genetics, extremities physiology, home-

odomain regeneration genetics, gene expression regulation, larva cytology, oncogene wound healing genetics, zebrafish growth and development.

Gerlach, G. and N. Lysiak (2006). **Kin recognition and inbreeding avoidance in zebrafish, *Danio rerio*, is based on phenotype matching**. *Animal Behaviour* 71(6): 1371-1377. ISSN: 0003-3472.
NAL Call Number: 410 B77
Descriptors: zebrafish, avoidance reactions, familiarity, freshwater fish, inbreeding, kin recognition, odor, olfaction, olfactory discrimination learning, phenotypes, reproductive behavior, sexual maturity, sexual selection, social environment, *Danio rerio*.

Gerlai, R. (2003). **Zebrafish: an uncharted behavior genetic model.** *Behavior Genetics* 33(5): 461-8. ISSN: 0001 8244.
NAL Call Number: QH301.B45
Abstract: The zebra fish has been a preferred subject of genetic analysis. It produces a large number of offspring that can be kept in small aquaria, it can be easily mutagenized using chemical mutagens (e.g., ethyl nitrosourea [ENU]), and high-resolution genetic maps exist that aid identification of novel genes. Libraries containing large numbers of mutant fish have been generated, and the genetic mechanisms of the development of zebra fish, whose embryo is transparent, have been extensively studied. Given the extensive homology of its genome with that of other vertebrate species including our own and given the available genetic tools, zebra fish has become a popular model organism. Despite this popularity, however, surprisingly little is known about its behavior. It is argued that behavioral analysis is a powerful tool with which the function of the brain may be studied, and the zebra fish will represent an excellent subject of such analysis. The present paper is a proof of concept study that uses pharmacological manipulation (exposure to alcohol) to show that the zebra fish is amenable to the behavioral genetic analysis of aggression and thus may allow us to reveal molecular mechanisms of this behavioral phenomenon relevant to vertebrates.
Descriptors: zebrafish, aggression physiology, behavioral genetics methods, genetic models, aggression drug effects, ethanol pharmacology, motor activity, species specificity.

Germana, A., S. Paruta, G.P. Germana, F.J. Ochoa Erena, G. Montalbano, J. Cobo, and J.A. Vega (2007). **Differential distribution of S100 protein and calretinin in mechanosensory and chemosensory cells of adult zebrafish (*Danio rerio*).** *Brain Research* 1162: 48-55. ISSN: 0006 8993.
NAL Call Number: QP376
Abstract: Calcium-binding proteins play a critical role in vertebrate sensory cells, and some of them have been detected in mechanosensory and chemosensory cells of bony and cartilaginous fishes. In this study immunohistochemistry and Western blot were used to investigate the occurrence and the distribution of S100 protein and calretinin in mechanosensory (neuromasts of the lateral line system; maculae and cristae ampullaris of the inner ear) as well as chemosensory (superficial and oral taste buds; olfactory epithelium) cells in adult zebrafish *(Danio rerio*). Specific protein bands with an estimated molecular weight of around 10 kDa and 30 kDa were detected by Western blot and were identified with S100 protein and calretinin, respectively. S100 protein and calretinin were observed segregated in mechanosensory and chemosensory cells, and the presence of S100 protein in a cell excluded that of calretinin, and vice versa. As a rule, the mechanosensory cells were S100 protein positive, whereas the chemosensory ones displayed calretinin immunoreactivity. Calretinin was also detected in nerve fibers supplying some of the investigated organs. In the olfactory epithelium, S100

protein immunoreactivity was present in the crypt olfactory sensory neurons, whereas calretinin immunoreactivity was widespread in olfactory sensory neurons and probably other olfactory cells. In this localization the co-expression of S100 protein and calretinin cannot be excluded. These results demonstrate the cell segregation of two specific calcium-binding proteins, and they enable to selectively label these cells by using easily reproducible immunohistochemical techniques associated to well-known antibodies.
Descriptors: zebrafish, calcium binding protein, vitamin D dependent metabolism, afferent neurons metabolism, s100 proteins metabolism.

Ghiselli, G. (2006). **SMC3 knockdown triggers genomic instability and p53-dependent apoptosis in human and zebrafish cells.** *Molecular Cancer* 5: 52. ISSN: 1476-4598.
Abstract: BACKGROUND: The structural maintenance of chromosome 3 (SMC3) protein is a constituent of a number of nuclear multimeric protein complexes that are involved in DNA recombination and repair in addition to chromosomal segregation. Overexpression of SMC3 activates a tumorigenic cascade through which mammalian cells acquire a transformed phenotype. This has led us to examine in depth how SMC3 level affects cell growth and genomic stability. In this paper the effect of SMC3 knockdown has been investigated. RESULTS: Mammalian cells that are SMC3 deficient fail to expand in a clonal population. In order to shed light on the underlying mechanism, experiments were conducted in zebrafish embryos in which cell competence to undergo apoptosis is acquired at specific stages of development and affects tissue morphogenesis. Zebrafish Smc3 is 95% identical to the human protein, is maternally contributed, and is expressed ubiquitously at all developmental stages. Antisense-mediated loss of Smc3 function leads to increased apoptosis in Smc3 expressing cells of the developing tail and notocord causing morphological malformations. The apoptosis and the ensuing phenotype can be suppressed by injection of a p53-specific MO that blocks the generation of endogenous p53 protein. Results in human cells constitutively lacking p53 or BAX, confirmed that a p53-dependent pathway mediates apoptosis in SMC3-deficient cells. A population of aneuploid cells accumulated in zebrafish embryos following Smc3-knockdown whereas in human cells the transient downregulation of SMC3 level lead to the generation of cells with amplified centrosome number. CONCLUSION: Smc3 is required for normal embryonic development. Its deficiency affects the morphogenesis of tissues with high mitotic index by triggering an apoptotic cascade involving p53 and the downstream p53 target gene bax. Cells with low SMC3 level display centrosome abnormalities that can lead to or are the consequence of dysfunctional mitosis and/or aneuploidy. Collectively the data support the view that SMC3 deficiency affects chromosomal stability leading to the activation of p53-dependent mitotic checkpoint.
Descriptors: zebrafish, apoptosis physiology, cell cycle cell cycle, chromosomal proteins, non histone genetics and physiology, genomic instability genetics, proteochondroitin sulfates genetics and physiology, tumor suppressor protein p53 metabolism, amino acid sequence, genetically modified, cultured cells, chromosome mapping, molecular cloning , embryo cytology, embryonic development genetics, developmental gene expression regulation, hct116 cells, molecular sequence data , messenger RNA, stored, amino acid sequence homology, transfection, zygote metabolism.

Ghosh, S., S. Moore, R.M. Bell, and M. Dush (2003). **Functional analysis of a phosphatidic acid binding domain in human Raf-1 kinase: mutations in the phosphatidate binding domain lead to tail and trunk abnormalities in developing zebrafish embryos.** *Journal of Biological Chemistry* 278(46): 45690-6. ISSN: 0021-9258.
NAL Call Number: 381J824

Abstract: Previously, we and others identified a 35-amino acid segment within human Raf-1 kinase that preferentially binds phosphatidic acid. The presence of phosphatidic acid was found to be necessary for the translocation of Raf-1 to the plasma membrane. We have now employed a combination of alanine-scanning and deletion mutagenesis to identify the critical amino acid residues in Raf-1 necessary for interaction with phosphatidic acid. Progressive mutations within a tetrapeptide motif (residues 398-401 of human Raf-1) reduced and finally eliminated binding of Raf-1 to phosphatidic acid. We then injected zebrafish embryos with RNA encoding wild-type Raf-1 kinase or a mutant version with triple alanine mutations in the tetrapeptide motif and followed the morphological fate of embryonic development. Embryos with mutant but not wild-type Raf-1 exhibited defects in posterior axis formation exemplified by bent trunk and tail structures. Molecular evidence for lack of signaling through mutated Raf-1 was obtained by aberrant in situ hybridization of the ntl (no tail) gene, which functions downstream of Raf-1. Our results demonstrate that a functional phosphatidate binding site is necessary for Raf-1 function in embryonic development.
Descriptors: zebrafish, phosphatidic acids chemistry, proto oncogene proteins C Raf chemistry and genetics, alanine chemistry, amino acid motifs and sequence, binding sites, drug dose response relationship, gene deletion , glutathione transferase metabolism, in situ hybridization, molecular sequence data, site directed mutagenesis mutation, phosphatidic acids metabolism, protein binding, tertiary protein structure, protein transport, proto oncogene proteins C Raf physiology, RNA metabolism, recombinant fusion proteins metabolism, reverse transcriptase PCR , amino acid sequence homology, tail embryology, genetic transcription.

Ghysen, A. and C. Dambly-Chaudiere (2007). **The lateral line microcosmos**. *Genes & Development* 21(17): 2118-2130. ISSN: 0890-9369.
NAL Call Number: QH426.G46
Descriptors: zebrafish, amphibiotic species, cell migration, developmental stages, embryonic development, freshwater fish, lateral line, polarity, regeneration, reviews, sense organs, sensory systems, neuromasts, amphibia, *Danio rerio*, amphibians.
Notes: A review.

Gibert, Y., D.R. McMillan, K. Kayes Wandover, A. Meyer, G. Begemann, and P.C. White (2005). **Analysis of the very large G-protein coupled receptor gene (Vlgr1/Mass1/USH2C) in zebrafish.** *Gene* 353(2): 200-6. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: Very Large G-protein coupled Receptor-1 (VLGR1/Mass1/USH2C) is the largest known cell surface protein in vertebrates. Mutations in VLGR1 are associated with audiogenic epilepsy in mice and Usher syndrome (sensorineural deafness and retinitis pigmentosa) in humans. We characterized the zebrafish VLGR1 gene (vlgr1). It is 51% identical to human VLGR1 in amino acid sequence, but is 64% identical in the 7-transmembrane and cytoplasmic domains. It is 6199 amino acids in size and is encoded by a 19.2 kb mRNA. All introns correspond in location and phase to those of the human and mouse genes. In situ hybridization studies of zebrafish embryos demonstrate vlgr1 expression in the developing central nervous system, particularly in the hypothalamus, epiphysis and in the rhombic lips. Expression in the eye is associated with the optic nerve. Further studies using zebrafish may help ascertain the role of Vlgr1 in neural development.
Descriptors: zebrafish , G protein Coupled receptors genetics, base sequence, DNA primers, complementary DNA, developmental gene expression regulation, in situ hybridization, molecular sequence data, complementary RNA genetics.

Giraldez, A.J., R.M. Cinalli, M.E. Glasner, A.J. Enright, J.M. Thomson, S. Baskerville, S.M. Hammond, D.P. Bartel, and A.F. Schier (2005). **MicroRNAs regulate brain morphogenesis in zebrafish.** *Science* 308(5723): 833-8. ISSN: 0036-8075.

NAL Call Number: 470Sci2

Abstract: MicroRNAs (miRNAs) are small RNAs that regulate gene expression posttranscriptionally. To block all miRNA formation in zebrafish, we generated maternal-zygotic dicer (MZdicer) mutants that disrupt the Dicer ribonuclease III and double-stranded RNA-binding domains. Mutant embryos do not process precursor miRNAs into mature miRNAs, but injection of preprocessed miRNAs restores gene silencing, indicating that the disrupted domains are dispensable for later steps in silencing. MZdicer mutants undergo axis formation and differentiate multiple cell types but display abnormal morphogenesis during gastrulation, brain formation, somitogenesis, and heart development. Injection of miR-430 miRNAs rescues the brain defects in MZdicer mutants, revealing essential roles for miRNAs during morphogenesis.

Descriptors: zebrafish, brain embryology, microRNAs physiology, morphogenesis , body patterning, cell differentiation, gastrula physiology, gene silencing, heart embryology, microRNAs genetics, microRNAs metabolism, mutation, neurons cytology, phenotype, RNA processing, post transcriptional, double stranded RNA metabolism, ribonuclease III genetics and metabolism, signal transduction, somites cytology and physiology, spinal cord embryology.

Giraldez, A.J., Y. Mishima, J. Rihel, R.J. Grocock, S. Van Dongen, K. Inoue, A.J. Enright, and A.F. Schier (2006). **Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs.** *Science* 312(5770): 75-9. ISSN: 0036-8075.

NAL Call Number: 470Sci2

Abstract: MicroRNAs (miRNAs) comprise 1 to 3% of all vertebrate genes, but their in vivo functions and mechanisms of action remain largely unknown. Zebrafish miR-430 is expressed at the onset of zygotic transcription and regulates morphogenesis during early development. By using a microarray approach and in vivo target validation, we find that miR-430 directly regulates several hundred target messenger RNA molecules (mRNAs). Most targets are maternally expressed mRNAs that accumulate in the absence of miR-430. We also show that miR-430 accelerates the deadenylation of target mRNAs. These results suggest that miR-430 facilitates the deadenylation and clearance of maternal mRNAs during early embryogenesis.

Descriptors: zebrafish, adenosine metabolism, physiology, embryonic development, developmental gene expression regulation, microRNAs physiology, polymers metabolism, messenger RNA metabolism, 3' untranslated regions, gene expression profiling, reporter genes, green fluorescent proteins genetics and metabolism, mutation, oligonucleotide array sequence analysis, protein biosynthesis, RNA stability, messenger RNA genetics, ribonuclease III genetics and metabolism, genetic transcription, up regulation, zygote physiology.

Giudicelli, F., E.M. Ozbudak, G.J. Wright, and J. Lewis (2007). **Setting the tempo in development: an investigation of the zebrafish somite clock mechanism.** *Public Library of Science Biology* 5(6): E150. ISSN: 1544-9173.

NAL Call Number: QH301.P56

Abstract: The somites of the vertebrate embryo are clocked out sequentially from the presomitic mesoderm (PSM) at the tail end of the embryo. Formation of each somite corresponds to one cycle of oscillation of the somite segmentation clock--a system of genes whose expression switches on and off periodically in the cells of the PSM. We have previ-

ously proposed a simple mathematical model explaining how the oscillations, in zebrafish at least, may be generated by a delayed negative feedback loop in which the products of two Notch target genes, her1 and her7, directly inhibit their own transcription, as well as that of the gene for the Notch ligand DeltaC; Notch signalling via DeltaC keeps the oscillations of neighbouring cells in synchrony. Here we subject the model to quantitative tests. We show how to read temporal information from the spatial pattern of stripes of gene expression in the anterior PSM and in this way obtain values for the biosynthetic delays and molecular lifetimes on which the model critically depends. Using transgenic lines of zebrafish expressing her1 or her7 under heat-shock control, we confirm the regulatory relationships postulated by the model. From the timing of somite segmentation disturbances following a pulse of her7 misexpression, we deduce that although her7 continues to oscillate in the anterior half of the PSM, it governs the future somite segmentation behaviour of the cells only while they are in the posterior half. In general, the findings strongly support the mathematical model of how the somite clock works, but they do not exclude the possibility that other oscillator mechanisms may operate upstream from the her7/her1 oscillator or in parallel with it.
Descriptors: zebrafish, basic helix loop helix transcription factors genetics, biological clocks physiology, membrane somites physiology, transcription factors genetics, genetically modified, basic helix loop helix transcription factors metabolism, developmental gene expression regulation, heat shock response physiology, membrane proteins metabolism, time factors, transcription factors metabolism, genetic transcription, metabolism.

Glenney, G.W. and G.D. Wiens (2007). **Early diversification of the TNF superfamily in teleosts: genomic characterization and expression analysis**. *Journal of Immunology* 178(12): 7955-7973. ISSN: 0022 1767.
NAL Call Number: 448.8J8232
Abstract: The TNF superfamily (TNFSF) of proteins are cytokines involved in diverse immunological and developmental pathways. Little is known about their evolution or expression in lower vertebrate species. Bioinformatic searches of Zebrafish, Tetraodon, and Fugu genome and other teleost expressed sequence tag databases identified 44 novel gene sequences containing a TNF homology domain. This work reveals the following: (1) teleosts possess orthologs of BAFF, APRIL, EDA, TWEAK, 4-1BBL, Fas ligand, LIGHT, CD40L, RANKL, and possibly TL1A; (2) the BAFF-APRIL subfamily is enriched by a third member, BALM, unique to fish; (3) orthologs of lymphotoxins alpha and beta were not clearly identified in teleosts and are substituted by a related ligand, TNF-New; (4) as many as four TRAIL-like genes are present in teleosts, as compared with only one in mammals; and (5) T cell activation ligands OX40L, CD27L, CD30L, and GITRL were not identified in any fish species. Finally, we characterize mRNA expression of TNFSF members CD40L, LIGHT, BALM, APRIL, Fas ligand, RANKL, TRAIL-like, and TNF-New in rainbow trout, *Oncorhynchus mykiss*, immune and nonimmune tissues. In conclusion, we identified a total of 14 distinct TNFSF members in fishes, indicating expansion of this superfamily before the divergence of bony fish and tetrapods, ~360-450 million years ago. Based on these findings, we extend a model of TNFSF evolution and the coemergence of the vertebrate adaptive immune system.
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Descriptors: zebrafish, bioinformatics, cytokines, evolution, genomes, immune system, lymphocyte transformation, messenger RNA, nucleotide sequences, proteins, T lymphocytes, rainbow trout, Tetraodontidae, trout, TNFSF evolution.

Goering, L.M., K. Hoshijima, B. Hug, B. Bisgrove, A. Kispert, and D.J. Grunwald (2003). **An interacting network of T-box genes directs gene expression and fate in the zebrafish mesoderm.** *Proceedings of the National Academy of Sciences of the United States of America* 100(16): 9410-15. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: T-box genes encode transcription factors that play critical roles in generating the vertebrate body plan. In many developmental fields, multiple T-box genes are expressed in overlapping domains, establishing broad regions in which different combinations of T-box genes are coexpressed. Here we demonstrate that three T-box genes expressed in the zebrafish mesoderm, no tail, spadetail, and tbx6, operate as a network of interacting genes to regulate region-specific gene expression and developmental fate. Loss-of-function and gain-of-function genetic analyses reveal three kinds of interactions among the T-box genes: combinatorial interactions that generate new regulatory functions, additive contributions to common developmental pathways, and competitive antagonism governing downstream gene expression. We propose that T-box genes, like Hox genes, often function within gene networks comprised of related family members.

Descriptors: zebrafish, developmental gene expression regulation, mesoderm metabolism, T-box domain proteins genetics and physiology, base sequence, genotype, genetic models, molecular sequence data, Myod protein metabolism, plasmids metabolism, T-box domain proteins metabolism, trans activation genetics.

Goldsmith, P., H. Baier, and W.A. Harris (2003). **Two zebrafish mutants, ebony and ivory, uncover benefits of neighborhood on photoreceptor survival.** *Journal of Neurobiology* 57(3): 235-45. ISSN: 0022-3034.

NAL Call Number: QP351.J55

Descriptors: zebrafish, apoptosis physiology, photoreceptors pathology and physiology, retinal degeneration genetics, alkylating agents toxicity, chemically induced blindness, cell communication physiology, chimera, ethylnitrosourea toxicity, immunohistochemistry, in situ nick end labeling, mutation, retinal degeneration physiopathology.

Gong, B., D. Liang, T.G. Chew, and R. Ge (2004). **Characterization of the zebrafish vascular endothelial growth factor A gene: comparison with vegf-A genes in mammals and Fugu.** *Biochimica Et Biophysica Acta* 1676(1): 33-40. ISSN: 0006 3002.

NAL Call Number: 381B522

Abstract: Vascular endothelial growth factor (VEGF-A) is a key angiogenic growth factor which regulates vertebrate embryonic vascularization, adult physiology such as wound healing and reproduction as well as many human diseases. To understand the evolution and regulation of this gene in vertebrates, we have isolated and characterized the zebrafish vegf-A gene and compared it with VEGF-A genes of human, mouse as well as an in silico isolated VEGF-A homologue from pufferfish. Our results indicate that the zebrafish vegf-A gene is organized similarly to mammalian and Fugu VEGF-A genes, with eight exons interrupted by seven introns. However, zebrafish vegf-A introns are generally larger than mammalian introns while Fugu VEGF-A introns are much smaller. Furthermore, zebrafish exon 6 (z6) has a unique sequence while Fugu's exon 6 is highly homologous to the mammalian counterparts. Alternative splicing generates multiple vegf-A mRNA isoforms in zebrafish with Vegf(121) as the dominant isoform in adult and Vegf(165) as the dominant isoform in early embryos. The exon z6 containing isoform Vegf(12345z678) is only detected in heart, muscle, and early embryos while another isoform Vegf-A(1234577)(a)(8) is only detected in heart. Furthermore, no conserved 5' flanking sequences between zebrafish and Fugu were observed

while numerous conserved regions exist between human and mouse in this area. These results suggest both conserved and diverged functions of VEGF-A from fish to mammals since the separation of these two groups from their common ancestor about 450 million years ago and a diverged regulation of this gene since the separation of zebrafish from Fugu. These data will be valuable for future studies of VEGF-A gene regulation and function in different vertebrates.

Descriptors: zebrafish, molecular evolution, *Takifugu* genetics, vascular endothelial growth factor A genetics, amino acid sequence, conserved sequence, DNA primers, genetic databases, gene components, gene expression, molecular sequence data, reverse transcriptase PCR, sequence alignment, DNA sequence analysis.

Gong, H.Y., C.J. Lin, M.H. Chen, M.C. Hu, G.H. Lin, Y. Zhou, L.I. Zon, and J.L. Wu (2004). **Two distinct teleost hepatocyte nuclear factor 1 genes, hnf1alpha/tcf1 and hnf1beta/tcf2, abundantly expressed in liver, pancreas, gut and kidney of zebrafish.** *Gene* 338(1): 35-46. ISSN: 0378-1119.

NAL Call Number: QH442.A1G4

Abstract: Two distinct forms of zebrafish hepatocyte nuclear factor 1 (hnf1) were identified and referred to as hnf1alpha/tcf1 and hnf1beta/tcf2. Both hnf1 genes were shown to be expressed abundantly in liver, pancreas, gut and kidney. Zebrafish HNF1alpha and HNF-1beta proteins contain all HNF1 signature domains including the dimerization domain, POU-like domain and atypical homeodomain. Sequence and phylogenetic analysis reveals that zebrafish hnf1alpha is closer to tetrapodian hnf1alpha than to tetrapodian hnf1beta and zebrafish hnf1beta is highly conserved with tetrapodian hnf1beta. Existences of hnf1alpha and hnf1beta in teleost zebrafish, tilapia and fugu suggest that hnf1 gene duplication might occur before the divergence of teleost and tetrapod ancestors. Zebrafish hnf1alpha and hnf-1beta genes were mapped to linkage group LG8 and LG15 in T51 panel by RH mapping and are composed of 10 and 9 exons, respectively. Zebrafish hnf1beta gene with at least 11 genes in LG15 was identified to maintain the conserved synteny with those of human in chromosome 17 and those of mouse in chromosome 11. Our results indicate that distinct hnf1alpha and hnf1beta genes in teleosts had been evolved from the hnf1 ancestor gene of chordate.

Descriptors: zebrafish, DNA binding proteins genetics, gene expression profiling, nuclear transcription factors genetics, amino acid and base sequence, molecular cloning, complementary DNA chemistry and genetics, embryonic development, exons, developmental gene expression regulation, hepatocyte nuclear factor 1, hepatocyte nuclear factor 1 alpha and beta, in situ hybridization, intestines metabolism, introns, kidney, liver and pancreas metabolism, molecular sequence data, phylogeny, radiation hybrid mapping, sequence alignment, DNA sequence analysis, amino acid sequence homology.

Gong, Y., C. Mo, and S.E. Fraser (2004). **Planar cell polarity signalling controls cell division orientation during zebrafish gastrulation.** *Nature* 430(7000): 689-93. ISSN: 0028-0836.

NAL Call Number: 472 N21

Abstract: Oriented cell division is an integral part of pattern development in processes ranging from asymmetric segregation of cell-fate determinants to the shaping of tissues. Despite proposals that it has an important function in tissue elongation, the mechanisms regulating division orientation have been little studied outside of the invertebrates *Caenorhabditis elegans* and *Drosophila melanogaster*. Here, we have analysed mitotic divisions during zebrafish gastrulation using in vivo confocal imaging and found that cells in dorsal tissues preferentially divide along the animal-vegetal axis of the embryo. Establishment of this

animal-vegetal polarity requires the Wnt pathway components Silberblick/Wnt11, Dishevelled and Strabismus. Our findings demonstrate an important role for non-canonical Wnt signalling in oriented cell division during zebrafish gastrulation, and indicate that oriented cell division is a driving force for axis elongation. Furthermore, we propose that non-canonical Wnt signalling has a conserved role in vertebrate axis elongation, orienting both cell intercalation and mitotic division.
Descriptors: zebrafish, cell polarity physiology, gastrula cytology and physiology, signal transduction physiology, cell division and size, mitotic spindle apparatus physiology, messenger RNA genetics and metabolism.

Gong, Z., H. Wan, T.L. Tay, H. Wang, M. Chen, and T. Yan (2003). **Development of transgenic fish for ornamental and bioreactor by strong expression of fluorescent proteins in the skeletal muscle**. *Biochemical and Biophysical Research Communications* 308(1): 58-63 . ISSN: 0006-291X.
NAL Call Number: 442.8B5236
Descriptors: trasgenic zebrafish, bioreactors, gene expression, green fluorescent protein, muscles, recombinant, *Danio rerio*, gene expression, mylz2 gene, red fluorescent protein, yellow fluorescent protein, recombinant DNA, genetic engineering.

Gong, Z. (2004). ***Fish Development and Genetics: The Zebrafish and Medaka Models.***, Vol. 2, World Scientific Publishing Co. Pte. Ltd.: Singapore, 675 p. ISBN: 9812388214.
NAL Call Number: QL638.C94 F57
Descriptors: zebrafish, Danio rerio, Oryzias latipes, development, developmental biology, collected papers and use as model, genetics, zebrafish.

Gong ZhiYuan, Wu YiLian, S.P. Mudumana and S. Lin (2004). **Transgenic fish for developmental biology studies**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd. Singapore, Singapore , p. 476-516. ISBN: 9812388214.
NAL Call Number: QL638.C94 F57
Abstract: Transgenic technology is the introduction of foreign DNA into a host organism so that the function and regulation of the inserted foreign DNA can be studied in the transgenic organism. Over the past decade, most of the key transgenic techniques have been developed in various fish models. In the present review, we focus on the transgenic studies in two experimental fish models, the zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*). These include transient transgenic assays, stable transgenic lines focusing on GFP transgenic fish and their applications, conditional activation of transgene expression using inducible promoters and binary transgenic systems (GAL4-UAS and Cre-loxP), cell lineage ablation, insertional mutagenesis, gene traps and the potential of gene targeting approaches in fish. Future prospects of transgenic fish studies are also discussed. Copyright © 2008 CABI
Descriptors: zebrafish , animal models, DNA, gene expression, genes, mutations, promoters, reviews, transgenics, *Danio rerio*, *Oryzias latipes.*

Gonzalez Nunez, V., R. Gonzalez Sarmiento, and R.E. Rodriguez (2003). **Cloning and characterization of a full-length pronociceptin in zebrafish: evidence of the existence of two different nociceptin sequences in the same precursor.** *Biochimica Et Biophysica Acta* 1629(1-3): 114-8. ISSN: 0006 3002.
NAL Call Number: 381B522
Abstract: We report the cloning and molecular characterization of a zebrafish pronociceptin

gene, which is expressed in brain and intestine. This fish precursor codes for two different nociceptin peptides, one of which presents an opioid sequence in its N-terminus, being more similar to dynorphin A than its mammalian counterparts. Our results represent a new tool to investigate the function of nociceptin and its receptor in relation to pain and drug addiction.
Descriptors: zebrafish, opioid peptides genetics, protein precursors genetics, opioid receptors genetics, amino acid sequence, brain metabolism, molecular cloning, intestines metabolism, molecular sequence data, sequence alignment.

Gopinath, A., L. Andrew Tseng, and K.E. Whitlock (2004). **Temporal and spatial expression of gonadotropin releasing hormone (GnRH) in the brain of developing zebrafish (*Danio rerio*).** *Gene Expression Patterns* 4(1): 65-70. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Gonadotropin releasing hormone (GnRH) is a neuroendocrine decapeptide found in all vertebrate animals. The best understood function of GnRH is its endocrine function as a releasing hormone acting on the pituitary. But GnRH also functions as a neuromodulator within the nervous system. In a given species, GnRH occurs in a variety of forms that fall into three general categories based on pattern of protein/gene expression in the brain and amino acid sequence. The salmon GnRH (sGnRH), is found in the terminal nerve, where it has a neuromodulatory function. The sGnRH form is also the hypothalamic form in many fishes, although some fishes have a species specific form in the hypothalamus. Finally, chicken-GnRH-II (cGnRH-II), the most highly conserved form, is found in the midbrain where its function remains unclear. Here we have cloned the sGnRH and cGnRH-II cDNAs from zebrafish. By conducting stage specific in situ hybridization in developing zebrafish embryos, we provide a description of the spatial and temporal expression patterns of these genes. The location of sGnRH and cGnRH-II expressing cells is in agreement with previous reports of GnRH in the brains of adult fishes. Our results provide the first developmental description of GnRH gene expression where cGnRH-II and sGnRH are initially expressed at the onset of the first day after fertilization. Unlike what has been reported in many adult fishes, we did not find sGnRH expressed in the hypothalamic population during development.
Descriptors: zebrafish, brain embryology and metabolism, developmental gene expression regulation, gonadotropin releasing hormone genetics, metabolism, gene expression profiling, in situ hybridization, molecular sequence data, phylogeny, protein sequence analysis, amino acid sequence homology, thalamus embryology and metabolism, time factors.

Gore, A.V., S. Maegawa, A. Cheong, P.C. Gilligan, E.S. Weinberg, and K. Sampath (2005). **The zebrafish dorsal axis is apparent at the four-cell stage.** *Nature* 438(7070): 1030-5. ISSN: 0028-0836.
NAL Call Number: 472 N21
Abstract: A central question in the development of multicellular organisms pertains to the timing and mechanisms of specification of the embryonic axes. In many organisms, specification of the dorsoventral axis requires signalling by proteins of the Transforming growth factor-beta and Wnt families. Here we show that maternal transcripts of the zebrafish Nodal-related morphogen, Squint (Sqt), can localize to two blastomeres at the four-cell stage and predict the dorsal axis. Removal of cells containing sqt transcripts from four-to-eight-cell embryos or injection of antisense morpholino oligonucleotides targeting sqt into oocytes can cause a loss of dorsal structures. Localization of sqt transcripts is independent of maternal Wnt pathway function and requires a highly conserved sequence in the 3' untranslated region. Thus, the dorsoventral axis is apparent by early cleavage stages and may require the

maternally encoded morphogen Sqt and its associated factors. Because the 3' untranslated region of the human nodal gene can also localize exogenous sequences to dorsal cells, this mechanism may be evolutionarily conserved.

Descriptors: zebrafish, body patterning, cytology, embryology, 3' untranslated regions genetics, base sequence, metabolism, molecular sequence data, oocytes metabolism, messenger RNA genetics and metabolism, beta catenin metabolism.

Goto Kazeto, R., K.E. Kight, Y. Zohar, A.R. Place, and J.M. Trant (2004). **Localization and expression of aromatase mRNA in adult zebrafish.** *General and Comparative Endocrinology* 139(1): 72-84. ISSN: 0016-6480.

NAL Call Number: 444.8G28

Abstract: Estradiol plays a key role in the control of many behavioral and physiological aspects of reproduction therefore the expression of cytochrome P450 aromatase (CYP19), the enzyme responsible for the conversion of androgens to estrogens, is of vital interest. The zebrafish, and many other teleosts, have two aromatase genes (CYP19A1 and CYP19A2) that are expressed predominantly in the ovary and brain, respectively, however, the physiological impact of extra-gonadal aromatase has been poorly described. In this study, in situ hybridizations of whole-mount and paraffin sections of adult zebrafish brains, pituitaries, and ovarian follicles showed that CYP19A2 was strongly expressed in the olfactory bulb (OB), ventral telencephalon (TEL), preoptic area (POA), and ventral/caudal hypothalamic zone (HT) of the brain, and in the anterior and posterior lobes of the pituitary. The regional distribution of the CYP19A2 mRNA did not vary with sex however transcript abundance varied within (male "high expressers" had much higher expression in the OB, TEL, and HT than in "low expressers") and between sexes (higher in OB, TEL, and HT of males than in females). In situ hybridizations of CYP19A1 failed to develop a signal in the brain or pituitary but were detectable by RT-PCR. CYP19A1 was highly expressed in Stage III B follicles (>500 nm) with significantly lower levels in the Stage IV follicles (>680 nm), Stage III A follicles (>350 nm), and Stage I and II follicles (350 microm) which were embedded in connective tissues. The differential expression of the aromatase genes, particularly CYP19A2 in the brain, suggests that the two aromatase genes play different roles in the reproductive behavior and/or physiology of bony fish.

Descriptors: zebrafish, aromatase metabolism, aromatase genetics, brain enzymology, in situ hybridization, isoenzymes genetics, isoenzymes metabolism, ovarian follicle enzymology and physiology, pituitary gland enzymology, messenger RNA metabolism, reverse transcriptase PCR, sex factors, tissue distribution, proteins genetics.

Gouttenoire, J., U. Valcourt, C. Bougault, E. Aubert Foucher, E. Arnaud, L. Giraud, and F. Mallein Gerin (2007). **Knockdown of the intraflagellar transport protein IFT46 stimulates selective gene expression in mouse chondrocytes and affects early development in zebrafish.** *Journal of Biological Chemistry* 282(42): 30960-73. ISSN: 0021-9258.

NAL Call Number: 381J824

Abstract: Bone morphogenetic proteins (BMPs) act as multifunctional regulators in morphogenesis during development. In particular they play a determinant role in the formation of cartilage molds and their replacement by bone during endochondral ossification. In cell culture, BMP-2 favors chondrogenic expression and promotes hypertrophic maturation of chondrocytes. In mouse chondrocytes we have identified a BMP-2-sensitive gene encoding a protein of 301 amino acids. This protein, named mIFT46, is the mouse ortholog of recently identified *Caenorhabditis elegans* and *Chlamydomonas reinhardtii* intraflagellar transport (IFT) proteins. After generation of a polyclonal antibody against mIFT46, we showed for the first

time that the endogenous protein is located in the primary cilium of chondrocytes. We also found that mIFT46 is preferentially expressed in early hypertrophic chondrocytes located in the growth plate. Additionally, mIFT46 knockdown by small interfering RNA oligonucleotides in cultured chondrocytes specifically stimulated the expression of several genes related to skeletogenesis. Furthermore, Northern blotting analysis indicated that mIFT46 is also expressed before chondrogenesis in embryonic mouse development, suggesting that the role of mIFT46 might not be restricted to cartilage. To explore the role of IFT46 during early development, we injected antisense morpholino oligonucleotides in *Danio rerio* embryos to reduce zebrafish IFT46 protein (zIFT46) synthesis. Dramatic defects in embryonic development such as a dorsalization and a tail duplication were observed. Thus our results taken together indicate that the ciliary protein IFT46 has a specific function in chondrocytes and is also essential for normal development of vertebrates.
Descriptors: zebrafish, cartilage embryology, chondrocytes metabolism, chondrogenesis physiology, developmental gene expression regulation physiology, growth plate embryology, intracellular signaling peptides and proteins metabolism, bone morphogenetic proteins pharmacology, *Caenorhabditis elegans* embryology and genetics, *Caenorhabditis elegans* proteins antagonists and inhibitors, *Caenorhabditis elegans* proteins genetics and metabolism, *Chlamydomonas reinhardtii* genetics and metabolism, chondrocytes cytology, chondrogenesis drug effects, cilia genetics and metabolism, developmental gene expression regulation drug effects, growth plate cytology, intracellular signaling peptides and proteins antagonists, inhibitors and mice, antisense oligonucleotides genetics and pharmacology, protozoan proteins antagonists, inhibitors, genetics and metabolism, small interfering RNA genetics and pharmacology, amino acid sequence homology, transforming growth factor beta pharmacology, proteins antagonists and inhibitors.

Grabher, C., J.S. Joly, and J. Wittbrodt (2004). **Highly efficient zebrafish transgenesis mediated by the meganuclease I-SceI.** *Methods in Cell Biology* 77: 381-401. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish , genetically modified genetics, Type II deoxyribonucleases site specific metabolism and genetics, gene transfer techniques, microinjections methods, microinjections instrumentation.

Granato, M. (2006). **Motor axon guidance in the zebrafish embryo**. *Genes and Genetic Systems* 81(2): 154. ISSN: 1341 7568.
NAL Call Number: QH426.G462
Descriptors: zebrafish, nervous system, neural coordination, molecular biophysics.

Gratton, P., G. Allegrucci, M. Gallozzi, C. Fortunato, F. Ferreri, and V. Sbordoni (2004). **Allozyme and microsatellite genetic variation in natural samples of zebrafish, *Danio rerio*.** *Journal of Zoological Systematics and Evolutionary Research* 42(1): 54-62. ISSN: 0947 5745.
NAL Call Number: QL351.Z4
Abstract: In this paper, we report the results from allozyme and microsatellite markers in natural populations of the zebrafish, a species of great significance in biological studies. Four zebrafish wild samples from West Bengal, India, were analysed in a preliminary survey of levels and patterns of genetic variation. Results indicate high levels of genetic variability and weak genetic structure, although the latter is consistent with the geographical features of the area under study, sampling sites being located in the Ganges and Brahmaputra delta region, which is characterized by high waterways connectivity.
Descriptors: zebrafish, *Danio rerio*, enzymes , allozyme variation, molecular genetics,

microsatellite variation, population genetics, allozyme and microsatellite genetic variation, biochemical variation, allozyme and microsatellite variation, West Bengal, India.
Language of Text: German.

Gray, C., I.M. Packham, F. Wurmser, N.C. Eastley, P.G. Hellewell, P.W. Ingham, D.C. Crossman, and T.J. Chico (2007). **Ischemia is not required for arteriogenesis in zebrafish embryos.** *Arteriosclerosis Thrombosis and Vascular Biology* 27(10): 2135-41. ISSN: 1079 5642.
Abstract: OBJECTIVE: The role of ischemia in collateral vessel development (arteriogenesis) is a contentious issue that cannot be addressed using mammalian models. To investigate this, we developed models of arteriogenesis using the zebrafish embryo, which gains sufficient oxygenation via diffusion to prevent ischemia in response to arterial occlusion. METHODS AND RESULTS: We studied gridlock mutant embryos that suffer a permanently occluded aorta and show that these restore aortic blood flow by collateral vessels. We phenocopied gridlock mutants by laser-induced proximal aortic occlusion in transgenic Fli1:eGFP/GATA1:dsRED embryos. Serial imaging showed these restore aortic blood flow via collateral vessels by recruitment of preexisting endothelium in a manner similar to gridlocks. Collateral aortic blood flow in gridlock mutants was dependent on both nitric oxide and myeloid cells. Confocal microscopy of transgenic gridlock/Fli1:eGFP mutants demonstrated no aberrant angiogenic response to the aortic occlusion. qPCR of HIF1alpha expression confirmed the absence of hypoxia in this model system. CONCLUSIONS: We conclude that NO and myeloid cell-dependent collateral vessel development is an evolutionarily ancient response to arterial occlusion and is able to proceed in the absence of ischemia.
Descriptors: zebrafish, aortic diseases physiopathology, arterial occlusive diseases physiopathology, arteries growth and development, collateral circulation, ischemia physiopathology, physiologic neovascularization, aortic diseases embryology, genetics and metabolism, arterial occlusive diseases embryology, genetics and metabolism, arteries embryology and metabolism, basic helix loop helix transcription factors genetics and metabolism, cell hypoxia, disease models, hypoxia inducible factor 1, alpha subunit metabolism, ischemia embryology, genetics and metabolism, confocal microscopy, mutation, myeloid cells metabolism, nitric oxide metabolism, nitric oxide synthase metabolism, proto oncogene protein C Fli 1 genetics and metabolism, messenger RNA metabolism, time factors.

Gregg, R.G., G.B. Willer, J.M. Fadool, J.E. Dowling, and B.A. Link (2003). **Positional cloning of the young mutation identifies an essential role for the Brahma chromatin remodeling complex in mediating retinal cell differentiation**. *Proceedings of the National Academy of Sciences, USA* 100(11): 6535-6540. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, cell differentiation, cell fate, chromatin remodeling, developmental genetics, differentiation, eyes, freshwater fish, genes, MAP kinase, mutations, pattern formation, phenotypes, retina, retinas, *Danio rerio*, Brahma gene, Brahma-related gene, zebra fish, Brahma-related gene, young gene, developmental genetics.

Gross, J.M. and J.E. Dowling (2005). **Tbx2b is essential for neuronal differentiation along the dorsal/ventral axis of the zebrafish retina.** *Proceedings of the National Academy of Sciences of the United States of America* 102(12): 4371-6. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: The mechanisms by which retinal neurons are patterned along the dorsal/ventral axis remain largely unknown, yet this patterning is integral for the topographic mapping of visual space. With an interest in elucidating the mechanisms that regulate the development

of this retinal axis, we have characterized a T-box family transcription factor, Tbx2b, during zebrafish retinogenesis. Tbx2b is expressed throughout all phases of retinal development with a striking asymmetry of distribution highest dorsally to lowest ventrally. To examine Tbx2b function during retinal development, two morpholino antisense oligonucleotides were created; one blocking the translational start site of Tbx2b and the other interfering with Tbx2b mRNA splicing. Injection of either of these morpholinos resulted in profound defects in the development of the dorsal retina. By using molecular markers for neuronal subtypes, the ventral retina contained all cell types, whereas in the dorsal retina, only retinal ganglion cells expressed markers of differentiation. The cells of the dorsal retina were postmitotic, however, as demonstrated by a lack of BrdUrd incorporation during the normal periods of retinal differentiation. Markers for dorsal and ventral retinal compartments were also expressed normally in Tbx2b morphants. Combined, these observations suggest that the cellular mechanisms regulating neuronal differentiation within the retina are asymmetric about the dorsal/ventral axis and that Tbx2b mediates this process within the dorsal retina.
Descriptors: zebrafish, DNA binding proteins genetics, proteins genetics and physiology, retina embryology, transcription factors genetics and physiology and physiology, body patterning genetics, cell differentiation, developmental gene expression regulation, in situ hybridization, morphogenesis genetics, neurons cytology, oligodeoxyribonucleotides, antisense genetics and pharmacology, retina cytology.

Gross, J.M., B.D. Perkins, A. Amsterdam, A. Egana, T. Darland, J.I. Matsui, S. Sciascia, N. Hopkins, and J.E. Dowling (2005). **Identification of zebrafish insertional mutants with defects in visual system development and function.** *Genetics* 170(1): 245-61. ISSN: 0016-6731.
NAL Call Number: 442.8G28
Abstract: Genetic analysis in zebrafish has been instrumental in identifying genes necessary for visual system development and function. Recently, a large-scale retroviral insertional mutagenesis screen, in which 315 different genes were mutated, that resulted in obvious phenotypic defects by 5 days postfertilization was completed. That the disrupted gene has been identified in each of these mutants provides unique resource through which the formation, function, or physiology of individual organ systems can be studied. To that end, a screen for visual system mutants was performed on 250 of the mutants in this collection, examining each of them histologically for morphological defects in the eye and behaviorally for overall visual system function. Forty loci whose disruption resulted in defects in eye development and/or visual function were identified. The mutants have been divided into the following phenotypic classes that show defects in: (1) morphogenesis, (2) growth and central retinal development, (3) the peripheral marginal zone, (4) retinal lamination, (5) the photoreceptor cell layer, (6) the retinal pigment epithelium, (7) the lens, (8) retinal containment, and (9) behavior. The affected genes in these mutants highlight a diverse set of proteins necessary for the development, maintenance, and function of the vertebrate visual system.
Descriptors: zebrafish, eye embryology, eye abnormalities genetics, ocular physiology, behavior, cell survival genetics, eye anatomy and histology, eye growth and development, eye abnormalities metabolism, crystalline lens abnormalities, mutation, photoreceptors pathology, retina pathology, zebrafish growth and development.

Gruber, J., H. Manninga, T. Tuschl, M. Osborn, and K. Weber (2005). **Specific RNAi mediated gene knockdown in zebrafish cell Lines.** *RNA Biology* 2(3): 101-5. ISSN: 1547-6286.
Abstract: Here we demonstrate highly efficient RNA interference in ZFL, SJD and ZF4 cell lines derived from adult and embryonic zebrafish *Danio rerio*. Microinjection of siRNAs resulted in silencing in almost 100% of cells while transfection using cationic liposomes

led to silencing in 30%. Use of siRNAs against zebrafish lamin A, lamin B2 and the motor protein Eg5, led to knockdown of the target genes with the specific phenotypes expected from prior studies in mammalian cells. In contrast injection of lamin A, GL2 and eGFP siRNAs into zebrafish embryos resulted in morphological defects, abnormal development and early death of most embryos. The results indicate unspecific responses to siRNAs in the embryo but a fully developed and active RNAi machinery in cell lines.
Descriptors: zebrafish, RNAi, ZfL, SJd, Zf4, cell lines, microinjection.

Grzmil, M., D. Whiting, J. Maule, C. Anastasaki, J.F. Amatruda, R.N. Kelsh, C.J. Norbury, and E.E. Patton (2007). **The INT6 Cancer Gene and MEK signaling pathways Converge during zebrafish development.** *Public Library of Science One* 2(9): E959. ISSN: 1817-101X.
Abstract: BACKGROUND: Int-6 (integration site 6) was identified as an oncogene in a screen of tumorigenic mouse mammary tumor virus (MMTV) insertions. INT6 expression is altered in human cancers, but the precise role of disrupted INT6 in tumorigenesis remains unclear, and an animal model to study Int-6 physiological function has been lacking. PRINCIPAL FINDINGS: Here, we create an in vivo model of Int6 function in zebrafish, and through genetic and chemical-genetic approaches implicate Int6 as a tissue-specific modulator of MEK-ERK signaling. We find that Int6 is required for normal expression of MEK1 protein in human cells, and for Erk signaling in zebrafish embryos. Loss of either Int6 or Mek signaling causes defects in craniofacial development, and Int6 and Erk-signaling have overlapping domains of tissue expression. SIGNIFICANCE: Our results provide new insight into the physiological role of vertebrate Int6, and have implications for the treatment of human tumors displaying altered INT6 expression.
Descriptors: zebrafish embryos, INT6, cancer gene, MEK signaling, development, in vivo model .

Gu, X., F. Xu, W. Song, X. Wang, P. Hu, Y. Yang, X. Gao, and Q. Zhao (2006). **A novel cytochrome P450, zebrafish Cyp26D1, is involved in metabolism of all-trans retinoic acid.** *Molecular Endocrinology* 20(7): 1661-72. ISSN: 0888-8809.
NAL Call Number: QP187.3M64M6
Abstract: Retinoid signaling is essential for development of vertebrate embryos, and its action is mainly through retinoic acid (RA) binding to its RA receptors and retinoid-X receptors, while the critical concentration and localization of RA in embryos are determined by the presence and activity of retinal dehydrogenases (for RA synthesis) and cytochrome P450 RAs (Cyp26s) (for degradation of RA). Previously, we identified a novel cyp26 gene (cyp26d1) in zebrafish that is expressed in hindbrain during early development. Using reverse-phase HPLC analyses, we show here that zebrafish Cyp26D1 expressed in 293T cells could metabolize all-trans RA, 9-cis RA, and 13-cis RA, but could not metabolize retinol or retinal. The metabolites of all-trans RA produced by Cyp26D1 were the same as that produced by Cyp26A1, which are mainly 4-hydroxy-all-trans-RA and 4-oxo-all-trans-RA. Performing mRNA microinjection into zebrafish embryos, we demonstrated that overexpression of Cyp26D1 in embryos not only caused the distance between rhombomere 5 and the first somite of the injected embryos to be shorter than control embryos but also resulted in left-right asymmetry of somitogenesis in the injected embryos. These alterations were similar to those caused by the overexpression of cyp26a1 in zebrafish embryos and to that which resulted from treating embryos with 1 microm 4-diethylamino-benzaldehyde (retinal dehydrogenase inhibitor), implying that cyp26d1 can antagonize RA activity in vivo. Together, our in vitro and in vivo results provided direct evidence that zebrafish Cyp26D1 is involved in RA metabolism.

Descriptors: zebrafish, cytochrome P450 enzyme system metabolism and genetics, tretinoin metabolism, amino acid sequence, conserved sequence, cytochrome P450 enzyme system isolation and purification, molecular sequence data, tertiary protein structure, retinaldehyde metabolism, amino acid sequence homology, substrate specificity, tissue distribution genetics, tretinoin analogs and derivatives, vitamin A metabolism.

Gu, X., F. Xu, X. Wang, X. Gao, and Q. Zhao (2005). **Molecular cloning and expression of a novel CYP26 gene (cyp26d1) during zebrafish early development.** *Gene Expression Patterns* 5(6): 733-9. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Proper restriction of retinoid signaling by Cyp26s is essential for development of vertebrate embryos while inappropriate retinoid signaling can cause teratogenesis. Here, we report cloning and expression analysis of a novel cyp26 gene (cyp26d1) isolated from zebrafish. The predicted protein encoded by cyp26d1 consists of 554 amino acids. It exhibits 54% amino acid identity with human Cyp26C1, 50% with zebrafish Cyp26B1 and 38% with zebrafish Cyp26A1. Whole-mount in situ hybridization shows that cyp26d1 is first expressed in sphere stage, then disappears at 50% epiboly and resumes its expression at 75% epiboly. During segmentation period, cyp26d1 message is found at presumptive hindbrain. Double in situ hybridization with krox20 and cyp26d1 reveals that cyp26d1 is expressed in presumptive rhombomere 2-4 (r2-r4) at 2-somite stage. At 3-somite stage, cyp26d1 gene is expressed in r6 and pharyngeal arch (pa) one in addition to its expression at r2 and r4. At 6-somite stage, cyp26d1 message is present in continuous bands at r2-r6 and in pa1. This expression pattern is maintained from 10-somite stage through 21-somite stage except that the expression level is greatly reduced at r2 and r4. At 21-somite stage, cyp26d1 is also found in a group of cells in telencephalon and diencephalons. At 25-31h post-fertilization (hpf), the zebrafish cyp26d1 expression domain is extended to eyes, otic vesicles and midbrain in addition to its expression in hindbrain, telencephalon, diencephalons, and pharyngeal arches. At 35-48hpf, the expression of cyp26d1 is mainly restricted to otic vesicles, pharyngeal arches and pectoral fins and the expression level is greatly reduced.
Descriptors: zebrafish, cytochrome P450 enzyme system genetics, developmental gene expression regulation, amino acid sequence, body patterning, molecular cloning, conserved sequence, complementary DNA genetics, enzymology, enzymologic gene expression regulation, molecular sequence data, morphogenesis, sequence alignment, amino acid sequence homology.

Guner, B. and R.O. Karlstrom (2007). **Cloning of zebrafish nkx6.2 and a comprehensive analysis of the conserved transcriptional response to Hedgehog/Gli signaling in the zebrafish neural tube.** *Gene Expression Patterns* 7(5): 596-605. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Sonic Hedgehog (Shh) signaling helps pattern the vertebrate neural tube, in part by regulating the dorsal/ventral expression of a number of homeodomain containing transcription factors. These Hh responsive genes have been divided into two classes, with Class II genes being activated by Hh signaling and Class I genes being repressed by Hh signaling. While the transcriptional response to varying Hh levels is well defined in chick and mouse, it is only partially described in zebrafish, despite the fact that zebrafish has emerged as a powerful genetic system for the study of neural patterning. To better characterize the Hh response in the zebrafish neural tube, we cloned the zebrafish Class II Hh target genes nkx2.9 and nkx6.2. We then analyzed the expression of a number of Class I and Class II Hh responsive genes in wild type, Hh mutant, and Hh over-expressing zebrafish embryos. We show that

expression of Class I and Class II genes is highly conserved in the vertebrate neural tube. Further, ventral-most Class II gene expression was completely lost in all Hh pathway mutants analyzed, indicating high levels of Hh signaling are blocked in all of these mutants. In contrast, more dorsally expressed genes were variably affected in different Hh pathway mutants, indicating mid-levels of Hh signaling are differentially affected. This comprehensive expression study provides an important tool for the characterization of Hh signaling in zebrafish and provides a sensitive assay for determining the degree to which newly identified zebrafish mutants affect Hh signaling.

Descriptors: zebrafish, developmental gene expression regulation, Hedgehog proteins metabolism, homeodomain proteins genetics and metabolism, neurons metabolism, transcription factors genetics and metabolism, genetic transcription, molecular cloning, complementary DNA genetics and metabolism, cytology, metabolism, in situ hybridization, neurons cytology, RNA probes, messenger RNA administration and dosage, messenger RNA genetics and metabolism, signal transduction.

Guo, S. (2004). **Linking genes to brain, behavior and neurological diseases: what can we learn from zebrafish?** *Genes, Brain, and Behavior* 3(2): 63-74. ISSN: 1601-1848.

Abstract: How our brain is wired and subsequently generates functional output, ranging from sensing and locomotion to emotion, decision-making and learning and memory, remains poorly understood. Dys-regulation of these processes can lead to neurodegenerative, as well as neuro-psychiatric, disorders. Molecular genetic together with behavioral analyses in model organisms identify genes involved in the formation of neuronal circuits, the execution of behavior and mechanisms involved in neuro-pathogenesis. In this review I will discuss the current progress and future potential for study in a newly established vertebrate model organism for genetics, the zebrafish *Danio rerio*. Where available, schemes and results of genetic screens will be reviewed concerning the sensory, motor and neuromodulatory monoamine systems. Genetic analyses in zebrafish have the potential to provide important insights into the relationship between genes, neuronal circuits and behavior in normal as well as diseased states.

Descriptors: zebrafish, behavior physiology, biogenic monoamines physiology, genetic models, nervous system diseases genetics, brain physiology, genetic screening, models, motor skills physiology, neuroimmunomodulation, sensation genetics, signal transduction genetics.

Guo, X., L. Cheng, Y. Liu, W. Fan, and D. Lu (2007). **Cloning, expression, and functional characterization of zebrafish Mist1.** *Biochemical and Biophysical Research Communications* 359(1): 20-6. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: The basic helix-loop-helix (bHLH) protein Mist1 is an important exocrine pancreas transcriptional factor expressed in the acinar cells of mammals. In the present study, we cloned the homologous Mist1 cDNA encoding a predicted protein of 184 amino acids in zebrafish. The typical bHLH domain of zebrafish Mist1 shares high identity with that of its orthologs in mouse, rat, and human. Expression analysis revealed that Mist1 maternal transcripts are distinct in the very beginning of embryogenesis and that endogenous Mist1 is chronologically expressed in polster, hatching gland, hindbrain and appears exclusively in the pancreas from 72 hpf onward. Knockdown of Mist1 conditionally causes mild morphological defects in embryos. In MO-treated embryos, midbrain-hindbrain boundary is missing and exocrine pancreas is significantly reduced and disorganized. These results suggest that Mist1 functions in an evolutionary conserved way as a key transcriptional regulator specific for exocrine pancreas development in zebrafish.

Descriptors: zebrafish, basic helix loop helix transcription factors chemistry and physiology, *Drosophila* proteins chemistry and physiology, embryonic development physiology, pancreas embryology and physiology, amino acid sequence, molecular cloning, molecular evolution, developmental gene expression regulation physiology, molecular sequence data, organ specificity, amino acid sequence homology, tissue distribution.

Guo, Y., H. Cheng, X. Huang, S. Gao, H. Yu, and R. Zhou (2005). **Gene structure, multiple alternative splicing, and expression in gonads of zebrafish Dmrt1.** *Biochemical and Biophysical Research Communications* 330(3): 950-7. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: Many basic cellular processes are shared across vast phylogenetic distances, whereas sex-determining mechanisms are highly variable between phyla although the existence of two sexes is nearly universal in the animal kingdom. The only molecular similarity in sex determination found so far between phyla is among the fly doublesex, worm mab-3, and vertebrate Dmrt1/DMY, which contain a zinc-finger-like DNA-binding motif, DM domain. Here we report that three isoforms of the zebrafish Dmrt1 were generated in gonads by multiple alternative splicing, which encoded predicted proteins with 267, 246, and 132 amino acids, respectively. By cDNA cloning and genomic structure analysis, we found that there were seven exons of Dmrt1, which were alternatively spliced to generate the Dmrt1 isoforms. Northern blotting analysis revealed that expression of zebrafish Dmrt1 was higher in testis than ovary. Real time fluorescent quantitative RT-PCR indicated that expression of isoform a of Dmrt1 was dominantly higher than those of Dmrt1 b and c. Furthermore, in situ hybridization to gonads sections showed that Dmrt1 was expressed in developing germ cells of both testis and ovary, suggesting that the Dmrt1 gene is not only associated with testis development, but also, may be important in ovary differentiation of zebrafish.
Descriptors: zebrafish, alternative splicing genetics, developmental gene expression regulation, gonads metabolism, transcription factors genetics, amino acid and base sequence, molecular cloning, complementary DNA genetics, germ cells cytology, germ cells metabolism, gonads cytology, gonads growth and development , molecular sequence data, protein isoforms chemistry, genetics and metabolism, messenger RNA genetics and metabolism, sequence alignment, transcription factors chemistry and metabolism.

Guo, Y., Q. Li, S. Gao, X. Zhou, Y. He, X. Shang, H. Cheng, and R. Zhou (2004). **Molecular cloning, characterization, and expression in brain and gonad of Dmrt5 of zebrafish.** *Biochemical and Biophysical Research Communications* 324(2): 569-75. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: The DM domain gene family has at least eight members with conserved DNA-binding DM domain, which encodes putative transcription factors related to the sexual regulator Dsx of *Drosophila* and Mab-3 of *Caenorhabditis elegans*. Although some of the DM genes are involved in sexual development, the function of most of these genes remains unclear. There is also few structural and functional analysis concerning DM domain genes of the model fish, zebrafish. We report here molecular cloning, sequence, and expression of zebrafish Dmrt5, which consists of two exons, and encodes a 440-amino acid protein with conserved DMA and DMB domains in addition to DM domain. Phylogenetic analysis shows that zebrafish Dmrt5 fits within the Dmrt5 clade of fish and mammals. Zebrafish Dmrt5 was expressed in early gastrula period, subsequently increased to a high level in late stage of gastrula period (bud stage) and lower until the hatch period. In situ hybridization analysis showed its expression in developing central nervous system of embryos, especially in mid-brain and mid-hind brain boundary. In adult, its expression was restricted in brain and

developing germ cells, especially in spermatogonia, spermatocytes, spermatids, and sperm cells, and in developing oocytes, including early perinucleolus stage oocyte, late yolk vesicle stage oocyte, and oil drop stage oocyte. These data suggest that zebrafish Dmrt5 have potentially important roles in gonadal development and may have contributed to the functional endocrine axis.

Descriptors: zebrafish, brain metabolism, gonads metabolism, transcription factors biosynthesis and genetics, proteins biosynthesis and genetics, amino acid and base sequence, molecular cloning, DNA primers chemistry, DNA binding proteins genetics, proteins metabolism, *Drosophila* proteins metabolism, exons, gastrula metabolism, in situ hybridization, mice, rats, genetic models, molecular sequence data, oocytes metabolism, phylogeny, tertiary protein structure, reverse transcriptase PCR, amino acid sequence homology, sex differentiation.

Guryev, V., E. Berezikov, and E. Cuppen (2005). **CASCAD: a database of annotated candidate single nucleotide polymorphisms associated with expressed sequences**. *Bio Med Central Genomics* 6(10) ISSN: 1471 2164.

Online: http://www.biomedcentral.com/content/pdf/1471-2164-6-10.pdf

NAL Call Number: QH447.B63

Abstract: Background: With the recent progress made in large-scale genome sequencing projects a vast amount of novel data is becoming available. A comparative sequence analysis, exploiting sequence information from various resources, can be used to uncover hidden information, such as genetic variation. Although there are enormous amounts of SNPs for a wide variety of organisms submitted to NCBI dbSNP and annotated in most genome assembly viewers like Ensembl and the UCSC Genome Browser, these platforms do not easily allow for extensive annotation and incorporation of experimental data supporting the polymorphism. However, such information is very important for selecting the most promising and useful candidate polymorphisms for use in experimental setups. Description: The CASCAD database is designed for presentation and query of candidate SNPs that are retrieved by in silico mining of high-throughput sequencing data. Currently, the database provides collections of laboratory rat (*Rattus norvegicus*) and zebrafish (*Danio rerio*) candidate SNPs. The database stores detailed information about raw data supporting the candidate, extensive annotation and links to external databases (e.g. GenBank, Ensembl, UniGene, and LocusLink), verification information, and predictions of a potential effect for non-synonymous polymorphisms in coding regions. The CASCAD website allows search based on an arbitrary combination of 27 different parameters related to characteristics like candidate SNP quality, genomic localization, and sequence data source or strain. In addition, the database can be queried with any custom nucleotide sequences of interest. The interface is crosslinked to other public databases and tightly coupled with primer design and local genome assembly interfaces in order to facilitate experimental verification of candidates. Conclusions: The CASCAD database discloses detailed information on rat and zebrafish candidate SNPs, including the raw data underlying its discovery. An advanced web-based search interface http://cascad.niob.knaw.nl allows universal access to the database content and allows various queries supporting many types of research utilizing single nucleotide polymorphisms. Copyright © 2008 CABI

Descriptors: zebrafish, databases, expressed sequence tags, genetic polymorphism, genome analysis, genomes, nucleotide sequences, single nucleotide polymorphism, Danio rerio, rats.

Guryev, V., M.J. Koudijs, E. Berezikov, S.L. Johnson, R.H. Plasterk, F.J. van Eeden, and E. Cuppen (2006). **Genetic variation in the zebrafish.** *Genome Research* 16(4): 491-7. ISSN: 1088-9051.

NAL Call Number: QP606.D46P34

Abstract: Although zebrafish was introduced as a laboratory model organism several decades ago and now serves as a primary model for developmental biology, there is only limited data on its genetic variation. An establishment of a dense polymorphism map becomes a requirement for effective linkage analysis and cloning approaches in zebrafish. By comparing ESTs to whole-genome shotgun data, we predicted >50,000 high-quality candidate SNPs covering the zebrafish genome with average resolution of 41 kbp. We experimentally validated approximately 65% of a randomly sampled subset by genotyping 16 samples from seven commonly used zebrafish strains. The analysis reveals very high nucleotide diversity between zebrafish isolates. Even with the limited number of samples that we genotyped, zebrafish isolates revealed considerable interstrain variation, ranging from 7% (inbred) to 37% (wild-derived) of polymorphic sites being heterozygous. The increased proportion of polymorphic over monomorphic sites results in five times more frequent observation of a three allelic variant compared with human or mouse. Phylogenetic analysis shows that comparisons between even the least divergent strains used in our analysis may provide one informative marker approximately every 500 nucleotides. Furthermore, the number of haplotypes per locus is relatively large, reflecting independent establishment of the different lines from wild isolates. Finally, our results suggest the presence of prominent C-to-U and A-to-I RNA editing events in zebrafish. Overall, the levels and organization of genetic variation between and within commonly used zebrafish strains are markedly different from other laboratory model organisms, which may affect experimental design and interpretation.

Descriptors: zebrafish, genome genetics, single nucleotide polymorphism, RNA editing genetics, chromosome mapping methods, genetic markers genetics, linkage genetics, species specificity genetics.

Guyon, J.R., A.N. Mosley, S.J. Jun, F. Montanaro, L.S. Steffen, Y. Zhou, V. Nigro, L.I. Zon, and L.M. Kunkel (2005). **Delta-sarcoglycan is required for early zebrafish muscle organization.** *Experimental Cell Research* 304(1): 105-15. ISSN: 0014 4827.

NAL Call Number: 442.8EX7

Abstract: Mutations in sarcoglycans (alpha-, beta-, gamma-, and delta-) have been linked with limb girdle muscular dystrophy (LGMD) types 2C-F in humans. We have cloned the zebrafish orthologue encoding delta-sarcoglycan and mapped the gene to linkage group 21. The predicted zebrafish delta-sarcoglycan protein is highly homologous with its human orthologue including conservation of two of the three predicted glycosylation sites. Like other members of the dystrophin-associated protein complex (DAPC), delta-sarcoglycan localizes to the sarcolemmal membrane of the myofiber in adult zebrafish, but is more apparent at the myosepta in developing embryos. Zebrafish embryos injected with morpholinos against delta-sarcoglycan were relatively inactive at 5 dpf, their myofibers were disorganized, and swim bladders uninflated. Immunohistochemical and immunoblotting experiments show that delta-, beta-, and gamma-sarcoglycans were all downregulated in the morphants, whereas dystrophin expression was unaffected. Whereas humans lacking delta-sarcoglycan primarily show adult phenotypes, our results suggest that delta-sarcoglycan plays a role in early zebrafish muscle development.

Descriptors: zebrafish, mice, skeletal muscle embryology and cytology, sarcoglycans genetics, sarcoglycans physiology and physiology, amino acid and base sequence, molecular

cloning, down regulation, dystrophin metabolism, molecular sequence data, sarcoglycans analysis, sarcolemma chemistry, myofibers.

Guyon, J.R., A.N. Mosley, Y. Zhou, K.F. O'Brien, X. Sheng, K. Chiang, A.J. Davidson, J.M. Volinski, L.I. Zon, and L.M. Kunkel (2003). **The dystrophin associated protein complex in zebrafish.** *Human Molecular Genetics* 12(6): 601-15. ISSN: 0964-6906.
NAL Call Number: RB155.5.H87
Abstract: Many cases of muscular dystrophy in humans are caused by mutations in members of the dystrophin associated protein complex (DAPC). Zebrafish are small vertebrates whose bodies are composed predominantly of skeletal muscle, making them attractive models for studying mammalian muscle disorders. Potential orthologs to most of the human DAPC proteins have been found in zebrafish by database screening. Expression of the sarcoglycans, dystroglycan and dystrophin has been confirmed by western blotting. Immunohistochemical and biochemical techniques localize these proteins to the muscle cell membrane in adult zebrafish. Morpholino (MO) experiments designed to inhibit the translation of dystrophin mRNA produce juvenile zebrafish that are less active than zebrafish injected with control morpholinos. Western blot analysis of the dystrophin morpholino-injected zebrafish shows concurrent reduction of dystrophin and the sarcoglycans, suggesting that these proteins, like those in mammals, are part of a complex whose integrity is dependent on dystrophin expression. These results indicate that the zebrafish is an excellent animal model in which to approach the study of dystrophin and its associated proteins.
Descriptors: zebrafish, dystrophin biosynthesis and chemistry, amino acid and base sequence, Western blotting, cell membrane metabolism, chromosome mapping, molecular cloning, cytoplasm metabolism, complementary DNA metabolism, databases , polyacrylamide gel electrophoresis, exons, gene library, immunoblotting, immunohistochemistry, genetic models, molecular sequence data, skeletal muscle metabolism, muscles metabolism, phenotype, PCR, protein biosynthesis, amino acid sequence homology.

Hackett, P.B., K.J. Clark, S.C. Ekker and J.J. Essner (2004). **Applications of transposable elements in fish for transgenesis and functional genomics**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd.: Singapore, Singapore, p. 532-580 . ISBN: 9812388214.
NAL Call Number: QL638.C94 F57
Abstract: Transgenic fish were first made more than 30 years ago. Since then a variety of methods and constructs have been tested for introducing genetic sequences into fish for scientific investigations as well as commercial purposes. Here we review transposable elements and their applications in fish. Transposons can be used to deliver genes to chromosomes to confer new traits or as insertional agents and traps to uncover the functions and expression patterns of natural genes in chromosomes. Two DNA transposons have been characterized for transposon-based gene transfer and insertional mutagenesis. The first is the Sleeping Beauty transposon system that was reconstituted from a Tc1/mariner-like relic in salmonid genomes after more than a 10 million year evolutionary sleep. The second is a naturally occurring transposon from medaka, the Tol2 transposon that belongs to the hAT family of mobile elements. In comparison with random integration of plasmid sequences and pseudotyped retroviral genomes, transposons have several advantages for genetic studies in fish. These include introduction, of a single, defined DNA sequence into a cellular chromosome, stable expression from the integrant for multiple generations, no absolute size restrictions on the transferred gene, ease in construction and use, and safety. Early experiments have

validated the versatility of the Sleeping Beauty transposon for all of these purposes. The applications of transposon systems surpass use just in fish; the Sleeping Beauty transposon system is being used in mice to discover functions of genes and is being developed for gene therapy in humans. Copyright © 2008 CABI
Descriptors: transgenic zebrafish, models, chromosomes, DNA, genes, genomes, nucleotide sequences, plasmids, techniques, traits, transposable elements, *Oryzias latipes*, Salmonidae.
Notes: A review.

Hadrys, T., V. Prince, M. Hunter, R. Baker, and S. Rinkwitz (2004). **Comparative genomic analysis of vertebrate Hox3 and Hox4 genes**. *Journal of Experimental Zoology* 302b(2): 147-164. ISSN: 0022-104X.
NAL Call Number: 410 J825
Descriptors: zebrafish, alternative splicing, comparative studies, DNA, genomes, HOX protein, nucleotide sequence, transcription, transcription factors, *Danio rerio*, Teleostei, Tetraodontidae, vertebrata, blowfishes, globefishes, Hox3 gene, Hox4 gene, Kreisler protein, Krox20 protein, pbx protein, puffers, rabbitfishes, swellfishes, toadfishes, Valentino protein, comparative genomics.

Haffner, C., M. Frauli, S. Topp, M. Irmler, K. Hofmann, J. Regula, L. BallyCuif, and C. Haass (2004). **Nicalin and its binding partner nomo are novel nodal signaling antagonists**. *European Molecular Biology Organization Journal* 23(15): 3041-3050. ISSN: 0261 4189.
NAL Call Number: QH506.E46
Abstract: Nodals are signaling factors of the transforming growth factor-beta (TGFbeta) superfamily with a key role in vertebrate development. They control a variety of cell fate decisions required for the establishment of the embryonic body plan. We have identified two highly conserved transmembrane proteins, Nicalin and Nomo ((No)under bar>dal (mo) under bar>dulator, previously known as pM5), as novel antagonists of Nodal signaling. Nicalin is distantly related to Nicastrin, a component of the Alzheimer's disease-associated gamma-secretase, and forms a complex with Nomo. Ectopic expression of both proteins in zebrafish embryos causes cyclopia, a phenotype that can arise from a defect in mesendoderm patterning mediated by the Nodal signaling pathway. Accordingly, downregulation of Nomo resulted in an increase in anterior axial mesendoderm and the development of an enlarged hatching gland. Inhibition of Nodal signaling by ectopic expression of Lefty was rescued by reducing Nomo levels. Furthermore, Nodal-as well as Activin-induced signaling was inhibited by Nicalin and Nomo in a cell-based reporter assay. Our data demonstrate that the Nicalin/Nomo complex antagonizes Nodal signaling during mesendodermal patterning in zebrafish. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, biochemistry and molecular biophysics, development, Alzheimer's disease, behavioral and mental disorders, nervous system disease, cyclopia, congenital disease, development, ectopic expression, nicalin, Nodal signaling.

Haftek, Z., G. Morvan Dubois, B. Thisse, C. Thisse, R. Garrone, and D. Le Guellec (2003). **Sequence and embryonic expression of collagen XVIII NC1 domain (endostatin) in the zebrafish.** *Gene Expression Patterns* 3(3): 351-4. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Endostatin, located in the NC1 domain of the collagen XVIII, is believed to inhibit the migration and proliferation of endothelial cells (Fed. Am. Soc. Exp. Biol. J. 15 (2001) 1044) and to play a role in axon guidance in *Caenorhabditis elegans* (J. Cell Biol. 152 (2001) 1219). Zebrafish is an attractive vertebrate model to determine the role of endostatin

and the entire molecule of collagen XVIII during vertebrate development. Therefore, we have investigated the expression pattern of COL18A1 in zebrafish embryos from the segmentation to the hatching period stages.

Descriptors: zebrafish embryos, endostatins genetics, amino acid and base sequence, endostatins biosynthesis, model, in situ hybridization, molecular sequence data, metabolism.

Hagemann, S. and S.E. Hammer (2006). **The implications of DNA transposons in the evolution of P elements in zebrafish (*Danio rerio*).** *Genomics* 88(5): 572-9. ISSN: 0888-7543.

NAL Call Number: QH445.2.G45

Abstract: We have carried out detailed analyses of *Drosophila* P-transposon-homologous Pdre elements in zebrafish (*Danio rerio*) and present a model to explain the evolutionary origin of the complete and terminally truncated copies. Analysis of the most complex terminally truncated element led to the identification of novel DNA transposons, referred to as demio, hexi, and lady elements. We also show that the existence of a P-homologous stable gene can be explained in two ways: it is a P-transposon source gene, and insertion into or recombination with another transposon can lead to mobilization by recruitment of structures necessary for transposition (known as the source gene hypothesis), or it is the result of a domestication event involving a P transposon (domestication hypothesis). Our results indicate that both scenarios are possible and that they reflect different stages of the P-element life cycle.

Descriptors: zebrafish, DNA transposable elements genetics, molecular evolution, genetics, base sequence, DNA genetics, genomics, genetic models, sequence deletion, transposases genetics.

Hahn YoonSoo, Yang SeungKyoung, and Chung JaeHoon (2005). **Structure and expression of the zebrafish mest gene, an ortholog of mammalian imprinted gene PEG1/MEST**. *Biochimica Et Biophysica Acta, Gene Structure and Expression* 1731(2): 125-132. ISSN: 0167 4781.

NAL Call Number: QH450.B56

Abstract: PEG1/MEST is a paternally expressed gene in placental mammals. Here, we report identification of zebrafish (*Danio rerio*) gene mest, an ortholog of mammalian PEG1/MEST. Zebrafish mest encodes a polypeptide of 344 amino acids and shows a significant similarity to mammalian orthologs. Zebrafish mest is present as a single copy in the zebrafish genome and is closely linked to copg2 as in mammals. It is notable that 10 of 11 intron positions in mest are conserved among mammalian PEG1/MEST genes, indicating that the genomic organization and linkage between mest and copg2 loci was established in ancient vertebrates. Zebrafish mest is expressed in blastula, segmentation, and larval stages, exhibiting gradually increased expression as the development proceeds. Allelic expression analysis in hybrid larvae shows that both parental alleles are transcribed. We also observed one-codon alternative splicing involving an alternative usage of the two consecutive splice acceptors of intron 1, generating two protein isoforms with different lengths of a single amino acid. Copyright © 2008 CABI

Descriptors: zebrafish, alleles, amino acid sequences, complementary DNA, gene expression, genes, genomes, genomic imprinting, nucleotide sequences, *Danio rerio*.

Hale, L.A., A. Tallafuss, Y.L. Yan, L. Dudley, J.S. Eisen, and J.H. Postlethwait (2006). **Characterization of the retinoic acid receptor genes Raraa, Rarab and Rarg during zebrafish development.** *Gene Expression Patterns* 6(5): 546-55. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Retinoic acid signaling is important for patterning the central nervous system,

paired appendages, digestive tract, and other organs. To begin to investigate retinoic acid signaling in zebrafish, we determined orthologies between zebrafish and tetrapod retinoic acid receptors (Rars) and examined the expression patterns of rar genes during embryonic development. Analysis of phylogenies and conserved syntenies showed that the three cloned zebrafish rar genes include raraa and rarab, which are co-orthologs of tetrapod Rara, and rarg, which is the zebrafish ortholog of tetrapod Rarg. We did not, however, find an ortholog of Rarb. RNA in situ hybridization experiments showed that rarab and rarg, are maternally expressed. Zygotic expression of raraa occurs predominantly in the hindbrain, lateral mesoderm, and tailbud. Zygotic expression of rarab largely overlaps that of raraa, except that in later stages rarab is expressed more broadly in the brain and in the pectoral fin bud and pharyngeal arches. Zygotic expression of zebrafish rarg also overlaps the other two genes, but it is expressed more strongly in the posterior hindbrain beginning in late somitogenesis as well as in neural crest cells in the pharyngeal arches. Thus, these three genes have largely overlapping expression patterns and a few gene-specific expression domains. Knowledge of these expression patterns will guide the interpretation of the roles these genes play in development.
Descriptors: zebrafish, retinoic acid receptor genetics, genetics, base sequence, DNA primers, in situ hybridization, phylogeny.

Hale, M.E., M. Kheirbek, J.E. Schriefer, and V.E. Prince (2004). **Hox gene misexpression and cell-specific lesions reveal functionality of homeotically transformed neurons**. *Journal of Neuroscience* 24(12): 3070-3076. ISSN: 0270 6474.
Abstract: Hox genes are critical for establishing the segmental pattern of the vertebrate hindbrain. Changes in their expression can alter neural organization of hindbrain segments and may be a mechanism for brain evolution. To test the hypothesis that neurons induced through changes in Hox gene expression can integrate into functional neural circuits, we examined the roles of ectopic Mauthner cells (M-cells) in the escape response of larval zebrafish. The activity of the paired Mauthner cells in rhombomere 4 (r4) has been shown to be critical for generating a high-performance startle behavior in response to stimulation of the tail (Liu and Fetcho, 1999). Previous studies have found that misexpression of particular Hox genes causes ectopic M-cells to be generated in r2 in addition to the r4 cells (Alexandre et al., 1996; McClintock et al., 2001). With calcium imaging, we found that the homeotically transformed neurons respond to startle stimuli. To determine the roles of ectopic and endogenous M-cells in the behavior, we lesioned the r2, r4, or both M-cells with cell-specific laser lesion and examined the effect on startle performance. Lesion of the normal M-cells did not decrease escape performance when the ectopic cells were present. These results indicate that the homeotically transformed Mauthner cells are fully functional in the escape circuit and are functionally redundant with normal M-cells. We suggest that such functional redundancy between neurons may provide a substrate for evolution of neural circuits.
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Descriptors: zebrafish, behavior, development, molecular genetics, nervous system: neural coordination, calcium imaging, imaging and microscopy techniques, cell specific laser lesion, cell specific lesions, escape response, high performance startle behavior, neural circuit evolution, startle performance, tail stimulation, Hox gene.

Hammer, S.E., S. Strehl, and S. Hagemann (2005). **Homologs of *Drosophila* P transposons were mobile in zebrafish but have been domesticated in a common ancestor of chicken and human.** *Molecular Biology and Evolution* 22(4): 833-44. ISSN: 0707-4038.
NAL Call Number: QH506.M642
Abstract: A substantial fraction of vertebrate and invertebrate genomes is composed of

mobile elements and their derivatives. One of the most intensively studied transposon families, the P elements of *Drosophila*, was thought to exist exclusively in the genomes of dipteran insects. Based on the data provided by the human genome project, in 2001 our group has identified a P element-homologous sequence in the human genome. This P element-homologous human gene, named Phsa, is 19,533 nucleotides long, comprises six exons and five introns, and encodes a protein of still unknown function with a length of 903 amino acid residues. The N-terminal THAP domain of the putative Phsa protein shows similarities to the site-specific DNA-binding domain of the *Drosophila* P element transposase. In the present study, FISH analysis and the screening of a human lambda genomic library revealed a single copy of Phsa located on the long arm of chromosome 4, upstream of a gene coding for the hypothetical protein DKFZp686L1814. The same gene arrangement was found for the homologous gene Pgga in the genome of chicken, thus, displaying Pgga at orthologous position on the long arm of chromosome 4. The single-copy gene status and the absence of terminal inverted repeats and target-site duplications indicate that Phsa and Pgga constitute domesticated stationary sequences. In contrast, a considerable number of P-homologous sequences with terminal inverted repeats and intact target-site duplications could be identified in zebrafish, strongly indicating that Pdre elements were mobile within the zebrafish genome. Pdre elements are the first P-like transposons identified in a vertebrate species. With respect to Phsa, gene expression studies showed that Phsa is expressed in a broad range of human tissues, suggesting that the putative Phsa protein plays a not yet understood but essential role in a specific metabolic pathway. We demonstrate that P-homologous DNA sequences occur in the genomes of 21 analyzed vertebrates but only as rudiments in the rodents. Finally, the evolutionary history of P element-homologous vertebrate sequences is discussed in the context of the "molecular domestication" hypothesis versus the "source gene hypothesis."

Descriptors: zebrafish, *Drosophila*, chickens genetics , DNA transposable elements, amino acid and base sequence, DNA primers, genome , in situ hybridization, fluorescence, molecular sequence data, phylogeny, PCR, amino acid sequence homology, Phsa.

Hampson, R. and S.M. Hughes (2003). **Acridine mutagenesis of zebrafish (*Danio rerio*).** *Mutation Research* 525(1-2): 1-9. ISSN: 0027-5107.

NAL Call Number: QH431.M8

Descriptors: zebrafish, aminacrine analogs and derivatives, aminacrine toxicity, mutagenesis, mutagens toxicity, nitrogen mustard compounds toxicity, genetic crosses, drug dose response relationship, drug effects, chemically induced fish diseases , fish diseases genetics, germ line mutation, phenotype, PCR methods.

Hanaoka, R., I.B. Dawid, and A. Kawahara (2007). **Cloning and expression of zebrafish genes encoding the heme synthesis enzymes uroporphyrinogen III synthase (UROS) and protoporphyrinogen oxidase (PPO).** *DNA Sequence* 18(1): 54-60. ISSN: 1042 5179.

NAL Call Number: QH435.D57

Abstract: Heme is synthesized from glycine and succinyl CoA by eight heme synthesis enzymes. Although genetic defects in any of these enzymes are known to cause severe human blood diseases, their developmental expression in mammals is unknown. In this paper, we report two zebrafish heme synthesis enzymes, uroporphyrinogen III synthase (UROS) and protoporphyrinogen oxidase (PPO) that are well conserved in comparison to their human counterparts. Both UROS and PPO formed pairs of bilateral stripes in the lateral plate mesoderm at the 15-somite stage. At 24 h post-fertilization (hpf), UROS and PPO were predominantly expressed in the intermediate cell mass (ICM) that is the major site of primitive

hematopoiesis. The expression of UROS and PPO was drastically suppressed in the bloodless mutants cloche and vlad tepes/gata 1 from 15-somite to 24hpf stages, indicating that both cloche and vlad tepes/gata 1 are required for the induction and maintenance of UROS and PPO expression in the ICM.
Descriptors: zebrafish, heme biosynthesis, protoporphyrinogen oxidase genetics, base sequence, molecular cloning, DNA primers, developmental gene expression regulation, enzymologic gene expression regulation, in situ hybridization, protoporphyrinogen oxidase chemistry, uroporphyrinogen III synthetase chemistry and genetics.

Hanna, R., Y. Pang, P. Thomas, and Y. Zhu (2006). **Cell-surface expression, progestin binding, and rapid nongenomic signaling of zebrafish membrane progestin receptors alpha and beta in transfected cells.** *Journal of Endocrinology* 190(2): 247-60. ISSN: 0022-0795.
Abstract: Recently, a unique family of membrane progestin receptors (mPRalpha, mPRbeta, and mPRgamma) was identified, which may be responsible for mediating rapid, nongenomic actions of progestins in a variety of target tissues. In this study, the mPRalpha and mPRbeta isoforms from zebrafish were shown to be rapidly and specifically activated by the maturation-inducing steroid (MIS) of this species, 4-pregnen-17,20beta-diol-3-one (17,20beta-DHP). The zebrafish mPRalpha and a previously uncharacterized mPRbeta isoform were stably expressed in nuclear progesterone receptor-deficient mammalian breast cancer cells, MDA-MB-231. Expression and surface localization of the receptors were verified by flow cytometry, biotin surface labeling, and Western blotting. Plasma membrane proteins from mPRalpha- or mPRbeta-transfected cells showed high affinity (mPRalpha, K(d) 7 nM; mPRbeta, K(d) 12 nM), saturable, displaceable, single-binding sites specific for 17,20beta-DHP, whereas negligible specific 17,20beta-DHP binding was observed in nontransfected cells. Progestin treatment caused significant activation of mitogen-activated protein kinase (MAPK) within 5 min in cells transfected with either of the receptors as measured by western blotting and flow cytometry. The rank order of the potencies of several progestins in activating MAPK via mPRalpha and mPRbeta was the same (17,20beta-DHP>progesterone >4-pregnen-17,20beta,21-triol-3-one). Interestingly, the MIS in zebrafish, 17,20beta-DHP, was also the most potent inhibitor, among the progestins tested, of adenylyl cyclase activity in cells transfected with either of the receptors. This progestin significantly decreased cAMP levels in both mPRalpha- and mPRbeta-transfected cells in a dose-responsive and time-dependent manner. In addition, signaling of the zebrafish mPRalpha was blocked by pertussis toxin, implying activation of a G(i) protein, while sensitivity to pertussis or cholera toxin was not shown with mPRbeta-mediated signaling, possibly indicating that this receptor activates a different pertussis toxin-insensitive G protein. The results of this study suggest that zebrafish mPRalpha and mPRbeta signal similarly upon progestin binding resulting in rapid activation of MAPK and downregulation of adenylyl cyclase activity.
Descriptors: zebrafish, cell membrane metabolism, MAP kinase signaling system physiology, progestins metabolism and analysis, biotinylation, Western blotting methods, tumor cell line, cyclic AMP analysis, flow cytometry, gene expression, oocytes chemistry, protein binding, radioligand assay, progesterone receptors genetics, progesterone receptors metabolism, recombinant recombinant proteins metabolism, transfection methods, proteins genetics and metabolism.

Hans, S., D. Liu, J. Christison, and M. Westerfield (2005). **Fgf8 in otic placode induction**. *Federation of American Societies for Experimental Biology Journal* 19(5, Suppl. S, Part 2): A1368. ISSN: 0892 6638.
NAL Call Number: QH301.F3

Abstract: The inner ear arises from a specialized set of cells, the otic placode, that forms at the lateral edge of the neural plate adjacent to the hindbrain. Previous studies indicate that fibroblast growth factors (Fgfs) are necessary for otic placode induction, because loss of both fgf3 and fgf8 results in near or total ablation of otic tissue in zebrafish. Furthermore, gain-of-function studies suggest that Fgf signaling is not only necessary but also sufficient for ofic induction, leading to the formation of ectopic otic tissue after misexpression of Fgf3 or Fgf8. However, Fgf signaling also contributes to the establishment of the dorsoventral axis of the embryo and can induce the formation of a partial secondary axis. Using a transgenic approach that allows us to misexpressfgf8 under the control of the zebrafish temperature-inducible hsp70 promoter,we readdressed the role of Fgff during ofic placode induction. We find that misexpression of fgf8 throughout the embryo fails to induce formation of ectopic otic vesicles and that misexpression of fgf8 at different stages has different consequences. Overexpression in the midgastrula leads to a reduced otic placode and subsequently to formation of a smaller otic vesicle, whereas overexpression of fgf8 at the end of gastrulation produces a larger otic placode and larger otic vesicle. We suggest that this change in the effect of fgf8 overexpression reflects the changing role of Fgfs during otic specification.
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Descriptors: zebrafish, development, endocrine system, chemical coordination and homeostasis, sense organs, zebrafish.

Hansen, I.A., T.T. To, S. Wortmann, T. Burmester, C. Winkler, S.R. Meyer, C. Neuner, M. Fassnacht, and B. Allolio (2003). **The pro-opiomelanocortin gene of The zebrafish (*Danio rerio*).** *Biochemical and Biophysical Research Communications* 303(4): 1121-8 . ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: The cDNA and the gene for pro-opiomelanocortin (POMC) in the zebrafish (*Danio rerio*) were isolated and analyzed. The gene consists of three exons and two short introns and has a similar overall structural organization as in Homo sapiens. Intron 1 (339 bp) divides the 5(‘) untranslated region from the coding region while intron 2 (1522 bp) is located between the signal peptide and the sequence encoding ACTH. Transcription starts 26 bp downstream of a TATA box and there is one polyadenylation signal in the 3(‘) untranslated region. The cDNA comprises of 964 bp with an open reading frame encoding a 222 amino acid hormone prepropeptide that is split into six putative hormones. Sequence comparison of zebrafish POMC to sequences of various other vertebrate species reveals four regions that are highly conserved during the evolution of vertebrates-the N-terminal region, ACTH, beta-MSH, and beta-endorphin, whereas the connecting peptides show a much higher degree of variability. Phylogenetic analysis of the POMC sequences of various vertebrate species resulted in the expected pattern of species evolution. In situ hybridization demonstrated POMC expression in a cluster of cells (corticotrophs) in the pituitary of the zebrafish as early as 23 h after fertilization. These findings will facilitate the use of the zebrafish as a model organism in the study of the physiological role of POMC-derived peptides.
Descriptors: zebrafish, pro opiomelanocortin genetics and biosynthesis, amino acid and base sequence, chromosome mapping, molecular cloning, molecular sequence data, phylogeny, messenger RNA biosynthesis, sequence alignment, metabolism, proteins biosynthesis.

Harden, M.V., L.A. Newton, R.C. Lloyd, and K.E. Whitlock (2006). **Olfactory imprinting is correlated with changes in gene expression in the olfactory epithelia of the zebrafish.** *Journal of Neurobiology* 66(13): 1452-66. ISSN: 0022-3034.

NAL Call Number: QP351.J55
Descriptors: zebrafish, gene expression physiology, developmental gene expression regulation physiology, imprinting psychology physiology, olfactory mucosa physiology, behavior developmental gene expression regulation drug effects, in situ hybridization methods, maze learning physiology, microarray analysis methods, odors, olfactory mucosa cytology, otx transcription factors genetics, otx transcription factors metabolism, time factors and metabolism.

Hashiguchi, Y. and M. Nishida (2005). **Evolution of vomeronasal-type odorant receptor genes in the zebrafish genome.** *Gene* 362: 19-28. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: Teleost fishes have two distinct families of odorant receptors, main odorant receptors (MORs) and vomeronasal receptors family 2 (V2Rs), both of which are expressed in the olfactory epithelium where they detect soluble chemicals. To obtain an insight into the evolution of the fish V2R gene family, V2R genes and their chromosomal locations were identified from zebrafish genome sequences. Eighty-eight putative V2R genes and pseudogenes were identified, most being tightly clustered into two chromosomal regions. The phylogenetic analysis revealed that V2R genes belonging to the same phylogenetic clade were located close to one another on the same chromosome. However, some V2R genes were also found in several different chromosome regions, either as singletons or small gene clusters. Recent large-scale duplications of V2R-containing chromosomal regions were detected in two V2R gene clusters. The evolution of the zebrafish V2R gene family can be explained by repeated tandem gene duplications within gene clusters and large-scale duplications among chromosomes.
Descriptors: zebrafish, molecular evolution, phylogeny, vomeronasal organ, odorant receptors genetics, multigene family, physical chromosome mapping, proteins classification.

Hashimoto, H., S. Uji, T. Kurokawa, Y. Washio, and T. Suzuki (2007). **Flounder and fugu have a single lefty gene that covers the functions of lefty1 and lefty2 of zebrafish during L-R patterning.** *Gene* 387(1-2): 126-32. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: The lefty gene encodes a member of the TGF-beta superfamily that regulates L-R axis formation during embryogenesis via antagonistic activity against Nodal, another TGF-beta superfamily member. Both mouse and zebrafish have two lefty genes, lefty1 and lefty2. Interestingly, the expression domains of mouse and zebrafish lefty are different from one another. At present, the orthology and functional diversity of the mouse and zebrafish lefty genes are not clear. Here, we report that flounder and two fugu species, Takifugu and Tetraodon, have a single lefty gene in their genomes. In addition, we provide evidence that the mouse lefty genes were duplicated on a single chromosome but the zebrafish lefty genes arose from a whole-genome duplication that occurred early in the divergence of ray-finned fishes. These independent origins likely explain the difference in the expression domains of the mouse and zebrafish lefty gene pairs. Furthermore, we found that the duplication corresponding to the zebrafish lefty2 gene was lost from the fugu genome, suggesting that loss of lefty2 in the fugu/flounder lineage occurred after its divergence from the zebrafish lineage. During L-R patterning, the single lefty gene of flounder covers two expression domains, the left side of the dorsal diencephalon and the left LPM, which are regulated separately by lefty1 and lefty2 in zebrafish. We infer that the lefty genes of the ray-finned fishes and mammals underwent independent gene duplication events that resulted in independent regulation of lefty expression.
Descriptors: zebrafish, physiology, flounder genetics, *Takifugu* genetics, transforming

growth factor beta genetics, amino acid sequence, bone morphogenetic proteins metabolism, embryology, developmental gene expression regulation, molecular sequence data, transforming growth factor beta physiology.

Hashimoto, Y., H. Suzuki, Y. Kageyama, K. Yasuda, and K. Inoue (2006). **Bruno-like protein is localized to zebrafish germ plasm during the early cleavage stages.** *Gene Expression Patterns* 6(2): 201-5. ISSN: 1567 133X.
NAL Call Number: QH450.B73
Abstract: Maternally supplied germ plasm is essential for germ lineage establishment in many species, but the molecular details are still largely unknown, especially in vertebrates, and identification of novel factors that localize to germ plasm is desirable. We previously reported that one of the components of zebrafish germ plasm is mRNA of the bruno-like (brul) gene, a homologue of bruno, which, in Drosophila, is known to participate in germ lineage establishment. Here, we show that not only mRNA but also protein of brul is localized to the zebrafish germ plasm at the ends of the cleavage furrows. In 4- and 8-cell stage embryos, Brul protein is localized to the periphery of the blastomeres, as well as to the ends of the cleavage furrows, forming numerous minute particles. These particles appear at the cortex of the fertilized egg within 10 min after fertilization. Surprisingly, these distinctive localizations, as well as the minute particles, completely disappeared by the 16-cell stage, although relatively weak expression was detected ubiquitously throughout embryogenesis. This is the first report of a protein that localizes to the germ plasm in zebrafish.
Descriptors: zebrafish, RNA binding proteins metabolism, blastocyst metabolism, cleavage stage, ovum metabolism, germ cells metabolism, immunohistochemistry.

Havis, E., I. Anselme, and S. Schneider Maunoury (2006). **Whole embryo chromatin immunoprecipitation protocol for the in vivo study of zebrafish development.** *BioTechniques* 40(1): 34-38 . ISSN: 0736 6205.
NAL Call Number: RB37.1B56
Descriptors: zebrafish, chromatin immunoprecipitation methods, embryonic development genetics, acetylation, early growth response protein 2 metabolism, ultrastructure, histones metabolism, promoter regions genetics, transcription factors metabolism.

He, C. and X. Chen (2005). **Transcription regulation of the vegf gene by the BMP/Smad pathway in the angioblast of zebrafish embryos.** *Biochemical and Biophysical Research Communications* 329(1): 324-30. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: Vascular endothelial growth factor (VEGF) is a mitogen that is critically involved in vasculogenesis, angiogenesis, and hematopoiesis. However, what and how transcription factors participate in the regulation of vegf gene expression are not fully understood. Here we report the cloning and sequencing of the zebrafish vegf promoter which revealed that the promoter contains a number of bone morphogenetic protein (BMP)-activated Smad binding elements (SBE), implicating Smad1 and Smad5 in the regulation of BMP-induced expression of vegf. Electrophoretic mobility shift assays of adding recombinant Smad proteins to the SBE-containing DNA oligonucleotides that represent portions of zebrafish vegf promoter resulted in mobility shift of the oligonucleotides. These changes demonstrate potential interactions between Smad1/5 and the vegf promoter. Reporter activity assays using the wild-type or SBE-deleted vegf promoters to drive the luciferase reporter gene expression revealed that Smad1 stimulated while Smad5 repressed the vegf promoter activity in zebrafish embryos. These data indicate that the BMP/Smad signaling pathway is involved in the regulation of

zebrafish vegf transcription. In addition, we demonstrate that transgenic expression of human BMP4 in zebrafish embryos induced an expansion of the posterior intermediate cell mass (ICM, also commonly called blood island), a population of cells containing endothelial and hematopoietic precursors. In the expanded ICM, vegf and VEGF receptor 2 (flk-1) were ectopically co-expressed, suggesting that an autocrine/paracrine regulation of vegf expression may exist and contribute to the BMP-induced hemangiogenic cell proliferation.
Descriptors: zebrafish, bone morphogenetic proteins metabolism, gene expression regulation, physiologic neovascularization, genetic transcription, vascular endothelial growth factor A biosynthesis and genetics, base sequence, bone morphogenetic proteins chemistry, cell proliferation, molecular cloning, complementary DNA metabolism, DNA binding proteins genetics, proteins metabolism, reporter genes, in situ hybridization, luciferases metabolism, molecular sequence data, site directed mutagenesis oligonucleotides chemistry, phosphoproteins metabolism, promoter regions genetics, protein binding, recombinant proteins chemistry, signal transduction, Smad proteins, Smad1 and Smad5 protein, time factors, trans activators metabolism, transgenes.

He, C.B., Y.L. Mu, Z.S. Wang, Z.C. Zhou, and W.D. Liu (2006). **Phylogenetic analysis of CC chemokine genes of fish**. *Journal of Fishery Sciences of China/Zhongguo Shuichan Kexue* 13(1): 119-127. ISSN: 1005-8737.
Descriptors: zebrafish, bioinformatics, CC chemokines, CCL17, CCL19, CCL21 and CCL22 protein, cell activation, cytokines, leukocytes, phylogeny, structure-function relationships, genomics, orthology , cichlidae, *Danio rerio*, fundulus, Ictalurus furcatus, melanochromis auratus, *Oncorhynchus mykiss*, *Paralabidochromis chilotes*, *Paralichthys olivaceus*, *Salmo salar*, Atlantic salmon, blue catfish, cichlids, Japanese flounder, Malawi golden cichlid, olive flounder, rainbow trout.

Helterline, D.L., D. Garikipati, D.L. Stenkamp, and B.D. Rodgers (2007). **Embryonic and tissue-specific regulation of myostatin-1 and -2 gene expression in zebrafish.** *General and Comparative Endocrinology* 151(1): 90-7. ISSN: 0016-6480.
NAL Call Number: 444.8G28
Abstract: Myostatin is a member of the TGF-beta superfamily and a potent negative regulator of muscle growth and development in mammals. Its expression is limited primarily to skeletal muscle in mammals, but occurs in many different fish tissues, although quantitative measurements of the embryonic and tissue-specific expression profiles are lacking. A recent phylogenetic analysis of all known myostatin genes identified a novel paralogue in zebrafish, zfMSTN-2, and prompted the reclassification of the entire subfamily to include MSTN-1 and -2 sister clades in the bony fishes. The differential expression profiles of both genes were therefore determined using custom RNA panels generated from pooled (100-150/sampling) embryos at different stages of development and from individual adult tissues. High levels of both transcripts were transiently present at the blastula stage, but were undetectable throughout gastrulation (7 hpf). Levels of zfMSTN-2 peaked during early somitogenesis (11 hpf), returned to basal levels during late somitogenesis and did not begin to rise again until hatching (72 hpf). By contrast, zfMSTN-1 mRNA levels peaked during late somitogenesis (15.5-19 hpf), returned to baseline at 21.5 hpf and eventually rose 25-fold by 72 hpf. In adults, both transcripts were present in a wide variety of tissues, including some not previously known to express myostatin. Expression of zfMSTN-1 was highest in brain, muscle, heart and testes and was 1-3 log orders above that in other tissues. It was also greater than zfMSTN-2 expression in most tissues, nevertheless, levels of both transcripts increased almost 600-fold in spleens of fish subjected to stocking stress. Myostatin expres-

sion was also detected in mouse spleens, suggesting that myostatin may influence immune cell development in mammals as well as fish. These studies indicate that zfMSTN-1 and -2 gene expression is differentially regulated in developing fish embryos and in adult tissues. The increased expression of both genes in spleens from stressed fish is further supportive of an immunomodulatory role and may explain increased disease susceptibility associated with stocking stress.

Descriptors: zebrafish, mice, gene expression profiling, developmental gene expression regulation, transforming growth factor beta genetics, messenger RNA genetics and metabolism, reverse transcriptase PCR, spleen metabolism, transforming growth factor beta metabolism, stocking stress, disease susceptibility.

Hendricks, M. and Suresh Jesuthasan (2004). **Form and function in the zebrafish nervous system**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd. Singapore, Singapore , p. 164-184. ISBN: 9812388214.

NAL Call Number: QL638.C94 F57

Descriptors: zebrafish, behavior, biological development, brain, cell differentiation, cytoskeleton, embryos, genetics, genomes, innervation, morphology, nervous system, neuroglia, neurons, neurotransmitters, perception, physiological functions, screening, sense organs, synapsis, *Danio rerio.*

Her, G.M., C.C. Chiang, W.Y. Chen, and J.L. Wu (2003). **In vivo studies of liver-type fatty acid binding protein (L-FABP) gene expression in liver of transgenic zebrafish (*Danio rerio*).** *Federation of European Biochemical Societies Journal* 538(1-3): 125-33. ISSN: 1742 464X.

NAL Call Number: QP501.E8

Abstract: Mammalian liver fatty acid binding protein (L-FABP) is a small cytosolic protein in various tissues including liver, small intestine and kidney and is thought to play a crucial role in intracellular fatty acid trafficking and metabolism. To better understand its tissue-specific regulation during zebrafish hepatogenesis, we isolated 5'-flanking sequences of the zebrafish L-FABP gene and used a green fluorescent protein (GFP) transgenic strategy to generate liver-specific transgenic zebrafish. The 2.8-kb 5'-flanking sequence of zebrafish L-FABP gene was sufficient to direct GFP expression in liver primordia, first observed in 2 dpf embryos and then continuously to the adult stage. This pattern of transgenic expression is consistent with the expression pattern of the endogenous gene. F2 inheritance rates of 42-51% in all the seven transgenic lines were consistent with the ratio of Mendelian segregation. Further, hhex and zXbp-1 morphants displayed a visible liver defect, which suggests that it is possible to establish an in vivo system for screening genes required for liver development.

Descriptors: zebrafish, carrier liver metabolism, neoplasm proteins, nerve tissue proteins, proteins, genetically modified , base sequence, molecular cloning, DNA primers, fatty acid binding proteins, green fluorescent proteins metabolism, PCR.

Her, G.M., C.C. Chiang, and J.L. Wu (2004). **Zebrafish intestinal fatty acid binding protein (I-FABP) gene promoter drives gut-specific expression in stable transgenic fish.** *Genesis* 38(1): 26-31. ISSN: 1526-954X.

NAL Call Number: QH426.D32

Descriptors: zebrafish, carrier developmental gene expression regulation, intestines embryology, promoter regions genetics, genetically modified embryology and metabolism, fatty acid

binding proteins, green fluorescent proteins, intestines cytology and metabolism, luminescent proteins, organ specificity genetics, recombinant proteins.

Hermanson, S., A.E. Davidson, S. Sivasubbu, D. Balciunas, and S.C. Ekker (2004). **Sleeping beauty transposon for efficient gene delivery**. *Methods in Cell Biology* 77: 349-362. ISSN: 0091 679X.
NAL Call Number: 442P92
Descriptors: zebrafish, molecular genetics, biochemistry and molecular biophysics, gene delivery, Southern blot genetic techniques, electrophoretic techniques, genetic techniques, inverse PCR.

Hernandez, L.P. (2003). **Hedgehog signaling affects growth and differentiation of pharyngeal cartilages**. *Integrative and Comparative Biology* 43(6): 1010. ISSN: 1540 7063.
NAL Call Number: QL1.I67
Descriptors: zebrafish, development, skeletal system, molecular genetics , muscular system, dental and oral system, evolution and adaptation, mutant analysis, chondrogenesis, Hedgehog signaling, overall pharyngeal structure, visceral skeleton evolution, cartilage growth and differentiation.

Herzog, W., C. Sonntag, B. Walderich, J. Odenthal, H.M. Maischein, and M. Hammerschmidt (2004). **Genetic analysis of adenohypophysis formation in zebrafish.** *Molecular Endocrinology* 18(5): 1185-95. ISSN: 0888-8809.
NAL Call Number: QP187.3M64M6
Abstract: The adenohypophysis consists of at least six different cell types, somatotropes, lactotropes, thyrotropes, melanotropes, corticotropes, and gonadotropes. In mouse, cloning of spontaneous mutations and gene targeting has revealed multiple genes required for different steps of adenohypophysis development. Here, we report the results of a systematic search for genes required for adenohypophysis formation and patterning in zebrafish. By screening F3 offspring of N-ethyl-N-nitrosourea-mutagenized founder fish, we isolated eleven mutants with absent or reduced expression of GH, the product of somatotropes, but a normally developing hypothalamus. Of such mutants, eight were further analyzed and mapped. They define four genes essential for different steps of adenohypophysis development. Two of them, lia and pia, affect the entire adenohypophysis, whereas the other two are required for a subset of adenohypophyseal cell types only. The third gene is zebrafish pit1 and is required for lactotropes, thyrotropes, and somatotropes, similar to its mouse ortholog, whereas the fourth, aal, is required for corticotropes, melanotropes, thyrotropes, and somatotropes, but not lactotropes. In conclusion, the isolated zebrafish mutants confirm principles of adenohypophysis development revealed in mouse, thereby demonstrating the high degree of molecular and mechanistic conservation among the different vertebrate species. In addition, they point to thus far unknown features of adenohypophysis development, such as the existence of a new lineage of pituitary cells, which partially overlaps with the Pit1 lineage. Positional cloning of the lia, pia, and aal genes might reveal novel regulators of vertebrate pituitary development.
Descriptors: zebrafish, cell lineage genetics, mutation genetics, anterior pituitary gland growth, development and metabolism, molecular cloning, developmental gene expression regulation, morphogenesis genetics.

Hikima, J., M.L. Lennard, M.R. Wilson, N.W. Miller, L.W. Clem, and G.W. Warr (2006). **Conservation and divergence of the E micro 3' enhancer in the IGH locus of teleosts**. *ImmunoGenetics* 58(2/3): 226-234. ISSN: 0093 7711.

NAL Call Number: QR184.I4

Abstract: The core region of the E micro 3' transcriptional enhancer that drives the expression of the teleost IGH locus has been characterized functionally in two species, the catfish (*Ictalurus punctatus*) and the zebrafish (*Danio rerio*). These studies have suggested important differences: Whereas the catfish enhancer acts through an E-box and two octamer motifs, the zebrafish enhancer exerts its major effects through two E-box motifs alone. In this study, the function of the catfish enhancer was reexamined in a broader comparative context within the teleosts. Electrophoretic mobility shift assays of motifs from catfish, zebrafish, and Fugu were conducted to determine their ability to bind catfish E-protein and Oct transcription factors. Transient expression assays were conducted using a region of the catfish core enhancer that includes a newly described hybrid octamer/E-box motif. Sequences homologous to the E micro 3' enhancer region from six teleosts were aligned to determine conserved regions ("phylogenetic footprinting"). These studies allowed the following conclusions to be drawn: (1) The important 3'E-box motif described in the zebrafish corresponds in the homologous region of the catfish enhancer to an Oct motif with a newly described negative regulatory function and (2) Comparison of the E micro 3' enhancer sequences of six teleosts indicates that while a variety of octamer and E-box motifs are found in this region, strict evolutionary conservation of the important functional elements of the teleost E micro 3' enhancer has not occurred. Copyright © 2008 CABI

Descriptors: zebrafish, gene expression, genes, loci, phylogenetics, transcription, *Danio rerio*, *fishes*, *Ictalurus punctatus.*

Hinits, Y., D.P. Osborn, J.J. Carvajal, P.W. Rigby, and S.M. Hughes (2007). **Mrf4 (myf6) is dynamically expressed in differentiated zebrafish skeletal muscle.** *Gene Expression Patterns* 7(7): 738-45. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Mrf4 (Myf6) is a member of the basic helix-loop-helix (bHLH) myogenic regulatory transcription factor (MRF) family, which also contains Myod, Myf5 and myogenin. Mrf4 is implicated in commitment of amniote cells to skeletal myogenesis and is also abundantly expressed in many adult muscle fibres. The specific role of Mrf4 is unclear both because mrf4 null mice are viable, suggesting redundancy with other MRFs, and because of genetic interactions at the complex mrf4/myf5 locus. We report the cloning and expression of an mrf4 gene from zebrafish, *Danio rerio*, which shows conservation of linkage to myf5. Mrf4 mRNA accumulates in a subset of terminally differentiated muscle fibres in parallel with myosin protein in the trunk and fin. Although most, possibly all, trunk muscle expresses mrf4, the level of mRNA is dynamically regulated. No expression is detected in muscle precursor cell populations prior to myosin accumulation. Moreover, mrf4 expression is not detected in head muscles, at least at early stages. As fish mature, mrf4 expression is pronounced in the region of slow muscle fibres.

Descriptors: zebrafish, myogenic regulatory factors biosynthesis, myogenic regulatory factors physiology, amino acid sequence, amnion cytology, cardiac myosins biosynthesis and physiology, gene expression regulation, molecular sequence data, muscles metabolism, myogenic regulatory factor 5 metabolism, myogenin metabolism, myosin light chains biosynthesis and physiology, phylogeny, amino acid sequence homology, tissue distribution.

Hirata, H., L. Saint Amant, G.B. Downes, W.W. Cui, W. Zhou, M. Granato, and J.Y. Kuwada (2005). **Zebrafish bandoneon mutants display behavioral defects due to a mutation in the glycine receptor beta-subunit.** *Proceedings of the National Academy of Sciences of the United States of America* 102(23): 8345-50. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: Bilateral alternation of muscle contractions requires reciprocal inhibition between the two sides of the hindbrain and spinal cord, and disruption of this inhibition should lead to simultaneous activation of bilateral muscles. At 1 day after fertilization, wild-type zebrafish respond to mechanosensory stimulation with multiple fast alternating trunk contractions, whereas bandoneon (beo) mutants contract trunk muscles on both sides simultaneously. Similar simultaneous contractions are observed in wild-type embryos treated with strychnine, a blocker of the inhibitory glycine receptor (GlyR). This result suggests that glycinergic synaptic transmission is defective in beo mutants. Muscle voltage recordings confirmed that muscles on both sides of the trunk in beo are likely to receive simultaneous synaptic input from the CNS. Recordings from motor neurons revealed that glycinergic synaptic transmission was missing in beo mutants. Furthermore, immunostaining with an antibody against GlyR showed clusters in wild-type neurons but not in beo neurons. These data suggest that the failure of GlyRs to aggregate at synaptic sites causes impairment of glycinergic transmission and abnormal behavior in beo mutants. Indeed, mutations in the GlyR beta-subunit, which are thought to be required for proper localization of GlyRs, were identified as the basis for the beo mutation. These data demonstrate that GlyRbeta is essential for physiologically relevant clustering of GlyRs in vivo. Because GlyR mutations in humans lead to hyperekplexia, a motor disorder characterized by startle responses, the zebrafish beo mutant should be a useful animal model for this condition.

Descriptors: zebrafish, behavior physiology, mutation genetics, glycine receptors chemistry and genetics, proteins genetics and metabolism, disease models, gene expression profiling , developmental gene expression regulation , molecular sequence data, muscles innervation and physiology, protein subunits genetics and metabolism, glycine receptors antagonists and inhibitors, rhombencephalon metabolism, spinal cord metabolism, strychnine pharmacology, synaptic transmission, touch physiology.

Hirate, Y. and H. Okamoto (2006). **Canopy1, a novel regulator of FGF signaling around the midbrain-hindbrain boundary in zebrafish.** *Current Biology* 16(4): 421-7. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: FGF signaling from the midbrain-hindbrain boundary (MHB, isthmus) plays a major role both in maintenance of the MHB and induction of the tectum and cerebellum. Since different levels of FGF signaling in the MHB result in a qualitative difference in inducing activity, FGF signaling in the MHB should be tightly regulated positively and negatively at multiple steps to ensure correct levels of FGF signaling. Factors that negatively regulate FGF signal around the MHB are reported. However, factors that ensure strong FGF signal in the MHB are largely unknown. Here we report the identification of Canopy1 (Cnpy1), a novel MHB-specific, Saposin-related protein that belongs to an evolutionarily conserved protein family. The cnpy1 gene was expressed specifically in the MHB of zebrafish embryos. Exogenous FGF8 induced expression of cnpy1 in the tectal primordial. Knockdown of cnpy1 resulted in MHB defects and impaired FGF signaling in a cell-autonomous manner. Cnpy1 is localized in the endoplasmic reticulum and interacts with FGFR1. This study highlights a positive-feedback loop between the FGFR pathway and Cnpy1 that may ensure the strength of FGF signaling in the MHB, leading to correct development of the tectum and cerebellum.

Descriptors: zebrafish, brain stem embryology, brain stem physiology, fibroblast growth factor 8 physiology, nerve tissue, amino acid sequence, endoplasmic reticulum chemistry, molecular sequence data, nerve tissue proteins chemistry, type 1 fibroblast growth factor receptors physiology, signal transduction, proteins chemistry.

Hirate, Y., H. Okamoto, and K. Yamasu (2003). **Structure of the zebrafish fasciclin I-related extracellular matrix protein (betaig-h3) and its characteristic expression during embryogenesis.** *Gene Expression Patterns* 3(3): 331-6. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: betaig-h3, which is structurally related to the insect fasciclin I, is assumed to act as a cell adhesion molecule through binding to cell-surface integrins. In this study, we obtained cDNA clones for the zebrafish orthologue of betaig-h3 and examined the expression of the gene (betaig-h3) in zebrafish embryos using in situ hybridization. Expression is first seen at the bud stage in the presomitic mesoderm. Throughout the somitogenesis stage, betaig-h3 is expressed in all the segmented somites, as well as in the presomitic mesoderm (S0 and S-I). High expression is observed in the dorsolateral part of the somite until the mid-somitogenesis stage. At late somitogenesis stages, the betaig-h3 expression in the dorsolateral somite fades away, while expression is upregulated in the ventromedial part of the somite that corresponds to the sclerotome. In embryos after completion of somitogenesis and fry after hatching, betaig-h3 continues to be expressed in the sclerotome. In addition, new expression starts in the mesenchyme cells in the head, pharyngeal arches, and pectoral fins. In the embryonic brain, expression is observed along the anterior and postoptic commissures, as well as along the optic nerve.

Descriptors: zebrafish, extracellular matrix transforming growth factor beta genetics, amino acid sequence, extracellular matrix proteins biosynthesis, in situ hybridization, molecular sequence data , sequence alignment, transforming growth factor beta biosynthesis.

Hirayama, J., L. Cardone, M. Doi, and P. Sassone Corsi (2005). **Common pathways in circadian and cell cycle clocks: light-dependent activation of Fos/AP-1 in zebrafish controls CRY-1a and WEE-1.** *Proceedings of the National Academy of Sciences of the United States of America* 102(29): 10194-9. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: The cell cycle and the circadian clock are endogenous pacemakers, which coexist in most eukaryotic cells and share a number of conceptual features. In the zebrafish, light directly regulates the timing of both clocks, although the signaling and transcriptional pathways that convey photic information to essential nuclear regulators have yet to be deciphered. We have previously established the Z3 cell line, which recapitulates the features of zebrafish circadian clock and represents an ideal system to study light-dependent signaling and gene regulation. We conducted a search for light-responsive transcription factors and found that AP-1 DNA binding is highly induced. Light induces the expression of zWee1, a cell cycle gene essential for G2/M transition, and zCry1a, a clock gene of the feedback regulatory loop. We have found consensus AP-1 sites in the regulatory regions of both zWee1 and zCry1a genes, and we show that light inducibility of both genes is abrogated by inhibition of AP-1 function. Light also elicits chromatin remodeling by stimulating hyperacetylation at Lys-14 of histone H3 at both zWee1 and zCry1a promoters, as assessed by chromatin immunoprecipitation assays by using anti-Fos antibody. These findings provide strong evidence that circadian and cell cycle clocks share unique light-responsive pathways in zebrafish.

Descriptors: zebrafish, biological clocks physiology, cell cycle proteins metabolism, flavoproteins metabolism, gene expression regulation physiology, light, nuclear proteins metabolism, protein tyrosine kinases metabolism, transcription factor AP 1 metabolism, Western blotting, cell cycle physiology, cell cycle cell line, chromatin immunoprecipitation, circadian rhythm physiology, molecular cloning, flavonuclear promoter regions genetics, protein tyrosine kinases genetics.

Hirayama, J., S. Cho, and P. Sassone Corsi (2007). **Circadian control by the reduction/oxidation pathway: catalase represses light-dependent clock gene expression in the zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 104(40): 15747-52. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: Light is the key entraining stimulus for the circadian clock, but several features of the signaling pathways that convert the photic signal to clock entrainment remain to be deciphered. Here, we show that light induces the production of hydrogen peroxide (H(2)O(2)) that acts as the second messenger coupling photoreception to the zebrafish circadian clock. Treatment of light-responsive Z3 cells with H(2)O(2) triggers the induction of zCry1a and zPer2 genes and the subsequent circadian oscillation of zPer1. Remarkably, the induction kinetics and oscillation profile in response to H(2)O(2) are identical to those initiated by light. Catalase (Cat), an antioxidant enzyme degrading H(2)O(2), shows an oscillating pattern of expression and activity, antiphasic to zCry1a and zPer2. Interestingly, overexpression of zCAT results in a reduced light-dependent zCry1a and zPer2 gene induction. In contrast, inhibition of zCAT function enhances light-mediated inducibility of these clock genes. These findings implicate the enzymatic function of zCAT enzyme in the negative regulation of light-dependent clock gene transcriptional activation. Our findings provide an attractive link between the regulation of the cellular reduction/oxidation (redox) state and the photic signaling pathways implicated in circadian control.

Descriptors: zebrafish, catalase genetics, circadian rhythm, enzymologic gene expression regulation radiation effects, eye hydrogen peroxide metabolism and pharmacology , light, oxidation reduction, reactive oxygen species, trans activators genetics.

Hirayama, J., I. Fukuda, T. Ishikawa, Y. Kobayashi, and T. Todo (2003). **New role of zCRY and zPER2 as regulators of sub-cellular distributions of zCLOCK and zBMAL proteins**. *Nucleic Acids Research* 31(3): 935-943. ISSN: 0305-1048.

NAL Call Number: QD341.A2N8

Descriptors: zebrafish, biological clocks, circadian rhythms, DNA, gene expression, genes, nucleotide sequence, proteins, repressors, transcription activation, cDNA, *Danio rerio*, BMAL protein, clock protein, CRY1a protein, CRY3 protein, PER2 protein, transcription factors, genetics and evolution.

Hirayama, J., H. Nakamura, T. Ishikawa, Y. Kobayashi, and T. Todo (2003). **Functional and structural analyses of cryptochrome: vertebrate CRY regions responsible for interaction with the CLOCK:BMAL1 heterodimer and its nuclear localization**. *Journal of Biological Chemistry* 278(37): 35620-35628. ISSN: 0021-9258.

NAL Call Number: 381J824

Descriptors: zebrafish, amino acid sequence, DNA, DNA-binding protein, deoxyribodipyrimidine photo-lyase, inhibitors, nucleotide sequence, transcription factors, cryptochromes, *Danio rerio*, BMAL1 protein, clock protein, CRY1, CRY1a and CRY3 protein, cryptochromes.

Hirsinger, E., F. Stellabotte, S.H. Devoto, and M. Westerfield (2004). **Hedgehog signaling is required for commitment but not initial induction of slow muscle precursors**. *Developmental Biology* 275(1): 143-157. ISSN: 0012-1606.

NAL Call Number: 442.8D49

Descriptors: zebrafish, cell differentiation, developmental stages, embryonic development,

freshwater fish, gene expression, morphogenesis, muscle tissue, organogenesis, *Danio rerio*, reproduction and development.

Hoegg, S., H. Brinkmann, J.S. Taylor, and A. Meyer (2004). **Phylogenetic timing of the fish-specific genome duplication correlates with the diversification of teleost fish**. *Journal of Molecular Evolution* 59(2): 190-203. ISSN: 0022-2844.
NAL Call Number: QH359.J6
Descriptors: zebrafish, evolution, freshwater fish, genetic diversity, genomes, monophenol monooxygenase, nucleotide sequence, phenotypes, phylogenetics, phylogeny, species diversity, gene duplication, molecular evolution, tyrosinase, Acipenser, Acipenseriformes, *Actinopterygii*, Cichlidae, *Danio rerio*, Elopomorpha, *Oryzias latipes*, Osteoglossiformes, Osteoglossomorpha, polypterus, Semionotiformes, cichlids, Medaka, ray-finned fishes, spiny rayed fishes, spoonfishes, sturgeons, taxonomy, systematics and evolutionary genetics.

Hoffman, T.L., A.L. Javier, S.A. Campeau, R.D. Knight, and T.F. Schilling (2007). **Tfap2 transcription factors in zebrafish neural crest development and ectodermal evolution.** *Journal of Experimental Zoology. Part B. Molecular and Developmental Evolution* 308(5): 679-91. ISSN: 1552-5007.
NAL Call Number: QL1.J87
Descriptors: zebrafish, ectoderm physiology, developmental gene expression regulation genetics, neural crest embryology, transcription factor ap 2 physiology, amino acid sequence, chordata genetics, DNA primers chemistry, embryology, gene expression profiling veterinary, keratins biosynthesis and drug effects, molecular sequence data, multigene family physiology, phylogeny, protein sequence analysis veterinary, transcription factor ap 2 biosynthesis and genetics and pharmacology, *Xenopus* proteins pharmacology.

Hogan, B.M., J.A. Danks, J.E. Layton, N.E. Hall, J.K. Heath, and G.J. Lieschke (2005). **Duplicate zebrafish PTH genes are expressed along the lateral line and in the central nervous system during embryogenesis.** *Endocrinology* 146(2): 547-51. ISSN: 0013 7227.
NAL Call Number: 448.8EN2
Abstract: PTH plays a critical role in calcium metabolism in tetrapods. The primary site of PTH expression is the parathyroid glands, although it is also detected in the thymus and hypothalamus. Fish lack anatomically distinct parathyroid glands, and the first animals to evolve parathyroid glands were the amphibians. However, fish do have PTH family ligands and receptors, which are functionally similar to their mammalian counterparts. We report the expression patterns of duplicate zebrafish pth genes during embryogenesis. Both zebrafish pth1 and pth2 transcripts are expressed along the lateral line before the migration of the lateral line primordium and later in development Pth protein is detected in lateral line neuromasts by immunohistochemistry. pth1 transcripts are also detected in the central nervous system in the ventral neural tube. These temporally and anatomically restricted expression patterns imply a novel role for PTH family hormones during embryonic development of the zebrafish and allow for the genetic dissection of PTH function in this model organism.
Descriptors: zebrafish, afferent pathways physiology, central nervous system physiology and embryology, developmental gene expression regulation, parathyroid hormone genetics, afferent pathways embryology, animal structures embryology and physiology, molecular cloning, mechanoreceptors physiology, parathyroid glands.

Hogan, B.M., J.E. Layton, U.J. Pyati, S.L. Nutt, J.W. Hayman, S. Varma, J.K. Heath, D. Kimelman, and G.J. Lieschke (2006). **Specification of the primitive myeloid precursor pool requires signaling through Alk8 in zebrafish.** *Current Biology* 16(5): 506-11. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Abstract: In the zebrafish embryo, primitive hematopoiesis initiates in two spatially distinct regions. Rostrally, the cells of the anterior lateral plate mesoderm (ALPM) give rise exclusively to cells of the myeloid lineage in a pu.1-dependent manner. Caudally, in the posterior lateral plate mesoderm (PLPM), the expression of gata1 defines a precursor pool that gives rise predominantly to the embryonic erythrocytes. The transcription factor scl acts upstream of both gata1 and pu.1 in these precursor pools, activating a series of conserved transcription factors that cell-autonomously specify either myeloid or erythroid fates. However, the mechanisms underlying the spatial separation of the hematopoietic precursor pools and the induction of differential gene expression within these pools are not well understood. We show here that the Bmp receptor lost-a-fin/alk8 is required for rostral pu.1 expression and myelopoiesis, identifying an early genetic event that distinguishes between the induction of anterior and posterior hematopoiesis. Introducing a constitutively active version of the Alk8 receptor led to increased pu.1 expression, but the role of alk8 was independent of the scl-dependent cell-fate pathway. Furthermore, the role of Alk8 in myelopoiesis was genetically separable from its earlier role in dorsal-ventral embryonic patterning.
Descriptors: zebrafish, Type I activin receptors physiology, myeloid progenitor cells metabolism, myelopoiesis, signal transduction, metabolism, Type I activin receptors genetics and metabolism, body patterning, bone morphogenetic protein receptors metabolism, cell differentiation, cultured cells, developmental gene expression regulation, mice, myeloid progenitor cells cytology, proto oncogene proteins genetics and metabolism, trans activators genetics and metabolism.

Hogan, B.M., L. Pase, N.E. Hall, and G.J. Lieschke (2006). **Characterisation of duplicate zinc finger like 2 erythroid precursor genes in zebrafish.** *Development Genes and Evolution* 216(9): 523-9. ISSN: 0949 944X.
NAL Call Number: QL951.R68
Abstract: In separate expression pattern and micro-array screens the zinc finger containing factor, znfl2, has been previously implicated in hematopoiesis. Here we analysed znfl2 expression in detail and performed genetic epistatic analysis in a series of hematopoietic mutants and transient gain-of-function models. znfl2 expression in the hematopoietic intermediate mesoderm and derived erythrocytes required early genes cloche and spadetail, but not gata1. Expression was up-regulated in scl gain-of-function embryos, identifying znfl2 as an early erythroid factor that is regulated upstream or independently of gata1. Furthermore, we identified a duplicate znfl2 gene in the genome (znfl2b) which was expressed in early mesendoderm and weakly in the lateral plate mesoderm, overlapping in expression with znfl2. The production of loss-of-function models for znfl2, znfl2b and znfl2/znfl2b together suggested that these erythrocyte specific zinc finger genes are dispensible for erythropoiesis.
Descriptors: zebrafish, erythropoiesis genetics, gene expression, transcription factors genetics and analysis, zinc fingers, gene duplication, messenger RNA analysis and metabolism.

Holley, S.A. (2006). **Anterior-posterior differences in vertebrate segments: specification of trunk and tail somites in the zebrafish blastula.** *Genes and Development* 20(14): 1831-7. ISSN: 0890 9369.
NAL Call Number: QH426.G46
Descriptors: zebrafish, body patterning physiology, tail embryology, blastula, bone mor-

phogenetic proteins metabolism Fgf metabolism, developmental gene expression regulation, mesoderm metabolism, signal transduction, skeleton, somites, transforming growth factor beta genetics and metabolism, vertebrates embryology, Wnt proteins genetics and metabolism.

Holloway, B. and M.C. Mullins (2004). **The zebrafish maternal-effect mutant Betty Boop fails to complete epiboly**. *Molecular Biology of the Cell* 15(Suppl. S): 388A. ISSN: 1059 1524.
NAL Call Number: QH604.C452
Descriptors: Betty Boop mutant zebrafish, molecular genetics, enzymology, development, morphogenesis, maternal effect, epiboly.

Holmen, J., L.A. Vollestad, K.S. Jakobsen, and C.R. Primmer (2005). **Cross-species amplification of zebrafish and central stoneroller microsatellite loci in six other cyprinids**. *Journal of Fish Biology* 66(3): 851-859. ISSN: 0022 1112.
NAL Call Number: QL614.J68
Abstract: One hundred and twenty *Danio rerio and Campostoma anomalum* microsatellite loci were tested for amplification in the cyprinids *Phoxinus phoxinus, Scardinius erythrophthalmus, Rutilus rutilus, Abramis brama, Carassius carassius and Gobio gobio*. The PCR amplification products were recorded in 35 to 50 loci, depending on focal species. Eight to 28 of the amplifying loci were also polymorphic, establishing potential sets of genetic markers for population genetics studies. Copyright © 2008 CABI
Descriptors: zebrafish, genetic markers, genetic polymorphism, loci, microsatellites, PCR, *Abramis brama*, *Carassius carassius*, Cyprinidae, *Danio rerio*, *Gobio gobio*, *Phoxinus phoxinus*, *Rutilus rutilus.*

Holzschuh, J., G. Hauptmann, and W. Driever (2003). **Genetic analysis of the roles of Hh, FGF8, and nodal signaling during catecholaminergic system development in the zebrafish brain.** *Journal of Neuroscience* 23(13): 5507-19. ISSN: 0270-6474.
Abstract: CNS catecholaminergic neurons can be distinguished by their neurotransmitters as dopaminergic or noradrenergic and form in distinct regions at characteristic embryonic stages. This raises the question of whether all catecholaminergic neurons of one transmitter type are specified by the same set of factors. Therefore, we performed genetic analyses to define signaling requirements for the specification of distinct clusters of catecholaminergic neurons in zebrafish. In mutants affecting midbrain- hindbrain boundary (MHB) organizer formation, the earliest ventral diencephalic dopaminergic neurons appear normal. However, after 2 d of development, we observed fewer cells than in wild types, which suggests that the MHB provides proliferation or survival factors rather than specifying ventral diencephalic dopaminergic clusters. In hedgehog (Hh) pathway mutants, the formation of catecholaminergic neurons is affected only in the pretectal cluster. Surprisingly, neither fibroblast growth factor 8 (FGF8) alone nor in combination with Hh signaling is required for specification of early developing dopaminergic neurons. We analyzed the formation of prosomeric territories in the forebrain of Hh and Nodal pathway mutants to determine whether the absence of specific dopaminergic clusters may be caused by early patterning defects ablating corresponding parts of the CNS. In Nodal pathway mutants, ventral diencephalic and pretectal catecholaminergic neurons fail to develop, whereas both anatomical structures form at least in part. This suggests that Nodal signaling is required for catecholaminergic neuron specification. In summary, our results do not support the previously suggested dominant roles for sonic hedgehog and Fgf8 in specification of the first catecholaminergic neurons, but instead indicate a novel role for Nodal signaling in this process.

Descriptors: mutant zebrafish, brain metabolism, catecholamines metabolism, Fgf metabolism, trans activators metabolism, body patterning physiology, brain cytology and embryology, cell differentiation physiology, diencephalon cytology, diencephalon embryology and metabolism, dopamine metabolism fibroblast growth factor 8, Fgf genetics, Hedgehog proteins, in situ hybridization, signal transduction physiology, trans activators genetics.

Hong, J.H., F.S. Hwang, A. Amsterdam, Y. Tian, T. Benjamin, N. Hopkins, and M.B. Yaffe (2006). **Regulation of mesenchymal stem cell differentiation by protein kinase signaling pathways and transcriptional modulation**. *Federation of American Societies for Experimental Biology Journal* 20(5, Part 2): A1337. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: Mesenchymal stem cells are a pluripotent cell type that can differentiate into several distinct lineages. Two key transcription factors, Runx2 and peroxisome proliferator-activated receptor gamma (PPAR gamma), drive mesenchymal stein cells to differentiate into either osteoblasts or adipocytes, respectively. How these two transcription factors are regulated in order to specify these alternate cell fates remains a pivotal question. We have found that a 14-3-3-binding protein, TAZ (Transcriptional co-Activator with PDZ-binding motif), functions as a molecular rheostat that modulates mesenchymal stem cell differentiation by co-activating Runx2-dependent gene transcription while repressing PPAR gamma-dependent gene transcription. By modulating TAZ expression in model cell lines, mouse embryonic fibroblasts, and primary mesenchymal stem cells in culture, and in zebrafish in vivo, we observe dramatic alterations in osteogenic versus adipogenic potential. Protein kinases downstream of PI 3-kinase appear to regulate the activity, molecular interactions, and subcellular localization of TAZ, establishing a signaling pathway linking receptors at the cell surface to nuclear events responsible for cell fate decisions. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, cell biology, enzymology, biochemistry and molecular biophysics, gene transcription regulation, mouse fibroblasts.

Hoptak Solga, A.D., K.A. Klein, A.M. DeRosa, T.W. White, and M.K. Iovine (2007). **Zebrafish short fin mutations in connexin 43 lead to aberrant gap junctional intercellular communication.** *Federation of European Biochemical Societies Journal* 581(17): 3297-302. ISSN: 1742 464X.
NAL Call Number: QP501.E8
Abstract: Mutations in the zebrafish connexin43 (cx43) gene cause the short fin phenotype, indicating that direct cell-cell communication contributes to bone length. Three independently generated cx43 alleles exhibit short segments of variable sizes, suggesting that gap junctional intercellular communication may regulate bone growth. Dye coupling assays showed that all alleles are capable of forming gap junction channels. However, ionic coupling assays revealed allele-specific differences in coupling efficiency and gating. For instance, oocyte pairs expressing the weakest allele exhibited much higher levels of coupling than either of the strong alleles. Therefore, measurable differences in Cx43 function may be correlated with the severity of defects in bone length.
Descriptors: zebrafish, cell communication genetics, connexin 43 genetics, gap junctions genetics, limb deformities, congenital genetics, bones of lower extremity abnormalities, HeLa cells, missense mutation physiology, patch clamp techniques, phenotype, transfection.

Horikawa, K., K. Ishimatsu, E. Yoshimoto, S. Kondo, and H. Takeda (2006). **Noise-resistant and synchronized oscillation of the segmentation clock**. *Nature* 441(7094): 719-723. ISSN: 0028-0836.

NAL Call Number: 472 N21

Abstract: Periodic somite segmentation in vertebrate embryos is controlled by the 'segmentation clock', which consists of numerous cellular oscillators. Although the properties of a single oscillator, driven by a hairy negative-feedback loop, have been investigated, the system-level properties of the segmentation clock remain largely unknown. To explore these characteristics, we have examined the response of a normally oscillating clock in zebrafish to experimental stimuli using in vivo mosaic experiments and mathematical simulation. We demonstrate that the segmentation clock behaves as a coupled oscillator, by showing that Notch-dependent intercellular communication, the activity of which is regulated by the internal hairy oscillator, couples neighbouring cells to facilitate synchronized oscillation. Furthermore, the oscillation phase of individual oscillators fluctuates due to developmental noise such as stochastic gene expression and active cell proliferation. The intercellular coupling was found to have a crucial role in minimizing the effects of this noise to maintain coherent oscillation. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, development, molecular genetics, biosynchronization, cell proliferation, intercellular communication, stochastic gene expression, segmentation clock, somite segmentation, cellular oscillator, developmental noise.

Horne Badovinac, S., M. Rebagliati, and D.Y. Stainier (2003). **A cellular framework for gut-looping morphogenesis in zebrafish.** *Science* 302(5645): 662-5. ISSN: 0036-8075.

NAL Call Number: 470Sci2

Abstract: Many vertebrate organs adopt asymmetric positions with respect to the midline, but little is known about the cellular changes and tissue movements that occur downstream of left-right gene expression to produce this asymmetry. Here, we provide evidence that the looping of the zebrafish gut results from the asymmetric migration of the neighboring lateral plate mesoderm (LPM). Mutations that disrupt the epithelial structure of the LPM perturb this asymmetric migration and inhibit gut looping. Asymmetric LPM migration still occurs when the endoderm is ablated from the gut-looping region, suggesting that the LPM can autonomously provide a motive force for gut displacement. Finally, reducing left-sided Nodal activity randomizes the pattern of LPM migration and gut looping. These results reveal a cellular framework for the regulation of organ laterality by asymmetrically expressed genes.

Descriptors: zebrafish, body patterning, developmental gene expression regulation, gut-looping, intestines embryology, nuclear proteins, cell movement, cues, endoderm physiology, guanylate kinase, homeodomain proteins genetics and physiology, isoenzymes, mesoderm cytology and physiology, morphogenesis, mutation, nucleoside phosphate kinase genetics and metabolism, antisense oligonucleotides, phenotype, protein kinase C genetics and physiology, transcription factors genetics and physiology.

Horng, J.L., L.Y. Lin, C.J. Huang, and P.P. Hwang (2005). **Expression and function of vacuolar-type proton pump in developing zebrafish *Danio Rerio* skin**. *Journal of the Fisheries Society of Taiwan* 32: 1. ISSN: 0379-4180.

NAL Call Number: SH135.T3

Descriptors: zebrafish, ATP, antibodies, freshwater fish, fry, gene expression, gills, osmoregulation, secretory products, skin, pH, *Danio rerio*, physiology, biochemistry, biophysics, Taiwan.

Hoshijima, K. and S. Hirose (2007). **Expression of endocrine genes in zebrafish larvae in response to environmental salinity.** *Journal of Endocrinology* 193(3): 481-91. ISSN: 0022-0795.

Abstract: We tested the capability of the endocrine system in zebrafish to respond to envi-

ronmental salinity challenges during larval stages. We reveal that the zebrafish larvae have a system in which several endocrine genes, including atrial natriuretic peptide (anp), renin, prolactin, growth hormone (gh) and parathyroid hormone 1 (pth1), respond at the transcription level to changes in environmental salinity and that the responses are gene specific. Both anp and renin are upregulated in larvae raised in dilute freshwater medium but are downregulated in concentrated medium. On the other hand, expression of prolactin and gh is strongly enhanced in the dilute medium, but shows little or no change under higher salinity conditions. Interestingly, PTH1 expression depends on Ca(2+) concentration, as observed in mammals. Thus, taken together with the advantages of a model organism, including accessibility to genetic approaches, we propose that zebrafish larvae are useful for a comprehensive study of the regulatory mechanisms of the endocrine system in ionic and osmotic homeostasis.

Descriptors: zebrafish, physiological adaptation, gene expression regulation, hormones genetics, metabolism, atrial natriuretic factor genetics, gene expression, growth hormone genetics, in situ hybridization, larva, models, osmosis, parathyroid hormone genetics, prolactin genetics, renin genetics, sodium chloride metabolism.

Hou, L., H. Arnheiter, and W.J. Pavan (2006). **Interspecies difference in the regulation of melanocyte development by Sox10 and MITF**. *Proceedings of the National Academy of Sciences of the United States of America* 103(24): 9081-9085. ISSN: 0027 8424.

NAL Call Number: 500N21P

Abstract: There is increasing indication that interspecific phenotypic differences result from variations in gene-regulatory interactions. Here we provide evidence that mice differ from zebrafish in the way they use homologous key components to regulate pigment cell differentiation. In both zebrafish and mice, one transcription factor, SOX10, controls the expression of another, MITF (microphthalmia-associated transcription factor), which in turn regulates a set of genes critical for pigment cell development and pigmentation. Mutations in either Sox10 or Mitf impair pigment cell development. In Sox10-mutant zebrafish, experimentally induced expression of Mitf fully rescues pigmentation. Using lineage-directed gene transfer, we show that, in the mouse, Mitf can rescue Sox10-mutant precursor cells only partially. In fact, retrovirally mediated, Sox10-independent Mitf expression in mouse melano-blasts leads to cell survival and expression of a number of pigment biosynthetic genes but does not lead to expression of tyrosinase, the rate-limiting pigment gene which critically depends on both Sox10 and Mitf. Hence, compared with fish, mice have evolved a regulation of tyrosinase expression that includes feed-forward loops between Sox10 and tyrosinase regulatory regions. The results may help to explain how some embryos, such as zebrafish, can achieve rapid pigmentation after fertilization, whereas others, such as mice, become pigmented only several days after birth. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, development, molecular genetics, integumentary system, chemical coordination and homeostasis, cell survival, pigmentation pattern, pigment cell differentiation.

Houwing, S., L.M. Kamminga, E. Berezikov, D. Cronembold, A. Girard, H. van den Elst, D.V. Filippov, H. Blaser, E. Raz, C.B. Moens, R.H. Plasterk, G.J. Hannon, B.W. Draper, and R.F. Ketting (2007). **A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in zebrafish.** *Cell* 129(1): 69-82. ISSN: 0092 8674.

NAL Call Number: QH573.C42

Abstract: Piwi proteins specify an animal-specific subclass of the Argonaute family that, in vertebrates, is specifically expressed in germ cells. We demonstrate that zebrafish Piwi (Ziwi)

is expressed in both the male and the female gonad and is a component of a germline-specifying structure called nuage. Loss of Ziwi function results in a progressive loss of germ cells due to apoptosis during larval development. In animals that have reduced Ziwi function, germ cells are maintained but display abnormal levels of apoptosis in adults. In mammals, Piwi proteins associate with approximately 29-nucleotide-long, testis-specific RNA molecules called piRNAs. Here we show that zebrafish piRNAs are present in both ovary and testis. Many of these are derived from transposons, implicating a role for piRNAs in the silencing of repetitive elements in vertebrates. Furthermore, we show that piRNAs are Dicer independent and that their 3' end likely carries a 2'O-Methyl modification.
Descriptors: zebrafish, germ cells cytology, untranslated RNA metabolism, chemistry, cytology, genome, germ cells chemistry and metabolism , ovary cytology, RNA interference, untranslated RNA genetics, RNA binding proteins metabolism, retroelements, testis cytology.

Hsiao, C.D., Y.C. Chen, W.D. Lin, N.Y. Chuang, Y.E. Shieh, Y.C. Wang, J.M. Ho, and P.P. Hwang (2005). **Construction of Integrated database for fish expressed sequence tags**. *Journal of the Fisheries Society of Taiwan* 32(1): 11. ISSN: 0379-4180.
NAL Call Number: SH135.T3
Descriptors: zebrafish, algorithms, bibliographic information , chromosomes, computer programs, DNA microarrays, data collections, databases, differential display, freshwater fish, gene expression, gene mapping, genomes, libraries, nucleotide sequence, population genetics, expressed sequence tags, *Danio rerio*, *Fugu*, *Oryzias latipes*, Salmonidae, Teleostei, Tilapia, African mouthbrooders, medaka, salmonids, genetics and evolution, Taiwan.

Hsiao, C.D., M.S. You, Y.J. Guh, M. Ma, Y.J. Jiang, and P.P. Hwang (2007). **A positive regulatory loop between foxi3a and foxi3b is essential for specification and differentiation of zebrafish epidermal ionocytes.** *Public Library of Science One* 2: E302. ISSN: 1817-101X.
Abstract: BACKGROUND: Epidermal ionocytes play essential roles in the transepithelial transportation of ions, water, and acid-base balance in fish embryos before their branchial counterparts are fully functional. However, the mechanism controlling epidermal ionocyte specification and differentiation remains unknown. METHODOLOGY/PRINCIPAL FINDINGS: In zebrafish, we demonstrated that Delta-Notch-mediated lateral inhibition plays a vital role in singling out epidermal ionocyte progenitors from epidermal stem cells. The entire epidermal ionocyte domain of genetic mutants and morphants, which failed to transmit the DeltaC-Notch1a/Notch3 signal from sending cells (epidermal ionocytes) to receiving cells (epidermal stem cells), differentiates into epidermal ionocytes. The low Notch activity in epidermal ionocyte progenitors is permissive for activating winged helix/forkhead box transcription factors of foxi3a and foxi3b. Through gain- and loss-of-function assays, we show that the foxi3a-foxi3b regulatory loop functions as a master regulator to mediate a dual role of specifying epidermal ionocyte progenitors as well as of subsequently promoting differentiation of Na(+),K(+)-ATPase-rich cells and H(+)-ATPase-rich cells in a concentration-dependent manner. CONCLUSIONS/SIGNIFICANCE: This study provides a framework to show the molecular mechanism controlling epidermal ionocyte specification and differentiation in a low vertebrate for the first time. We propose that the positive regulatory loop between foxi3a and foxi3b not only drives early ionocyte differentiation but also prevents the complete blockage of ionocyte differentiation when the master regulator of foxi3 function is unilaterally compromised.
Descriptors: zebrafish, regulatory loop, foxi3a, foxi3b, differentiation, epidermal ionocytes.

Hsiao, N.W., D. Samuel, Y.N. Liu, L.C. Chen, T.Y. Yang, G. Jayaraman, and P.C. Lyu (2003). **Mutagenesis study on the zebra fish SOX9 high-mobility group: comparison of sequence and non-sequence specific HMG domains.** *Biochemistry* 42(38): 11183-93. ISSN: 0006 2960.
NAL Call Number: 381 B523
Abstract: A unique class of proteins, containing high-mobility group (HMG) domain(s), recognizes unusual DNA structures and/or bends specific to AT-rich linear double-stranded DNA. The DNA binding feature of these proteins is exhibited in the HMG domain(s). Although the sequence specific and non-sequence specific HMG domains exhibit very high degrees of sequence similarity, the reasons for the difference between their DNA recognition mechanisms are unclear. A series of zebra fish SOX9 HMG domain mutants was prepared in an effort to elucidate the importance of various residues on protein stability and DNA binding. This study is the first of a comprehensive mutagenesis study on a sequence specific HMG domain. Comparing how various residues influence sequence specific and non-sequence specific HMG domains helps us to rationalize their mode of action. Positively charged amino acids concentrated at the surface of sequence specific HMG domains recognize specific, linear AT-rich DNA segments. After the negative charges at the surface of the DNA are neutralized, the hydrophobic residues of the protein may intercalate DNA. Phenylalanine at position 12 plays a crucial role in the sequence specific HMG domain. The differences in pI values, the instability index, and DNA contact regions between sequence and non-sequence specific HMG domains are associated with their functional modes.
Descriptors: zebrafish, high mobility group proteins chemistry, genetics and metabolism , transcription factors genetics and metabolism, amino acid and base sequence, circular dichroism, *Escherichia coli* metabolism, molecular models, molecular sequence data, site directed mutagenesis oligonucleotide probes genetics and metabolism, protein denaturation, secondary protein structure, tertiary protein structure, sequence alignment, amino acid sequence homology, transcription factors chemistry.

Hsieh, S.L., R.W. Liu, C.H. Wu, W.T. Cheng, and C.M. Kuo (2003). **cDNA nucleotide sequence coding for stearoyl-CoA desaturase and its expression in the zebrafish (*Danio rerio*) embryo.** *Molecular Reproduction and Development* 66(4): 325-33. ISSN: 1040-452X.
NAL Call Number: QP251.M64
Descriptors: zebrafish, complementary DNA genetics, developmental gene expression regulation, stearoyl coa desaturase genetics, amino acid and base sequence, Northern blotting, molecular cloning, in situ hybridization, molecular sequence data, phylogeny, messenger RNA genetics and metabolism, reverse transcriptase PCR, sequence alignment, stearoyl coa desaturase chemistry.

Hsieh, Y.C., M.S. Chang, J.Y. Chen, J.J. Yen, I.C. Lu, C.M. Chou, and C.J. Huang (2003). **Cloning of zebrafish BAD, a BH3-only proapoptotic protein, whose overexpression leads to apoptosis in COS-1 cells and zebrafish embryos.** *Biochemical and Biophysical Research Communications* 304(4): 667-75 . ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: The BH3-only proapoptotic protein, BAD, was cloned from zebrafish embryos and its properties were characterized. Zebrafish BAD (zBAD) is a protein with 147 amino acids that contains a BH3 domain and a putative 14-3-3 binding site with the sequence of RPRSRS(84)AP, corresponding to S(136) in mouse BAD (mBAD). zBAD shares 34%, 28%, and 29% amino acid sequence identity to the human, mouse, and rat BAD, respectively. RT-PCR analysis revealed that the expression of zBAD gene is found in various parts of zebrafish tissues. The treatment with the z-VAD fmk, a broad-range caspase inhibitor, in

COS-1 cells significantly increased the expression of zebrafish BAD fusion proteins (GFP-zBAD and HA-zBAD), indicating that zebrafish BAD fusion proteins may be cleaved by caspase(s). zBAD was shown to induce apoptosis when it was overexpressed in COS-1 cells. In addition, zBAD was also expressed in muscle cells under the muscle-specific promoter from zebrafish alpha-actin gene. Abnormality in the skeletal muscles and the loss of green fluorescence signal in the same region were observed. Taken together, our results indicate that zBAD could induce apoptosis in vitro and in vivo and may have biological implications in apoptosis during zebrafish development.
Descriptors: zebrafish embryos, apoptosis physiology, carrier proteins metabolism, actins genetics and metabolism, amino acid chloromethyl ketones metabolism, amino acid sequence, COScells, carrier molecular cloning, cysteine proteinase inhibitors metabolism, reporter genes, molecular sequence data, recombinant fusion proteins metabolism, sequence alignment, bcl associated death protein.

Hsu, H.J., G. Lin, and B.C. Chung (2004). **Parallel early development of zebrafish interrenal glands and pronephros: differential control by wt1 and ff1b.** *Endocrine Research* 30(4): 803. ISSN: 0743 5800.
NAL Call Number: RC648.A1E48
Descriptors: zebrafish, adrenal cortex embryology, kidney embryology, transcription factors physiology, Wt1, cholesterol side chain cleavage enzyme genetics, embryonic development physiology, gene expression regulation physiology, transcription factors metabolism.

Hsu, K., A.T. Look, and J.P. Kanki (2004). **Lessons from transgenic zebrafish expressing the green fluorescent protein (GFP) in the myeloid lineage.** *Methods in Cell Biology* 77: 333-47. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, genetically modified embryology, green fluorescent proteins analysis, myeloid cells metabolism, genetically modified metabolism, cell lineage, cytology, immunology, developmental gene expression regulation genetics , gene transfer techniques, green fluorescent myeloid cells immunology, promoter regions genetics, proto oncogene tissue distribution immunology, trans activators genetics.

Hu, C.Y., C.H. Yang, W.Y. Chen, Y.H. Chen, C.J. Huang, and H.J. Tsai (2004). **The change in The early retinal development of zebrafish after Egr1 gene knockdown**. *Investigative Opthalmology and Visual Science* 45(Suppl. 1): U320. ISSN: 0146 0404.
Abstract: Purpose: Egrl (early growth response I)gene has been proposed to be associated-with abnormal eye growth. Our aim is to identify the change in early retinal development after Egrl gene knockdown. Methods: We microinjected the anti-sense mRNA inhibitor, Egrl-morpholino (Gene Tools), into the 1- to 4-cell zebrafish embryos. Chordin morpholino and the standard control oligo were also injected separately as positive and negative controls. The expression rate was calculated according to their morphologic changes. At 72 hours post-fertilization (hpf), larvae were fixed in 4% paraformaldehyde, embedded in paraffin, sectionedinto 5 lint slices, stained and observed under light microscope. Immunohistochemical staining was applied to identify amacrine cells. Results: Hundreds of zebrafish embryos have been microinjected. Seven different dosage of Egrl-morpholino was tried, and the most appropriate dose according to its efficacy and lethality at 24-hpf was 13.5 ng per embryo. More than 70% of survival embryos at 48-hpf had altered phenotype, yet only <1% of live negative control did. The most common defects of Egrl morphants were small eyes as well as enlarged pericardium. Western blot analysis showed the absence of 55-kD Egrl protein

in morphants. Histological examination of 72-hpf morphants revealed smaller eyes with a decreased cell number and a thinner inner plexiform, layer. Immunohistochemical study suggested a decrease of amacrine cells. Conclusions: Egrl gene plays an important role in the early retinal development, especially for the amacrine cells. However, the mechanism of abnormal eye growth is still to be determined. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, retinal development, molecular genetics, sense organs, sensory reception, eye growth, phenotype.

Hu, C.Y., C.H. Yang, W.Y. Chen, C.J. Huang, H.Y. Huang, M.S. Chen, and H.J. Tsai (2006). **Egr1 gene knockdown affects embryonic ocular development in zebrafish.** *Molecular Vision* 12: 1250-8. ISSN: 1090-0535.
NAL Call Number: QP475 .M65
Abstract: PURPOSE: To identify the changes in zebrafish embryonic ocular development after early growth response factor 1 (Egr1) gene knockdown by Egr1-specific translation inhibitor, morpholino oligonucleotides (MO). METHODS: Two kinds of Egr1-MO were microinjected separately with various dosages into one to four celled zebrafish embryos to find an optimal dose generating an acceptable mortality rate and high frequency of specific phenotype. Chordin-MO served as the positive control; a 5 mismatch MO of Egr1-MO1 and a nonspecific MO served as negative controls. We graded the Egr1 morphants according to their gross abnormalities, and measured their ocular dimensions accordingly. Western blot analysis and synthetic Egr1 mRNA rescue experiments confirmed whether the deformities were caused by Egr1 gene knockdown. Histological examination and three kinds of immunohistochemical staining were applied to identify glutamate receptor one expression in retinal ganglion cells and amacrine cells, to recognize acetylated alpha-tubulin expression which indicated axonogenesis, and to label photoreceptor cells with zpr-1 antibody. RESULTS: After microinjection of 8 ng Egr1-MO1 or 2 ng Egr1-MO2, 81.8% and 97.3% of larvae at 72 h postfertilization had specific defects, respectively. The gross phenotype included string-like heart, flat head, and deformed tail. The more severely deformed larvae had smaller eyes and pupils. Co-injection of 8 ng Egr1-MO1 and supplementary 12 pg synthetic Egr1 mRNA reduced the gross abnormality rate from 84.4% to 29.7%, and decreased the severity of deformities. Egr1 protein appeared in the wildtype and rescued morphants, but was lacking in the Egr1 morphants with specific deformities. Lenses of Egr1 morphants were smaller and had some residual nucleated lens fiber cells. Morphants' retinal cells arranged disorderly and compactly with thin plexiform layers. Immunohistochemical studies showed that morphants had a markedly decreased number of mature retinal ganglion cells, amacrine cells, and photoreceptor cells. Retinal axonogenesis was prominently reduced in morphants. CONCLUSIONS: The Egr1 gene plays an important role in zebrafish embryonic oculogenesis. Ocular structures including lens and retina were primitive and lacked appropriate differentiation. Such arrested retinal and lenticular development in Egr1 morphants resulted in microphthalmos.
Descriptors: zebrafish, early growth response protein 1 genetics, eye embryology, gene deletion, embryonic development drug effects, eye abnormalities embryology, eye abnormalities pathology, eye abnormalities prevention and control, gene expression, immunohistochemistry methods, crystalline lens embryology, crystalline lens pathology, antisense oligonucleotides pharmacology, protein biosynthesis drug effects, messenger RNApharmacology, retina embryology, retina pathology, staining and labeling.

Hu, C.Y., C.H. Yang, C.T. Tu, W.Y. Chen, C.J. Huang, and H.J. Tsai (2005). **Knockdown of zebrafish pitx3 gene affects the early development of lens**. *Investigative Ophthalmology and Visual Science* 46(Suppl. S): 1882. ISSN: 0146 0404.

Abstract: Purpose: The homeobox gene, PITX3, is associated with the anterior segment mesenchymal dysgenesis and congenital cataract in humans. In this study, we attempt to investigate its effect on lens development by way of gene knockdown in zebrafish. Methods: We microinjected Pitx3 morpholino oligo-nucleotides (MO), a translational inhibitor specific for Pitx3, into 1- to 4-celled zebrafish embryos. In order to observe their lenses in vivo, some morphants and normal controls were treated with 0.003% 1-phenyl-2-thiourea (PTU) every day to inhibit melanogenesis. At 72 hours post-fertilization (hpf), their left eyes were photographed for measuring ocular dimensions. The results were analyzed with 2-tailed independent t-test. Some larvae untreated with PTU were fixed for histological examination. We also performed Western blot and immunohistochemical stainto show the amount and location of alpha-A-crystallin, the major lenticular structural protein, in 72-hpf controls and Pitx3 morphants. Results: After microinjection of 3, 6, 7.5, or 9 ng of Pitx3-MO per embryo, more than 94% of morphants survived at 24 hpf and less than 8% of survived morphants had deformed body axis at 72 hpf. Grossly morphants had less bulging lenses, or even were aphakic. Image analysis proved that morphants' eyes and lenses were significantly smaller than those of the controls, but their anterior chamber depth and retinal thickness did not differ. Western blot analysis showed that the 20-kD alpha-A-crystallin was less in morphants. Histological examination revealed that morphants had smaller lenses, but their retinal structure was similar to that of controls. Immunohistochemical stain showed decreased alpha-A-crystallin in the lens of Pitx3 morphant. Conclusions: Knockdown of Pitx3 gene did not significantly affect the early gross development, except for arrested lens development and microphthalmia which was associated with inadequate lenticular alpha-A-crystallin synthesis. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, development, molecular genetics, sense organs, lens development, melanogenesis, retinal thickness, microphthalmia.

Huang, K.S., Y.C. Lin, K.C. Su, and H.Y. Chen (2007). **An electroporation microchip system for the transfection of zebrafish embryos using quantum dots and GFP genes for evaluation.** *Biomedical Microdevices* 9(5): 761-8. ISSN: 1387 2176.

NAL Call Number: R856A1

Abstract: This study focuses on the design and experimental verification of an electroporation (EP) microchip system for the transfection of zebrafish (*Danio rerio*). For generating suitable pulses, a circuit is used to provide voltages between 0 and 700 V, with nearly 0-3,500 V/cm electric field. In addition, a proposed EP microchip, designed in a modular fashion, is fabricated using micro electromechanical system (MEMS) technology to allow for rapid and convenient replacement of each component. A numerical simulation is carried out to analyze the uniformity and strength of the EP electric fields generated in the microchip. Trypan blue dye, water-soluble quantum dots (MUA-QDs) and genes coding for green fluorescence protein (pEGFP-N1 plasmids) were employed to verify the successful delivery and transfection of zebrafish embryos. The experimental results show that the optimum delivery rate of trypan blue dyes and MUA-QDs were respectively up to 62 and 36% by using the proposed EP system. The successfully transfected embryos with the pEGFP-N1 plasmid used exhibit green fluorescence in the zebrafish embryos. The approach in the transfection of zebrafish embryos will provide many potential usages for cellular imaging areas, gene therapy research and medical applications.

Descriptors: zebrafish embryos, electroporation instrumentation, green fluorescent micro-

chip analytical procedures instrumentation, quantum dots, transfection instrumentation, equipment design, microchemistry instrumentation, electron transmission microscopy, reproducibility of results, ultraviolet spectrophotometry.

Hughes, I., I. Thalmann, R. Thalmann, and D.M. Ornitz (2006). **Mixing model systems: using zebrafish and mouse inner ear mutants and other organ systems to unravel the mystery of otoconial development.** *Brain Research* 1091(1): 58-74. ISSN: 0006 8993.
NAL Call Number: QP376
Abstract: Human vestibular dysfunction is an increasing clinical problem. Degeneration or displacement of otoconia is a significant etiology of age-related balance disorders and Benign Positional Vertigo (BPV). In addition, commonly used antibiotics, such as aminoglycoside antibiotics, can lead to disruption of otoconial structure and function. Despite such clinical significance, relatively little information has been compiled about the development and maintenance of otoconia in humans. Recent studies in model organisms and other mammalian organ systems have revealed some of the proteins and processes required for the normal biomineralization of otoconia and otoliths in the inner ear of vertebrates. Orchestration of extracellular biomineralization requires bringing together ionic and proteinaceous components in time and space. Coordination of these events requires the normal formation of the otocyst and sensory maculae, specific secretion and localization of extracellular matrix proteins, as well as tight regulation of the endolymph ionic environment. Disruption of any of these processes can lead to the formation of abnormally shaped, or ectopic, otoconia, or otoconial agenesis. We propose that normal generation of otoconia requires a complex temporal and spatial control of developmental and biochemical events. In this review, we suggest a new hypothetical model for normal otoconial and otolith formation based on matrix vesicle mineralization in bone which we believe to be supported by information from existing mutants, morphants, and biochemical studies.
Descriptors: zebrafish, models, otolithic membrane growth and development, otolithic membrane physiology, physiologic calcification, calcium metabolism, endolymph physiology, epithelium physiology, mutant strains physiology, olfactory marker protein metabolism, otolithic membrane cytology.

Hultman, K.A., N. Bahary, L.I. Zon, and S.L. Johnson (2007). **Gene Duplication of the zebrafish kit ligand and partitioning of melanocyte development functions to kit ligand a.** *Public Library of Science Genetics* 3(1): E17. ISSN: 1553-7390.
Abstract: The retention of particular genes after the whole genome duplication in zebrafish has given insights into how genes may evolve through partitioning of ancestral functions. We examine the partitioning of expression patterns and functions of two zebrafish kit ligands, kit ligand a (kitla) and kit ligand b (kitlb), and discuss their possible coevolution with the duplicated zebrafish kit receptors (kita and kitb). In situ hybridizations show that kitla mRNA is expressed in the trunk adjacent to the notochord in the middle of each somite during stages of melanocyte migration and later expressed in the skin, when the receptor is required for melanocyte survival. kitla is also expressed in other regions complementary to kita receptor expression, including the pineal gland, tail bud, and ear. In contrast, kitlb mRNA is expressed in brain ventricles, ear, and cardinal vein plexus, in regions generally not complementary to either zebrafish kit receptor ortholog. However, like kitla, kitlb is expressed in the skin during stages consistent with melanocyte survival. Thus, it appears that kita and kitla have maintained congruent expression patterns, while kitb and kitlb have evolved divergent expression patterns. We demonstrate the interaction of kita and kitla by morpholino knockdown analysis. kitla morphants, but not kitlb morphants, phenocopy the null allele of kita,

with defects for both melanocyte migration and survival. Furthermore, kitla morpholino, but not kitlb morpholino, interacts genetically with a sensitized allele of kita, confirming that kitla is the functional ligand to kita. Last, we examine kitla overexpression in embryos, which results in hyperpigmentation caused by an increase in the number and size of melanocytes. This hyperpigmentation is dependent on kita function. We conclude that following genome duplication, kita and kitla have maintained their receptor-ligand relationship, coevolved complementary expression patterns, and that functional analysis reveals that most or all of the kita receptor's function in the embryo are promoted by its interaction with kitla.
Descriptors: zebrafish, gene duplication, melanocytes metabolism, stem cell factor genetics, base sequence, DNA primers, molecular evolution, hyperpigmentation genetics, molecular sequence data, phylogeny, messenger RNA genetics.

Hurlstone, A.F.L., A.G. Haramis, E. Wienholds, H. Begthel, J. Korving, F.V. Eeden, E. Cuppen, D. Zivkovic, R.H.A. Plasterk, and H. Clevers (2003). **The Wnt/ beta -catenin pathway regulates cardiac valve formation**. *Nature* 425(6958): 633-637. ISSN: 0028-0836.
NAL Call Number: 472 N21
Descriptors: zebrafish, cell differentiation, circulatory system, development, embryonic development, gastrulation, gene expression, heart, muscles, *Danio rerio*, Apc gene, dkk1 gene, Wnt protein, beta -catenin signalling, beta -catenin, cardiac valve formation, genetics and evolution, developmental genetics.

Hussain, M.U., M. Kremer, G. Zoidl, and R. Dermietzel (2003). **Transcriptional and translational regulation of zebrafish connexin 55.5 (zf.Cx.55.5) and connexin 52.6 (zf.Cx52.6).** *Cell Communication and Adhesion* 10(4-6): 227-31. ISSN: 1061 5385.
Abstract: Zebrafish connexin 55.5 (zf.Cx55.5) and connexin 52.6 (zf.Cx52.6) show highly restricted expression patterns in the nervous system. Both connexins are confined to subsets of neurons in the fish retina. In order to get initial answers to the questions of pattern definition in neuronal subsets, we elucidated molecular mechanisms responsible for their expression. Different upstream DNA fragments were subcloned into a pGL3-basic vector and transiently transfected in HeLa and N2A cells. Luciferase activity showed the presence of two putative promoter elements in zfCx55.5 and a promoter element in zfCx52.6 that showed different promoter activities in HeLa and N2A cells. Moreover, fusion constructs of zfCx55.5 with EGFP revealed the presence of a new isoform with an additional short exon I.
Descriptors: zebrafish, cell communication physiology, connexins metabolism, promoter regions genetics, recombinant fusion proteins metabolism, cell communication genetics, cultured cells, connexins genetics, reporter green fluorescent proteins, HeLa cells, luminescent proteins genetics and metabolism, recombinant fusion proteins genetics.

Hwang, G., F. Mller, M.A. Rahman, D.W. Williams, P.J. Murdock, K.J. Pasi, G. Goldspink, H. Farahmand, and N. Maclean (2004). **Fish as bioreactors: transgene expression of human coagulation factor VII in fish embryos**. *Marine Biotechnology* 6(5): 485-492. ISSN: 1436 2228.
NAL Call Number: TP248.27.M37M37
Abstract: A plasmid containing human coagulation factor VII (hFVII) complementary DNA regulated by a cytomegalovirus promoter was microinjected into fertilized eggs of zebrafish, African catfish, and tilapia. The active form of hFVII was detected in the fish embryos by various assays. This positive expression of human therapeutic protein in fish embryos demonstrates the possibility of exploitation of transgenic fish as bioreactors.
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Descriptors: zebrafish, blood and lymphatics: transport and circulation, molecular genetics , plasmid, coagulation factor VIII, bioreactor.

Hwang, P.P., T.C. Pan, B.K. Liao, C.J. Huang, and L.Y. Lin (2005). **Epithelial Ca channel expression and Ca uptake in developing zebrafish**. *Federation of American Societies for Experimental Biology Journal* 19(5, Suppl. S, Part 2): A1584. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: The purpose of the present work was to study the involvement of the ECaC (epithelial calcium channel) in the Ca2+ uptake mechanism in developing zebrafish (*Danio rerio*). Using rapid amplification of cDNA ends (RACE), full-length cDNA encoding the ECaC (2578 bp, encoding 709 amino acids) of zebrafish was cloned and sequenced. The zECaC was found to be ubiquitously expressed in all tissues examined, and began to be expressed in the skin covering the yolk sac of embryos at 24 h post-fertilization (hpf). zECaC-expressing cells expanded to cover the skin of the entire yolk sac following embryonic development, and began to occur in the gill filaments at 96 hpf, and thereafter zECaC-expressing cells rapidly increased in both gills and yolk sac skin. In correspondence to the profile of ECaC expression, the Ca2+ influx and content began to increase at 36 similar to 72 hpf. Incubating zebrafish embryos in low-Ca2+ (0.02 mM) fresh water caused upregulation of the whole-body Ca2+ influx and zECaC expression in both gills and skin. Co-localization of zECaC mRNA and the Na-K-ATPase alpha subunit (a marker for mitochondria-rich, MR, cells) indicated that only a portion of the MR cells expressed zECaCmRNA. These results suggest that the zECaC plays a key role in Ca2+ absorption in developing zebrafish. The present work was financially supported by the Academia Sinica. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics, enzymology, marine ecology, environmental sciences, cloning, genetic techniques, rapid amplification of cDNA ends, race analysis, genetic techniques.

Ibabe, A., E. Bilbao, and M.P. Cajaraville (2005). **Expression of peroxisome proliferator-activated receptors in zebrafish (*Danio rerio*) depending on gender and developmental stage.** *Histochemistry and Cell Biology* 123(1): 75-87. ISSN: 0948-6143.
NAL Call Number: QH613.H57
Abstract: Peroxisome proliferator-activated receptors (PPARs) are members of the superfamily of nuclear hormone receptors involved in embryo development and differentiation of several tissues in mammals. The aim of the present study was to investigate the possible differential expression of the three PPAR subtypes (PPARalpha, PPARbeta, and PPARgamma) in relation to gender and developmental stage in zebrafish. For this purpose PPAR expression was assessed by immunohistochemistry in 7-day-old larvae, 1-month-old juveniles, and 1-year-old adults. Additionally, the activity of peroxisomal acyl-CoA oxidase (AOX), a gene regulated by PPARs, and the volume density of catalase-immunolabeled liver peroxisomes (V(VP)) was examined. No significant gender-related differences were detected in the tissue distribution of the three PPAR subtypes or in peroxisomal AOX activity and V(VP). The percentage of PPARbeta-positive hepatocytes was significantly higher in females than in males suggesting a specific regulatory role of this subtype in female zebrafish. The three PPAR subtypes were already expressed at the larval stage, with a similar tissue distribution pattern to that found in adults. For all stages, PPARalpha and PPARgamma were expressed at higher levels than PPARbeta, and PPARbeta immunolabeling was stronger in juveniles than in larval or adult stages. The percentages of hepatocyte nuclei immunolabeled for PPARs was higher in early developmental stages than in adults, similarly to AOX activity and V(VP). In con-

clusion, our results indicate that PPAR expression, the activity of its target gene AOX, and peroxisomal biogenesis are developmentally modulated in zebrafish.
Descriptors: zebrafish, peroxisome proliferator activated receptors metabolism, age factors, Western blotting, cell nucleus metabolism, hepatocytes metabolism, immunohistochemistry, organ specificity, oxidoreductases metabolism, PPAR alpha metabolism, PPAR gamma metabolism, PPAR beta metabolism, peroxisomes enzymology, sex factors.

Ichiyanagi, K. and N. Okada (2006). **Genomic alterations upon integration of zebrafish L1 elements revealed by the TANT method.** *Gene* 383: 108-16. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: Transposable elements, or transposons, constitute substantial portions of eukaryotic genomes and have contributed to the diversity and functions of the genomes. Bioinformatic analysis of target junctions of genomic transposon copies can provide insights into their mobility mechanisms and consequent genomic alterations, but definitive identification of the target junctions remains difficult despite the steady accumulation of genomic sequence information. To overcome this difficulty, we recently developed a method termed "the target analysis of nested transposons" (TANT), which anatomizes junction features of numerous genomic copies of transposons that reside within other transposons. Whereas the mammalian long interspersed nuclear element (LINE)-1 (L1), a retrotransposon, has been proposed to make a considerable impact on host genomes, the mobility and impact of non-mammalian L1s are poorly understood. In the present study, we analyzed genomic copies of zebrafish L1 elements by using the TANT method. Some copies exhibited the features of integration that are similar to those of mammalian L1s. The zebrafish L1 retrotransposition reaction, however, frequently truncated the target-site DNA by up to 0.6 kb and produced a new sequence at LINE-target junctions. Moreover, our data suggest that L1 retrotransposition can be used to repair double-strand DNA breaks (DSBs). These results imply that L1s have had considerable impact on the evolution of the zebrafish genome.
Descriptors: zebrafish, long interspersed nucleotide elements, base sequence, DNA transposable elements genetics, genomics methods, genetic models, molecular sequence data, nucleic acid sequence homology.

Ichiyanagi, K., H. Nishihara, D.D. Duvernell, and N. Okada (2007). **Acquisition of endonuclease-especificity during evolution of L1 retrotransposon**. *Molecular Biology and Evolution* 24(9): 2009-2015. ISSN: 0737-4038.
NAL Call Number: QH506.M642
Descriptors: zebrafish, amino acids, DNA, endonuclease, evolution, fitness, freshwater fish, genomes, phylogenetics, retrotransposition, retrotransposons, substrate specificity, *Danio rerio*, evolution, genetics and evolution.

Imamura, S. and S. Kishi (2005). **Molecular cloning and functional characterization of zebrafish ATM.** *International Journal of Biochemistry and Cell Biology, The* 37(5): 1105-16. ISSN: 1357-2725.
NAL Call Number: QD415.A1I5
Abstract: Ataxia-telangiectasia mutated (ATM) is the gene product mutated in ataxia-telangiectasia (A-T), which is an autosomal recessive disorder with symptoms including neurodegeneration, cancer predisposition and premature aging. ATM is thought to play a pivotal role in signal transduction in response to genotoxic DNA damage. To study the physiological and developmental functions of ATM using the zebrafish model system, we cloned the zebrafish homolog cDNA of human ATM (hATM), zebrafish ATM (zATM), analyzed

the expression pattern of zATM during early development, and further developed the system to study loss of zATM function in zebrafish embryos. Employing information available from the zebrafish genomic database, we utilized a PCR-based approach to isolate zATM cDNA clones. Sequence analysis of zATM showed a high level homology in the functional domains of hATM. The putative FAT, phosphoinositide 3-kinase-like, and FATC domains of zATM, which regulate ATM kinase activity and functions, were the most highly conserved regions, exhibiting 64-94% amino acid identity to the corresponding domains in hATM, while exhibiting approximately 50% amino acid identity outside these domains. The zATM gene is expected to consist of 62 coding exons, and we have identified at least 55 exons encompassing more than 100kb of nucleotide sequence, which encodes about 9 kb of cDNA. By in situ hybridization, zATM mRNA was detected ubiquitously with a dramatic increase at the 18-somite stage, then more specifically in the eye, brain, trunk, and tail at later stages. To inhibit zATM expression and function, we designed and synthesized splice-blocking antisense-morpholino oligonucleotides targeting the phosphoinositide 3-kinase-like domain. We demonstrated that this knockdown of zATM caused abnormal development upon ionizing radiation-induced DNA damage. Our data suggest that the ATM gene is structurally and functionally conserved in vertebrates from zebrafish to human.

Descriptors: zebrafish, cell cycle proteins genetics and physiology, DNA binding proteins genetics, proteins genetics and physiology, protein serine threonine kinases genetics and physiology, tumor suppressor proteins genetics and physiology and physiology, aging, amino acid sequence, ataxia telangiectasia genetics, base sequence, cell cycle proteins chemistry, molecular cloning, DNA damage, DNA binding proteins genetics, proteins chemistry, disease models, mice, molecular sequence data, protein serine threonine kinases chemistry, tumor suppressor proteins chemistry.

Imamura, S., N. Ojima, and M. Yamashita (2003). **Cold-inducible expression of the cell division cycle gene CDC48 and its promotion of cell proliferation during cold acclimation in zebrafish cells.** *Federation of European Biochemical Societies Journal* 549(1-3): 14-20. ISSN: 1742 464X.

NAL Call Number: QP501.E8

Abstract: A member of the ATPases associated with diverse cellular activities (AAA) family, the cell division cycle gene CDC48/VCP (valosin-containing protein)/p97, was cloned from zebrafish and found to be a major cold-inducible protein in fish cells. CDC48 mRNA levels increased significantly after reducing the temperature from 30 to 15 degrees C for 25 days. CDC48 protein levels also increased 2.5-fold after 30 days at cold temperatures. When fish cells overexpressing CDC48 were exposed to a temperature of 15 degrees C, cell proliferation was markedly enhanced in comparison with control cells. By contrast, expression of a mutant molecule with a tyrosine-805 to alanine substitution at the C-terminal phosphorylation site inhibited cell proliferation and induced apoptosis at low temperatures. Therefore, CDC48 may promote cell cycling and cell proliferation via C-terminal tyrosine phosphorylation during cold acclimation in fish cells.

Descriptors: zebrafish, acclimatization genetics, cell cycle cell cycle, cold, gene expression regulation physiology, amino acid sequence, cell division, molecular cloning, complementary DNA isolation and purification, molecular sequence data, phosphorylation, messenger RNA analysis, sequence alignment, genetic transcription, transfection.

Imanishi, Y., L. Yang, I. Sokal, S. Filipek, K. Palczewski, and W. Baehr (2004). **Diversity of guanylate cyclase-activating proteins (GCAPs) in teleost fish: characterization of three novel GCAPs (GCAP4, GCAP5, GCAP7) from zebrafish (*Danio rerio*) and prediction of eight**

GCAPs (GCAPI-8) in puffertish (*Fugu rubripes*). *Journal of Molecular Evolution* 59(2): 204-217. ISSN: 0022 2844.
NAL Call Number: QH359.J6
Abstract: The guanylate cyclase-activating proteins (GCAPs) are Ca2+-binding proteins of the calmodulin (CaM) gene superfamily that function in the regulation of photoreceptor guanylate cyclases (GCs). In the mammalian retina, two GCAPs (GCAP 1-2) and two transmembrane GCs have been identified as part of a complex regulatory system responsive to fluctuating levels of free Ca2+. A third GCAP, GCAP3, is expressed in human and zebrafish (*Danio rerio*) retinas, and a guanylate cyclase-inhibitory protein (GCIP) has been shown to be present in frog cones. To explore the diversity of GCAPs in more detail, we searched the pufferfish (*Fugu rubripes*) and zebrafish (*Danio rerio*) genomes for GCAP-related gene sequences (fuGCAPs and zGCAPs, respectively) and found that at least five additional GCAPs (GCAP4-8) are predicted to be present in these species. We identified genomic contigs encoding fuGCAP1-8. fuGCIP, zGCAP1-5, zGCAP7 and zGCIP. We describe cloning, expression and localization of three novel GCAPs present in the zebrafish retina (zGCAP4, zGCAP5, and zGCAP7). The results show that recombinant zGCAP4 stimulated bovine rod outer segment GC in a Ca2+-dependent manner. RT-PCR with zGCAP specific primers showed specific expression of zGCAPs and zGCIP in the retina, while zGCAP1 mRNA is also present in the brain. In situ hybridization with anti-sense zGCAP4, zGCAP5 and zGCAP7 RNA showed exclusive expression in zebrafish cone photoreceptors. The presence of at least eight GCAP genes suggests an unexpected diversity within this subfamily of Ca2+-binding proteins in the teleost retina, and suggests additional functions for GCAPs apart from stimulation of GC. Based on genome searches and EST analyses, the mouse and human genomes do not harbor GCAP4-8 or GCIP genes.
Descriptors: zebrafish, *Danio rerio, Fugu rubripes*, proteins, guanylate cyclase activatin G proteins, molecular genetics.

Inbal, A., S.H. Kim, J. Shin, and L. Solnica Krezel (2007). **Six3 represses nodal activity to establish early brain asymmetry in zebrafish.** *Neuron* 55(3): 407-15. ISSN: 0896-6273.
Abstract: The vertebrate brain is anatomically and functionally asymmetric; however, the molecular mechanisms that establish left-right brain patterning are largely unknown. In zebrafish, asymmetric left-sided Nodal signaling within the developing dorsal diencephalon is required for determining the direction of epithalamic asymmetries. Here, we show that Six3, a transcription factor essential for forebrain formation and associated with holoprosencephaly in humans, regulates diencephalic Nodal activity during initial establishment of brain asymmetry. Reduction of Six3 function causes brain-specific deregulation of Nodal pathway activity, resulting in epithalamic laterality defects. Based on misexpression and genetic epistasis experiments, we propose that Six3 acts in the neuroectoderm to establish a prepattern of bilateral repression of Nodal activity. Subsequently, Nodal signaling from the left lateral plate mesoderm alleviates this repression ipsilaterally. Our data reveal a Six3-dependent mechanism for establishment of correct brain laterality and provide an entry point to understanding the genetic regulation of Nodal signaling in the brain.
Descriptors: zebrafish, brain embryology, cerebral dominancephysiology, embryonic development physiology, eye, homeodomain, nerve tissue, epistasis, genetic, epithalamus embryology, transforming growth factor beta antagonists and inhibitors.

Inbal, A., J. Topczewski, and L. Solnica Krezel (2006). **Targeted gene expression in the zebrafish prechordal plate.** *Genesis* 44(12): 584-8. ISSN: 1526-954X.
NAL Call Number: QH426.D32

Descriptors: transgenic zebrafish, endoderm metabolism, gene expression, nervous system embryology, trans activators genetics, genetically modified, genetic crosses, in situ hybridization, nervous system metabolism.

Ingham, P.W. and H.R. Kim (2005). **Hedgehog signalling and the specification of muscle cell identity in the zebrafish embryo.** *Experimental Cell Research* 306(2): 336-42. ISSN: 0014 4827.

NAL Call Number: 442.8EX7

Abstract: Signalling by members of the Hedgehog family of secreted proteins plays a central role in the development of many animal species. In the zebrafish embryo, the specification of myoblast fates is controlled by Hedgehog signals emanating from axial midline structures. Distinct muscle cell identities are induced by varying levels of signalling activity. The SET domain transcription factor, Blimp1, is a key target of Hedgehog signalling in this process.

Descriptors: zebrafish embryos, developmental gene expression regulation physiology, skeletal muscle embryology, signal transduction physiology, trans activators physiology, cell differentiation, DNA binding proteins genetics , proteins, embryonic induction, Hedgehog proteins, nuclear proteins, repressor proteins metabolism, transcription factors metabolism, proteins.

Ings, J.S. and G.J. Van Der Kraak (2006). **Characterization of the mRNA expression of StAR and steroidogenic enzymes in zebrafish ovarian follicles.** *Molecular Reproduction and Development* 73(8): 943-54. ISSN: 1040-452X.

NAL Call Number: QP251.M64

Abstract: The objective of this study was to investigate the levels of expression of steroid biosynthetic enzymes and steroidogenic acute regulatory protein (StAR) at different stages of ovarian follicular development in zebrafish (*Danio rerio*), and to investigate the sites within the steroid biosynthetic pathway that may be regulated by gonadotropins. Ovarian follicles of sexually mature fish were separated into primary, previtellogenic, vitellogenic, and mature stages and the expression of StAR, P450 side chain cleavage (P450scc), 3beta-hydroxysteroid dehydrogenase (3beta-HSD), P450 hydroxylase/lyase (P450c17), 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1), 17beta-hydroxysteroid dehydrogenase type 3 (17beta-HSD3), and P450 aromatase (P450aromA) was determined by Real time RT-PCR. The expression of all genes changed significantly as follicles grew, with a decrease in the expression of StAR, P450scc, 3beta-HSD and P450c17 with maturation, and an increase in the expression of 17beta-HSD3 during vitellogenesis and 17beta-HSD1 and P450aromA during previtellogenesis. In vitro incubation of vitellogenic follicles demonstrated that the expression of StAR, 17beta-HSD3, and P450aromA increased in response to hCG, and decreased in the absence of hCG. In contrast, the expression of P450scc, 3beta-HSD, P450c17, and 17beta-HSD1 remained constant between treatments and over time. Testosterone and estradiol production in the culture medium was stimulated by human chorionic gonadotropin (hCG). These experiments aid in the characterization of the roles and regulation of steroids throughout ovarian development, and suggest that gonadotropins play a key role in the regulation of StAR, 17beta-HSD3, and P450aromA in zebrafish.

Descriptors: zebrafish, ovarian follicle physiology, phosphoproteins metabolism, messenger RNA metabolism, aromatase genetics and metabolism, cholesterol side chain cleavage enzyme genetics and metabolism, chorionic gonadotropin metabolism, hydroxysteroid dehydrogenases genetics and metabolism, lyases genetics and metabolism, phosphoproteins genetics.

Inoue, F., S. Nagayoshi, S. Ota, M.E. Islam, N. Tonou Fujimori, Y. Odaira, K. Kawakami, and K. Yamasu (2006). **Genomic organization, alternative splicing, and multiple regulatory regions of the zebrafish fgf8 gene.** *Development Growth and Differentiation* 48(7): 447-62. ISSN: 0012 1592.

NAL Call Number: QL951.E4

Abstract: Fgf8 is among the members of the fibroblast growth factor (FGF) family that play pivotal roles in vertebrate development. In the present study, the genomic DNA of the zebrafish fgf8 gene was cloned to elucidate the regulatory mechanism behind the temporally and spatially restricted expression of the gene in vertebrate embryos. Structural analysis revealed that the exon-intron organization of fgf8 is highly conserved during vertebrate evolution, from teleosts to mammals. Close inspection of the genomic sequence and reverse transcription-polymerase chain reaction analysis revealed that zebrafish fgf8 encodes two splicing variants, corresponding to Fgf8a and Fgf8b, among the four to seven splicing variants known in mammals. Misexpression of the two variants in zebrafish embryos following mRNA injection showed that both variants have dorsalizing activities on zebrafish embryos, with Fgf8b being more potent. Reporter gene analysis of the transcriptional regulation of zebrafish fgf8 suggested that its complicated expression pattern, which is considered essential for its multiple roles in development, is mediated by combinations of different regulatory regions in the upstream and downstream regions of the gene. Furthermore, comparison of the genomic sequence of fgf8 among different vertebrate species suggests that this regulatory mechanism is conserved during vertebrate evolution.

Descriptors: zebrafish, alternative splicing genetics, FGF 8 genetics, regulatory sequences, nucleic acid genetics , base sequence, conserved sequence genetics, exons genetics, fibroblast growth factor 8 metabolism, developmental gene expression regulation, green fluorescent proteins genetics and metabolism, in situ hybridization, introns genetics, molecular sequence data, protein isoforms genetics and metabolism, reverse transcriptase PCR, nucleic acid sequence homology, transcription initiation site.

Iovine, M.K. (2007). **Conserved mechanisms regulate outgrowth in zebrafish fins.** *Nature Chemical Biology* 3(10): 613-8. ISSN: 1552-4450.

Abstract: Regulation of size is one of the fundamental problems in biology. One general strategy has been to identify molecules required for cell growth and cell proliferation within an organ. This has been particularly revealing, identifying cell-autonomous pathways involved in cell growth, survival and proliferation. In order to identify pathways regulating overall limb growth and morphology, experiments have evaluated gene expression, transplanted and removed tissues, and knocked out genes. This work has provided a vast amount of information identifying molecular mechanisms regulating limb axis formation, outgrowth, and pattern formation. Using the zebrafish fin, genetic, cellular and molecular strategies have also been employed to follow both normal patterns of fin growth and growth in fin mutants. This review will focus on cellular and molecular regulation of the outgrowth and patterning of the zebrafish caudal fin during regeneration, and will emphasize similarities to other systems. Future perspectives describe opportunities using the zebrafish fin to reveal mechanisms underlying the regulation of final size.

Descriptors: zebrafish, embryonic induction physiology, extremities physiology, caudal fin, morphogenesis physiology, regeneration physiology, body patterning genetics, body patterning physiology, bone development genetics, bone development physiology, embryonic induction genetics, extremities embryology, gene expression regulation genetics and physiology, invertebrates, morphogenesis genetics, mutation, regeneration genetics.

Notes: A review.

Ishikawa, T.O., K.J. Griffin, U. Banerjee, and H.R. Herschman (2007). **The zebrafish genome contains two inducible, functional cyclooxygenase-2 genes.** *Biochemical and Biophysical Research Communications* 352(1): 181-7. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: Cyclooxygenase is a key enzyme in prostanoid biosynthesis. Mammalian species have two cyclooxygenases, constitutively expressed cyclooxygenase-1 (Cox-1) and inducible cyclooxygenase-2 (Cox-2). Cox-1 and/or Cox-2 have been also identified in other vertebrates, including fish. We identified a second zebrafish Cox-2 gene orthologue, Cox-2b. All of the functionally important amino acids for cyclooxygenase enzymes are conserved in Cox-2b. The 3' untranslated region of the Cox-2b message contains AU rich elements characteristic of regulation at the level of mRNA stability. Constitutive tissue expression patterns for Cox-2a and Cox-2b are distinct, but overlap. Both Cox-2a and Cox-2b expression are inducible in the kidney when fish are exposed to tetradecanoylphorbol acetate. Like Cox-2a, Cox-2b protein, expressed in COS cells is functionally active. Thus, the zebrafish genome contains two functional, inducible Cox-2 genes. Database searching demonstrates that some fish genomes contain multiple Cox-1 or Cox-2 cyclooxygenase genes, suggesting alternate duplication and retention of this gene.

Descriptors: zebrafish, cyclooxygenase 2 genetics and metabolism, genome genetics, amino acid and base sequence, COS cells, cell line, *Cercopithecus aethiops*, conserved sequence, cyclooxygenase 2 chemistry, isoenzymes chemistry, isoenzymes genetics, isoenzymes metabolism, molecular sequence data, phylogeny, sequence alignment.

Itoh, N. (2007). **The Fgf families in humans, mice, and zebrafish: Their evolutional processes and roles in development, metabolism, and disease.** *Biological and Pharmaceutical Bulletin* 30(10): 1819-25. ISSN: 0918 6158.

NAL Call Number: QP501

Abstract: Fibroblast growth factors (Fgfs) were originally isolated as growth factors for fibroblasts. However, Fgfs are now recognized as polypeptide growth factors of ca. 150-250 amino acid residues with diverse biological activities and expression profiles. The Fgf signaling system has been identified in multicelluar but not in unicellular organisms. In contrast to the only two Fgf genes and one Fgf receptor (Fgfr) gene in Caenorhabditis elegans, both the human and mouse Fgf and Fgfr gene families comprise twenty-two and four members, respectively. Their evolutional processes indicate that the Fgf and Fgfr gene families greatly co-expanded during the evolution of early vertebrates. The expansion of the Fgf and Fgfr gene families has enabled this signaling system to acquire diversity of function and a nearly ubiquitous involvement in many developmental and physiological processes. The zebrafish fgf gene family comprises twenty-seven members with several paralogs generated by an additional genome duplication. The mouse and zebrafish are useful models for studying gene functions. Fgf knockout mice have been generated. Several Fgf knockout mice die in the embryonic or early postnatal stages, indicating crucial roles for these genes in various developmental processes. However, other Fgf knockout mice survive with subtle phenotypic alterations. Their functions might be redundant. Studies using zebrafish embryos with mutated or knockdown fgfs also indicate that fgfs play crucial roles in development in that species. Although most Fgfs act in development in a paracrine and/or autocrine manner, some have potential roles in metabolism in an endocrine manner. In humans, Fgf signaling disorders result in hereditary diseases and cancers.

Descriptors: zebrafish, disease, evolution, FGFs genetics and physiology, metabolism genetics, knockout mice, mutation physiology, fibroblast receptors growth factor genetics and physiology.

Itzkowitz, M. and M.K. Iovine (2007). **Single gene mutations causing exaggerated fins also cause non-genetic changes in the display behavior of male zebrafish**. *Behaviour* 144(7): 787-795 . ISSN: 0005-7959.
Descriptors: zebrafish mutants, animal morphology, display behavior, fins, freshwater fish, genes, phylogenetics, point mutation, *Danio rerio.*

Ivanov, I.P., A.J. Pittman, C.B. Chien, R.F. Gesteland, and J.F. Atkins (2007). **Novel antizyme gene in *Danio rerio* expressed in brain and retina.** *Gene* 387(1-2): 87-92. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: The synthesis of the protein antizyme requires a +1 ribosomal frameshift event. The frameshifting serves as a regulatory sensor. Antizyme homologs have been identified in diverse organisms ranging from yeast to human and characterized in a disparate subset. Most vertebrates have multiple antizyme paralogs. Here we present identification in the zebrafish *Danio rerio* of a heretofore unknown member of the antizyme gene family. This novel antizyme does not correspond to any of the known orthologous groups in vertebrates and unlike most other antizymes is preferentially expressed in the retinal ganglion cell layer of the eye. In addition to the retina, it is also expressed in the brain and somites.
Descriptors: zebrafish brain metabolism, enzyme inhibitors metabolism, ornithine decarboxylase antagonists and inhibitors, retina metabolism, amino acid sequence, developmental gene expression regulation, molecular sequence data, phylogeny, protein biosynthesis.

Iwashita, M., M. Watanabe, M. Ishii, T. Chen, S.L. Johnson, Y. Kurachi, N. Okada, and S. Kondo (2006). **Pigment pattern in jaguar/obelix zebrafish is caused by a Kir7.1 mutation: implications for the regulation of melanosome movement.** *Public Library of Science Genetics* 2(11): E197. ISSN: 1553-7390.
Abstract: Many animals have a variety of pigment patterns, even within a species, and these patterns may be one of the driving forces of speciation. Recent molecular genetic studies on zebrafish have revealed that interaction among pigment cells plays a key role in pattern formation, but the mechanism of pattern formation is unclear. The zebrafish jaguar/obelix mutant has broader stripes than wild-type fish. In this mutant, the development of pigment cells is normal but their distribution is altered, making these fish ideal for studying the process of pigment pattern formation. Here, we utilized a positional cloning method to determine that the inwardly rectifying potassium channel 7.1 (Kir7.1) gene is responsible for pigment cell distribution among jaguar/obelix mutant fish. Furthermore, in jaguar/obelix mutant alleles, we identified amino acid changes in the conserved region of Kir7.1, each of which affected K(+) channel activity as demonstrated by patch-clamp experiments. Injection of a bacterial artificial chromosome containing the wild-type Kir7.1 genomic sequence rescued the jaguar/obelix phenotype. From these results, we conclude that mutations in Kir7.1 are responsible for jaguar/obelix. We also determined that the ion channel function defect of melanophores expressing mutant Kir7.1 altered the cellular response to external signals. We discovered that mutant melanophores cannot respond correctly to the melanosome dispersion signal derived from the sympathetic neuron and that melanosome aggregation is constitutively activated. In zebrafish and medaka, it is well known that melanosome aggregation and subsequent melanophore death increase when fish are kept under constant light conditions. These observations indicate that melanophores of jaguar/obelix mutant fish have a defect in the signaling pathway downstream of the alpha2-adrenoceptor. Taken together, our results suggest that the cellular defect of the Kir7.1 mutation is directly responsible for the pattern change in the jaguar/obelix mutant.
Descriptors: zebrafish, melanosomes metabolism, mutation genetics, pigmentation physi-

ology, potassium channels, inwardly rectifying genetics, metabolism, adrenergic agonists pharmacology, adrenergic antagonists pharmacology, amino acid sequence, biological transport drug effects, molecular cloning, gene expression regulation drug effects, melanophores drug effects and metabolism, melanosomes drug effects, molecular sequence data, phenotype, pigmentation drug effects, potassium channels, inwardly rectifying chemistry and metabolism , secondary protein structure, messenger RNA genetics and metabolism, adrenoceptor alpha 2 agonists receptors, inhibitors and metabolism, sequence alignment, signal transduction drug effects.

Jackman, W.R. and D.W. Stock (2006). **Transgenic analysis of Dlx regulation in fish tooth development reveals evolutionary retention of enhancer function despite organ loss**. *Proceedings of the National Academy of Sciences, USA* 103(51): 19390-19395. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, dentition, enhancers, evolution, evolutionary genetics, feeding, fishery regulations, freshwater fish, genetic control, mutation, mutations, pharynx, preservation, teeth, *Astyanax mexicanus*, *Danio rerio*, Mexican tetra, development and cell cycle, genetics, feeding habits.

Jang, W.S., E.J. Kim, H. Ro, K.E. Kim, T.L. Huh, C.H. Kim, and M. Rhee (2003). **Expression of a novel type I keratin, DAPK-1 in the dorsal aorta and pronephric duct of the zebrafish embryos.** *Gene* 312: 145-50. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: We isolated a novel cytokeratin gene of zebrafish (*Danio rerio*), DAPK-1 closely related to other vertebrate type I cytokeratin genes. Zygotic transcription starts at the sphere stage. After the mid-blastula stage, DAPK-1 is expressed in all surface cells, notably in those of the outer enveloping layer. DAPK-1 messages are also present specifically during the segmentation, pharyngula, and hatching periods. In particular, after 24 h post-fertilization, its expression is restricted to the developing eye region, otic vesicle, pectoral fin, dorsal aorta, and pronephric duct. In the mindbomb mutant embryo that has defects in the dorsal aorta development, DAPK-1 transcripts are not detected in the dorsal aorta and pronephric duct. The characteristic expression pattern of DAPK-1 may facilitate more detailed studies related to the morphogenesis of dorsal aorta and pronephric duct.
Descriptors: zebrafish , aorta metabolism, keratins genetics, kidney metabolism, amino acid sequence, aorta embryology, complementary DNA chemistry, genetics, isolation and purification, embryonic development, developmental gene expression regulation, kidney embryology, molecular sequence data, mutation, phylogeny, sequence alignment, DNA sequence analysis.

Jarvis, R.B. and J.F. Knowles (2003). **DNA damage in zebrafish larvae induced by exposure to low-dose rate gamma-radiation: detection by the alkaline comet assay.** *Mutation Research* 541(1-2): 63-9. ISSN: 0027-5107.
NAL Call Number: QH431.M8
Abstract: This study has determined the sensitivity of the alkaline comet assay for the detection of strand breaks in the DNA of cells taken from a whole organism rather than a single cell type as in previously reported studies. The assay has been performed on cells from whole zebrafish larvae irradiated for 1 or 24 h at dose rates of 0.4, 1.2 or 7.2 mGy/h. Zebrafish larvae exposed to only 1.2 mGy/h of gamma-radiation for 1h showed a statistically significant increase in DNA damage compared to controls. This represents a high sensitivity of this animal model for DNA damage and of the comet assay protocol used for detecting such

damage. Increasing the exposure time from 1 to 24 h caused significant increases in DNA damage in zebrafish larvae, although the modest size of these increases in damage for the relatively large increases (24 times) in total absorbed dose indicates that dose rate may be the major factor in determining the level of DNA damage observed under the conditions of these experiments.
Descriptors: zebrafish larva, comet assay methods, DNA radiation effects and damage, gamma rays, larva radiation effects.

Jekosch, K. (2004). **The zebrafish genome project: sequence analysis and annotation.** *Methods in Cell Biology* 77: 225-39. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, genome, computational biology, genetic databases, DNA sequence analysis.

Jensen, A.M. and M. Westerfield (2004). **Zebrafish mosaic eyes is a novel FERM protein required for retinal lamination and retinal pigmented epithelial tight junction formation.** *Current Biology* 14(8): 711-7. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Abstract: Polarization is a common feature of many types of cells, and we are beginning to understand how cells become polarized. The role of cell polarity in development and tissue morphogenesis, however, is much less well understood. Our previous analysis of the mosaic eyes (moe) mutations revealed that moe is required for retinal lamination and also suggested that zebrafish moe function is required in the retinal pigmented epithelium (RPE) for the proper localization of adjacent retinal cell divisions at the apical neuroepithelial surface.
To understand the function of moe in the RPE, we cloned the moe locus and show that it encodes a novel FERM (for 4.1 protein, ezrin, radixin, moesin) domain-containing protein. Expression of moe in the eye, kidney, and brain reflects phenotypes found in moe(-) mutants, including RPE and retinal lamination defects, edema, and small or absent brain ventricles. We show that moe function is required for tight junction formation in the RPE. We suggest that moe may be a necessary component of the crumbs pathway that regulates apical cell polarity and also may play a role in photoreceptor morphogenesis.
Descriptors: zebrafish, cell polarity physiology, eye phylogeny, pigment epithelium of eye cytology, retina cytology, tight junctions physiology, amino acid sequence, cluster analysis, eye, gene expression, immunohistochemistry, in situ hybridization, membrane proteins, molecular sequence data, oligodeoxyribonucleotides, antisense, tertiary protein structure, retina physiology, sequence alignment, DNA sequence analysis, .

Jensen, P.J., J.D. Gitlin, and M.O. Carayannopoulos (2006). **Glut1 deficiency links nutrient availability and apoptosis during embryonic development**. *Journal of Biological Chemistry* 281(19): 13382-13387. ISSN: 0021 9258.
NAL Call Number: 381J824
Abstract: GLUT1 is essential for human brain development and function, as evidenced by the severe epileptic encephalopathy observed in children with GLUT1 deficiency syndrome resulting from inherited loss- of- function mutations in the gene encoding this facilitative glucose transporter. To further elucidate the pathophysiology of this disorder, the zebrafish orthologue of human GLUT1 was identified, and expression of this gene was abrogated during early embryonic development, resulting in a phenotype of aberrant brain organogenesis consistent with the observed expression of Glut1 in the embryonic tectum and specifically rescued by human GLUT1 mRNA. Affected embryos displayed impaired glucose uptake

concomitant with increased neural cell apoptosis and subsequent ventricle enlargement, trigeminal ganglion cell loss, and abnormal hindbrain architecture. Strikingly, inhibiting expression of the zebrafish orthologue of the proapoptotic protein Bad resulted in complete rescue of this phenotype, and this occurred even in the absence of restoration of apparent glucose uptake. Taken together, these studies describe a tractable system for elucidating the cellular and molecular mechanisms of Glut1 deficiency and provide compelling in vivo genetic evidence directly linking nutrient availability and activation of mitochondria- dependent apoptotic mechanisms during embryonic brain development. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, molecular genetics, epleptic encephalopathy, nervous system, chemical coordination and homeostasis, nervous system disease, etiology, in situ hybridization, histology and cytology techniques, genetic techniques.

Jessen, J.R. and L. Solnica Krezel (2004). **Identification and developmental expression pattern of van gogh-like 1, a second zebrafish strabismus homologue.** *Gene Expression Patterns* 4(3): 339-44. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Cell movement plays a central role in both normal embryogenesis and the development of diseases such as cancer. Therefore, identification and analysis of proteins controlling cell movement is of special importance. The zebrafish trilobite locus encodes a Van Gogh/Strabismus homologue, which regulates diverse cell migratory behaviors during embryogenesis. Trilobite is most similar to human Van Gogh-like 2 (VANGL2)/Strabismus 1 and mouse Loop-tail associated protein/Lpp1. Both human and mouse genomes encode a second Strabismus homologue referred to as VANGL1/Strabismus 2 and Lpp2, respectively. This prompted us to ask whether another van gogh/strabismus gene, one more closely related to human VANGL1, exists in the zebrafish genome. This paper describes the identification of zebrafish vangl1 and provides the first spatiotemporal expression and functional analysis of a vertebrate vangl1 homologue. Our data indicate that vangl1 and trilobite/vangl2 are expressed in largely non-overlapping domains during embryogenesis. Injection of synthetic vangl1 RNA partially suppressed the gastrulation defect in trilobite mutant embryos, suggesting that Vangl1 and Trilobite/Vangl2 have similar biochemical activities.

Descriptors: zebrafish, membrane amino acid sequence, carrier chromosome mapping, molecular cloning, metabolism, developmental gene expression regulation, membrane proteins metabolism, molecular sequence data, phylogeny, sequence alignment.

Ji, W., W.L. Zhou, R. Abruzzese, W. Guo, A. Blake, S. Davis, S. Davis, and I. Polejaeva (2005). **A method for determining zygosity of transgenic zebrafish by Taqman real-time PCR**. *Analytical Biochemistry* 344(2): 240-246. ISSN: 0003 2697.

NAL Call Number: 381 An13

Abstract: When producing a genetically modified organism, intended genes are often integrated into a target genome by random insertions. Subsequently, it is often desirable to know the gene copy number of the transgenic organism and the zygosity of its offspring. Because of the random insertions, the estimation can be made only by quantitative measurement of the genes. Even though TaqMan real-time PCR has been used in gene expression analysis, it is routinely used to quantify differences larger than twofold or more than one PCR cycle. In this study, we employed TaqMan quantitative PCR to determine zygosity of transgenic fluorescent zebrafish in which a homozygote and a hemizygote differ by only twofold. We measured relative quantities of the transgene by taking the threshold cycle (Ct) of both the transgene and an internal control zebrafish genomic DNA. Using scatterplots and statisti-

cal inference, we demonstrated that homozygotes and hemizygotes could be differentiated unambiguously when multiple measurements were taken. We discuss the relationship between the repetitive measurements and TaqMan precision with a statistical model. The result illustrates that the method can be extended to some areas that require even higher precision such as determining the polyploidy of an organism. Copyright © 2008 CABI
Descriptors: genetically modified transgenic zebrafish, accuracy, DNA, genes, genomes, homozygosity, mathematical models, PCR, quantitative analysis, techniques, transposable elements, *Danio rerio*.

Jia, H., I.N. King, S.S. Chopra, H. Wan, T. Ni, C. Jiang, X. Guan, S.a.m. Wells, D. Srivastava, and T. Zhong (2007). **Vertebrate heart growth is regulated by functional antagonism between Gridlock and Gata5**. *Proceedings of the National Academy of Sciences, USA* 104(35): 14008-14013. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, antagonism, biochemical analysis, cell number, cell size, developmental stages, embryonic development, embryos, freshwater fish, growth, heart, helix-loop-helix proteins, mutations, phylogenetics, protein interaction, transcription factors, cardiomyocytes, *Danio rerio.*

Jia, S., M. Omelchenko, D. Garland, V. Vasiliou, J. Kanungo, M. Spencer, Y. Wolf, E. Koonin, and J. Piatigorsky (2007). **Duplicated gelsolin family genes in zebrafish: a novel scinderin-like gene (scinla) encodes the major corneal crystallin.** *Federation of American Societies for Experimental Biology Journal* 21(12): 3318-28. ISSN: 0892-6638.
NAL Call Number: QH301.F3
Abstract: We have previously identified a gelsolin-like protein (C/L-gelsolin) as a corneal crystallin in zebrafish. Here we show by phylogenetic analysis that there are at least six genes encoding gelsolin-like proteins based on their gelsolin domains in zebrafish: gsna and gsnb group with the vertebrate gelsolin gene, scina and scinb group with the scinderin (adseverin) gene, and scinla (C/L-gelsolin) and scinlb are novel scinderin-like genes. RT-PCR showed that scinla, scinlb, and gsnb are preferentially expressed in the adult cornea whereas gsna is expressed to a similar extent in cornea, lens, brain, and heart; scina and scinb expression were detectable only in whole zebrafish and not in these adult tissues. Quantitative RT-PCR and 2-dimensional polyacrylamide gel electrophoresis followed by MALDI/TOF mass spectroscopy confirmed high expression of beta-actin and scinla, moderate expression of scinlb, and very low expression of gsna and gsnb in the cornea. Finally, transgenic zebrafish carrying a green fluorescent protein reporter transgene driven by a 4 kb scinla promoter fragment showed expression in the cornea, snout, dorsal fin, and tail fin of 3-day-old zebrafish larvae. Our data suggest that scinla and scinlb are diverged paralogs of the vertebrate scinderin gene and show that scinla encodes the zebrafish corneal crystallin previously called C/L-gelsolin.
Descriptors: zebrafish, corneal crystallin, gelsolin, genes, scinderin-like, gene, scint1a.

Jia, S., Z. Ren, X. Li, Y. Zheng, and A. Meng (2008). **Smad2 and smad3 are required for mesendoderm induction by transforming growth factor- beta /nodal signals in zebrafish**. *Journal of Biological Chemistry* 283(4): 2418-2426. ISSN: 0021-9258.
NAL Call Number: 381J824
Descriptors: zebrafish, activin, amino acids, embryogenesis, mesendoderm, pattern formation, signal transduction, Smad2 and Smad3 protein, transcription factors, transforming growth factor- beta, *Danio rerio.*

Jian Qing, J. Bai Jun, Ye Xing, and L. Xia Shi (2004). **Cloning of Mylz2 promoter and generation of green fluorescence transgenic zebra fish**. *Journal of Fishery Sciences of China* 11(5): 391-395. ISSN: 1005 8737.

Abstract: The genomic DNA of zebra fish [*Danio rerio*] was extracted from liver tissue and the myosin light chain2 (Mylz2) promoter DNA was amplified from DNA by polymerase chain reaction (PCR) with two special primers. After purification, the amplified fragment was ligated into pGEM-T Easy Vector and transformed into *Escherichia coli* DH5 alpha . The sequencing and analysis of the cloned gene showed the similitude between the cloned fragment and the zebra fish myosin light chain2 (Mylz2) promoter. All of the important cis-elements such as TATA-Box, E-Box and MFF2 binding sites are the same in number and location, except for the MFF2 binding site at the 527th base position from 5' end, which indicates that the cloned fragment is the Mylz2 promoter. The cloned Mylz2 promoter was inserted into the vector (pEGFP-1) to construct the green fluorescence protein expression vector. The linearized expression vector was delivered into one or two cells stage zebra fish embryo by micro-injection. Under fluorescence microscope, green fluorescence has been observed in muscle tissue as early as the embryo blood-circling stage. In one-month-old fish, fluorescence is strong enough to be seen with the naked eye under ultraviolet light in the dark. Copyright © 2008 CABI

Descriptors: transgenic zebrafish, green fluorescence protein expression vector, DNA, fluorescence, *Danio rerio*, fishes.

Language of Text: Chinese.

Jiang YunJin (2004). **Vertebrate somite development, Notch signaling and others**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd. Singapore, Singapore , p. 294-338. ISBN: 9812388214.

NAL Call Number: QL638.C94 F57

Abstract: The molecular basis of somite development: the periodic generation of somites, rostrocaudal (RC) polarization in formed somites, somite furrow formation and somite differentiation has been substantially explored among different vertebrates for last few decades, enabling us to understand it from a more mechanistic way. The work on chicken c-hairy1 cycling, mouse knock-outs of Notch components and zebrafish somite mutants has demonstrated a vital role of Notch signaling in somite segmentation. A mechanism involving cyclical activation of transcription and delayed negative feedback regulation is emerging. Fgf8 and Wnt3a gradients are important in positioning somite boundaries and, probably, in coordinating tail growth and segmentation. In addition to segmentation, Notch signaling is also essential for RC polarity and boundary formation in collaboration with a variety of genes, including Mesp, Eph, ephrin, Protocadherin (Papc), Foxc and T-box genes. Zebrafish has played an indispensable role in recent progress. Studies of other species will also be discussed in a comparative and complementary way. Copyright © 2008 CABI

Descriptors: zebrafish embryos, cell cycle, embryonic development, gene expression, genes, molecular genetics, somite mutants, regulatory genes, Notch signal transduction, transcription, *Danio rerio.*

Jin, H. and L. Wang (2003). **Electronic cloning and analysis of zebrafish ILF2 gene**. *Journal of China Institute of Metrology* 14(1): 70-73. ISSN: 1004-1540.

Descriptors: zebrafish, amino acid sequence, freshwater fish, genes, nucleotides, *Danio rerio*, genetics and evolution.

Jin, H.-J., L.-X. Xiang, and J.-Z. Shao (2007). **Molecular cloning and identification of macrophage migration inhibitory factor (MIF) in teleost fish**. *Developmental & Comparative Immunology* 31(11): 1131-1144. ISSN: 0145-305X.
NAL Call Number: QR180.D4
Descriptors: zebrafish, 3' untranslated regions, cell migration, chromosome 1, cytokines, DNA, exons, freshwater fish, immune system, introns, kidney, lipopolysaccharides, macrophage migration inhibitory factor, macrophages, marine fish, migration inhibition, nucleotide sequence, open reading frames, phylogenetics, phylogeny, PCR, Southern blotting, spleen, genomics, mRNA, *Danio rerio*, *Fundulus heteroclitus*, *Haplochromis chilotes*, *Ictalurus punctatus*, *Oncorhynchus mykiss*, *Salmo salar*, Teleostei, Tetraodon, *Tetraodon nigroviridis*, Atlantic salmon, channel catfish, Mummichog, rainbow trout, immunity, genetics and evolution.

Jopling, C. and J. Den Hertog (2005). **Fyn/Yes and non-canonical Wnt signalling converge on RhoA in vertebrate gastrulation cell movements**. *European Molecular Biology Organization Reports* 6(5): 426-431. ISSN: 1469 221X.
NAL Call Number: QH506.E463
Abstract: Convergent extension (CE) cell movements during gastrulation mediate extension of the anterior - posterior body axis of vertebrate embryos. Non-canonical Wnt5 and Wnt11 signalling is essential for normal CE movements in vertebrate gastrulation. Here, we show that morpholino (MO)- mediated double knockdown of the Fyn and Yes tyrosine kinases in zebrafish embryos impaired normal CE cell movements, resembling the silberblick and pipetail mutants, caused by mutations in wnt11 and wnt5, respectively. Co-injection of Fyn/ Yes- and Wnt11- or Wnt5-MO was synergistic, but wnt11 or wnt5 RNA did not rescue the Fyn/ Yes knockdown or vice versa. Remarkably, active RhoA rescued the Fyn/ Yes knockdown as well as the Wnt11 knockdown, indicating that Fyn/ Yes and Wnt11 signalling converged on RhoA. Our results show that Fyn and Yes act together with noncanonical Wnt signalling via RhoA in CE cell movements during gastrulation. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics , gastrulation, tyrosine kinase, morpholino mediated double knockdown.

Jozefowicz, C., J. McClintock, and V. Prince (2003). **The fates of zebrafish Hox gene duplicates.** *Journal of Structural and Functional Genomics* 3(1-4): 185-94. ISSN: 1345-711X.
Abstract: In his 1970 book, Susumu Ohno stressed the importance of gene duplication in the evolution of the vertebrate genome and body plan. He elaborated the idea that duplication events provide novel genetic material on which evolution may act. Data are accumulating to show that extensive duplication events, perhaps incorporating the duplication of entire genomes, occurred in the lineage leading to teleost fishes. These duplications may have been pivotal in the explosive radiation of this highly successful vertebrate group. Thus, the teleosts provide us with an ideal opportunity to investigate the fates and functions of duplicated genes. A convenient system for these studies is the zebrafish, *Danio rerio*, which has become a popular genetic and embryological model.
Descriptors: zebrafish, molecular evolution, gene duplication, homeobox genes, chordata, nonvertebrate genetics, multigene family, phylogeny, embryological model.

Ju, B., H. Huang, K.Y. Lee, and S. Lin (2004). **Cloning zebrafish by nuclear transfer.** *Methods in Cell Biology* 77: 403-11. ISSN: 0091-679X.
NAL Call Number: 442P92

Descriptors: zebrafish, cloning, organism methods, nuclear transfer techniques, cell culture techniques, cell nucleus genetics, cytology, gene targeting methods.

Ju, B.S., H.G. Huang, K.Y. Lee and S. Lin (2004). **Cloning the zebrafish**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd. Singapore, Singapore , p. 517-531. ISBN: 9812388214.
NAL Call Number: QL638.C94 F57
Abstract: Although the efforts started 40 years ago, resources dedicated to fish cloning and achievements made so far lag far behind those in other vertebrates, especially mammals. In this review, we summarize the fish cloning work carried out by a number of laboratories and our data of generating cloned zebrafish from long-term cultured embryonic fibroblast cells. This success lays the foundation for developing cloning-based gene-trapping and homologous recombination technologies for gene function studies in zebrafish. In addition, fish cloning should contribute to studies addressing basic issues such as development and aging of cloned animals. Copyright © 2008 CABI
Descriptors: zebrafish, aging, cell cloning, genes, techniques, *Danio rerio.*
Notes: A review.

Ju, Z., M.C. Wells, A. Martinez, L. Hazlewood, and R.B. Walter (2005). **An in silico mining for simple sequence repeats from expressed sequence tags of zebrafish, medaka, Fundulus, and Xiphophorus.** *In Silico Biology* 5(5-6): 439-63. ISSN: 1434-3207.
Abstract: Teleost fish genome projects involving model species are resulting in a rapid accumulation of genomic and expressed DNA sequences in public databases. The expressed sequence tags (ESTs) collected in the databases can be mined for the analysis of both structural and functional genomics. In this study, we in silico analyzed 49,430 unigenes representing a total of 692,654 ESTs from four model fish for their potential use in developing simple sequence repeats (SSRs), or microsatellites. After bioinformatical mining, a total of 3,018 EST derived SSRs (EST-SSRs) were identified for 2,335 SSR containing ESTs (SSR-ESTs). The frequency of identified SSR-ESTs ranged from 1.5% for Xiphophorus to 7.3% for zebrafish. The dinucleotide repeat motif is the most abundant SSR, accounting for 47%, 52%, 64%, and 78% for medaka, Fundulus, zebrafish, and Xiphophorus, respectively. Simulation analysis suggests that a majority of these EST-SSRs have sufficient flanking sequences for polymerase chain reaction (PCR) primer design. Comparative DNA sequence analyses of SSR-ESTs identified several cross-species SSRs and sequences that may be used as cross-reference genes in comparative studies. For example, the flanking sequences of one SSR (CTG) n within the pituitary tumor-transforming gene (PTTG) 1 interacting protein (PTTGIP), showed conservation spanning the medaka, Fundulus, human, and mouse genomes. This study provides a large body of information on EST-SSRs that can be useful for the development of polymorphic markers, gene mapping, and comparative genome analysis. Functional analysis of these SSR-ESTs may reveal their role in metabolism and gene evolution of these model species.
Descriptors: zebrafish, Cyprinodontiformes genetics, Fundulidae genetics , microsatellite repeats, *Oryzias* genetics, amino acid and base sequence, cluster analysis, DNA genetics, nucleic acid databases, expressed sequence tags, genomics, molecular sequence data, sequence alignment, species specificity.

Kajihara, M., H. Sone, M. Amemiya, Y. Katoh, M. Isogai, H. Shimano, N. Yamada, and S. Takahashi (2003). **Mouse MafA, homologue of zebrafish somite Maf 1, contributes to the specific transcriptional activity through the insulin promoter.** *Biochemical and Biophysical Research Communications* 312(3): 831-42. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: Large Maf transcription factors, which are members of the basic leucine zipper (b-Zip) superfamily, have been reported to be involved in embryonic development and cell differentiation. Previously, we isolated a novel zebrafish large Maf cDNA, somite Maf1 (SMaf1), which possesses transactivational activity within its N-terminus domain. To elucidate SMaf1 function in mammals, we tried to isolate the mouse homologue of zebrafish SMaf1. We isolated the mouse homologue of zebrafish SMaf1, which is the same molecule as the recently reported MafA. MafA mRNA was detected in formed somites, head neural tube, and liver cells in the embryos. In the adult mouse, MafA transcript was amplified in the brain, lung, spleen, and kidney by RT-PCR. MafA mRNA was also detectable in beta-cell line. Next, we analyzed the transcriptional activity of MafA using rat insulin promoters I and II (RIPI and II), since a part of RIP sequence was similar to the Maf recognition element (MARE) and MafA was expressed in pancreatic beta cells. MafA was able to activate transcription from RIPII, but not RIPI, in a dose dependent manner and the activity was dependent on RIPE3b/C1 sequences. In addition, the amount of MafA protein was regulated by glucose concentration. These results indicate that MafA is the homologue of zebrafish SMaf1 and acts as a transcriptional activator of the insulin gene promoter through the RIPE3b element.

Descriptors: zebrafish, homeodomain proteins, insulin genetics and metabolism, proto oncogene proteins chemistry and metabolism, *Saccharomyces cerevisiae* proteins chemistry and metabolism, trans activation genetics, physiology, chemistry and metabolism, transcription factors chemistry and metabolism, amino acid sequence, chickens, C type lectins, Maf transcription factors, large, c57bl inbred mice, molecular sequence data, organ specificity, promoter regions genetics, proto oncogene *Saccharomyces cerevisiae* sensitivity and specificity, sequence alignment, protein sequence analysis, amino acid sequence homology, somites chemistry and metabolism, tissue distribution, transcription factors genetics, *Xenopus*.

Kajimura, S., K. Aida, and C. Duan (2005). **Understanding hypoxia-induced gene expression in early development: in vitro and in vivo analysis of HIF-1-regulated zebrafish IGFBP-1 gene expression**. *Integrative and Comparative Biology* 45(6): 1023. ISSN: 1540 7063.

NAL Call Number: QL1.I67

Descriptors: zebrafish, metabolism, development, molecular genetics, gene expression, respiration, point mutagenesis, deletion mutagenesis, genetic techniques, early development.

Kajimura, S., K. Aida, and C. Duan (2006). **Understanding hypoxia-induced gene expression in early development: in vitro and in vivo analysis of hypoxia-inducible factor 1-regulated zebra fish insulin-like growth factor binding protein 1 gene expression.** *Molecular and Cellular Biology* 26(3): 1142-55. ISSN: 0270-7306.

NAL Call Number: QH506.M664

Abstract: Insulin-like growth factor binding protein 1 (IGFBP-1) is a hypoxia-inducible gene that plays an important role in regulating embryonic growth and development under hypoxic stress. The molecular mechanisms underlying hypoxia-induced IGFBP-1 gene expression in the embryonic tissues are not well understood. Here we report that the hypoxia-inducible factor 1 (HIF-1) pathway is established in early embryogenesis and mediates hypoxia-induced IGFBP-1 expression. Hypoxia increased the HIF-1 activity, and

HIF-1alpha overexpression or CoCl2 treatment resulted in elevated IGFBP-1 expression in zebra fish embryos. Although the zebra fish IGFBP-1 promoter contains 13 consensus hypoxia response elements (HREs), deletion and mutational analysis revealed that only the HRE positioned at -1090/-1086 is required for the hypoxia and HIF-1 induction. Further experiments revealed that there is an HIF-1 ancillary sequence (HAS) adjacent only to the functional HRE. Mutation of this HAS greatly reduced the responsiveness of the IGFBP-1 promoter to hypoxia and HIF-1. The HAS does not directly bind to HIF-1 or affect the binding of the HRE to HIF-1. The HAS is bound to a nuclear protein(s), and this HAS binding activity is reduced by hypoxia. These results suggest that HIF-1 mediates hypoxia-induced IGFBP-1 gene expression in early development by selectively interacting with the -1090/-1086 HRE and its adjacent HAS.
Descriptors: mutant zebrafish, anoxia genetics, developmental gene expression regulation, hypoxia inducible factor 1 metabolism, insulin like growth factor binding protein 1 genetics, consensus sequence, metabolism, embryonic development genetics, promoter regions genetics, response elements.

Kajimura, S., K. Aida, and C. Duan (2005). **Insulin-like growth factor-binding protein-1 (IGFBP-1) mediates hypoxia-induced embryonic growth and developmental retardation**. *Proceedings of the National Academy of Sciences of the United States of America* 102(4): 1240-1245. ISSN: 0027 8424.
NAL Call Number: 500N21P
Abstract: Although reduced fetal growth in response to hypoxia has been appreciated for decades, we have a poor understanding of the effects of hypoxia on embryonic development and the underlying cellular and molecular mechanisms. Here we show that hypoxia treatment not only resulted in embryonic growth retardation but also caused significant delay in developmental speed and the timing of morphogenesis in vital organs of zebrafish. Hypoxia strongly induced the expression of insulin-like growth factor (IGF)binding protein (IGFBP)-1, a secreted protein that binds IGFs in extracellular environments. Hypoxia did not change the expression levels of IGFs, IGF receptors, or other IGFBPs. The hypothesis that elevated IGFBP-1 mediates hypoxia-induced embryonic growth retardation and developmental delay by binding to and inhibiting the activities of IGFs was tested by loss- and gain-of-function approaches. Knockdown of IGFBP-1 significantly alleviated the hypoxia-induced growth retardation and developmental delay. Overexpression of IGFBP-1 caused growth and developmental retardation under normoxia. Furthermore, reintroduction of IGFBP-1 to the IGFBP-1 knocked-down embryos restored the hypoxic effects on embryonic growth and development. When tested in vitro with cultured zebrafish embryonic cells, IGFBP-1 itself had no mitogenic activity, but it inhibited IGF-1- and IGF-2-stimulated cell proliferation. This inhibitory effect was abolished when IGF-1 or IGF-2 was added in molar excess, suggesting that IGFBP-1 inhibits embryonic growth and development by binding to and inhibiting the activities of IGFs. The induction of IGFBP-1 expression may be a conserved physiological mechanism to restrict the IGF-stimulated growth and developmental process under hypoxic stress. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, molecular genetics, growth retardation, congenital disease, hypoxia, anoxia , metabolic disease, cell proliferation, developmental delay, embryonic growth retardation, extracellular environment.

Kakizawa, T., S. Nishio, G. Triqueneaux, S. Bertrand, J. Rambaud, and V. Laudet (2007). **Two differentially active alternative promoters control the expression of the zebrafish orphan nuclear receptor gene Rev-erbalpha.** *Journal of Molecular Endocrinology* 38(5): 555-68.

ISSN: 0952-5041.
NAL Call Number: QP187.3.M64J68
Abstract: The orphan nuclear receptor Rev-erbalpha (NR1D1) plays an important role in the regulation of the circadian pacemaker and its expression has been shown to be regulated with a robust circadian rhythm in zebrafish and mammals. In addition, in zebrafish its expression has been shown to be developmentally regulated. In order to analyze the mechanisms of the zfRev-erbalpha gene regulation, we have isolated its 5'-upstream region. We found that two promoters control the zfRev-erbalpha expression. The first one (ZfP1) is characterized by a very high degree of sequence identity with the mammalian P1 promoter and contains, as the mammalian P1, a functional Rev-erbalpha-binding site (RevDR2). Inhibition of zfRev-erbalpha activity in zebrafish embryos using antisense-morpholino knockdown results in an increase of zfRev-erbalpha gene expression suggesting that zfRev-erbalpha is repressing its own transcription in vivo. In addition, we show that ROR orphan receptors also regulate in vitro and in vivo zfRev-erbalpha gene expression through the same RevDR2 element. In contrast, the second promoter ZfP2 is strikingly different from the mammalian P2: its sequence is not conserved between zebrafish and mammals and is not regulated by the same transcription factors. Together, these data suggest that ZfP1 is orthologous to the mammalian P1 promoter, whereas zebrafish ZfP2 has no mammalian ortholog and does not function like ZfP1 to control Rev-erbalpha expression.
Descriptors: zebrafish, developmental gene expression regulation, promoter regions genetics physiology, cytoplasmic receptors and nuclear genetics, base sequence, COS cells, *Cercopithecus aethiops* molecular sequence data, cytoplasmic receptors and nuclear metabolism, retinoic acid receptors genetics and physiology, response elements, genetic transcription, transfection.

Kallivretaki, E., R. Eggen, S. Neuhauss, M. Alberti, U. Kausch, and H. Segner (2006). **Aromatase in zebrafish: a potential target for endocrine disrupting chemicals.** *Marine Environmental Research* 62(Suppl): S187-90. ISSN: 0141-1136.
NAL Call Number: QH545.W3M36
Abstract: In zebrafish, two isoforms of the aromatase gene exist, namely cyp19a1 and cyp19a2, expressed predominantly in the gonads and brain, respectively. In this study, we focus on characterizing the specificity of antibodies against the aromatase isoforms, and on (xeno)estrogen-induced changes of individual cyp19a2 mRNA concentrations in the brains of adult male zebrafish. Among three polyclonal antibodies studied, the one against CYP19A2 was found to be specific in Western blots and immunohistochemistry. Real-time RT-PCR analyses revealed strong interindividual variation of cyp19a2 levels in the brains of adult male zebrafish. After a three-week-exposure to (xeno)estrogens, mean values of cyp19a2 mRNA levels tended to increase, with significant induction at 200 ng 17beta-estradiol/L, but interindividual variation of cyp19a2 expression was maintained.
Descriptors: zebrafish, aromatase gene, cyp19a1, cyp19a2, expression, gonad, brain, xenoestrogen-induced changes.

Kanai, T., K. Imai, and H. Nakayasu (2007). **Distribution of a brain-specific extracellular matrix protein in developing and adult zebrafish.** *Brain Research* 1129(1): 53-62. ISSN: 0006 8993.
NAL Call Number: QP376
Abstract: A monoclonal antibody (IgG) that recognizes a 53-kDa zebrafish brain protein was isolated and used to characterize the distribution of this protein in zebrafish. (1) The antigen was found only in the brain and not in any other tissues such as muscle, dermis and cartilage. Within the brain, the antibody recognized extracellular matrix (ECM) outside

neuronal cells. (2) Digestion by hyaluronidase released the antigen from brain tissue, and the monoclonal antibody staining was also decreased by the digestion by hyaluronidase. (3) The pattern of antigen distribution is not perineuronal, as the density of the antigen at the periphery of the cells was practically identical to that of the empty intercellular spaces. Therefore, this monoclonal antibody does not recognize the perineuronal glycocortex. (4) The antigen is distributed only in limited areas of the brain, namely in the periphery of the forebrain, the hypothalamus, the optic tectum, the interpeduncular nucleus, the cerebellum and the ventricular rim of the medulla. In the optic tectum, the antibody strongly stained the most superficial layer, and in the cerebellum, it stained the molecular but not the granular layer. These patterns of distribution are very different from those of other typical brain ECM proteins and suggest that this protein may play quite distinct roles in brain development and maintenance.
Descriptors: zebrafish, brain metabolism, extracellular matrix proteins metabolism, neurons metabolism, antibody specificity immunology, brain growth and development and ultrastructure, tumor cell line, extracellular matrix proteins immunology, isolation and purification, hybridomas, immunohistochemistry, mice, inbred Balb C mice, neurons ultrastructure, neuropil metabolism and ultrastructure, subcellular fractions, proteins immunology, proteins isolation and purification.

Kaneko, M. and G.M. Cahill (2005). **Light-dependent development of circadian gene expression in transgenic zebrafish.** *Public Library of Science Biology* 3(2): E34. ISSN: 1544-9173.
NAL Call Number: QH301.P56
Abstract: The roles of environmental stimuli in initiation and synchronization of circadian oscillation during development appear to vary among different rhythmic processes. In zebrafish, a variety of rhythms emerge in larvae only after exposure to light-dark (LD) cycles, whereas zebrafish period3 (per3) mRNA has been reported to be rhythmic from day 1 of development in constant conditions. We generated transgenic zebrafish in which expression of the firefly luciferase (luc) gene is driven by the zebrafish per3 promoter. Live larvae from these lines are rhythmically bioluminescent, providing the first vertebrate system for high-throughput measurement of circadian gene expression in vivo. Circadian rhythmicity in constant conditions was observed only after 5-6 d of development, and only if the fish were exposed to LD signals after day 4. Regardless of light exposure, a novel developmental profile was observed, with low expression during the first few days and a rapid increase when active swimming begins. Ambient temperature affected the developmental profile and overall levels of per3 and luc mRNA, as well as the critical days in which LD cycles were needed for robust bioluminescence rhythms. In summary, per3-luc zebrafish has revealed complex interactions among developmental events, light, and temperature in the expression of a clock gene.
Descriptors: zebrafish, circadian rhythm genetics , gene expression regulation, light, luciferases genetics and metabolism, genetically modified, fireflies enzymology and genetics, reporter genes, molecular sequence data, nuclear promoter regions genetics, messenger RNA genetics.

Kanungo, J., S.K. Swamynathan, and J. Piatigorsky (2004). **Abundant corneal gelsolin in zebrafish and the 'four-eyed' fish, *Anableps anableps*: possible analogy with multifunctional lens crystallins.** *Experimental Eye Research* 79(6): 949-56. ISSN: 0014 4835.
NAL Call Number: QP474
Abstract: The cornea accumulates high proportions (can be up to 50%) of taxon-specific, water-soluble, cytoplasmic proteins (often enzymes) that have been considered analogous to the multifunctional lens crystallins. We have shown that gelsolin (an actin-severing protein)

is the major water-soluble corneal protein of the zebrafish (*Danio rerio*) and the 'four-eyed' fish (*Anableps anableps*). Each Anableps eye contains one lens, an aquatic ventral cornea with an epithelium comprising 5-7 cell layers, and an air-exposed flatter dorsal cornea with an epithelium comprising >20 cell layers and appreciably enriched with glycogen. Gelsolin accounts for 38 and 21% of the dorsal and ventral cornea, respectively, suggesting that the abundance of gelsolin in the cornea is not incompatible with its function in air. The thicker, glycogen-enriched, air-exposed dorsal cornea may protect against UV irradiation and desiccation. Gelsolin comprises approximately 50% of the 5 cell-layer thick aquatic corneal epithelium of zebrafish. Reported zebrafish ESTs have indicated the presence of a second gelsolin gene in this species. We show by RT-PCR that the abundant corneal gelsolin (also expressed weakly in lens) (C/L-gelsolin) is also expressed in early development and differs from a ubiquitously expressed gelsolin (U-gelsolin) that is not specialized for cornea. Microinjection tests showed that overexpression of C/L-gelsolin dorsalizes the embryo and can lead to axis duplication, while interruption of C/L-gelsolin expression with a specific morpholino oligonucleotide ventralizes the embryo and interferes with brain and eye development. The evidence that C/L-gelsolin participates in the bone morphogenetic protein (BMP)/Smad dorsal-ventral signaling pathway is reviewed. Finally, we speculate that soluble C/L-gelsolin:actin complexes in the cornea may be analogous to soluble alphaA:alphaB-crystallin complexes in the lens. Together, our data are consistent with an analogy between the abundance of gelsolin in fish corneas and taxon-specific multifunctional crystallins in lenses.
Descriptors: zebrafish , cornea metabolism, crystallins metabolism, gelsolin metabolism, cornea anatomy and histology, crystallins genetics, fishes anatomy and histology, gene expression, in situ hybridization, crystalline lens metabolism, messenger RNA genetics, reverse transcriptase PCR methods.

Kappler, J.A., C.J. Starr, D.K. Chan, R. Kollmar, and A.J. Hudspeth (2004). **A nonsense mutation in the gene encoding a zebrafish myosin VI isoform causes defects in hair-cell mechanotransduction.** *Proceedings of the National Academy of Sciences of the United States of America* 101(35): 13056-61. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, hair cells metabolism, inner ear and lateral-line, myosin heavy chains genetics, protein isoforms genetics, nonsense codon, confocal microscopy, molecular sequence data, mutation, myosin heavy chains metabolism, phenotype, metabolism.

Kapuscinski, A.R. (2005). **Current scientific understanding of the environmental biosafety of transgenic fish and shellfish**. *Revue Scientifique Et Technique Office International Des Epizooties* 24(1): 309-322. ISSN: 0253 1933.
NAL Call Number: SF781.R4
Abstract: A fluorescent zebrafish was the first genetically engineered animal to be marketed, and biotechnologists are developing many transgenic fish and shellfish. Biosafety science is not sufficiently advanced to be able to draw scientifically reliable and broadly trusted conclusions about the environmental effects of these animals. The science is best developed for identifying hazards posed by environmental spread of a transgenic fish or shellfish and least developed for assessing potential ecological harms of spread. Environmental spread of certain transgenic fish or shellfish could be an indirect route of entry into the human food supply. The management of predicted environmental risks is in its infancy and has thus far focused on the first step of the risk management process, i.e. risk reduction, via a few confinement methods. There is a critical need to improve scientific methods of environmental safety assessment and management and to gather empirical data needed to substantiate biosafety

conclusions and to effectively manage transgenic fish and shellfish. Scientists and potentially affected parties should participate in prioritising the knowledge gaps to be addressed. Copyright © 2008 CABI
Descriptors: transgenic zebrafish and shellfish, biosafety, environment, genetic engineering, risk assessment and reduction, shellfish culture, fishes.
Language of Text: Spanish;French.

Karchner, S.I., D.G. Franks, and M.E. Hahn (2005). **AHR1B, a new functional aryl hydrocarbon receptor in zebrafish: tandem arrangement of ahr1b and ahr2 genes**. *Biochemical Journal* 392(1): 153-161. ISSN: 0264 6021.
NAL Call Number: 382B52
Abstract: The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that regulates gene expression following activation by TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) or a variety of other synthetic and natural compounds. Previous studies have identified two AHR genes, AHR1 and AHR2, in zebrafish (*Danio rerio*), a widely used model species for studying vertebrate development and an emerging model in developmental toxicology. Zebrafish AHR2 binds TCDD with high affinity, is transcriptionally active and has a major role in mediating the developmental toxicity of TCDD. Zebrafish AHR1 lacks the ability to bind TCDD and activate transcription, and has no known function. In the present study, we report a new zebrafish AHR, designated AHR1B, which shares 34% amino acid sequence identity with AHR1 (AHR1A). The ahr1b gene resides on chromosome 22, adjacent to ahr2, whereas the ahr1a gene is located on chromosome 16. AHR1B is expressed in embryos as early as 24 hours post-fertilization and increases through the next 2 days, but expression is not inducible by TCDD. In contrast with the previously identified AHR1A, in vitro-expressed AHR1B protein exhibits specific, high-affinity binding of [3H]TCDD. Furthermore, AHR1B is able to activate the transcription of a reporter gene under the control of AHR response elements with an efficacy comparable with that of AHR2, but with a higher EC50. We speculate that AHR1B may have a physiological role, such as in embryonic development, whereas AHR2 mediates the response to xenobiotics. Copyright © 2008 CABI
Descriptors: zebrafish embryos, amino acid sequences, biochemical receptors, chromosomes, genes, hydrocarbons, transcription factors, *Danio rerio.*

Kassen, S.C., V. Ramanan, J.E. Montgomery, C. T Burket, C.G. Liu, T.S. Vihtelic, and D.R. Hyde (2007). **Time course analysis of gene expression during light-induced photoreceptor cell death and regeneration in albino zebrafish.** *Developmental Neurobiology* 67(8): 1009-31. ISSN: 1932 8451.
Abstract: Constant intense light causes apoptosis of rod and cone photoreceptors in adult albino zebrafish. The photoreceptors subsequently regenerate from proliferating inner nuclear layer (INL) progenitor cells that migrate to the outer nuclear layer (ONL) and differentiate into rods and cones. To identify gene expression changes during this photoreceptor regeneration response, a microarray analysis was performed at five time points during the light treatment. The time course included an early time point during photoreceptor death (16 h), later time points during progenitor cell proliferation and migration (31, 51, and 68 h) and a 96 h time point, which likely corresponds to the initial photoreceptor differentiation. Mean expression values for each gene were calculated at each time point relative to the control (0 h light exposure) and statistical analysis by one-way ANOVA identified 4567 genes exhibiting significant changes in gene expression along the time course. The genes within this data set were clustered based on their temporal expression patterns and proposed functions. Quantitative real-time PCR validated the microarray expression profiles for selected genes, including

stat3 whose expression increased markedly during the light exposure. Based on immunoblots, both total and activated Stat3 protein expression also increased during the light treatment. Immunolocalization of Stat3 on retinal tissue sections demonstrated increased expression in photoreceptors and Muller glia by 16 h of light exposure. Some of the Stat3-positive Muller cells expressed PCNA at 31 h, suggesting that Stat3 may play a role in signaling a subset of Muller cells to proliferate during the regeneration response.

Descriptors: zebrafish, gene expression regulation, light, photoreceptors physiology, albinism genetics, albinism veterinary, genetically modified, apoptosis physiology, apoptosis radiation effects, cell death physiology, cell death radiation effects, cell division, retina cones radiation effects, fish diseases genetics, reporter genes, green fluorescent kinetics, oligonucleotide array sequence analysis, photoreceptors radiation effects, retina radiation effects, retinal degeneration genetics, retinal rods radiation effects.

Katoh, M. and M. Katoh (2003). **Evolutionary conservation of CCND1-ORAOV1-FGF19-FGF4 locus from zebrafish to human.** *International Journal of Molecular Medicine* 12(1): 45-50. ISSN: 1107-3756.

Abstract: The CCND1-ORAOV1-FGF19-FGF4-FGF3-FLJ10261-FADD-PPFIA1-EMS1 locus on human chromosome 11q13 is frequently amplified in esophageal cancer, breast cancer, and bladder tumors. FGF19, FGF4 and FGF3 genes are implicated in embryogenesis and carcinogenesis. We proposed in 2002 the hypothesis that mouse Fgf15 might be the ortholog of human FGF19 based on comparative genomics. Here, we identified zebrafish fgf19 and oraov1 genes by using bioinformatics to demonstrate the hypothesis. Zebrafish fgf19 gene, consisting of three exons, was located around nucleotide position 121802-124963 of zebrafish genome draft sequence AL929586.12 in the reverse orientation. Zebrafish fgf19 (209 aa) was more homologous to chicken fgf19 and human FGF19 than to rodent Fgf15. Zebrafish oraov1 gene, consisting of five exons, was located around nucleotide position 112172-115838 of AL929586.12 in the reverse orientation. Zebrafish oraov1 protein (141 aa) was more homologous to human ORAOV1 than to rodent Oraov1. The CCND1-ORAOV1-FGF19-FGF4 locus was well conserved between human and zebrafish genomes in the order of genes, in the direction of genes, and in the exon-intron structure. Rat Ccnd1-Oraov1-Fgf15-Fgf4 locus was synthenic to mouse Ccnd1-Oraov1 (also known as 2210010N10Rik)-Fgf15-Fgf4 locus. Fgf15, homologous to human FGF19 and zebrafish fgf19, was located on the synthenic locus of human FGF19 and zebrafish fgf19 within rodent genomes. Based on the evolutionary conservation of the CCND1-ORAOV1-FGF19-FGF4 locus from zebrafish to human, it was concluded that Fgf15 gene is the rodent ortholog of human FGF19 gene.

Descriptors: zebrafish, cyclin D1 genetics, Fgf genetics, synteny, amino acid and base sequence, chicken genetics, computational biology, molecular evolution, molecular sequence data, phylogeny, rats, FGFS genetics.

Katoh, M. and M. Katoh (2004). **Identification and characterization of human FHDC1, mouse Fhdc1 and zebrafish fhdc1 genes in silico.** *International Journal of Molecular Medicine* 13(6): 929-34. ISSN: 1107-3756.

Abstract: Formin homology proteins, implicated in organogenesis and carcinogenesis, are actin regulators with scaffold function. FMNL1, FMNL2, FMNL3, DIAPH1, DIAPH2, DIAPH3, DAAM1 and DAAM2 are FDD-type Formin homology proteins, while FHOD1, FHOD3, GRID2IP, Fmn1 and Fmn2 are non-FDD-type Formin homology proteins. Here, we identified human FHDC1 gene and vertebrate FHDC1 orthologs by using bioinformatics. The complete coding sequence of human FHDC1 cDNA was determined by assembling

3'-recombinated FLJ35083 chimeric cDNA and 5'-truncated KIAA1727 (AB051514.1) partial cDNA. The complete coding sequence of mouse Fhdc1 cDNA was determined by assembling 3'-truncated CD555494 EST and 5'-truncated 6330505N24 (AK031946.1) partial cDNA. The complete coding sequence of zebrafish fhdc1 cDNA was determined by assembling fhdc1 exons within zebrafish genome clone DKEY-4A14 (BX571710.4). FHDC1 gene was located at human chromosome 4q31.3, and Fhdc1 gene at mouse chromosome 3F1. Human FHDC1 (1143 aa) showed 73.3% total amino-acid identity with mouse Fhdc1 (1148 aa), and 43.4% total amino-acid identity with zebrafish Fhdc1 (1165 aa). FDCH1-FDCH5 domains were identified as novel conserved regions among vertebrate FHDC1 orthologs. Human FHDC1, mouse Fhdc1, and zebrafish Fhdc1 were non-FDD-type Formin homology proteins with FH1 and FH2 domains in the N-terminal part as well as with FDCH1, FDCH2, FDCH3, FDCH4, and FDCH5 domains in the C-terminal part. This is the first report on the identification and characterization of the human FHDC1, mouse Fhdc1 and zebrafish fhdc1 genes.
Descriptors: zebrafish, expressed sequence tags, amino acid and base sequence, chromosome mapping, computational biology, cytoskeletal proteins, complementary DNA genetics, mice, molecular sequence data, protein isoforms genetics, sequence alignment.

Kaur, S., M.S.S.S. Abu Abab, S.Y. Yeo, and R. Ramchandran (2007). **Expression pattern for unc5b, an axon guidance gene in embryonic zebrafish development.** *Gene Expression* 13(6): 321-27. ISSN: 1052-2166.
NAL Call Number: QH450.G465
Abstract: Branching processes such as nerves and vessels share molecular mechanisms of path determination. Our study focuses on unc5b, a member of the unc5 axon guidance gene family. Here, we have cloned the full-length zebrafish ortholog of unc5b, mapped its chromosome location in the zebrafish genome, and compared its expression patterns to robo4, another axon guidance family member. In situ show that unc5b is expressed predominantly in sensory structures such as the eye, ear, and brain. Both unc5b and robo4 show robust expression in all three compartments of the embryonic brain, namely forebrain, midbrain, and hindbrain. In particular, the hindbrain rhombomere expression displays interesting patterns in that robo4 is expressed in medial rhombomere cell clusters when compared to unc5b expressed in lateral rhombomere clusters. A similar medial-lateral theme is observed in other neural structures such as the neural tube. Our expression analysis provides a starting point for studying the role of axon guidance genes in embryonic hindbrain patterning.
Descriptors: zebrafish, axons physiology, embryonic development genetics, cell surface receptors physiology, amino acid sequence, body patterning genetics, molecular cloning developmental gene expression regulation, molecular sequence data, phylogeny, cell surface receptors genetics and metabolism, rhombencephalon embryology, amino acid sequence homology.

Kaur, S., M.D. Castellone, V.M. Bedell, M. Konar, J.S. Gutkind, and R. Ramchandran (2006). **Robo4 signaling in endothelial cells implies attraction guidance mechanisms**. *Journal of Biological Chemistry* 281(16): 11347-11356. ISSN: 0021 9258.
NAL Call Number: 381J824
Abstract: Roundabouts (robo) are cell-surface receptors that mediate repulsive signaling mechanisms at the central nervous system midline. However, robos may also mediate attraction mechanisms in the context of vascular development. Here, we have performed structure-function analysis of roundabout4 (Robo4), the predominant robo expressed in vasculature and found by gain of function approaches in vitro that Robo4 activates Cdc42

and Rac1 Rho GTPases in endothelial cells. Indeed, complementary robo4 gene knockdown approaches in zebrafish embryos show lower amounts of active Cdc42 and Rac1 and angioblasts isolated from these knockdown embryos search actively for directionality and guidance cues. Furthermore, Robo4-expressing endothelial cells show morphology and phenotype, characteristic of Rho GTPase activation. Taken together, this study suggests that Robo4 mediates attraction-signaling mechanisms through Rho GTPases in vertebrate vascular guidance. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, development, molecular genetics, biochemistry and molecular biophysics, enzymology, phenotype, vascular development, guidance cue, attraction guidance mechanism.

Kawaguchi, M., S. Yasumasu, J. Hiroi, T. Suzuki, A. Shimizu, and I. Iuchi (2004). **The exon-intron structures of fish hatching enzyme genes**. *Zoological Science* 21(12): 1274. ISSN: 0289 0003.

NAL Call Number: 442P92

Descriptors: zebrafish, freshwater ecology, ecology, environmental sciences, molecular genetics, enzymology, reverse transcriptase PCR, genetic techniques, in situ hybridization, histology and cytology techniques.

Kawahara, A., Y.S. Che, R. Hanaoka, H. Takeda, and I.B. Dawid (2005). **Zebrafish GADD45beta genes are involved in somite segmentation.** *Proceedings of the National Academy of Sciences of the United States of America* 102(2): 361-6. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: Somites in vertebrates are periodic segmented structures that give rise to the vertebrae and muscles of body. Somites are generated from presomitic mesoderm (PSM), but it is not fully understood how cellular differentiation and segment formation are achieved in the anterior PSM. We report here that zebrafish gadd45beta1 and gadd45beta2 genes are periodically expressed as paired stripes adjacent to the neural tube in the anterior PSM region where presomitic cells mature. In mammals, it is known that GADD45 (growth arrest and DNA damage) family proteins play a role in cell-cycle control. We found that both knockdown and overexpression of gadd45beta genes caused somite defects with different consequences for marker gene expression. Knockdown of gadd45beta genes with antisense morpholino oligonucleotides caused a broad expansion of mesp-a in the PSM, and both cyclic expression of her1 and segmented expression of MyoD were disorganized. On the other hand, injection of gadd45beta1 or gadd45beta2 suppressed expression of mesp-a and her1 in anterior PSM and MyoD in paraxial mesoderm. These results indicate that regulated expression of gadd45beta genes in the anterior PSM is required for somite segmentation.

Descriptors: zebrafish, cleavage stage, ovum physiology, somites physiology, amino acid sequence, intracellular signaling peptides and proteins, MAP kinase kinase kinase 4 physiology, mesoderm physiology, molecular sequence data, proteins chemistry, signal transduction.

Kawakami, K. (2004). **Transgenesis and gene trap methods in zebrafish by using the Tol2 transposable element.** *Methods in Cell Biology* 77: 201-22. ISSN: 0091-679X.

NAL Call Number: 442P92

Descriptors: genetically modified zebrafish genetics, DNA transposable elements genetics, chemistry, metabolism, gene expression regulation genetics, gene transfer techniques, genetic techniques, green fluorescent proteins analysis and genetics, insertional mutagenesis.

Kawakami, Y., C. Rodriguez Esteban, M. Raya, H. Kawakami, M. Marti, I. Dubova, and J.C. Izpisua Belmonte (2006). **Wnt/ beta -catenin signaling regulates vertebrate limb regeneration**. *Genes & Development* 20(23): 3232-3237. ISSN: 0890-9369.
NAL Call Number: QH426.G46
Descriptors: zebrafish, amphibiotic species, appendages, embryonic development, freshwater fish, limbs, regeneration, signal transduction, spatial distribution, temporal variations, Wnt protein, catenin, *Ambystoma*, *Danio rerio*, *Xenopus*, clawed frogs, genetics and evolution.

Kawamura, A., S. Koshida, H. Hijikata, T. Sakaguchi, H. Kondoh, and S. Takada (2005). **Zebrafish hairy/enhancer of split protein links FGF signaling to cyclic gene expression in the periodic segmentation of somites.** *Genes and Development* 19(10): 1156-61. ISSN: 0890-9369.
NAL Call Number: QH426.G46
Abstract: Notch and fibroblast growth factor (FGF) signaling pathways have been implicated in the establishment of proper periodicity of vertebrate somites. Here, we show evidence that a Hes6-related hairy/Enhancer of split-related gene, her13.2, links FGF signaling to the Notch-regulated oscillation machinery in zebrafish. Expression of her13.2 is induced by FGF-soaked beads and decreased by an FGF signaling inhibitor. her13.2 is required for periodic repression of the Notch-regulated genes her1 and her7, and for proper somite segmentation. Furthermore, Her13.2 augments autorepression of her1 in association with Her1 protein. Therefore, FGF signaling appears to maintain the oscillation machinery by supplying a binding partner, Her13.2, for Her1.
Descriptors: zebrafish, body patterning physiology, developmental gene expression regulation physiology, signal transduction physiology, somites metabolism, transcription factors metabolism, proteins metabolism, basic helix loop helix transcription factors, body patterning genetics, FGFs metabolism and pharmacology, developmental gene expression regulation genetics, signal transduction genetics, transcription factors biosynthesis and genetics.

Kedinger, V., F. Alpy, C. Tomasetto, C. Thisse, B. Thisse, and M.C. Rio (2005). **Spatial and temporal distribution of the TRAF4 genes during zebrafish development.** *Gene Expression Patterns* 5(4): 545-52. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: The tumor necrosis factor-associated factor 4 (TRAF4) is a particular member of the TRAF protein family since it is not involved in the Tumor Necrosis Factor (TNF) and Interleukin-1 (IL-1) signaling pathways. In the present study, we cloned two zebrafish orthologs of the human traf4, traf4a and traf4b, which are the first TRAFs described in zebrafish. During embryogenesis, traf4b expression is present in a weak ubiquitous manner. In contrast, traf4a exhibits a highly specific expression pattern in the sensorial and neural cells, and the somites of embryos. This gene is tightly regulated during embryogenesis. Together, our data show that traf4 is conserved during evolution, and traf4a is the zebrafish ortholog of traf4.
Descriptors: zebrafish, developmental gene expression regulation, TRAF tumor necrosis factor receptor associated peptides and amino acid sequence, conserved sequence, mice, molecular sequence data , morphogenesis, phylogeny, sequence alignment , amino acid sequence homology, TNF receptor associated factor 4, zebrafish classification.

Keller, M.J. and A.B. Chitnis (2007). **Insights into the evolutionary history of the vertebrate zic3 locus from a teleost-specific zic6 gene in the zebrafish, *Danio rerio*.** *Development Genes and Evolution* 217(7): 541-547. ISSN: 0949 944X.
NAL Call Number: QL951.R68

Abstract: The Zic gene family of zinc-finger transcription factors includes five orthologues, zic1-5, that are common to the Euteleostian vertebrates (fish, frogs, birds, and mammals). The Zic genes have been implicated as regulators of a number of critical developmental processes, including neurulation, neuronal differentiation, neural crest specification, the establishment of left-right asymmetry, and regulation of cell proliferation. The different Zic genes encode proteins that are expressed in broadly overlapping spatial domains, have conserved DNA-binding domains that recognize a common motif, are capable of physical interactions, and can co-regulate one another's transcription. Thus, the transcriptional regulation of individual proteins and their effects on downstream targets must be assessed within the context of co-expression with other family members. We describe a novel gene, zic6, that is specific to the teleost fishes and lacks the lateral and rostral expression domains typical of the other Zic family members. We present evidence that zic6 is an ancestral locus arising by chromosomal duplication early in the Euteleostomi that was subsequently lost in the terrestrial vertebrates.
Descriptors: zebrafish, fish, evolution, gene expression, Zic, synteny, internet resource.

Kelsh, R.N. (2005). **Transgenic fish: complementary models to mammalian systems for studying gene function**. *Transgenic Research* 14(4): 489. ISSN: 0962 8819.
NAL Call Number: QH442.6T74
Descriptors: zebrafish, molecular genetics, cell migration, gene function, transgene, cell movement.

Ken, C.F., T.H. Jung, and C.T. Lin (2005). **Characterization of superoxide dismutase promoter from diatom *Thalassiosira weissflogii***. *Journal of the Fisheries Society of Taiwan* 32(1): 8. ISSN: 0379-4180.
NAL Call Number: SH135.T3
Descriptors: zebrafish, antioxidants, biological stress, biomarkers, cloning, computer programs, diatoms, expression vectors, gene expression, green fluorescent protein, hydrogen peroxide, light effects, oxidative stress, phytoplankton, population genetics, promoters, proteins, reactive oxygen species, reporter gene, superoxide dismutase, biomarkers, *Danio rerio.*

Ken, C.F., C.T. Lin, Y.D. Wen, and J.L. Wu (2007). **Replacement of buried cysteine from zebrafish Cu/Zn superoxide dismutase and enhancement of its stability via site-directed mutagenesis.** *Marine Biotechnology* 9(3): 335-42. ISSN: 1436-2228.
NAL Call Number: TP248.27.M37M37
Abstract: Zebrafish Cu/Zn-superoxide dismutase (ZSOD1) has one free cysteine (Cys-7) in a first beta-strand with lower thermostability. We predicted the stability would be increased with single-point mutation at 70 degrees C via the I-Mutant 2.0 server, and generated a mutant SOD with replacement of the free Cys to Ala (ZSODC7A) by site-directed mutagenesis. The mutant was expressed and purified from the *Escherichia coli* strain AD494(DE3) pLysS and the yield was 2 mg from 0.4 L of culture. The ZSODC7A was heated at 90 degrees C. In a time-dependent assay, the time interval for 50% inactivation was 32 min, and its thermal inactivation rate constant K (d) was 2 x 10(-2) min(-1). The mutant was still activated in broad pH range (2.3-12), and had only a moderate effect under sodium dodecyl sulfate treatment. The calculated specific activity of the mutant was 3980 U/mg, twice that of wild-type ZSOD1. In addition, we soaked fish larva with equal enzyme units of either ZSOD1 or ZSODC7A for 2 h, and then stressed them with 100 ppm of paraquat to induce oxidative injury. The survival rate was significant.
Descriptors: zebrafish, cysteine genetics, site directed mutagenesis superoxide dismutase

chemistry and genetics, amino acid sequence, enzyme stability, enzymologic gene expression regulation, heat, larva drug effects, paraquat toxicity.

Kennedy, B.N., Y. Alvarez, S.E. Brockerhoff, G.W. Stearns, B. Sapetto Rebow, M.R. Taylor, and J.B. Hurley (2007). **Identification of a zebrafish cone photoreceptor-specific promoter and genetic rescue of achromatopsia in the nof mutant.** *Investigative Ophthalmology and Visual Science* 48(2): 522-9. ISSN: 0146-0404.

Abstract: PURPOSE: To identify in vivo a promoter fragment that specifically directs transgene expression in all zebrafish cone photoreceptors. This promoter subsequently enables GFP labeling of cones for facile morphologic analysis and purification and genetic rescue of achromatopsia. METHODS: Promoter fragments of the zebrafish cone transducin alpha (TalphaC) gene were subcloned upstream of EGFP and microinjected into one- to two-cell-stage embryos. Promoter activity was assessed by fluorescence microscopy in wholemounts and retinal cryosections, and cone photoreceptors were purified by flow cytometry. Visual physiology was assessed by the optokinetic response (OKR) assay. RESULTS: A 3.2-kb promoter fragment from zebrafish TalphaC specifically directed robust transgene expression in retinal cone photoreceptors and pineal photoreceptors. With this promoter, a stable transgenic line expressing EGFP in all zebrafish cone photoreceptors types was generated, and populations of cones were purified. Achromatopsia in the nof mutant was rescued using the identified promoter fragment to direct transgenic expression of wild-type cone transducin in mutant cones. CONCLUSIONS: A 3.2-kb TalphaC promoter fragment replicates the temporal and spatial pattern of endogenous TalphaC expression. The integrity of cones can be readily assessed in an EGFP transgenic line generated with this promoter, enabling downstream genetic and chemical screens for cone determinants.

Descriptors: zebrafish, color vision defects therapy, retina cones metabolism, developmental gene expression regulation physiology, gene therapy, promoter regions genetics, transducin genetics, genetically modified, nonsense codon, color vision defects genetics, flow cytometry, green fluorescent proteins metabolism, fluorescence microscopy, optokinetic nystagmus physiology, plasmids.

Khersonsky, S.M., D.W. Jung, T.W. Kang, D.P. Walsh, H.S. Moon, H. Jo, E.M. Jacobson, V. Shetty, T.A. Neubert, and Y.T. Chang (2003). **Facilitated forward chemical genetics using a tagged triazine library and zebrafish embryo screening.** *Journal of the American Chemical Society* 125(39): 11804-805. ISSN: 0002-7863.

NAL Call Number: 381 AM33

Descriptors: zebrafish, preclinical drug evaluation methods, triazines chemistry, combinatorial chemistry techniques methods.

Kida, Y.S., T. Sato, K.Y. Miyasaka, A. Suto, and T. Ogura (2007). **Daam1 regulates the endocytosis of EphB during the convergent extension of the zebrafish notochord.** *Proceedings of the National Academy of Sciences of the United States of America* 104(16): 6708-13. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: Convergent extension (CE) movement of cells is one of the fundamental processes that control the organized morphogenesis of tissues and organs. The molecular events connecting the noncanonical Wnt pathway and CE movement, however, are not well understood. We show that subcellular localization of Daam1, an essential component of noncanonical Wnt signaling, changes dynamically during notochord formation. In the early phases, Daam1 complexes with EphB receptors and Disheveled 2. This complex is incor-

porated into endocytic vesicles in a dynamin-dependent manner, thereby resulting in the removal of EphB from the cell surface with subsequent switching of cell adhesiveness. In the next step, Daam1 colocalizes with the actin cytoskeleton to induce morphological extension of cells. We elucidate the molecular mechanism underlying the CE movement of notochord cells with Daam1 as a dynamic coordinator of endocytosis and cytoskeletal remodeling.
Descriptors: zebrafish, signal transducing adaptor, endocytosis physiology, notochord embryology, Eph family receptors metabolism, signal transducing adaptor proteins metabolism, cell adhesion physiology, cell line, cytoskeleton chemistry and metabolism, notochord chemistry and metabolism, phosphoproteins metabolism and physiology, Eph family receptors physiology, subcellular fractions chemistry and metabolism.

Kidd, K.R., K. Greer, J.B. Hoying, and B.M. Weinstein (2004). **Molecular analysis of arterial-venous differentiation using oligonucleotide microarray technology in the zebrafish**. *Federation of American Societies for Experimental Biology Journal* 18(4-5): Abst. 533.3. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: Arterial-venous (A-V) differentiation is an integral part of the growth and establishment of functional vascular beds. Hemodynamic forces and directional blood flow are known to influence A-V differentiation, but recent identification of molecular differences between arterial and venous endothelial cells in embryonic vessels prior to the onset of flow indicates that genetic programs are critical to this fate determination. Previous studies in our laboratory have shown that a hierarchical relationship exists between hedgehog, vascular endothelial growth factor (vegf) and notch to regulate A-V differentiation. In the current study, we implement microarray analysis and the extensive genetic models and experimental tools developed in our laboratory to build upon this signaling cascade and identify further molecular components involved in regulating A-V differentiation. Towards this goal, we have successfully arrayed and established a protocol for using a 15,000 gene, zebrafish oligo (50-mer) set purchased from MWG. Preliminary studies have shown positive differential gene expression between vascular mutant lines. We are currently implementing an integrated loop experimental design and an ANOVA analysis method to identify differential vascular gene expression patterns in response to specific perturbations of hedgehog, vegf, and notch signaling, with the longer-term goal of functionalizing these genes and incorporating them within our existing molecular pathway. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, methods and techniques, molecular genetics, oligonucleotide microarray, genetic techniques, arterial venous differentiation, vascular gene expression.

Kiener, T.K., I. Sleptsova Friedrich, and W. Hunziker (2007). **Identification, tissue distribution and developmental expression of tjp1/zo-1, tjp2/zo-2 and tjp3/zo-3 in the zebrafish, *Danio rerio.*** *Gene Expression Patterns* 7(7): 767-76. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: The tight junction (TJ) or zona occludens (ZO) proteins TJP1/ZO-1, TJP2/ZO-2 and TJP3/ZO-3 belong to the membrane associated guanylate kinase-like (MAGUK) protein family and link TJ integral membrane proteins to the actin cytoskeleton. TJPs also serve as scaffolds for signaling proteins and transcription factors that regulate vesicular traffic and cell proliferation and differentiation. Here, we report the identification of two tjp1/zo-1 (tjp1.1 and tjp1.2) and tjp2/zo-2 (tjp2.1 and tjp2.2) genes each and one tjp3/zo-3 gene, and characterize their tissue specific distribution and developmental expression in zebrafish embryos. Transcripts for all five TJPs are maternally supplied and localized expression due to embry-

onic transcription is observed following the midblastula transition stage of development. In addition to a widespread distribution, individual genes show tissue specific expression patterns and a dynamic regulation during the developmental stages from 2 cells to 4 dpf analyzed. The most noticeable differences in expression patterns are observed in the posterior part of the embryo during somitogenesis. While all TJPs are expressed in the pronephric ducts and epidermis by 18 hpf, tjp1.1 is highly expressed in the hypochord and blood island, tjp1.2 in the somites and the posterior part of the notochord, tjp2.1 in the somites and the ventral part of the spinal cord, and tjp2.2 in the somites only. Individual TJPs are also strongly expressed in different layers of the eye and, at later stages, in central nervous system (CNS) tissues. Interestingly, the differential tissue and developmental expression of the two tjp1 and tjp2 genes indicates that the duplicated genes have been adapted for distinct transcriptional regulations during evolution.

Descriptors: zebrafish, carrier proteins biosynthesis, gene expression profiling, developmental gene expression regulation, membrane proteins biosynthesis, phosphoproteins biosynthesis, proteins biosynthesis, molecular cloning and evolution, gene duplication, genetic models, phylogeny, tissue distribution, genetic transcription.

Kikuta, H., D. Fredman, S. Rinkwitz, B. Lenhard, and T.S. Becker (2007). **Retroviral enhancer detection insertions in zebrafish combined with comparative genomics reveal genomic regulatory blocks a fundamental feature of vertebrate genomes.** *Genome Biology* 8(Suppl 1): S4. ISSN: 1465-6906.

NAL Call Number: QH431.G452

Abstract: A large-scale enhancer detection screen was performed in the zebrafish using a retroviral vector carrying a basal promoter and a fluorescent protein reporter cassette. Analysis of insertional hotspots uncovered areas around developmental regulatory genes in which an insertion results in the same global expression pattern, irrespective of exact position. These areas coincide with vertebrate chromosomal segments containing identical gene order; a phenomenon known as conserved synteny and thought to be a vestige of evolution. Genomic comparative studies have found large numbers of highly conserved noncoding elements (HCNEs) spanning these and other loci. HCNEs are thought to act as transcriptional enhancers based on the finding that many of those that have been tested direct tissue specific expression in transient or transgenic assays. Although gene order in hox and other gene clusters has long been known to be conserved because of shared regulatory sequences or overlapping transcriptional units, the chromosomal areas found through insertional hotspots contain only one or a few developmental regulatory genes as well as phylogenetically unrelated genes. We have termed these regions genomic regulatory blocks (GRBs), and show that they underlie the phenomenon of conserved synteny through all sequenced vertebrate genomes. After teleost whole genome duplication, a subset of GRBs were retained in two copies, underwent degenerative changes compared with tetrapod loci that exist as single copy, and that therefore can be viewed as representing the ancestral form. We discuss these findings in light of evolution of vertebrate chromosomal architecture and the identification of human disease mutations.

Descriptors: zebrafish, enhancer elements genetics, genome, genomics methods, insertional mutagenesis, Retroviridae genetics.

Kim, H.J., S. Sumanas, S. Palencia Desai, Y. Dong, J.N. Chen, and S. Lin (2006). **Genetic analysis of early endocrine pancreas formation in zebrafish.** *Molecular Endocrinology* 20(1): 194-203. ISSN: 0888-8809.

NAL Call Number: QP187.3M64M6

Abstract: Endocrine pancreas of zebrafish consist of at least four different cell types that function similarly to mammalian pancreatic islet. No mutants specifically affecting formation of the endocrine pancreas have been identified during the previous large-scale mutagenesis screens in zebrafish due to invisibility of a pancreatic islet. We combined in situ hybridization method to visualize pancreatic islet with an ethyl-nitroso-urea mutagenesis screen to identify novel genes involved in pancreatic islet formation in zebrafish. We screened 900 genomes and identified 11 mutations belonging to nine different complementation groups. These mutants fall into three major phenotypic classes displaying severely reduced insulin expression, reduced insulin expression with abnormal islet morphology, or abnormal islet morphology with relatively normal number of insulin expressing cells. Seven of these mutants do not have any other visible phenotypes associated. These mutations affect different processes in pancreatic islet development. Additional analysis on glucagon and somatostatin cell specification revealed that somatostatin cells are specified at a separate domain from insulin cells whereas glucagon cells are specified adjacent to insulin cells. Furthermore, glucagon cells and somatostatin cells are always associated with insulin cells in mutants that have scattered insulin expression. These data indicate that there are separate mechanisms regulating endocrine cell migration, proliferation, and differentiation. Further study on these mutants will reveal important information on novel genes involved in pancreatic islet cell specification and morphogenesis.

Descriptors: zebrafish, insulin metabolism, pancreas embryology, glucagon metabolism, in situ hybridization, Islets of Langerhans cytology and embryology, morphogenesis, mutagenesis, mutation, pancreas cytology, phenotype.

Kim, H.J., J.R. Schleiffarth, J. Jessurun, S. Sumanas, A. Petryk, S. Lin, and S.C. Ekker (2005). **Wnt5 signaling in vertebrate pancreas development**. *Biomed Central Biology* 3 ISSN: 1741 7007.

NAL Call Number: QH1.B565

Abstract: Background: Signaling by the Wnt family of secreted glycoproteins through their receptors, the frizzled (Fz) family of seven-pass transmembrane proteins, is critical for numerous cell fate and tissue polarity decisions during development.Results: We report a novel role of Wnt signaling in organogenesis using the formation of the islet during pancreatic development as a model tissue. We used the advantages of the zebrafish to visualize and document this process in living embryos and demonstrated that insulin-positive cells actively migrate to form an islet. We used morpholinos (MOs), sequence-specific translational inhibitors, and time-lapse imaging analysis to show that the Wnt-5 ligand and the Fz-2 receptor are required for proper insulin-cell migration in zebrafish. Histological analyses of islets in Wnt5a(-/-) mouse embryos showed that Wnt5a signaling is also critical for murine pancreatic insulin-cell migration.Conclusion: Our results implicate a conserved role of a Wnt5/Fz2 signaling pathway in islet formation during pancreatic development. This study opens the door for further investigation into a role of Wnt signaling in vertebrate organ development and disease. Copyright©Thomson Reuters 2009

Descriptors: zebrafish mutants, development, molecular genetics, digestive system, endocrine system, time lapse imaging, microscopy techniques, cell migration, pancreatic development.

Kimura, Y., Y. Okamura, and S. Higashijima (2006). **Alx, a zebrafish homolog of Chx10, marks ipsilateral descending excitatory interneurons that participate in the regulation of spinal locomotor circuits.** *Journal of Neuroscience* 26(21): 5684-97. ISSN: 0270-6474.

Abstract: Recent molecular genetic studies suggest that the expression of transcription factors in the developing spinal cord helps determine the morphological and physiological

properties of neurons. Using the zebrafish preparation, we have examined the properties of neurons marked by alx, a zebrafish homolog of mammalian Chx10. We performed morphological and physiological studies using transgenic zebrafish expressing fluorescent reporter constructs in cells that had at any time point expressed alx (alx neurons). Our data reveal that zebrafish alx neurons are all ipsilateral descending neurons that are positive for vesicular glutamate transporter 2, suggesting that they are glutamatergic excitatory interneurons. Patch recordings show that earlier-born neurons are active during stronger movements such as escapes and fast swimming (strong movement class), whereas later-born ones are involved in sustained weak swimming (weak movement class). Paired recordings between alx neurons and motoneurons show that neurons of the strong movement class make frequent monosynaptic excitatory connections onto motoneurons. Thus, neurons of this class are likely premotor interneurons that regulate motoneuron activity during escapes and fast swimming. We also show the existence of a monosynaptic connection between an alx neuron of the weak movement class and a motoneuron. Collectively, our data demonstrate that alx marks ipsilateral descending neurons that are involved in the regulation of motoneuron activity during forms of locomotion, such as escape and swimming.

Descriptors: zebrafish, efferent pathways physiology, eye proteins metabolism, homeodomain proteins metabolism, interneurons physiology, locomotion physiology, nerve net physiology, spinal cord physiology, proteins metabolism, excitatory postsynaptic potentials physiology, feedback physiology, tissue distribution.

Kitambi, S.S. and G. Hauptmann (2007). **The zebrafish orphan nuclear receptor genes nr2e1 and nr2e3 are expressed in developing eye and forebrain.** *Gene Expression Patterns* 7(4): 521-8. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Mammalian Nr2e1 (Tailless, Mtll or Tlx) and Nr2e3 (photoreceptor-specific nuclear receptor, Pnr) are highly related orphan nuclear receptors, that are expressed in eye and forebrain-derived structures. In this study, we analyzed the developmental expression patterns of zebrafish nr2e1 and nr2e3. RT-PCR analysis showed that nr2e1 and nr2e3 are both expressed during embryonic and post-embryonic development. To examine the spatial distribution of nr2e1 and nr2e3 during development whole-mount in situ hybridization was performed. At tailbud stage, initial nr2e1 expression was localized to the rostral brain rudiment anterior to pax2.1 and eng2 expression at the prospective midbrain-hindbrain boundary. During subsequent stages, nr2e1 became widely expressed in fore- and midbrain primordia, eye and olfactory placodes. At 24hpf, strong nr2e1 expression was detected in telencephalon, hypothalamus, dorsal thalamus, pretectum, midbrain tectum, and retina. At 2dpf, the initially widespread nr2e1 expression became more restricted to distinct regions within the fore- and midbrain and to the retinal ciliary margin, the germinal zone which gives rise to retina and presumptive iris. Expression of nr2e3 was exclusively found in the developing retina and epiphysis. In both structures, nr2e3 expression was found in photoreceptor cells. The developmental expression profile of zebrafish nr2e1 and nr2e3 is consistent with evolutionary conserved functions in eye and rostral brain structures.

Descriptors: zebrafish, eye embryology, gene expression , prosencephalon embryology, cytoplasmic receptors and nuclear genetics, amino acid sequence, metabolism, eye metabolism, molecular sequence data, phylogeny, prosencephalon metabolism, sequence alignment.

Kitevski, B. and M. Pyron (2003). **Female zebrafish (*Danio rerio*) do not prefer mutant longfin males**. *Journal of Freshwater Ecology* 18(3): 501-502. ISSN: 0270 5060.

NAL Call Number: QH541.5F7J68

Abstract: When we presented longfin and normal male zebrafish to females in an aquarium experiment, females did not spend more time adjacent to longfin than normal males and did not interact more with longfin than normal males. When we tested for effects of longfin mutants on male-male interactions by comparing aggressive interactions between two normal males to interactions between a normal male and a longfin mutant male, there was no difference in male-male aggression. These results suggest that neither male nor female zebrafish use fin size in evaluating male status. Copyright © 2008 CABI
Descriptors: zebrafish, aggression, mating preferences, mutants, longfin mutant male, female preference, *Danio rerio.*

Klee, E.W., K.J. Shim, M.A. Pickart, S.C. Ekker, and L.B. Ellis (2005). **AMOD: a morpholino oligonucleotide selection tool**. *Nucleic Acids Research* 33(suppl 2): w506-w511. ISSN: 0305-1048.
NAL Call Number: QD341.A2N8
Descriptors: zebrafish, algorithms, antisense oligonucleotides, base composition, databases, freshwater fish, genomes, nucleic acids, nucleotide sequence, nucleotides, oligonucleotides, population genetics, site selection, translation initiation, genomics, *Danio rerio*.
Notes: A review.

Kloosterman, W.P., F.A. Steiner, E. Berezikov, E.d. Bruijn, J.v.d. Belt, M. Verheul, E. Cuppen, and R.H.A. Plasterk (2006). **Cloning and expression of new microRNAs from zebrafish**. *Nucleic Acids Research* 34(9): 2558-2569. ISSN: 0305 1048.
NAL Call Number: QD341.A2N8
Abstract: MicroRNAs (miRNAs) play an important role in development and regulate the expression of many animal genes by post-transcriptional gene silencing. Here we describe the cloning and expression of new miRNAs from zebrafish. By high-throughput sequencing of small-RNA cDNA libraries from 5-day-old zebrafish larvae and adult zebrafish brain we found 139 known miRNAs and 66 new miRNAs. For 65 known miRNAs and for 11 new miRNAs we also cloned the miRNA star sequence. We analyzed the temporal and spatial expression patterns for 35 new miRNAs and for 32 known miRNAs in the zebrafish by whole mount in situ hybridization and northern blotting. Overall, 23 of the 35 new miRNAs and 30 of the 32 known miRNAs could be detected. We found that most miRNAs were expressed during later stages of development. Some were expressed ubiquitously, but many of the miRNAs were expressed in a tissue-specific manner. Most newly discovered miRNAs have low expression levels and are less conserved in other vertebrate species. Our cloning and expression analysis indicates that most abundant and conserved miRNAs in zebrafish are now known. Copyright © 2008 CABI
Descriptors: zebrafish larvae, brain, complementary DNA, DNA cloning, gene expression, RNA, transcription, *Danio rerio*, *cDNA libraries.*

Kloosterman, W.P., E. Wienholds, R.F. Ketting, and R.H. Plasterk (2004). **Substrate requirements for let-7 function in the developing zebrafish embryo.** *Nucleic Acids Research* 32(21): 6284-91. ISSN: 0305-1048.
NAL Call Number: QD341.A2N8
Abstract: MicroRNAs (miRNAs) are involved in the regulation of gene expression at the post-transcriptional level by base pairing to the 3'-UTR (untranslated region) of mRNAs. The let-7 miRNA was first discovered in Caenorhabditis elegans and is evolutionarily conserved. We used zebrafish embryos as a vertebrate in vivo system to study substrate requirements for function of let-7. Injection of a double-stranded let-7 miRNA into the

zygotes of zebrafish and frogs causes specific phenotypic defects. Only the antisense strand of the let-7 duplex has biological activity. In addition, co-injected mRNA of gfp fused to the 3'-UTR of a zebrafish lin-41 ortholog (a presumed target of let-7) is silenced by let-7. Point mutant studies revealed that the two let-7 target sites in the lin-41 3'-UTR are both essential and sufficient for silencing. let-7 and mir221 together, but not either of them alone, can silence a construct with one of the let-7 target sites replaced by a target site for mir221, showing that two different miRNAs can provide the required cooperative effect. let-7 target sites can be moved around: they are also functional when positioned in the coding sequence or even in the 5'-UTR of gfp. We took advantage of reporter and phenotypic assays to analyze the activity of all possible point mutant derivatives of let-7 and found that only the 5' region is critical for function of let-7.
Descriptors: zebrafish, gene silencing, microRNAs pharmacology, 3' and 5' untranslated regions, DNA mutational analysis, anatomy and histology, metabolism, embryonic development, reporter genes, microRNAs administration and dosage, microRNAs genetics, microinjections, zebrafish anatomy and histology.

Kloosterman, W.P., E. Wienholds, E. DeBruijn, S. Kauppinen, and R.H.A. Plasterk (2006). **In situ detection of MicroRNAs in animal embryos using LNA-modified oligonucteotide probes**. *Nature Methods* 3(1): 27-29. ISSN: 1548 7091.
NAL Call Number: QH315
Abstract: MicroRNAs (miRNAs) are 20-23 nucleotide (nt) RNA molecules that regulate gene expression post-transcriptionally. A key step toward understanding the function of the hundreds of miRNAs identified in animals is to determine their expression during development. Here we performed a detailed analysis of conditions for in situ detection of miRNAs in the zebrafish embryo using Locked nucleic acid (LNA)-modified DNA probes and report expression patterns for 15 miRNAs in the mouse embryo. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, methods and techniques, development, molecular genetics, locked nucleic acid (LNA) modified DNA probe, gene expression, micro RNA's.

Kluever, N., M. Kondo, A. Herpin, H. Mitani, and M. Schartl (2005). **Divergent expression patterns of Sox9 duplicates in teleosts indicate a lineage specific subfunctionalization**. *Development Genes and Evolution* 215(6): 297-305. ISSN: 0949-944X.
NAL Call Number: QL951.R68
Descriptors: zebrafish, cartilage, data processing, embryogenesis, evolutionary genetics, freshwater fish, gene mapping, linkage analysis, nucleotide sequence, phylogenetics, phylogeny, Sox9 protein, testes, transcription, transcription factors, *Danio rerio*, *Gasterosteus aculeatus*, *Oryzias latipes*, Teleostei, Alaskan stickleback, medaka, threespine stickleback, taxonomy, systematics and evolutionary genetics.

Knaut, H., C. Werz, R. Geisler, and C. Nusslein Volhard (2003). **A zebrafish homologue of the chemokine receptor Cxcr4 is a germ-cell guidance receptor.** *Nature* 421(6920): 279-82. ISSN: 0028-0836.
NAL Call Number: 472 N21
Abstract: Germ cells preserve an individual's genetic information and transmit it to the next generation. Early in development germ cells are set aside and undergo a specialized developmental programme, a hallmark of which is the migration from their site of origin to the future gonad. In *Drosophila*, several factors have been identified that control germ-cell migration to their target tissues; however, the germ-cell chemoattractant or its receptor have

remained unknown. Here we apply genetics and in vivo imaging to show that odysseus, a zebrafish homologue of the G-protein-coupled chemokine receptor Cxcr4, is required specifically in germ cells for their chemotaxis. odysseus mutant germ cells are able to activate the migratory programme, but fail to undergo directed migration towards their target tissue, resulting in randomly dispersed germ cells. SDF-1, the presumptive cognate ligand for Cxcr4, shows a similar loss-of-function phenotype and can recruit germ cells to ectopic sites in the embryo, thus identifying a vertebrate ligand-receptor pair guiding migratory germ cells at all stages of migration towards their target.

Descriptors: zebrafish, chemotaxis, germ cells cytology, germ cells metabolism, Cxcr4 receptors metabolism, Cxc chemokines genetics and metabolism, heterotrimeric GTP binding proteins metabolism, mutation genetics, messenger RNA genetics and metabolism, Cxcr4 receptors genetics.

Ko, Y.P., B. Kobbe, M. Paulsson, and R. Wagener (2005). **Zebrafish (*Danio rerio*) matrilins: shared and divergent characteristics with their mammalian counterparts.** *Biochemical Journal, The* 386(Pt 2): 367-79. ISSN: 0264 6021.

NAL Call Number: 382B52

Abstract: We have cloned the cDNAs of the zebrafish (*Danio rerio*) members of the matrilin family of extracellular adaptor proteins. In contrast to mammals, no orthologue of matrilin-2 was found in zebrafish, either by RT (reverse-transcriptase) PCR using degenerated primers or by screening the databases (Ensembl and NCBI); however, two forms of matrilin-3, matrilin-3a and -3b, were present. The identity with the mammalian matrilins is from more than 70% for the VWA (von Willebrand factor A)-like domains to only 28% for the coiled-coil domains of matrilin-3a and -3b. In all zebrafish matrilins we found a greater variety of splice variants than in mammals, with splicing mainly affecting the number of EGF (epidermal growth factor)-like repeats. The exon-intron organization is nearly identical with that of mammals, and also the characteristic AT-AC intron interrupting the exons coding for the coiled-coil domain is conserved. In the matrilin-3b gene a unique exon codes for a proline- and serine/threonine-rich domain, possibly having mucin-like properties. The matrilin-1 and -3a genes were mapped to chromosome 19 and 20 respectively by the radiation hybrid method. The temporal and spatial expression of zebrafish matrilins is similar to that seen in the mouse. Zebrafish matrilin-4 is highly expressed as early as 24 hpf (h post fertilization), whereas the other matrilins show peak expression at 72 hpf. By immunostaining of whole mounts and sections, we found that matrilin-1 and -3a show predominantly skeletal staining, whereas matrilin-4 is more widespread, with the protein also being present in loose connective tissues and epithelia.

Descriptors: zebrafish , extracellular matrix glycovariation genetics, alternative splicing genetics, cell line, molecular cloning methods, conserved sequence genetics, exons genetics, extracellular matrix proteins immunology, gene expression regulation genetics, developmental gene expression regulation genetics , glycoproteins immunology, immune sera metabolism, introns genetics, kidney chemistry, cytology, embryology and metabolism, molecular sequence data, peptides geneticsand immunology, radiation hybrid mapping methods, recombinant proteins genetics.

Koch, R., G.J. Rauch, S. Humphray, T.R. Geisler, and R. Plasterk (2004). **Bacterial artificial chromosome (BAC) clones and the current clone map of the zebrafish genome.** *Methods in Cell Biology* 77: 295-304. ISSN: 0091-679X.

NAL Call Number: 442P92

Descriptors: zebrafish, artificial chromosomes, bacterial genetics, contig mapping methods, genomic library, genome.

Kochilas, L.K., V. Potluri, A. Gitler, K. Balasubramanian, and A.J. Chin (2003). **Cloning and characterization of zebrafish tbx1.** *Gene Expression Patterns* 3(5): 645-51. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Tbx1 is one of the genes within the DiGeorge Critical Region (DGCR) and has been recently identified as the critical gene for the cardiovascular anomalies in the DiGeorge mouse models. We have cloned, sequenced and analyzed the zebrafish (*Danio rerio*) tbx1 cDNA. It encodes a protein of 460 amino acids that shares 64% identity and 67% similarity with the human TBX1 orthologue at the amino acid level. Although maternal expression was detected by RT-PCR, only zygotic expression could be detected by whole-mount in situ hybridization. Expression of zebrafish tbx1 by whole-mount in situ hybridization was first detected at 40% epiboly, 5.0 hours post fertilization (hpf) in the dorsal blastoderm margin. Through the stage of embryonic shield formation, tbx1 expression is restricted to the hypoblast, in the region of cells fated to become head and lateral plate mesoderm and pharyngeal endoderm. At 18 hpf, when the heart tube is beginning to assemble, three domains of tbx1 expression can be seen: cardiac precursors, pharyngeal arch precursors and otic vesicle. These three domains will remain the sites of tbx1 expression to varying degrees through at least 72 hpf. By 51 hpf, tbx1 expression can be seen in the cardiac outflow tract, the ventricle and the atrium, although by 72 hpf cardiac expression is strongest in the cardiac outflow tract. This newly identified tbx1 expression pattern in cardiac regions other than the cardiac outflow tract offers a new insight into the role of the tbx1 transcription factor in cardiac development.
Descriptors: zebrafish, T-box domain proteins chemistry and genetics, amino acid sequence, molecular cloning, complementary DNA genetics, gene expression, heart embryology, mice, molecular sequence data, reverse transcriptase PCR, sequence alignment, amino acid sequence homology.

Koduru, S., S.R. Vegiraju, S.K. Nadimpalli, K. von Figura, R. Pohlmann, and A. Dennes (2006). **The early vertebrate *Danio rerio* Mr 46000 mannose-6-phosphate receptor: biochemical and functional characterisation.** *Development Genes and Evolution* 216(3): 133-43. ISSN: 0949 944X.
NAL Call Number: QL951.R68
Abstract: Mannose-6-phosphate receptors (MPRs) have been identified in a wide range of species from humans to invertebrates such as molluscs. A characteristic of all MPRs is their common property to recognize mannose-6-phosphate residues that are labelling lysosomal enzymes and to mediate their targeting to lysosomes in mammalian cells by the corresponding receptor proteins. We present here the analysis of full-length sequences for MPR 46 from zebrafish (*Danio rerio*) and its functional analysis. This is the first non-mammalian MPR 46 to be characterised. The amino acid sequences of the zebrafish MPR 46 displays 70% similarity to the human MPR 46 protein. In particular, all essential cysteine residues, the transmembrane domain as well as the cytoplasmic tail residues harbouring the signals for endocytosis and Golgi-localizing, gamma-ear-containing, ARF-binding protein (GGA)-mediated sorting at the trans-Golgi network, are highly conserved. The zebrafish MPR 46 has the arginine residue known to be essential for mannose-6-phosphate binding and other additional characteristic residues of the mannose-6-phosphate ligand-binding pocket. Like the mammalian MPR 46, zebrafish MPR 46 binds to the multimeric mannose-6-phosphate ligand phosphomannan and can rescue the missorting of lysosomal enzymes in mamma-

lian MPR-deficient cells. The conserved C-terminal acidic dileucine motif (DxxLL) in the cytoplasmic domain of zebrafish MPR 46 essential for the interaction of the GGAs with the receptor domains interacts with the human GGA1-VHS domain. Interestingly, the serine residue suggested to regulate the interaction between the tail and the GGAs in a phosphorylation-dependent manner is substituted by a proline residue in fish.
Descriptors: zebrafish, IGF type 2 receptor genetics, IGF type 2 receptor physiology, ADP ribosylation factors metabolism, adaptor proteins, vesicular transport metabolism, amino acid sequence, cathepsin D metabolism, cattle, chickens genetics, molecular cloning, fishes genetics, gene expression profiling, mice, molecular sequence data, protein binding, protein transport and metabolism, amino acid sequence homology, tissue distribution, *Xenopus* genetics.

Kohli, G., S. Hu, E. Clelland, T. Di Muccio, J. Rothenstein, and C. Peng (2003). **Cloning of transforming growth factor-beta 1 (TGF-beta 1) and its type II receptor from zebrafish ovary and role of TGF-beta 1 in oocyte maturation.** *Endocrinology* 144(5): 1931-41. ISSN: 0013 7227.
NAL Call Number: 448.8EN2
Abstract: TGF-beta is a multifunctional factor involved in regulating a variety of cellular activities. In mammals, TGF-beta is known to regulate reproduction, including ovarian functions. The role of TGF-beta in lower vertebrates, such as fish, is poorly understood. To examine the role of TGF-beta in fish reproduction, cDNAs encoding TGF-beta 1 and the type II TGF-beta receptor (T beta RII) were cloned from the zebrafish ovary using PCR-based strategies. The mature peptide region of the zebrafish TGF-beta 1 shows 70-85% identity with TGF-beta 1 from other species. The zebrafish T beta RII cDNA sequence is the first to be reported from a fish species, and it shows a high level of conservation at the kinase domain. Using RT-PCR, we have detected mRNA expression of TGF-beta 1, T beta RII, as well as its downstream signaling molecules Smad2, 3, and 4 in ovarian follicles at different stages of development. In addition, we have examined the effect of TGF-beta 1 on oocyte maturation. TGF-beta 1 significantly inhibited both gonadotropin- and 17 alpha, 20 beta-dihydroxyprogesterone-induced oocyte maturation in a dose- and time-dependent manner. These findings demonstrate, for the first time, that TGF-beta 1 plays a role in regulating oocyte maturation in fish and suggest that a TGF-beta/Smad signaling pathway is present in the zebrafish ovary.
Descriptors: zebrafish, molecular cloning, oocytes physiology, ovary metabolism, receptors, transforming growth factor beta physiology, transforming growth factor beta physiology, metabolism, proteins, amino acid sequence genetics, base sequence genetics, cell aging physiology, DNA binding proteins genetics, proteins metabolism, molecular sequence data, receptors, transforming growth factor beta genetics, Smad2, Smad3 and Smad4 protein, trans activators metabolism, transforming growth factor beta genetics, transforming growth factor beta1.

Kojima, D. (2004). **Physiological role of a non-visual opsin, val-opsin, in the developing zebrafish**. *Zoological Science* 21(12): 1210. ISSN: 0289 0003.
NAL Call Number: 442P92
Descriptors: zebrafish, development, molecular genetics, endocrine system, sensory reception, reverse transcriptase PCR, in situ hybridization, histology and cytology techniques, genetic techniques.

Komen, H. and G.H. Thorgaard (2007). **Androgenesis, gynogenesis and the production of clones in fishes: a review**. *Aquaculture* 269(1/4): 150-173. ISSN: 0044 8486.
NAL Call Number: SH1.A6
Abstract: Fish species that have external fertilization can be reproduced by induced parthenogenesis. The nuclear content of either the sperm or egg is destroyed by UV or gamma irradiation, and the treated gamete then is fused with an untreated egg or sperm to form a haploid embryo. This is subsequently made diploid by inhibition of either the second meiotic division or the first cell division. After first cell division blockage, the resulting individual is a so-called doubled haploid (DH). DH individuals carry only the duplicated set of chromosomes inherited from the untreated egg or sperm and are, by definition, fully homozygous. In the first part of this review, we discuss the latest insights into the mechanisms underlying the process of making meiotic diploids and DH individuals, and review the problems associated with making and characteristics of doubled haploids and clones in fishes. In the second part of this review, we explore the use of doubled haploids and clones in quantitative trait locus mapping and selective breeding. Copyright © 2008 CABI
Descriptors: zebrafish embryos, androgenesis, animal cloning, DNA, DNA cloning, embryonic development, fertility, fish eggs, genetic load, genomes, gynogenesis, haploids, heat shock, homozygotes, inbreeding depression, irradiation, phenotypes, phenotypic variation, quantitative trait loci, reviews, selective breeding, sex ratio, survival, tetraploidy, carp, *Danio rerio*, *Oreochromis niloticus*, rainbow trout.
Notes: A review.

Komoike, Y., A. Kawamura, N. Shindo, C. Sato, J. Satoh, R. Shiurba, and T. Higashinakagawa (2005). **Zebrafish Polycomb group gene ph2alpha is required for epiboly and tailbud formation acting downstream of FGF signaling.** *Biochemical and Biophysical Research Communications* 328(4): 858-66. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: We analyzed Polycomb group gene ph2alpha functionally in zebrafish embryos by a gene knock-down procedure using morpholino antisense oligos. Inhibition of ph2alpha message translation resulted in abnormal epibolic movements as well as a thick tailbud or incomplete covering of the yolk plug. At the 24hpf stage, morphants had short trunks and tails, phenotypes similar to those with disturbances in FGF signaling. Accordingly, we looked at the effects of ph2alpha expression upstream and downstream of the FGF pathway. Treatment with SU5402, an inhibitor of Fgfrs, or injection of dominant-negative Fgfr1 DNA markedly reduced ph2alpha expression in the tailbud. In addition, cells expressing mRNAs for no tail, spadetail, myoD, and papc, which are involved in FGF-related development of posterior mesoderm, were distributed abnormally. Collectively, the data argue that ph2alpha is required for epiboly and tailbud formation, acting downstream of the FGF signaling pathway.
Descriptors: zebrafish, carrier proteins metabolism, FGFr 1 metabolism, developmental gene expression regulation physiology, repressor proteins metabolism, carrier gene silencing, limb bud, repressor signal transduction physiology, tissue distribution.

Kong, X.H., X.Z. Wang, X.N. Gan, J.B. Li, and S.P. He (2007). **Phylogenetic relationships of Cyprinidae (Teleostei: Cypriniformes) inferred from the partial S6K1 gene sequences and implication of indel sites in intron 1**. *Science in China Series c (Life Sciences)* 50(6): 780-788. ISSN: 1006-9305.
Descriptors: zebrafish, Bayesian analysis, evolution, exons, introns, phylogeny, PCR,

Acheilognathinae, Catostomidae, Cyprinidae, Cypriniformes, *Danio rerio*, gobioninae, Leuciscinae, *Myxocyprinus asiaticus*, *Rasbora trilineata*, Schizothoracinae.

Koo, B.K., K.J. Yoon, K.W. Yoo, H.S. Lim, R. Song, J.H. So, C.H. Kim, and Y.Y. Kong (2005). **Mind bomb-2 is an e3 ligase for notch ligand**. *Journal of Biological Chemistry* 280(23): 22335-22342 . ISSN: 0021 9258.

NAL Call Number: 381J824

Abstract: The zebrafish gene, mind bomb (mib), encodes a protein that positively regulates of the Delta-mediated Notch signaling. It interacts with the intracellular domain of Delta to promote its ubiquitination and endocytosis. In our search for the mouse homologue of zebrafish mind bomb, we cloned two homologues in the mouse genome: a mouse orthologue (mouse mib1) and a paralogue, named mind bomb-2 (mib2), which is evolutionarily conserved from Drosophila to human. Both Mib1 and Mib2 have an E3 ubiquitin ligase activity in their C-terminal RING domain and interact with Xenopus Delta (XD) via their N-terminal region. Mib2 is also able to ligate ubiquitin to XD and shift the membrane localization of Delta to intracellular vesicles. Importantly, Mib2 rescues both the neuronal and vascular defects in the zebrafish mib(ta52b) mutants. In contrast to the functional similarities between Mib1 and Mib2, mib2 is highly expressed in adult tissues, but almost not at all in embryos, whereas mib1 is abundantly expressed in both embryos and adult tissues. These data suggest that Mib2 has functional similarities to Mib1, but might have distinct roles in Notch signaling as an E3 ubiquitin ligase. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, molecular genetics, enzymology, cell biology, gene cloning, genetic techniques, endocytosis.

Kopeika, J., T. Zhang, D.M. Rawson, and G. Elgar (2005). **Effect of cryopreservation on mitochondrial DNA of zebrafish (*Danio rerio*) blastomere cells.** *Mutation Research* 570(1): 49-61. ISSN: 0027-5107.

NAL Call Number: QH431.M8

Abstract: Cryopreservation has been extensively used in human reproductive medicine, aquaculture and conservation programmes for endangered species. However, despite the growing successes of cryopreservation, post-thaw recovery of reproductive and embryonic cells very often remains poor. Many studies have been devoted to the mechanisms of cryodamage. It is known that cryopreservation causes extensive damage to membranes; reduce the metabolic activity of cells; and disturbs the mitochondrial bioenergetical processes of cells. But few investigations on the genetic stability of cells during cryopreservation have been performed, and the role of any genetic impact cryopreservation needs to be determined. Some indirect data in the literature suggests that progress in this field might come from investigating freezing damage to mitochondrial DNA (mtDNA), nuclear DNA and other genome-related structures. In this study, zebrafish (*Danio rerio*) blastomeres were treated in three different ways: control suspension of blastomere cells in phosphate buffered saline; equilibration of blastomeres with 2M dimethyl sulfoxide (Me2SO) for 1h at room temperature and cryopreservation using Me2SO as a cryoprotectant. Mitochondrial DNA was analysed in fresh cells and after the different treatments. Two different loci of mtDNA were amplified with the help of PCR and sequenced. The sequences were analysed and nuclear base substitutions were counted for both control and treated samples. The results showed that cryopreservation significantly increased the frequency of mutations (0.78+/-0.27% in comparison to 0.16+/-0.25% of control), whilst 2M Me2SO treatment did not bring a significant increase in frequency of mutations (0.24+/-0.28%). The distributions of the mutation locations were analysed. More investigations are needed to determine whether

optimisation of cryopreservation protocol is possible to reduce these adverse effects; whether such mutations interfere with overall function of the cells; whether similar changes also occur in the nuclear DNA and whether such mutations happen in other species. Meanwhile, it is important to be cautious in making judgements of the effect of cryopreservation technique in assisted reproduction. This is the first report on the effect of cryopreservation on mtDNA.
Descriptors: zebrafish , blastomeres, cryopreservation, DNA, mitochondrial, mitochondria genetics, base sequence, DNA mutational analysis, molecular sequence data, mutation, sequence alignment.

Kopp, R., T. Schwerte, and B. Pelster (2005). **Cardiac performance in the zebrafish breakdance mutant.** *Journal of Experimental Biology* 208(Pt 11): 2123-34. ISSN: 0022-0949.
NAL Call Number: 442.8B77
Abstract: In the Tubingen screen a breakdance mutant of zebrafish (bre) was described as an arrhythmia, in which the ventricle beats only with every second atrial contraction (2:1 rhythm). Surprisingly, a careful analysis of the effect of the breakdance mutation on cardiac performance of the zebrafish during development between 3 d.p.f. and 14 d.p.f revealed that homozygous bre mutants did not always show the 2:1 rhythm. Cardiac activity was continuously recorded for a period of 20 min in each larva, and during this period we observed that heart rate randomly switched between the 2:1 rhythm and a 1:1 rhythm. Furthermore, at 28 degrees C and at 31 degrees C the expression of the 2:1 rhythm decreased with development. At 31 degrees C this was in part due to a significantly reduced survival rate of mutants beyond 4 d.p.f. Besides development, temperature had a marked effect on the expression of the 2:1 rhythm, and during the first days of development the expression of the 2:1 rhythm was significantly higher at elevated incubation temperatures. By contrast, in the 2:1 beating heart ventricular contraction rate was about 80 beats min(-1) throughout development irrespective of the temperature, and even in the 1:1 rhythm mutants showed a significant bradycardia at all three temperatures (25 degrees C, 28 degrees C or 31 degrees C). Compared to wild-type animals, cardiac output was significantly lower in bre mutants. Pressure traces recorded in the ventricle of mutants revealed a prolonged relaxation phase, indicating that the second pacemaker current could not be conveyed to the ventricle (AV-block). This phenotype is comparable to the human Long QT Syndrome, an arrhythmia caused by a modification of an ion channel involved in cardiac repolarization. The bradycardia and the modified temperature sensitivity of heart rate suggested that the activity of the pacemaker cells was also affected by this mutation.
Descriptors: breakdance mutant zebrafish, arrhythmia, fish diseases genetics, heart atria and ventricles physiopathology, arrhythmia genetics and physiopathology, blood pressure physiology, cardiac output genetics, fish diseases physiopathology, heart rate genetics, homozygote, mutation, heart stroke volume genetics, temperature, time factors.

Kotani, T. and K. Kawakami (2004). **The transposon-mediated gene trap method in zebrafish**. *Protein, Nucleic Acid, Enzyme [Tanpakushitsu Kakusan Koso]* 49(13): 2111-6. ISSN: 0039-9450.
NAL Call Number: QD431.T3
Descriptors: zebrafish, DNA transposable elements genetics, gene transfer techniques, genetic vectors, green fluorescent insertional mutagenesis methods.
Language of Text: Japanese.

Kotani, T., S. Nagayoshi, A. Urasaki, and K. Kawakami (2006). **Transposon-mediated gene trapping in zebrafish.** *Methods* 39(3): 199-206. ISSN: 0882-4010.
NAL Call Number: QP519.7.M47
Abstract: The Tol2 transposon system can create chromosomal insertions in the zebrafish germ lineage very efficiently. We constructed a Tol2-based gene trap vector, T2KSAG, which contains a splice accepter, the GFP gene and the polyA signal. In the pilot screen for gene trapping using T2KSAG, we identified 38 fish lines expressing GFP in specific organs and tissues. In the SAGp53A line, GFP is expressed in the forebrain and midbrain, and the insertion of the gene trap construct captured a transcript of the kab gene encoding a zebrafish homolog of the human KARP (Ku86 autoantigen related protein)-binding protein (KAB). In the SAGm18B line, GFP is expressed in the central nervous system, and the insertion captured a transcript of a gene for succinyl CoA:3-oxoacid CoA-transferase (SCOT). Here, we describe how we performed the gene trap screen and characterized the gene trap insertions and will discuss the outcome of the pilot screen.
Descriptors: zebrafish, DNA transposable elements, insertional mutagenesis methods, base sequence, carrier proteins genetics and metabolism, Coenzyme A transferases genetics and metabolism, reporter genes, green fluorescent proteins analysis and genetics, molecular sequence data, messenger RNA analysis and metabolism.

Kotoulas, G., E. Sarropoulou, L.K. Tsigenopoulos , B. Luro, M. Tsalavouta, A. Magoulas, and A.G. National Cent. for Marine Research (2003). **Bridging genomes: An integrated genomic approach toward genetic improvement of aquacultured fish species Bridge-Map-FP5 project, contract Q5RS-2001-01797.** *7th Hellenic Symposium on Oceanography and Fisheries,May 6, 2003-May 9, 2003, Chersonissos, Greece,* 175-176 p.
Descriptors: zebrafish, biological charts, clones, DNA, fish culture, genetics, genomes, indicators, mapping, monitoring , phenotypic variations, selective breeding, *Danio rerio*, *Sparus aurata*, gilthead seabream, population genetics.
Notes: 70 abstracts.

Koudijs, M.J., M.J. den Broeder, A. Keijser, E. Wienholds, S. Houwing, E.M. van Rooijen, R. Geisler, and F.J. van Eeden (2005). **The zebrafish mutants dre, uki, and lep encode negative regulators of the Hedgehog signaling pathway.** *Public Library of Science Genetics* 1(2): E19. ISSN: 1553-7390.
Abstract: Proliferation is one of the basic processes that control embryogenesis. To identify factors involved in the regulation of proliferation, we performed a zebrafish genetic screen in which we used proliferating cell nuclear antigen (PCNA) expression as a readout. Two mutants, hu418B and hu540A, show increased PCNA expression. Morphologically both mutants resembled the dre (dreumes), uki (ukkie), and lep (leprechaun) mutant class and both are shown to be additional uki alleles. Surprisingly, although an increased size is detected of multiple structures in these mutant embryos, adults become dwarfs. We show that these mutations disrupt repressors of the Hedgehog (Hh) signaling pathway. The dre, uki, and lep loci encode Su(fu) (suppressor of fused), Hip (Hedgehog interacting protein), and Ptc2 (Patched2) proteins, respectively. This class of mutants is therefore unique compared to previously described Hh mutants from zebrafish genetic screens, which mainly show loss of Hh signaling. Furthermore, su(fu) and ptc2 mutants have not been described in vertebrate model systems before. Inhibiting Hh activity by cyclopamine rescues uki and lep mutants and confirms the overactivation of the Hh signaling pathway in these mutants. Triple uki/dre/lep mutants show neither an additive increase in PCNA expression nor enhanced embryonic phenotypes, suggesting that other negative regulators, possibly Ptc1,

prevent further activation of the Hh signaling pathway. The effects of increased Hh signaling resulting from the genetic alterations in the uki, dre, and lep mutants differ from phenotypes described as a result of Hh overexpression and therefore provide additional insight into the role of Hh signaling during vertebrate development.
Descriptors: zebrafish, mutation, signal transduction genetics, trans activators genetics, body size, cell proliferation, dwarfism Hedgehog proteins, proliferating cell nuclear antigen genetics.

Kovalenko, D., X. Yang, R.J. Nadeau, L.K. Harkins, and R. Friesel (2003). **Sef Inhibits fibroblast growth factor signaling by Inhibiting FGFR1 tyrosine phosphorylation and subsequent ERK activation**. *Journal of Biological Chemistry* 278(16): 14087-14091. ISSN: 0021-9258.
NAL Call Number: 381J824
Descriptors: zebrafish, cell differentiation, cell division, freshwater fish, gene expression, growth regulators, inhibitors, receptors, tyrosine , *Danio rerio*, fibroblast growth factor signaling, gene regulation, Sef gene.

Kratz, E., P.M. Eimon, K. Mukhyala, H. Stern, J. Zha, A. Strasser, R. Hart, and A. Ashkenazi (2006). **Functional characterization of the Bcl-2 gene family in the zebrafish.** *Cell Death and Differentiation* 13(10): 1631-40. ISSN: 1350 9047.
NAL Call Number: QH671
Abstract: Members of the Bcl-2 protein family control the intrinsic apoptosis pathway. To evaluate the importance of this family in vertebrate development, we investigated it in the zebrafish (*Danio rerio*). We found that the zebrafish genome encodes structural and functional homologs of most mammalian Bcl-2 family members, including multi-Bcl-2-homology (BH) domain proteins and BH3-only proteins. Apoptosis induction by gamma-irradiation required zBax1 and zPuma, and could be prevented by overexpression of homologs of prosurvival Bcl-2 family members. Surprisingly, zebrafish Bax2 (zBax2) was homologous to mammalian Bax by sequence and synteny, yet demonstrated functional conservation with human Bak. Morpholino knockdown of both zMcl-1a and zMcl-1b revealed their critical role in early embryonic zebrafish development, and in the modulation of apoptosis activation through the extrinsic pathway. These data indicate substantial functional similarity between zebrafish and mammalian Bcl-2 family members, and establish the zebrafish as a relevant model for studying the intrinsic apoptosis pathway.
Descriptors: zebrafish, genes, Bcl 2, apoptosis genetics, apoptosis radiation effects, base sequence, gamma rays, developmental gene expression regulation, multigene family, oligodeoxyribonucleotides, antisense genetics, phylogeny, RNA interference.

Krens, S.F., S. He, H.P. Spaink, and B.E. Snaar Jagalska (2006). **Characterization and expression patterns of the MAPK family in zebrafish.** *Gene Expression Patterns* 6(8): 1019-26. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: The mitogen activated protein kinases (MAPK) family pathway is conserved in evolution through the plant and animal kingdoms. These proteins have been implicated in diverse cellular processes including cell growth, proliferation, differentiation, survival and development. In this study we annotated and cloned members of the zebrafish MAPK gene-family, containing the ERK, JNK and p38 subfamilies. Their sequences were compared to orthologs of other vertebrates (human, mouse and rat) and the temporal and spatial expression levels of the zebrafish mapk genes were determined during early zebrafish development. Semi-quantitative reverse transcriptase-PCR analysis revealed that most mapk genes are

expressed throughout zebrafish development. Erk2,3 and p38a were expressed at a constant level throughout zebrafish embryogenesis, whereas erk1,4,5,6,7 and p38b showed specific temporal expression patterns. The spatial expression patterns were obtained by whole mount in situ hybridization at 24 h post fertilization (hpf) and 48 hpf embryos. The expression patterns were localized in specific regions at both stages and were tightly regulated during embryogenesis. For p38b, no staining was detected at 24 and 48 hpf. However, its expression was demonstrated at blastula-stage. Together, we identified the zebrafish orthologs of the zebrafish MAPK gene family and determined their specific spatial and temporal expression and distribution patterns during zebrafish embryogenesis.
Descriptors: zebrafish embryo, developmental gene expression regulation, mitogen activated protein kinases genetics and metabolism, amino acid sequence, molecular cloning embryonic development, gene expression, molecular sequence data, multigene family genetics, phylogeny, amino acid sequence homology.

Krens, S.F.G., H.P. Spaink, and B. Snaar-Jagalska (2006). **Functions of the MAPK family in vertebrate-development**. *Federation of European Biochemical Societies Letters* 580(21): 4984-4990. ISSN: 0014-5793.
NAL Call Number: QD415.F41
Descriptors: zebrafish embryo, amphibiotic species, developmental stages, embryonic development, evolution, freshwater fish, growth, Anura, *Danio rerio*, frogs.

Krushna Padhi, B., M.A. Akimenko, and M. Ekker (2006). **Independent expansion of the keratin gene family in teleostean fish and mammals: an insight from phylogenetic analysis and radiation hybrid mapping of keratin genes in zebrafish.** *Gene* 368: 37-45. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: The sequence and chromosomal distribution of keratin genes of zebrafish were compared with that of other fishes and mammals to provide an insight into the evolution of this gene family in vertebrates. By comparative sequence analysis and radiation hybrid mapping, we identified 16 type I and 7 type II keratin genes in the zebrafish genome. This contrasts with mammals, where type I and type II keratin genes are similar in number. The keratin genes are scattered in the fish genome, contrasting with the two clusters of keratin genes in mammalian genomes. Compared to genes from two species of pufferfish, the zebrafish type I keratin genes underwent an expansion by independent tandem duplications. Expression profiles based on EST counts suggest that some of the tandemly duplicated type I keratin genes from zebrafish either underwent sub-functionalization or acquired new expression domains. The chromosomal arrangement of keratins 8, keratin18, and a second type II keratin, as a cluster of three genes, has remained conserved in vertebrate evolution, except for duplication of the three-gene cluster in some teleosts. This contrasts with other members of the keratin gene family, which diverged independently between fish and mammals.
Descriptors: zebrafish, expressed sequence tags, keratins genetics, multigene family, phylogeny, radiation hybrid mapping, molecular evolution, gene expression profiling.

Kubota, Y., Y. Oike, S. Satoh, Y. Tabata, Y. Niikura, T. Morisada, M. Akao, T. Urano, Y. Ito, T. Miyamoto, S. Watanabe, and T. Suda (2005). **Isolation and expression patterns of genes for three angiopoietin-like proteins, Angptl1, 2 and 6 in zebrafish.** *Gene Expression Patterns* 5(5): 679-85. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Angiopoietin-like proteins (Angptls) are known to possess biological activities

not only in the vascular system, but in the other mammalian tissues; however, their expression patterns and function in embryogenesis have not been extensively characterized. Here, we identify three zebrafish genes (Zangptl1, Zangptl2 and Zangptl6) highly homologous to mammalian Angptl1/ARP1, Angptl2/ARP2 and Angptl6/AGF, and describe their adult and embryonic temporal and spatial expression patterns. Zangptl1 is expressed faintly in the somites, while Zangptl2 is first detected in the yolk sac extension, spinal cord and branchial arches and is later expressed in the liver primordium and pectoral fin buds. Zangptl6 is expressed in the notochord. In addition to its embryonic expression, Zangptl2 is induced in adult fish during fin regeneration.

Descriptors: zebrafish, angiopoietins biosynthesis, blood proteins biosynthesis, developmental gene expression regulation, intercellular signaling peptides and proteins biosynthesis, proteins biosynthesis, molecular cloning, in situ hybridization, limb bud metabolism, phylogeny, regeneration, reverse transcriptase PCR , time factors, tissue distribution.

Kucenas, S.C., J.A. Cox, and M.M. Voigt (2003). **Transgenic zebrafish: an approach to study the roles of P2X receptors in development.** *Society for Neuroscience Abstract Viewer and Itinerary Planner* ISSN: 1558-3635.

Abstract: P2X receptors are ATP ligand-gated non-selective cation channels with a high permeability for calcium. Members of this receptor family are believed to be involved in sensory neurotransmission and can act to modulate neurotransmitter release by acting at presynaptic terminals. Most studies of these receptors have utilized adult animals. In order to better understand the role of P2X receptors in the development of the nervous system, we chose to use the lower vertebrate *Danio rerio*. Due to this model's external fertilization and development, this system has been widely employed as a dynamic model of development. The zebrafish has 9 unique P2X receptor subunits and in situ experiments have shown that 5 of these are expressed in the central nervous system of the developing embryo. Of these 5, 4 seem to be localized to the same subsets of cells; Rohon-Beard Cells of the spinal cord and cranial sensory ganglia. We propose to study why neurons within the developing nervous system require multiple P2X subunit genes and what physiologic effect they impart to the system. To accomplish this goal, we chose to use a transgenic approach utilizing Bacterial Artificial Chromosomes (BAC). A zebrafish BAC library was screened and clones containing P2X receptor subunit genes of interest were isolated and modified by replacing exon 1 of the subunit gene with an eGFP variant coding region. This strategy allows for GFP expression to occur in cells normally expressing the subunit gene. In initial studies, injection of a modified P2X3.2 BAC yielded fish that have an expression pattern consistent with in situ data, in that Rohon-Beard Cells and trigeminal neurons were labeled. These results validate this approach to labeling, in vivo, neurons expressing P2X receptor subunit genes. This is the first step in elucidating the function and physiology of the P2X receptors during development. Copyright©Thomson Reuters 2009

Descriptors: transgenic zebrafish, molecular genetics, nervous system, genetic techniques, p2X.

Notes: Abstract No.250.1.

Kuo, M.W., S.W. Lou, J. Postlethwait, and B.C. Chung (2005). **Chromosomal organization, evolutionary relationship, and expression of zebrafish GnRH family members.** *Journal of Biomedical Science* 12(4): 629-39. ISSN: 1021-7770.

NAL Call Number: R850.A1

Abstract: Multiple forms of gonadotropin-releasing hormone (GnRH) are found in different vertebrates. In this study, we have cloned cDNA encoding the full-length gnrh3 and

gnrh2 from zebrafish brain and characterized their structure and expression patterns. We performed phylogenetic analysis and compared conserved syntenies in the region surrounding the GnRH genes from human, chicken, pufferfish, and zebrafish genores. The gnrh3 and gnrh2 genes were mapped to LG17 and LG21, respectively. The zebrafish genome appears to lack an ortholog to human GNRH1, and the human genome appears to lack an ortholog of gnrh3. Expression of gnrh3 began in the olfactory pit at 24-26 h postfertilization and expanded to the olfactory bulb during early larval stage. Expression of gnrh2 is always in the midbrain. In addition, GnRH is also expressed in boundary cells surrounding seminiferous cysts of the testis. Thus, this detailed phylogenetic, chromosomal comparison, and expression study defines the identity and the evolutionary relationship of two zebrafish gnrh genes. We propose a model describing the evolution of gnrh genes involving ancestral duplication of the genes followed by selective loss of one gene in some teleosts.

Descriptors: zebrafish, chromosomes ultrastructure, gene expression regulation, gonadotropin releasing hormone analogs and derivatives and genetics, oligopeptides genetics, pyrrolidonecarboxylic acid analogs and derivatives, brain metabolism, chromosome mapping, molecular cloning, complementary DNA metabolism, molecular evolution, gonadotropin releasing hormone biosynthesis, immunohistochemistry, in situ hybridization, genetic models, oligopeptides biosynthesis, phylogeny , species specificity, tissue distribution.

Kurita, K., S.M. Burgess, and N. Sakai (2004). **Transgenic zebrafish produced by retroviral infection of in vitro-cultured sperm.** *Proceedings of the National Academy of Sciences of the United States of America* 101(5): 1263-7. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: Transgenic modification of sperm before fertilization has distinct advantages over conventional transgenic methods. The primary advantage is that the mosaicism inherent in those other techniques is avoided. A culture system using primary cultures of zebrafish male germ cells, in which the differentiation from spermatogonia to functional sperm can occur in vitro, provides the opportunity for genetic modification of sperm in vitro. Here, we report the production of transgenic zebrafish from cultured sperm. The sperm were differentiated from premeiotic germ cells infected with a pseudotyped retrovirus in vitro. The collected sperm were used to perform successful in vitro fertilizations, and transgenic embryos were identified. The transgenic fish transmitted the proviral integration to the next generation in a Mendelian fashion. We report the generation of a transgenic animal by cultured sperm and open the door to many exciting possibilities for the rapid generation of transgenic lines in model organisms such as zebrafish or other animal systems that are otherwise intractable to transgenesis.

Descriptors: transgenic zebrafish, genetically modified, fertilization in vitro, Retroviridae genetics, cultured cells , spermatozoa, transgenes.

Kurita, R., Y. Tabata, H. Sagara, K. Arai, and S. Watanabe (2004). **A novel smoothelin-like, actin-binding protein required for choroidal fissure closure in zebrafish.** *Biochemical and Biophysical Research Communications* 313(4): 1092-100. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: A gene expressed in the choroidal fissure of the zebrafish eye was isolated. This gene, designated #61, contained significant homology with the previously reported actin-binding protein smoothelin. During zebrafish embryogenesis, #61 expression was first detected in the lateral mesoderm of the mid-trunk region, and then strong expression was observed in the choroid fissure of the eye and in a part of the brain at 30 hpf. Abrogation of #61 activity by an antisense morpholino oligonucleotide resulted in the failure of closure

of the choroid fissure at 30 hpf. In addition, hemorrhage was observed at the caudal side of the eye. Detailed analysis indicated that leakage of blood may have arisen from the hyaloid vessels and the primordial midbrain channels. On the other hand, retinal differentiation and optic nerve formation seemed normal. Taken together, our data suggest that gene #61 may play a role in the formation of hyaloid vessels and subsequent choroid fissure closure.
Descriptors: zebrafish, choroid embryology, microfilament amino acid and base sequence, blood vessels embryology, molecular cloning, cytoskeletal complementary DNA genetics, developmental gene expression regulation drug effects, molecular sequence data, muscle antisense oligoribonucleotides genetics and pharmacology, optic nerve embryology, retina embryology, amino acid sequence homology.

Kurosawa, G., N. Takamatsu, M. Takahashi, M. Sumitomo, E. Sanaka, K. Yamada, K. Nishii, M. Matsuda, S. Asakawa, H. Ishiguro, K. Miura, Y. Kurosawa, N. Shimizu, Y. Kohara, and H. Hori (2006). **Organization and structure of hox gene loci in medaka genome and comparison with those of pufferfish and zebrafish genomes.** *Gene* 370: 75-82. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: We isolated BAC clones that cover the entire hox gene loci in the medaka fish *Oryzias latipes.* The BAC clones were characterized by the Southern hybridization with many hox gene probes isolated in our previous study and by PCR using primers designed for selective amplification of respective hox genes. Then, the BAC clones have been subjected to shotgun sequencing. The results revealed the organization of the entire hox gene loci. Forty-six hox genes in total are encoded in seven clusters as follows: 10 hox genes in Aa cluster; 5 in Ab; 9 in Ba; 4 in Bb; 10 in Ca; 6 in Da; and 2 in Db. Together with the information on the hox gene loci registered in the Fugu genome database and in the *Danio* genome database, the physical maps of three fish genomes were constructed and compared one another. Not only numbers of hox genes but also the distances between the neighboring hox genes are highly similar between medaka and fugu. As for six clusters, Aa, Ab, Ba, Bb, Ca and Da that are commonly present in the three fishes, only few or no differences were found in each cluster. Thus, the hox gene sets should have been well conserved once they had been established in respective species.
Descriptors: zebrafish, genome genetics, homeodomain multigene family genetics, *Oryzias* genetics, quantitative trait loci genetics, Southern blotting methods, chromosome mapping methods, artificial bacterial chromosomes genetics, genetic databases, nucleic acid sequence homology, *Takifugu* genetics.

Kwan, T.T., R. Liang, S. Lin, S.C. Ekker, C.M. Verfaillie, and A.Y. Leung (2005). **Natural regression of primitive hematopoiesis in zebrafish embryos is mediated by a death receptor gene**. *Experimental Hematology* 33(7, Suppl. 1): 79. ISSN: 0301 472X.
NAL Call Number: RB145.E97
Descriptors: zebrafish, development, molecular genetics, tune l method, genetic techniques, modified cyanomethemoglobin method, histology and cytology techniques, development, apoptosis, primitive hematopoiesis, axial circulation.

Kwok, H.F., W.K. So, Y. Wang, and W. Ge (2005). **Zebrafish gonadotropins and their receptors: I. Cloning and characterization of zebrafish follicle-stimulating hormone and luteinizing hormone receptors--evidence for their distinct functions in follicle development.** *Biology of Reproduction* 72(6): 1370-81. ISSN: 0006 3363.
NAL Call Number: QD415.F4

Abstract: In the present study, we cloned and characterized zebrafish FSH receptor (Fshr) and LH receptor (Lhr). Both fshr and lhr were abundantly expressed in the zebrafish gonads; however, they could also be detected in the kidney and liver, respectively. When overexpressed in mammalian cell lines together with a cAMP-responsive reporter gene, zebrafish Fshr responded to goldfish pituitary extract but not hCG, whereas Lhr could be activated by both. It was further demonstrated that Fshr was specific to bFSH, while Lhr could be stimulated by both bovine FSH and LH. Low level of fshr expression could be detected in the immature ovary, but the level steadily increased during vitellogenesis of the first cohort of developing follicles. In contrast, the expression of lhr could barely be detected in the immature ovary, but it became detectable at the beginning of vitellogenesis and steadily increased afterward with the peak level reached at the full-grown stage. At the follicle level, the expression of fshr was very weak in the follicles of primary growth stage but significantly increased with the follicles entering vitellogenesis. However, after reaching the maximal level in the midvitellogenic follicles, the level of fshr expression dropped slightly but significantly at the full-grown stage. In comparison, the expression of lhr obviously lagged behind that of fshr. Its expression became detectable only when the follicles started to accumulate yolk granules, but the level rose steadily afterward and reached the peak at the full-grown stage before oocyte maturation. These results suggest differential roles for Fshr and Lhr in zebrafish ovarian follicle development.
Descriptors: zebrafish, ovarian follicle growth and development, FSH and LH receptors genetics, amino acid and base sequence, cultured cells, chorionic gonadotropin pharmacology , molecular cloning, developmental gene expression regulation, gonads metabolism, molecular sequence data, FSH receptors drug effects and metabolism, sexual maturation genetics.

Lahiri, K., D. Vallone, S.B. Gondi, C. Santoriello, T. Dickmeis, and N.S. Foulkes (2005). **Temperature regulates transcription in the zebrafish circadian clock.** *Public Library of Science Biology* 3(11): E351. ISSN: 1544-9173.
NAL Call Number: QH301.P56
Abstract: It has been well-documented that temperature influences key aspects of the circadian clock. Temperature cycles entrain the clock, while the period length of the circadian cycle is adjusted so that it remains relatively constant over a wide range of temperatures (temperature compensation). In vertebrates, the molecular basis of these properties is poorly understood. Here, using the zebrafish as an ectothermic model, we demonstrate first that in the absence of light, exposure of embryos and primary cell lines to temperature cycles entrains circadian rhythms of clock gene expression. Temperature steps drive changes in the basal expression of certain clock genes in a gene-specific manner, a mechanism potentially contributing to entrainment. In the case of the per4 gene, while E-box promoter elements mediate circadian clock regulation, they do not direct the temperature-driven changes in transcription. Second, by studying E-box-regulated transcription as a reporter of the core clock mechanism, we reveal that the zebrafish clock is temperature-compensated. In addition, temperature strongly influences the amplitude of circadian transcriptional rhythms during and following entrainment by light-dark cycles, a property that could confer temperature compensation. Finally, we show temperature-dependent changes in the expression levels, phosphorylation, and function of the clock protein, CLK. This suggests a mechanism that could account for changes in the amplitude of the E-box-directed rhythm. Together, our results imply that several key transcriptional regulatory elements at the core of the zebrafish clock respond to temperature.
Descriptors: zebrafish, circadian rhythm, genetic transcription, body temperature, calibra-

tion, cell line, gene expression, light, molecular sequence data, temperature, transcription factors metabolism.

Lai, S.L., C.N. Chang, P.J. Wang, and S.J. Lee (2005). **Rho mediates cytokinesis and epiboly via ROCK in zebrafish.** *Molecular Reproduction and Development* 71(2): 186-96. ISSN: 1040-452X.
NAL Call Number: QP251.M64
Descriptors: zebrafish, ADP ribose transferases administration and dosage, *Botulinum* toxins administration and dosage, cell movement drug effects, protein serine threonine kinases metabolism, Rhoa GTP binding protein metabolism, cell movement physiology, cytology, embryology, gastrula cytology and physiology, intracellular signaling peptides and proteins, signal transduction drug effects and physiology.

Lam, C.S., I. Sleptsova Friedrich, A.D. Munro, and V. Korzh (2003). **SHH and FGF8 play distinct roles during development of noradrenergic neurons in the locus coeruleus of the zebrafish.** *Molecular and Cellular Neuroscience* 22(4): 501-15. ISSN: 1044-7431.
Abstract: Several signaling pathways have been implicated in the development of dopaminergic and serotonergic neurons. Here, we analyzed the formation of noradrenergic (NAergic) cells in the locus coeruleus (LC) of zebrafish. In the sonic hedgehog (shh) mutant, cells positive for tyrosine hydroxylase, a marker for putative NAergic cells in the LC were reduced. Similarly, the inhibition of translation of all hh genes and the perturbation of Shh signaling by forskolin resulted in a decrease in the number of cells. Conversely, when SHH was overexpressed, an increase in number was observed. Thus, Shh is involved in maintaining the appropriate number of cells in the LC. While elevated levels of bone morphogenetic protein 4 (BMP4) did not attenuate tyrosine hydroxylase-positive cells, exogenous fibroblast growth factor 8 (FGF8) rescued NAergic neurons in the acerebellar (ace) mutant, providing direct in vivo evidence that Fgf8 is required for the induction of NAergic neurons in the LC.
Descriptors: zebrafish embryo, embryology, Fgf deficiency, locus coeruleus embryology, neurons metabolism, norepinephrine biosynthesis, protein serine threonine kinases, growth factor receptors, trans activators deficiency, Type I activin receptors genetics and metabolism, apoptosis genetics, bone morphogenetic protein receptors, Type I, cell survival genetics, down regulation genetics, cytology, fibroblast growth factor 8, Fgf genetics, developmental gene expression regulation genetics, Hedgehog proteins, locus coeruleus cytology and metabolism, mutation genetics, neurons cytology, protein biosynthesis drug effects and genetics, protein synthesis inhibitors pharmacology, trans activators genetics, tyrosine 3 monooxygenase metabolism, up regulation genetics, metabolism.

Lam, S.H., Y.L. Wu, V.B. Vega, L.D. Miller, J. Spitsbergen, Y. Tong, H. Zhan, K.R. Govindarajan, S. Lee, S. Mathavan, K.R. Murthy, D.R. Buhler, E.T. Liu, and Z. Gong (2006). **Conservation of gene expression signatures between zebrafish and human liver tumors and tumor progression.** *Nature Biotechnology* 24(1): 73-5. ISSN: 1087-0156.
NAL Call Number: QH442.B5
Abstract: The zebrafish (*Danio rerio*) has been long advocated as a model for cancer research, but little is known about the real molecular similarities between zebrafish and human tumors. Comparative analysis of microarray data from zebrafish liver tumors with those from four human tumor types revealed molecular conservation at various levels between fish and human tumors. This approach provides a useful strategy for identifying an expression signature that is strongly associated with a disease phenotype.
Descriptors: zebrafish, humans, neoplastic gene expression regulation, experimental liver

neoplasms genetics and metabolism, neoplasm proteins genetics and metabolism, amino acid sequence, carcinogens, conserved sequence, disease progression, gene expression profiling , experimental chemically induced liver neoplasms,, molecular sequence data, amino acid sequence homology, species specificity.

Lamason, R.L., M.A. Mohideen, J.R. Mest, A.C. Wong, H.L. Norton, M.C. Aros, M.J. Jurynec, X. Mao, V.R. Humphreville, J.E. Humbert, S. Sinha, J.L. Moore, P. Jagadeeswaran, W. Zhao, G. Ning, I. Makalowska, P.M. McKeigue, D. O'donnell, R. Kittles, E.J. Parra, N.J. Mangini, D.J. Grunwald, M.D. Shriver, V.A. Canfield, and K.C. Cheng (2005). **SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans.** *Science* 310(5755): 1782-6. ISSN: 0036-8075.

NAL Call Number: 470Sci2

Abstract: Lighter variations of pigmentation in humans are associated with diminished number, size, and density of melanosomes, the pigmented organelles of melanocytes. Here we show that zebrafish golden mutants share these melanosomal changes and that golden encodes a putative cation exchanger slc24a5 (nckx5) that localizes to an intracellular membrane, likely the melanosome or its precursor. The human ortholog is highly similar in sequence and functional in zebrafish. The evolutionarily conserved ancestral allele of a human coding polymorphism predominates in African and East Asian populations. In contrast, the variant allele is nearly fixed in European populations, is associated with a substantial reduction in regional heterozygosity, and correlates with lighter skin pigmentation in admixed populations, suggesting a key role for the SLC24A5 gene in human pigmentation.

Descriptors: zebrafish, antiporters genetics, skin pigmentation genetics, African continental ancestry group genetics, alanine genetics, alleles, amino acid sequence, antiporters chemistry and physiology, Asian continental ancestry group genetics, calcium metabolism, European continental ancestry group genetics, evolution, gene frequency, haplotypes, heterozygote, ion transport, melanins analysis, melanosomes chemistry and ultrastructure, mice, molecular sequence data, multifactorial inheritance, mutation, pigment epithelium of eye chemistry and ultrastructure, single nucleotide polymorphism, selection genetics, threonine genetics, variation genetics.

Notes:

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Lambrechts, D. and P. Carmeliet (2004). **Genetics in zebrafish, mice, and humans to dissect congenital heart disease: insights in the role of VEGF.** *Current Topics in Developmental Biology* 62: 189-224. ISSN: 0070 2153.

NAL Call Number: QL951.C8

Abstract: Heart development and the establishment of a functional circulatory circuit are complex biological processes in which subtle perturbations may result in catastrophic consequences of cardiovascular birth defects. Studies in model organisms, most notably the mouse and the zebrafish, have identified genes that also cause these life-threatening defects when mutated in humans. Gradually, a framework for the genetic pathway controlling these events is now beginning to emerge. However, the puzzling phenotypic variability of the cardiovascular disease phenotype in humans and the recent identification of phenotypic modifiers using model organisms indicates that other genetic loci might interact to modify the disease phenotype. To illustrate this, we review the role of vascular endothelial growth factor (VEGF) during vascular and cardiac development and stress how zebrafish and mouse genetic studies have helped us to understand the role this growth factor has in human disease, in particular in the Di-George syndrome.

Descriptors: zebrafish, disease models, congenital heart defects genetics and metabolism, vascular endothelial growth factor A metabolism and genetics, blood vessels embryology, blood vessels growth and development, blood vessels metabolism, congenital heart defects embryology, congenital heart defects pathology, mice, neurons metabolism and pathology.

Lang, M.R., L.A. Lapierre, M. Frotscher, J.R. Goldenring, and E.l.a.W. Knapik (2006). **Secretory COPII coat component Sec23a is essential for craniofacial chondrocyte maturation**. *Nature Genetics* 38(10): 1198-1203. ISSN: 1061-4036.
NAL Call Number: QH426.N37
Descriptors: zebrafish, body length, cartilage, chondrocytes, chondrogenesis, collagen, deposits, dysplasia, endoplasmic reticulum, extracellular matrix, fins, freshwater fish, genetic abnormalities, golgi apparatus, leucine, morphogenesis, mutations, nonsense mutation, protein transport, sexual maturity, stop codon, symptoms, *Danio rerio*.

Langenau, D.M., A.A. Ferrando, D. Traver, J.L. Kutok, J.P. Hezel, J.P. Kanki, L.I. Zon, A.T. Look, and N.S. Trede (2004). **In vivo tracking of T cell development, ablation, and engraftment in transgenic zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 101(19): 7369-74. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: Transgenic zebrafish that express GFP under control of the T cell-specific tyrosine kinase (lck) promoter were used to analyze critical aspects of the immune system, including patterns of T cell development and T cell homing after transplant. GFP-labeled T cells could be ablated in larvae by either irradiation or dexamethasone added to the water, illustrating that T cells have evolutionarily conserved responses to chemical and radiation ablation. In transplant experiments, thymocytes from lck-GFP fish repopulated the thymus of irradiated wild-type fish only transiently, suggesting that the thymus contains only short-term thymic repopulating cells. By contrast, whole kidney marrow permanently reconstituted the T lymphoid compartment of irradiated wild-type fish, suggesting that long-term thymic repopulating cells reside in the kidney.
Descriptors: zebrafish, genetically modified immunology, dexamethasone pharmacology, immunohistochemistry, in situ hybridization, molecular sequence data, reverse transcriptase PCR, T lymphocytes drug effects and immunology.

Langenau, D.M., M.D. Keefe, N.Y. Storer, J. Guyon, J.L. Kutok, X. Le, W. Goessling, D. Neuberg, L.M. Kunkel, and L.I. Zon (2007). **Effects of RAS on the genesis of embryonal rhabdomyosarcoma**. *Genes & Development* 21(11): 1382-1395. ISSN: 0890-9369.
NAL Call Number: QH426.G46
Descriptors: zebrafish, cancer, carcinology, differentiation, embryonic development, freshwater fish, genes, muscles, phylogenetics, rhabdomyosarcoma, stem cells, subpopulations, tumor cells, tumors, tumors, satellite cells, *Danio rerio.*

Laplante, M., H. Kikuta, M. Konig, and T.S. Becker (2006). **Enhancer detection in the zebrafish using pseudotyped murine retroviruses.** *Methods* 39(3): 189-98. ISSN: 0882-4010.
NAL Call Number: QP519.7.M47
Abstract: Vectors based on murine retroviruses are among the most efficient means to insert reporter constructs into the context of a vertebrate chromosome with the aim to visualize cis-regulatory information available to a basal promoter at the site of insertion. In combination with using the zebrafish embryo as a readout for the activity of regulatory elements, enhancer detection becomes a powerful technique for gene discovery and for the mapping of

the extent of regulatory domains in a vertebrate genome. Our laboratory has performed the only large-scale enhancer detection screen to date in any vertebrate and we describe in this paper the methods we developed to generate viral particles, to insert reporter constructs into the zebrafish germ line, the screening of detection events in heterozygous F1 embryos, and the isolation of genomic sequence flanking the inserted vector for the purpose of genomic mapping. Given sufficient scale, the technology described here can be used to obtain cis-regulatory information across the entire zebrafish genome for any given basal promoter.
Descriptors: zebrafish, mice, genetically modified genetics, enhancer elements genetics, reporter genes, genetic engineering methods, genetic vectors, retroviridae genetics, molecular cloning, computational biology, genomics methods, DNA sequence analysis, transfection instrumentation and methods.

Lardelli, M. (2003). **The evolutionary relationships of zebrafish genes tbx6, tbx16/spadetail and mga.** *Development Genes and Evolution* 213(10): 519-22. ISSN: 0949 944X.
NAL Call Number: QL951.R68
Abstract: The T-box (Tbx) genes encode a family of transcription factors required for development of vertebrate embryos. In an attempt to discover human orthologues of the zebrafish (*Danio rerio*) tbx6 and tbx16/spadetail genes, we found that the most similar human (*Homo sapiens*) gene is the orthologue of mouse (*Mus musculus)* Mga (MAX gene associated). We have identified the zebrafish orthologue of Mga using analyses of sequence similarity and the orthologies of syntenic genes. Zebrafish mga maps close to dtk (developmental receptor tyrosine kinase), the orthologue of human TYRO3 (TYRO3 protein tyrosine kinase). Like its human and mouse orthologues, zebrafish mga lacks the three conserved introns within the T-box coding sequences that are characteristic of the vertebrate T-box gene family. This suggests that these genes are derived from an ancient reverse transcription event. The human genome does not appear to possess orthologues of zebrafish tbx6 or tbx16/spadetail.
Descriptors: zebrafish, phylogeny, T-box domain transcription factors genetics, amino acid and base sequence, complementary DNA genetics, molecular sequence data, sequence alignment, DNA sequence analysis.

Larsson, T.A., F. Olsson, G. Sundstrom, S. Brenner, B. Venkatesh, and D. Larhammar (2005). **Pufferfish and zebrafish have five distinct NPY receptor subtypes, but have lost appetite receptors Y1 and Y5.** *Annals of the New York Academy of Sciences* 1040: 375-7. ISSN: 0077 8923.
NAL Call Number: 500 N484
Abstract: The two neuropeptide Y (NPY) receptors Y1 and Y5 stimulate feeding in mammals, but are missing in the euteleosts, zebrafish and pufferfish (*Takifugu rubripes*). Both species have five other subtypes called Y2, Y7, Ya, Yb, and Yc. RT-PCR studies in pufferfish show that all five are expressed in the brain and may mediate NPY effects on feeding. Y2, Ya, and Yb are also broadly expressed in peripheral organs. These results reveal interesting differences in the NPY system of teleosts and mammals that may have arisen in the genetic turmoil involving the basal ray-fin fish tetraploidization.
Descriptors: zebrafish, neuropeptide Y receptor deficiency and genetics, tetraodontiformes physiology and genetics, appetite regulation genetics.

Lassiter, C.S. and E. Linney (2007). **Embryonic expression and steroid regulation of brain aromatase cyp19a1b in zebrafish (*Danio Rerio*).** *Zebrafish* 4(1): 49-58. ISSN: 1545-8547.
Abstract: Estradiol is produced from testosterone by the aromatase gene, cyp19. In the zebrafish *Danio rerio*, brain aromatase, cyp19a1b, is highly expressed during development.

We report the developmental expression pattern of cyp19a1b using whole mount in situ hybridization and describe hormonal effects on the gene using RT-PCR. Expression is up-regulated between 24 and 48 hours postfertilization (hpf). Localized expression of cyp19a1b is first detected at 48 hpf in the preoptic area, hypothalamus, terminal nerve, and olfactory bulb. The gene is itself induced by estradiol in a positive feedback loop. Testosterone exposure also induces the cyp19a1b gene in zebrafish; however, a majority of this induction is blocked by an estrogen receptor antagonist. The expression pattern of aromatase in the brain and its control by steroid hormones is well conserved among the vertebrate lineage.
Descriptors: zebrafish, steroid regulation, brain aromatase, cyp19a1b, conserved in vertebrates.

Lau, B., S. Bretaud, Y. Huang, E. Lin, and S. Guo (2006). **Dissociation of food and opiate preference by a genetic mutation in zebrafish.** *Genes, Brain, and Behavior* 5(7): 497-505. ISSN: 1601-1848.
Abstract: Both natural rewards and addictive substances have the ability to reinforce behaviors. It has been unclear whether identical neural pathways mediate the actions of both. In addition, little is known about these behaviors and the underlying neural mechanisms in a genetically tractable vertebrate, the zebrafish *Danio rerio*. Using a conditioned place preference paradigm, we demonstrate that wildtype zebrafish exhibit a robust preference for food as well as the opiate drug morphine that can be blocked by the opioid receptor antagonist naloxone. Moreover, we show that the too few mutant, which disrupts a conserved zinc finger-containing gene and exhibits a reduction of selective groups of dopaminergic and serotonergic neurons in the basal diencephalon, displays normal food preference but shows no preference for morphine. Pretreatment with dopamine receptor antagonists abolishes morphine preference in the wildtype. These studies demonstrate that zebrafish display measurable preference behavior for reward and show that the preference for natural reward and addictive drug is dissociable by a single-gene mutation that alters subregions of brain monoamine neurotransmitter systems. Future genetic analysis in zebrafish shall uncover further molecular and cellular mechanisms underlying the formation and function of neural circuitry that regulate opiate and food preference behavior.
Descriptors: zebrafish, addictive behavior genetics, behavior physiology, reward, analysis of variance, brain drug effects, brain metabolism, conditioning, classical drug effects, conditioning, classical physiology, dopamine metabolism, drug dose response relationship, food preferences physiology, morphine administration and dosage, morphine antagonists and inhibitors, mutation, naloxone pharmacology, narcotic antagonists pharmacology, administration and dosage, nerve tissue serotonin metabolism, signal transduction genetics, vision genetics and physiology, zinc fingers.

Lee, C. and A. Smith (2004). **Molecular cytogenetic methodologies and a bacterial artificial chromosome (BAC) probe panel resource for genomic analyses in zebrafish.** *Methods in Cell Biology* 77: 241-54. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, chromosome mapping methods, artificial bacterial chromosomes genetics and chemistry, genomics methods, in situ hybridization, fluorescence methods, artificial bacterial chromosomes chemistry, DNA probes chemistry.

Lee, C.T., T. Risom, and W.M. Strauss (2006). **Micro RNAs in mammalian development**. *Birth Defects Research* 78(2): 129-139. ISSN: 1542 0752.
NAL Call Number: QL991.T4

Abstract: Development in mammals is a complex process requiring gene expression to be spatially and temporally well-regulated. Factors modulate gene functioning by controlling transcription, translation, or mRNA degradation. microRNAs (miRNAs) are a group of small RNA molecules (similar to 22 nucleotides) that attenuate gene activity posttranscriptionally by suppressing translation or destabilizing mRNAs. miRNAs have been recently validated to regulate many animal developmental events including proliferation, differentiation, and apoptosis. Many miRNAs display intriguing expression and functioning patterns throughout these pathways. Here we will review achievements to date about studies of how miRNAs affect a variety of animal developmental transitions, from the formation of early embryos to the generation of highly specialized tissues. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, developmental transitions, molecular genetics, cell biology, apoptosis, cell proliferation, tissue formation.
Notes: A review.

Lee, E.Y. and H.S. Kim (2006). **Cloning and characterization of zebrafishMicrosomal Epoxide hydrolase based on bioinformatics.** *Journal of Molecular Catalysis* 34(2): 129-135. ISSN: 1598 642X.
NAL Call Number:
Abstract: A gene encoding for a putative microsomal epoxide hydrolase (mEH) of a zebrafish, *Danio rerio*, was cloned and characterized. The putative mEH protein of D. rerio exhibited sequence similarity with mammalian mEH and some other bacterial EHs. A structural model for the putative mEH was constructed using homology modeling based on the crystallographic templates, l qo 7 and l ehy. The catalytic triad consisting of Asp_233, Glu_413, and His_440 was identified, and the characteristic features such as two tyrosine residues and oxyanion hole were found to be highly conserved.
Descriptors: zebrafish, epoxy compounds, cloning, bioinformatics.
Notes: Email:hskim@ks.ac.kr.

Lee, J.W. and J.M. Gross (2007). **Laminin beta 1 and gamma 1 containing laminins are essential for basement membrane integrity in the zebrafish eye**. *Investigative Ophthalmology and Visual Science* 48(6): 2483-2490. ISSN: 0146-0404.
Abstract: PURPOSE. In this study recessive zebrafish mutations in the genes encoding laminin beta 1 (lamb1) and laminin Gamma 1 (lamc1) were used to determine the functions of these laminin proteins during ocular basement membrane formation and during zebrafish eye development. METHODS. Ocular defects in lamb1 and lamc1 mutants were characterized by using a combination of histology, immunohistochemistry, in situ hybridization, and transmission electron microscopy. RESULTS. The results demonstrated that zebrafish lamb1 and lamc1 mutants possess defects in two ocular basement membranes - the lens capsule and the inner limiting membrane - whereas Bruch's membrane is largely unaffected. lamb1 and lamc1 mutants possess severe lens dysplasias that result from a compromise in lens capsule integrity. Inner limiting membrane continuity is irregular in these mutants, and these irregularities result in small retinal ectopias that extend from the retina into the interstitial space between the retina and the lens. At late embryonic stages (e.g., 5-7 days after fertilization), retinal lamination defects are also observed in a subset of laminin mutants. CONCLUSIONS. The results demonstrate that laminin beta 1 and - Gamma 1 containing laminins are essential for the integrity of the lens capsule basement membrane and inner limiting membrane in the zebrafish eye. Copyright © 2008 CABI
Descriptors: zebrafish, eye lens, genes, genetics, laminins, molecular biology, molecular genetics, mutations, retina, *Danio rerio.*

Lee, K.W., S.M. Ho, C.H. Wong, S.E. Webb, and A.L. Miller (2004). **Characterization of mid-spindle microtubules during furrow positioning in early cleavage period zebrafish embryos.** *Zygote* 12(3): 221-30. ISSN: 0967-1994.

NAL Call Number: QH491.Z94

Abstract: We report evidence to suggest that during the first few meroblastic cell divisions in zebrafish embryos a dynamic population of central-spindle microtubules serve a crucial function in positioning the cleavage furrow at the surface of the blastoderm. Originating from the mid-zone of the mitotic spindle they develop into what we term a mid-spindle 'pre-furrowing microtubule array' that expands upward and outward from the spindle mid-zone towards the blastodisc surface. We suggest that this structure transmits positional information to the blastodisc cortex that results in the correctly positioned assembly of the cytokinetic contractile apparatus. We also propose that the pre-furrowing microtubule array then develops into a furrow-ingression microtubule array that helps direct and assemble the deepening furrow as it cuts its way through the blastodisc. Due to the location of its origin, the pre-furrowing microtubule array serves to successfully separate the daughter nuclei and thus equally divide the blastoderm. Furthermore, co-localization with elements of the cortical endoplasmic reticulum and their inositol 1,4,5-trisphosphate receptors suggests that the pre-furrowing microtubule array may also play a role in organizing localized Ca2+ transients that have been shown to be essential to the furrow positioning, propagation and deepening process during cytokinesis in zebrafish embryos.

Descriptors: zebrafish embryos, cleavage stage, ovum physiology, microtubules physiology, mitotic spindle apparatus physiology, calcium channels metabolism, endoplasmic reticulum metabolism, immunohistochemistry, cytoplasmic receptors and nuclear metabolism, time factors.

Lee, K.W., S.E. Webb, and A.L. Miller (2006). **Requirement for a localized, IP3R-generated Ca2+ transient during the furrow positioning process in zebrafish zygotes.** *Zygote* 14(2): 143-55. ISSN: 0967-1994.

NAL Call Number: QH491.Z94

Abstract: We report that the first localized Ca(2+) transient visualized in the blastodisc cortex of post-mitotic zebrafish zygotes has unique features. We confirm that this initial 'furrow positioning' Ca(2+) transient precedes the physical appearance of the first cleavage furrow at the blastodisc surface and that it has unique dynamics, which distinguish it from the subsequent furrow propagation transients that develop from it. This initial transient displays a distinct rising phase that peaks prior to the initiation of the two linear, subsurface, self-propagating Ca(2+) waves that constitute the subsequent furrow propagation transient. Through the carefully timed introduction of the Ca(2+) buffer, dibromo-BAPTA, we also demonstrate the absolute requirement of this initial rising phase Ca(2+) transient in positioning the furrow at the blastodisc surface: no rising phase transient, no cleavage furrow. Likewise, the introduction of the inositol 1,4,5-trisphosphate receptor (IP3R) antagonist, 2-aminoethoxydiphenyl borate, eliminates both the rising phase transient and the appearance of the furrow at the cell surface. On the other hand, antagonists of the ryanodine receptor and NAADP-sensitive channels, or simply bathing the zygote in Ca(2+)-free medium, have no effect on the generation of the rising phase positioning transient or the appearance of the furrow at the surface. This suggests that like the subsequent propagation and deepening/zipping Ca(2+) transients, the rising phase furrow positioning transient is also generated specifically by Ca(2+) released via IP3Rs. We propose, however, that despite being generated by a similar Ca(2+) release mechanism, the unique features of this initial transient suggest that it might be a distinct signal with a specific function associated with positioning the cleavage

furrow at the blastodisc surface.
Descriptors: zebrafish, calcium signaling physiology, cleavage stage, ovum metabolism, inositol 1,4,5 trisphosphate receptors physiology, zygote metabolism, aequorin.

Lee, K.Y. (2004). **Developing a gene-trapping approach for gene identification using nuclear transfer in zebrafish**. *Journal of Animal Science and Technology* 46(2): 155-164. ISSN: 1598 9429.
Abstract: This study involves identifying and cloning trapped genes from cultured cells carrying the gene-trap constructs and generating cloned zebrafish using these cells for functional study. Gene-trapping studies in gene-trapped cells were carried out in initial and cloned zebrafish carrying gene-trap events, and were successfully produced based on the nuclear transplantation technique. Two kind of retroviral gene-trap constructs were adopted. The first one (SA/GFP-TP) carries a GFP reporter gene containing a splicing acceptor and an internal neo gene. The second one (Neo-TP) contains a promoter-less neo gene located in the LTR sequence of a retroviral vector. The infected cells were subjected to drug selection (neomycin treatment) because the two constructs carry the neomycin resistant gene. All those cells survived the neomycin treatment should carry the proviral insertions. For Neo-TP, isolated DNA from the neomycin-resistant fibroblast cells infected by Neo-TP, was digested with EcoR1 restriction enzyme and transformed into bacteria after ligation. This procedure led to the isolation of seven clones carrying flanking cellular DNA with a typical retroviral integration signature sequence. These clones contained genomic DNA ranging from 1 kb to 7 kb and sequences of 300-600 bp were obtained from each of the rescued plasmids. Database searching showed that all of them share high homology to zebrafish sequences. For fish cloning using tagged cells, initially, nucleus donors directly selected from a mixture of cells (Neo-TP cells) were used. A total of 44 embryos (3.7%) out of 1179 transplants reached blastula stage; 8 of these embryos (0.8%) hatched and 3 (0.3%) of them survived to adulthood. One out of three lived cloned zebrafish had an amplified fragment and was labelled with 32P. Copyright © 2008 CABI
Descriptors: zebrafish, embryos, genes, mutagenesis, nuclei, nucleotide sequences, plasmids, transfer, *Danio rerio.*
Language of Text: Korean.

LeGood, J.A., K. Joubin, A.J. Giraldez, N. BenHaim, S. Beck, Y. Chen, A.F. Schier, and D.B. Constam (2005). **Nodal stability determines signaling range**. *Current Biology* 15(1): 31-36. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Abstract: Secreted TGFbeta proteins of the Nodal family pattern the vertebrate body axes and induce mesoderm and endoderm (1). Nodal proteins can act as morphogens (2), but the mechanisms regulating their activity and signaling range are poorly understood. In particular, it has been unclear how inefficient processing or rapid turnover of the Nodal protein influences autocrine and paracrine signaling properties (3,4). Here, we evaluate the role of Nodal processing and stability in tissue culture and zebrafish embryos. Removal of the pro domain potentiates autocrine signaling but reduces Nodal stability and signaling range. Insertion of an N-glycosylation site present in several related TGFbeta proteins increases the stability of mature Nodal. The stabilized form of Nodal acts at a longer range than the wild-type form. These results suggest that increased proteolytic maturation of Nodal potentiates autocrine signaling, whereas increased Nodal stability extends paracrine signaling. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, biochemistry and molecular biophysics, development, tissue culture, culturing techniques, autocinne signaling, paracrine signaling.

Lehmann, C. and W.W. Naumann (2005). **Axon pathfinding and the floor plate factor Reissner's substance in wildtype, cyclops and one-eyed pinhead mutants of *Danio rerio*.** *Brain Research* 154(1): 1-14. ISSN: 0006 8993.
NAL Call Number: QP376
Abstract: The ventral median floor plate (FP) is a well-examined embryonic structure, which is involved in neuron differentiation and axon outgrowth. The FP of different vertebrates expresses the glycoprotein Reissner's substance (RS). This glycoprotein is also produced by the dorsal median subcommissural organ (SCO). We examined if the dorsal SCO and the ventral FP are interdependent for the expression of RS and looked for indications for a role of RS in axon outgrowth. Therefore, we examined zebrafish embryos of wildtype (wt) and the mutants cyclops(tf219) (cyc) and one-eyed pinhead(tz257) (oep), which both lack the FP. Our studies demonstrate that the FP is not necessary in order to induce the expression of RS in the SCO. The pattern of the anti-RS immunolabelling in the mutants is, however, changed compared to wt zebrafish embryos. As a consequence of the lacking FP and the degenerated ventricle system in cyc and oep mutants, a Reissner's fibre (RF) is not formed. Our studies confirm earlier results about the axon growth in cyc mutants, and provide the first detailed data about the aberrant axon growth in oep mutants. The modified outgrowth of the medial longitudinal fascicle in both mutants could be associated with the lack of RS/RF in the rhombencephalon and spinal cord. The neurites of the posterior commissure follow the aberrant position of the SCO in oep mutants. Our results suggest that both the RS of the ventral FP/flexural organ (FO) and the RS of the dorsal SCO have an influence on the outgrowth of axons and formation of commissures.
Descriptors: zebrafish, cell adhesion molecules, neuronal metabolism, central nervous system embryology, growth cones metabolism, nervous system malformations genetics, subcommissural organ metabolism, cell adhesion molecules, neuronal secretion, cell communication genetics, cell differentiation genetics, central nervous system cytology and metabolism, cues, functional laterality genetics, growth cones ultrastructure, growth substances metabolism, immunohistochemistry, mutation genetics, neural pathways embryology, rhombencephalon embryology, spinal cord embryology, subcommissural organ secretion.

Lemmens, R., A. Van Hoecke, N. Hersmus, V. Geelen, I. D'Hollander, V. Thijs, L. Van Den Bosch, P. Carmeliet, and W. Robberecht (2007). **Overexpression of mutantsuperoxide dismutase 1 causes a motor axonopathy in the zebrafish.** *Human Molecular Genetics* 16(19): 2359-65. ISSN: 0964-6906.
NAL Call Number: RB155.5.H87
Abstract: The development of small animal models is of major interest to unravel the pathogenesis and treatment of neurodegenerative diseases, especially because of their potential in large-scale chemical and genetic screening. We have investigated the zebrafish as a model to study amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disorder characterized by the selective loss of motor neurons, caused by mutations in superoxide dismutase 1 (SOD1) in a subset of patients. Overexpression of mutant human SOD1 in zebrafish embryos induced a motor axonopathy that was specific, dose-dependent and found for all mutations studied. Moreover, using this newly established animal model for ALS, we investigated the role of a known modifier in the disease: vascular endothelial growth factor (VEGF). Lowering VEGF induced a more severe phenotype, whereas upregulating VEGF rescued the mutant SOD1 axonopathy. This novel zebrafish model underscores the potential of VEGF

for the treatment of ALS and furthermore will permit large-scale genetic and chemical screening to facilitate the identification of new therapeutic targets in motor neuron disease.
Descriptors: mutant zebrafish, overexpression, superoxide dismutase 1, amyotrophic lateral sclerosis, ALS, motor axonopathy.

Lennard Richard, M.L., E. Bengten, M.R. Wilson, N.W. Miller, G.W. Warr, and J. Hikima (2007). **Comparative genomics of transcription factors driving expression of the immunoglobulin heavy chain locus in teleost fish**. *Journal of Fish Biology* 71(Sb): 153-173. ISSN: 0022-1112.
NAL Call Number: QL614.J68
Descriptors: zebrafish, DNA, enhancers, evolutionary conservation, freshwater fish, genetic diversity, genomes, genomic analysis, immunoglobulins, ligands, nucleotide sequence, Oct-2 protein, phylogeny, population genetics, transcription factors, *Danio rerio*, Teleostei.

Lesaffre, B., A. Joliot, A. Prochiantz, and M. Volovitch (2007). **Direct non-cell autonomous Pax6 activity regulates eye development in the zebrafish.** *Neural Development* 2: 2. ISSN: 1749-8104.
Abstract: ABSTRACT: BACKGROUND: Modifications in Pax6 homeogene expression produce strong eye phenotypes. This suggested to us that eye development might be an appropriate model to verify if homeoprotein intercellular passage has important functions in early development. Similar to other homeoproteins, Pax6 has two domains that enable secretion and internalization by live cells and, thus, intercellular passage. In principle, a straightforward way to test the hypothesis would be to mutate one of the two sequences to produce a 'cell autonomous only' Pax6. However, this was not possible because these sequences are in the homeodomain and their modification would affect Pax6 transcriptional properties. We have thus developed an approach aimed at blocking Pax6 only in the extracellular milieu of developing zebrafish embryos. RESULTS: A first strategy was to inject a one-cell embryo with a mRNA encoding a secreted single-chain anti-Pax6 antibody. A second, complementary, strategy was to inject a Pax6 antibody in the blastula extracellular milieu. In both cases, 'dissymmetric eyes', 'one eye only' and 'no eye' phenotypes were produced. In most cases, lens phenotypes paralleled retina malformations. Although eye phenotypes were analyzed 30 hours post-fertilization, there was a strong correlation between early eye field asymmetry, early asymmetry in Pax6 expression and later-occurring eye malformations. Several controls were introduced, demonstrating that the effect is specific to Pax6 and cannot be explained by intracellular antibody activities. CONCLUSION: This study supports the hypothesis that the Pax6 transcription factor is also a signaling molecule with direct non-cell autonomous activity.
Descriptors: zebrafish, Pax6 homeogene, eye phenotypes, mRNA, transcription factor.

Leskow, F.C., B.A. Holloway, H. Wang, M.C. Mullins, and M.G. Kazanietz (2006). **The zebrafish homologue of mammalian chimerin Rac-GAPs is implicated in epiboly progression during development.** *Proceedings of the National Academy of Sciences of the United States of America* 103(14): 5373-8. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: In this paper, we report an in vivo model for the chimerins, a family of Rac GTPase-activating proteins (Rac-GAPs) that are uniquely regulated by the lipid second messenger diacylglycerol and have been implicated in the control of actin dynamics, migration, and proliferation. We cloned the zebrafish homologue of mammalian alpha2-chimerin (chn1) and determined that it possesses Rac-GAP activity and a C1 domain with phorbol

ester/diacylglycerol-binding capability. chn1 morpholino knockdown embryos exhibit severe abnormalities, including the development of round somites, lack of yolk extension, and a kinked posterior notochord. These zebrafish morphants show Rac hyperactivation and progress faster through epiboly, leading to tailbud-stage embryos that have a narrow axis and an enlarged tailbud with expanded bmp4 and shh expression. Phenotypic rescue was achieved by mRNA microinjection of chn1 or an active chimerin Rac-GAP domain into the yolk syncytial layer but not by a chn1 mutant deficient in Rac-GAP activity, suggesting that the lack of chn1 Rac-GAP activity in the yolk syncytial layer was causative of the misbalance in morphogenetic movements. Our results reveal a crucial role for chn1 in early development and implicate Rac as a key regulator of morphogenetic movements during zebrafish epiboly.
Descriptors: zebrafish, cell division, chimerin proteins chemistry and genetics, chimerin, base sequence, COS cells, *Cercopithecus aethiops*, molecular cloning, DNA primers, in situ hybridization, molecular sequence data, site directed mutagenesis.

Leung, A.Y., J.C. Leung, L.Y. Chan, E.S. Ma, T.T. Kwan, K.N. Lai, A. Meng, and R. Liang (2005). **Proliferating cell nuclear antigen (PCNA) as a proliferative marker during embryonic and adult zebrafish hematopoiesis.** *Histochemistry and Cell Biology* 124(2): 105-11 . ISSN: 0948-6143.
NAL Call Number: QH613.H57
Abstract: We investigated the expression of proliferative cell nuclear antigen (PCNA) in zebrafish to delineate the proliferative hematopoietic component during adult and embryonic hematopoiesis. Immunostaining for PCNA and enhanced green fluorescence protein (eGFP) was performed in wild-type and fli1-eGFP (endothelial marker) and gata1-eGFP (erythroid cell marker) transgenic fish. Expression of PCNA mRNA was examined in wild-type and chordin morphant embryos. In adult zebrafish kidney, the renal tubules are surrounded by endothelial cells and it is separated into hematopoietic and excretory compartments. PCNA was expressed in hematopoietic progenitor cells but not in mature neutrophils, eosinophils or erythroid cells. Some PCNA+ cells are scattered in the hematopoietic compartment of the kidney while others are closely associated with renal tubular cells. PCNA was also expressed in spermatogonial stem cells and intestine crypts, consistent with its role in cell proliferation and DNA synthesis. In embryos, PCNA is expressed in the brain, spinal cord and intermediate cell mass (ICM) at 24 h-post fertilization. In chordin morphants, PCNA is significantly upregulated in the expanded ICM. Therefore, PCNA can be used to mark cell proliferation in zebrafish hematopoietic tissues and to identify a population of progenitor cells whose significance would have to be further investigated.
Descriptors: zebrafish, cytology, hematopoiesis, extramedullary physiology, kidney cytology, proliferating cell nuclear antigen metabolism, biological markers metabolism, cell proliferation, green fluorescent proteins metabolism, hematopoietic stem cells cytology and metabolism, immunoenzyme techniques, in situ hybridization, kidney metabolism, proliferating cell nuclear antigen genetics, messenger RNA metabolism.

Leung, Y.F., P. Ma, and J.E. Dowling (2007). **Gene expression profiling of zebrafish embryonic retinal pigment epithelium in vivo.** *Investigative Ophthalmology and Visual Science* 48(2): 881-90. ISSN: 0146-0404.
Abstract: PURPOSE: Eye development and photoreceptor maintenance is dependent on the retinal pigment epithelium (RPE), a thin layer of cells that underlies the neural retina. Despite its importance, development of RPE has not been studied by a genomic approach. In this study, a microarray expression-profiling methodology was established for studying RPE development. METHODS: The intact retina with RPE attached was dissected from devel-

oping embryos, and differentially expressed genes in RPE were inferred by comparing the dissected tissues with retinas without RPE, in microarray and statistical analyses. RESULTS: Of the probesets used, 8810 were significantly expressed in RPE at 52 hours postfertilization (hpf), of which 1443 may have biologically meaningful expression levels. Further, 78 and 988 probesets were found to be significantly over- or underexpressed in RPE, respectively, compared with retina. Also, 79.2% (38/48) of the known overexpressed probesets were independently validated as RPE-related transcripts. CONCLUSIONS: The results strongly suggest that this methodology can obtain in vivo RPE-specific gene expression from the zebrafish embryos and identify novel RPE markers.

Descriptors: zebrafish, metabolism, developmental gene expression regulation physiology, pigment epithelium of eye embryology, eye gene expression profiling methods, oligonucleotide array sequence analysis methods, RNA isolation and purification, retina embryology.

Levin, E.D., R.H. Ali, S.D.L. Upehurch, and E. Linney (2004). **Morpholino modeling of chlorpyrifos-induced learning deficits in zebrafish.** *Birth Defects Research* 70(5): 340. ISSN: 1542 0752.

NAL Call Number: QL991.T4

Descriptors: zebrafish, development, nervous system, pharmacology, toxicology, zebrafish .

Lewis, J. (2003). **Autoinhibition with transcriptional delay: a simple mechanism for the zebrafish somitogenesis oscillator.** *Current Biology* 13(16): 1398-408. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: BACKGROUND: The pattern of somites is traced out by a mechanism involving oscillating gene expression at the tail end of the embryo. In zebrafish, two linked oscillating genes, her1 and her7, coding for inhibitory gene regulatory proteins, are especially implicated in genesis of the oscillations, while Notch signaling appears necessary for synchronization of adjacent cells. RESULTS: I show by mathematical simulation that direct autorepression of her1 and her7 by their own protein products provides a mechanism for the intracellular oscillator. This mechanism operates robustly even when one allows for the fact that gene regulation is an essentially noisy (stochastic) process. The predicted period is close to the observed period (30 min) and is dictated primarily by the transcriptional delay, the time taken to make an mRNA molecule. Through its coupling to her1/her7 expression, Notch signaling can keep the rapid oscillations in adjacent cells synchronized. When the coupling parameters are varied, however, the model system can switch to oscillations of a much longer period, resembling that of the mouse or chick somitogenesis oscillator and governed by the delays in the Notch pathway. Such Notch-mediated synchronous oscillations are predicted even in the absence of direct her1/her7 autoregulation, through operation of the standard Notch signaling pathway that is usually assumed simply to give lateral inhibition. CONCLUSIONS: Direct autorepression of a gene by its own product can generate oscillations, with a period determined by the transcriptional and translational delays. Simple as they are, such systems show surprising behaviors. To understand them, unaided intuition is not enough: we need mathematics.

Descriptors: zebrafish, transcription factors genetics, chick embryo, feedback, developmental gene expression regulation, in situ hybridization, membrane proteins genetics and metabolism, mesoderm metabolism, mice, mutation, periodicity, Notch receptors, signal transduction, somites metabolism, species specificity, transcription factors metabolism, genetic transcription, metabolism.

Lewis, R.S. and A.C. Ward (2004). **Conservation, duplication and divergence of the zebrafish stat5 genes.** *Gene* 338(1): 65-74. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: There are seven mammalian signal transducer and activator of transcription (Stat) proteins that act downstream of cytokine and growth factor receptors to mediate rapid changes in gene expression. The mammalian Stat5a and Stat5b genes show high sequence identity and lie adjacent in a head-to-head configuration next to the Stat3 gene, apparently the result of a relatively recent mammal-specific gene duplication event. We have identified and characterized two stat5 homologues that are expressed in zebrafish, named stat5.1 and stat5.2. The stat5.1 gene shows a high level of conservation with the single stat5 gene found in other teleosts and lies next to the stat3 gene, in the same relative orientation as the mammalian Stat5b gene. In contrast, the stat5.2 gene lies on a different chromosome to stat5.1 and stat3, and has diverged from the stat5 genes of other teleosts, with no apparent orthologue. Together, these data suggest that the ancestral Stat5 gene has undergone two independent gene duplication events to generate a stat5.2 paralogue in zebrafish and a Stat5a paralogue in mammals.
Descriptors: zebrafish, DNA binding proteins genetics, gene duplication, milk trans activators genetics, variation genetics, amino acid sequence, conserved sequence genetics, DNA chemistry, DNA genetics, DNA isolation and purification, complementary DNA chemistry, complementary DNA genetics, isolation and purification, exons, introns, gene order , molecular sequence data, phylogeny, radiation hybrid mapping, STAT5 transcription factor, sequence alignment, DNA sequence analysis, amino acid sequence homology, *Takifugu* genetics.

Li, L., H. Katsuyama, N. Do, M. Saito, H. Tanii, and K. Saijoh (2007). **Abundant expression of nucleosome assembly protein 1 (NAP1) gene in goldfishscale with lateral Line**. *Journal of Toxicological Sciences* 32(4): 359-365. ISSN: 0388-1350.
NAL Call Number: RA1190
Descriptors: zebrafish, blast, environmental factors, freshwater fish, gene expression, gills, homology, lateral line, Nap1 gene, nucleosomes, phylogenetics, pollutants, pollution monitoring, Scales, toxicants, toxicity tests, *Carassius auratus*, *Danio rerio*, goldfish.

Li, P., S. Temple, Y. Gao, T.J. Haimberger, C.W. Hawryshyn, and L. Li (2005). **Circadian rhythms of behavioral cone sensitivity and long wavelength opsin mRNA expression: a correlation study in zebrafish.** *Journal of Experimental Biology* 208(Pt 3): 497-504. ISSN: 0022-0949.
NAL Call Number: 442.8B77
Abstract: Using a behavioral assay based on visually mediated escape responses, we measured long-wavelength-sensitive red cone (LC) sensitivities in zebrafish. In a 24 h period, the zebrafish were least sensitive to red light in the early morning and most sensitive in the late afternoon. To investigate if the fluctuation of behavioral cone sensitivity correlates with opsin gene expression, we measured LC opsin mRNA expression at different times in the day and night under different lighting conditions. Under a normal light-dark cycle, the expression of LC opsin mRNA determined by real-time RT-PCR was low in the early morning and high in the late afternoon, similar to the fluctuation of behavioral cone sensitivity. This rhythm of LC opsin mRNA expression, however, dampened out gradually in constant conditions. After 24 h of constant light (LL), the expression of LC opsin mRNA dropped to levels similar to those determined in the early morning in control animals. By contrast, when the zebrafish were kept in constant dark (DD), the expression of LC opsin mRNA increased, to levels about

30-fold higher than the expression in the early morning in control animals. This day-night fluctuation in LC opsin mRNA expression was correlated to changes in opsin density in the outer segment of cone photoreceptor cells. Microspectrophotometry (MSP) measurements found significant differences in red cone outer segment optical density with a rhythm following the behavioral sensitivity. Furthermore, dopamine modulated the circadian rhythms in expression of LC opsin mRNA. Administration of dopamine increased LC opsin mRNA expression, but only in the early morning.
Descriptors: zebrafish, circadian rhythm physiology, retina cones physiology, opsin biosynthesis, behavior physiology, circadian rhythm drug effects, retina cones drug effects and metabolism, dopamine pharmacology, gene expression regulation physiology, light, messenger RNA metabolism, vision physiology.

Li, Y.C., H.W. Yeh, J.H. Lu, and J.K. Lu (2005). **The impacts of tbx5 gene expression in the heart development of zebrafish (*Danio rerio*)**. *Journal of the Fisheries Society of Taiwan* 32(1): 15. ISSN: 0379-4180.
NAL Call Number: SH135.T3
Descriptors: zebrafish embryos, biological development, developmental stages, embryonic development, gene expression, genetics, heart, mutations, transcription, *Danio rerio.*

Li, Y.U., J. Xiang, and C. Duan (2005). **Insulin-like growth factor-binding protein-3 plays an important role in regulating pharyngeal skeleton and inner ear formation and differentiation**. *Journal of Biological Chemistry* 280(5): 3613-3620. ISSN: 0021-9258.
NAL Call Number: 381J824
Descriptors: zebrafish, auditory organs, cartilage, cell differentiation, defects, developmental stages, embryonic development, gene products, growth, morphogenesis, *Danio rerio*.

Li Yang, M. Chia Jer, R. Bartfai, A. Christoffels, Yue GenHua, Ding Ke, Y. Ho Mei, J.A. Hill, E. Stupka, and L. Orban (2004). **Comparative analysis of the testis and ovary transcriptomes in zebrafish by combining experimental and computational tools**. *Comparative and Functional Genomics* 5(5): 403-418. ISSN: 1531 6912. DOI: 10.1002/cfg.418.
NAL Call Number: QH447
Abstract: Studies on the zebrafish model have contributed to our understanding of several important developmental processes, especially those that can be easily studied in the embryo. However, our knowledge on late events such as gonad differentiation in the zebrafish is still limited. Here we provide an analysis on the gene sets expressed in the adult zebrafish testis and ovary in an attempt to identify genes with potential role in (zebra)fish gonad development and function. We produced 10 533 expressed sequence tags (ESTs) from zebrafish testis or ovary and downloaded an additional 23 642 gonad-derived sequences from the zebrafish EST database. We clustered these sequences together with over 13 000 kidney-derived zebrafish ESTs to study partial transcriptomes for these three organs. We searched for genes with gonad-specific expression by screening macroarrays containing at least 2600 unique cDNA inserts with testis-, ovary- and kidney-derived cDNA probes. Clones hybridizing to only one of the two gonad probes were selected, and subsequently screened with computational tools to identify 72 genes with potentially testis-specific and 97 genes with potentially ovary-specific expression, respectively. PCR-amplification confirmed gonad-specificity for 21 of the 45 clones tested (all without known function). Our study, which involves over 47 000 EST sequences and specialized cDNA arrays, is the first analysis of adult organ transcriptomes of zebrafish at such a scale. The study of genes expressed in adult zebrafish testis and ovary will provide useful information on regulation of gene expression in teleost gonads and

might also contribute to our understanding of the development and differentiation of reproductive organs in vertebrates. Copyright © 2008 CABI
Descriptors: zebrafish embryos, complementary DNA, gene expression, genes, gonads, ovaries, testes, sex differences, sex differentiation, *Danio rerio.*

Lien, C.L., M. Schebesta, S. Makino, G.J. Weber, and M.T. Keating (2006). **Gene expression analysis of zebrafish heart regeneration.** *Public Library of Science Biology* 4(8): E260. ISSN: 1544-9173.
NAL Call Number: QH301.P56
Abstract: Mammalian hearts cannot regenerate. In contrast, zebrafish hearts regenerate even when up to 20% of the ventricle is amputated. The mechanism of zebrafish heart regeneration is not understood. To systematically characterize this process at the molecular level, we generated transcriptional profiles of zebrafish cardiac regeneration by microarray analyses. Distinct gene clusters were identified based on temporal expression patterns. Genes coding for wound response/inflammatory factors, secreted molecules, and matrix metalloproteinases are expressed in regenerating heart in sequential patterns. Comparisons of gene expression profiles between heart and fin regeneration revealed a set of regeneration core molecules as well as tissue-specific factors. The expression patterns of several secreted molecules around the wound suggest that they play important roles in heart regeneration. We found that both platelet-derived growth factor-a and -b (pdgf-a and pdgf-b) are upregulated in regenerating zebrafish hearts. PDGF-B homodimers induce DNA synthesis in adult zebrafish cardiomyocytes. In addition, we demonstrate that a chemical inhibitor of PDGF receptor decreases DNA synthesis of cardiomyocytes both in vitro and in vivo during regeneration. Our data indicate that zebrafish heart regeneration is associated with sequentially upregulated wound healing genes and growth factors and suggest that PDGF signaling is required.
Descriptors: zebrafish, heart physiology, regeneration genetics, cell proliferation, gene expression profiling, SIS genes, inflammation mediators metabolism, intercellular signaling peptides and proteins metabolism, matrix metalloproteinases metabolism, molecular sequence data, cardiac myocytes cytology and metabolism, oligonucleotide array sequence analysis, platelet derived growth factor genetics and metabolism, signal transduction, up regulation.

Liepinsh, E., R. Barbals, E. Dahl, A. Sharipo, E. Staub, and G. Otting (2003). **The death-domain fold of The ASC PYRIN domain, presenting a basis for PYRIN/PYRIN recognition**. *Journal of Molecular Biology* 332(5): 1155-1163. ISSN: 0022-2836.
NAL Call Number: 442.8 J8224
Descriptors: zebrafish, amino acid sequence, DNA, freshwater fish, molecular biology, nucleotide sequence, phylogeny, proteins, *Danio rerio*, physiology, biochemistry, biophysics.

Lieschke, G.J. and P.D. Currie (2007). **Animal models of human disease: zebrafish swim into view.** *Nature Reviews. Genetics* 8(5): 353-67. ISSN: 1471-0056.
NAL Call Number: QH426
Abstract: Despite the pre-eminence of the mouse in modelling human disease, several aspects of murine biology limit its routine use in large-scale genetic and therapeutic screening. Many researchers who are interested in an embryologically and genetically tractable disease model have now turned to zebrafish. Zebrafish biology allows ready access to all developmental stages, and the optical clarity of embryos and larvae allow real-time imaging of developing pathologies. Sophisticated mutagenesis and screening strategies on a large scale, and with an economy that is not possible in other vertebrate systems, have generated

zebrafish models of a wide variety of human diseases. This Review surveys the achievements and potential of zebrafish for modelling human diseases and for drug discovery and development.

Descriptors: zebrafish, disease models, genetically modified, drug design, preclinical drug evaluation, genetic inborn diseases genetics, genetic inborn diseases pathology, biological models.

Notes: A review.

Lin, C.Y., Y.H. Chen, H.C. Lee, and H.J. Tsai (2004). **Novel cis-element in intron 1 represses somite expression of zebrafish myf-5.** *Gene* 334: 63-72. ISSN: 0378-1119.

NAL Call Number: QH442.A1G4

Abstract: Myf-5 is a basic helix-loop-helix (bHLH) transcription factor that controls muscle differentiation. During early embryogenesis, myf-5 expression is transient, somite- and stage-specific. However, the negative regulation of myf-5 is poorly understood. We constructed a plasmid [(-9977/-1)/E1/I1/E2/GFP] that contains the sequence -9977 to -1, exon 1 (E1), intron 1 (I1), and exon 2 (E2) of zebrafish (*Danio rerio*) myf-5 and a reporter GFP gene. This plasmid was microinjected into zebrafish zygotes. Surprisingly, the somite-specific expression rate of reporter GFP in the transgenic embryos was extremely low (2%, n=392), compared to that of (-9977/-1)/GFP (92%, n=210). Dramatic repression of myf-5 expression was also observed in embryos microinjected with plasmids in which the sequence -8600/-1, -2937/-1 or -290/-1 was linked to E1/I1/E2/GFP. Thus, intron 1 contains a silencer that specifically represses the activity of myf-5. Functional analysis of intron 1 showed a strong, negative, cis-regulatory element was located at +502/+835. Its function was orientation- and position-dependent. The repressive capability of this silencer was completely dependent on two core motifs, IE1 (+502/+527) and IE2 (+816/+835), and a 156-bp spanning sequence that lies between them. This is the first study to identify a novel, cis-acting silencer in intron 1 that is crucial to negatively regulating zebrafish myf-5 expression.

Descriptors: zebrafish, enhancer elements genetics, introns genetics, somites metabolism, embryonic development, developmental gene expression regulation, green fluorescent proteins, in situ hybridization, luminescent proteins genetics and metabolism, microinjections, fluorescence microscopy, plasmids administration, dosage and genetics, recombinant fusion proteins genetics and metabolism.

Lin, F., D.S. Sepich, S. Chen, J. Topczewski, C. Yin, L. Solnica Krezel, and H. Hamm (2005). **Essential roles of G{alpha}12/13 signaling in distinct cell behaviors driving zebrafish convergence and extension gastrulation movements.** *Journal of Cell Biology* 169(5): 777-87. ISSN: 0021-9525.

NAL Call Number: 442.8J828

Abstract: Galpha(12/13) have been implicated in numerous cellular processes, however, their roles in vertebrate gastrulation are largely unknown. Here, we show that during zebrafish gastrulation, suppression of both Galpha(12) and Galpha(13) signaling by overexpressing dominant negative proteins and application of antisense morpholino-modified oligonucleotide translation interference disrupted convergence and extension without changing embryonic patterning. Analyses of mesodermal cell behaviors revealed that Galpha(12/13) are required for cell elongation and efficient dorsalward migration during convergence independent of noncanonical Wnt signaling. Furthermore, Galpha(12/13) function cell-autonomously to mediate mediolateral cell elongation underlying intercalation during notochord extension, likely acting in parallel to noncanonical Wnt signaling. These findings provide the first evidence that Galpha(12) and Galpha(13) have overlapping and

essential roles in distinct cell behaviors that drive vertebrate gastrulation.
Descriptors: zebrafish embryos, embryonic development physiology, GTP binding protein alpha subunits, G12 G13 metabolism, gastrula metabolism, mesoderm metabolism, body patterning and cell communication physiology, cell line, cell movement physiology, cell shape physiology, GTP binding protein alpha subunits, G12 G13 genetics, gastrula cytology, developmental gene expression regulation physiology, intercellular signaling peptides and proteins metabolism, mesoderm cytology, molecular sequence data, G protein coupled receptors genetics and metabolism, signal transduction physiology, Wnt proteins.

Lin, F., D.S. Sepich, S. Chen, L. Solnica-Krezel, and H.E. Hamm (2006). **Ga12/13 signaling regulates epiboly by inhibiting e-eadherin function**. *Federation of American Societies for Experimental Biology Journal* 20(4, Part 1): A544. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: Epiboly is the first morphogenetic movement of the zebrafish embryo, which spreads and thins the blastoderm to completely enclose the yolk cell. Normal epiboly coordinates movements of the deep cells, the enveloping layer, and the yolk syncytial layer. E-cadherin has been shown to be vital for this epibolic movement, as embryos harboring mutations in E-cadherin gene or injected with E-cadherin MOs displayed severe epiboly delay or arrest. However, how the function of E-cadherin is regulated remains unknown. Here we show that epiboly is impaired in embryos with excessive or reduced G alpha(12/13) expression. In particular, deep cells migrated slower towards the vegetal pole, while epibolic movement of enveloping layer, dorsal forerunner cells and yolk syncytial nuclei progressed normally. Similar phenotypic changes were found in E-cadherin mutant embryos, suggesting a possible linkage between Ga-12/13 and E-cadherin signaling pathways. Indeed, suppression of G alpha(12) or G alpha(13) partially rescued, while increased G alpha(13) signaling exacerbated epiboly defects of the E-cadherin mutants. Moreover, immunoprecipitation studies indicated that G alpha(13) formed a complex with the C-terminus of E-cadherin. These results thus provide the first in vivo evidence that G alpha(12/13) signaling may affect epiboly probably by negatively regulating E-cadherin functions. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, molecular genetics, development, immunoprecipitation, immunologic techniques, epiboly.

Lin, T.Y., Y.C. Lin, N.C. Hwang, C.D. Hsiao, and P.P. Hwang (2005). **Whole genome identification of ATP6 gene family in zebrafish**. *Journal of the Fisheries Society of Taiwan* 32(1): 103. ISSN: 0379-4180.
NAL Call Number: SH135.T3
Descriptors: zebrafish, ATP6 gene, bioinformatics, carbonic anhydrase, databases, digestion, erythrocytes, genomes, gills, histochemistry, homeostasis, immunohistochemistry, ion exchange, kidney, Na super(+), H super(+)-exchanging ATPase, pharynx, phylogenetics, PCR, sodium channels, *Danio rerio.*

Lin, W.H., C.H. Wu, Y.C. Chen, and W.Y. Chow (2006). **Embryonic expression of zebrafish AMPA receptor genes: zygotic gria2alpha expression initiates at the midblastula transition.** *Brain Research* 1110(1): 46-54. ISSN: 0006 8993.
NAL Call Number: QP376
Abstract: The AMPA-preferring receptors (AMPARs) mediate rapid excitatory synaptic transmission in the central nervous system of vertebrates. Expression profiles of 8 AMPAR genes were studied by RT-PCR analyses to elucidate the properties of AMPARs during early

zebrafish development. Transcripts of all AMPAR genes are detected at the time of fertilization, suggesting maternal transcriptions of zebrafish AMPAR genes. The amounts of gria1 and gria2 transcripts are several-fold higher than that of gria3 and gria4 between 10 and 72 hpf (hour postfertilization). The edited gria2alpha transcript decreases during gastrulation period, suggesting that zygotic expression of gria2alpha begins around the time of midblastula transition. Relative to the amount of beta-actin, the amounts of AMPAR transcripts increase significantly after the completion of neurulation. The amounts of gria2 transcripts exceed the total amounts of the remaining AMPAR transcripts after 36 hpf, suggesting increases in the representation of low Ca2+ permeable AMPARs during neuronal maturation. Many but not all of the known mammalian protein-protein interaction motifs are preserved in the C-terminal domains (CTD) of zebrafish AMPARs. Before 16 hpf, the embryos express predominantly the alternative splice forms encoding longer CTD. Representations of the short CTD splice forms of gria2 and gria4alpha increase after 24 hpf, when neurulation is nearly completed.

Descriptors: zebrafish, blastula physiology , developmental gene expression regulation physiology, AMPA receptors metabolism RNA editing physiology, messenger RNA metabolism, AMPA receptors genetics, reverse transcriptase PCR methods, protein sequence analysis.

Lin, W., N. Jing, M.A. Basson, A. Dierich, J. Licht, and S.L. Ang (2005). **Synergistic activity of Sef and Sprouty proteins in regulating the expression of Gbx2 in the mid-hindbrain region**. *Genesis* 41(3): 110-115. ISSN: 1526 954X.

NAL Call Number: QH426.D32

Abstract: Sef and Sprouty proteins function as feedback antagonists of fibroblast growth factor (Fgf) signaling in zebrafish embryos. To study the role of Sef in mice, we generated Sef homozygous mutant animals. These animals are viable and show normal expression of mid-hindbrain genes at embryonic days 8.5 and 9.5. To investigate the possibility of functional synergism between Sef and Sprouty proteins, we electroporated Sprouty2(Y55A), which functions in a dominant-negative manner in tissue culture cells into the mid-hindbrain region of wildtype and Sef mutant embryos. The expression pattern of Gbx2, a downstream target of Fgf signaling, was expanded or shifted in electroporated embryos, and this effect was significantly enhanced in the Sef mutant background. Altogether, our results demonstrate that Sef and Sproutys function synergistically to regulate Gbx2 expression in the anterior hindbrain. genesis. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, nervous system, neural coordination, molecular genetics, electroporation , genetic techniques, tissue culture, culturing techniques.

Lin, Y.C., L.C. Hsieh, M.W. Kuo, J. Yu, H.H. Kuo, W.L. Lo, R.J. Lin, A.L. Yu, and W.H. Li (2007). **Human TRIM71 and Its nematode homologue are targets of let-7 microRNA and Its zebrafish orthologue Is essential for development.** *Molecular Biology and Evolution* 24(11): 2525-34. ISSN: 0707-4038.

NAL Call Number: QH506.M642

Abstract: Animal microRNAs (miRNAs) are short RNAs that function as posttranscriptional regulators of gene expression by binding to the target mRNAs. Noting that some miRNAs are highly conserved in evolution, we explored the possibility of evolutionary conservation of their targets. We identified human orthologues of experimentally verified let-7 miRNA target genes in Caenorhabditis elegans and used the luciferase reporter system to examine whether these human genes are still the targets of let-7 miRNA. We found that in some cases, the miRNA-target relationship has indeed been conserved in human. Interestingly, human TRIM71, an orthologue of C. elegans let-7-target lin-41 gene, can be repressed

by hsa-let-7a and hsa-let-7c. This repression was abolished when both predicted let-7 target sites of TRIM71 were mutated. Moreover, the zebrafish lin-41 orthologue was also repressed by let-7 to a similar degree as was TRIM71. When the expression of zebrafish lin-41 orthologue was silenced by microinjection of RNA interference or morpholino into zebrafish zygotes, retarded embryonic development was observed, providing direct evidence for an essential role of lin-41 in zebrafish development. Taken together, our results suggest that the regulation of TRIM71 expression by let-7 has been evolutionarily conserved and that TRIM71 likely plays an important role in development.
Descriptors: zebrafish, human TRIM71, nematode, homologue, let-7 microRNA, orthologue, development.

Link, B.A., M.P. Gray, R.S. Smith, and S.W. John (2004). **Intraocular pressure in zebrafish: comparison of inbred strains and identification of a reduced melanin mutant with raised IOP.** *Investigative Ophthalmology and Visual Science* 45(12): 4415-22. ISSN: 0146-0404.
Abstract: PURPOSE: The goal was to establish an accurate method for measuring intraocular pressure (IOP) in living adult zebrafish and to determine the average IOPs of genetically different strains and mutants. The overall purpose was to develop the zebrafish system for studies of IOP and glaucoma. Elevated IOP and anterior segment dysgenesis are two known risk factors for glaucoma. Because albino mammals have increased IOP and are prone to anterior segment anomalies, the study was focused on a reduced melanin zebrafish mutant, brass. METHODS: Servo-null electrophysiology was used to measure IOP in anesthetized adult zebrafish. A pulled-glass microelectrode was used to penetrate the cornea into the anterior chamber, and pressure transduction was recorded. IOP, histology, and visual behavioral assays were used to assess the brass mutant. RESULTS: Significant differences in IOP were detected between genetically distinct zebrafish strains. IOP averages were highest in the long fin (LF) strain (20.5 +/- 1.2 mm Hg) and lowest in the Oregon AB (AB) strain (10.8 +/- 0.3 mm Hg). In brass mutants, which show a reduced melanin phenotype, IOPs were elevated and mild iris hypoplasia was noted. No defects were observed within the retina or in visual function. CONCLUSIONS: In the present study, an accurate method was developed for measuring IOP in adult zebrafish. Both the ability to measure IOP in the genetically powerful zebrafish model and the conservation of elevated IOP and anterior segment anomalies between albino mammals and hypopigmented fish are important. They strongly support the use of zebrafish for studying the complex genetics of elevated IOP and glaucoma.
Descriptors: zebrafish, intraocular pressure, melanins deficiency, mutation, inbred strains, electrophysiology instrumentation, equipment design, microelectrodes, phenotype, species specificity.

Linney, E. and A.J. Udvadia (2004). **Construction and detection of fluorescent, germline transgenic zebrafish.** *Methods in Molecular Biology* 254: 271-88. ISSN: 1064-3745.
Descriptors: transgenic zebrafish, genetically modified, luminescent fluorescence, reporter genes, green fluorescent proteins, luminescent proteins metabolism, transgenes.

Liu, B., R. Joel Duff, R.L. Londraville, J.A. Marrs, and Q. Liu (2007). **Cloning and expression analysis of cadherin7 in the central nervous system of the embryonic zebrafish.** *Gene Expression Patterns* 7(1-2): 15-22. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Cadherin cell adhesion molecules exhibit unique expression patterns during development of the vertebrate central nervous system. In this study, we obtained a full-length cDNA of a novel zebrafish cadherin using reverse transcriptase-polymerase chain

reaction (RT-PCR) and 5' and 3' rapid amplification of cDNA ends (RACE). The deduced amino acid sequence of this molecule is most similar to the published amino acid sequences of chicken and mammalian cadherin7 (Cdh7), a member of the type II cadherin subfamily. cadherin7 message (cdh7) expression in embryonic zebrafish was studied using in situ hybridization and RT-PCR methods. cdh7 expression begins at about 12h postfertilization (hpf) in a small patch in the anterior neural keel, and along the midline of the posterior neural keel. By 24 hpf, cdh7 expression in the brain shows a distinct segmental pattern that reflects the neuromeric organization of the brain, while its expression domain in the spinal cord is continuous, but confined to the middle region of the spinal cord. As development proceeds, cdh7 expression is detected in more regions of the brain, including the major visual structures in the fore- and midbrains, while its expression domain in the hindbrain becomes more restricted, and its expression in the spinal cord becomes undetectable. cdh7 expression becomes reduced in 3-day old embryos. Our results show that cdh7 expression in the zebrafish developing central nervous system is both spatially and temporally regulated.
Descriptors: zebrafish, cadherins genetics, central nervous system embryology, central nervous system metabolism, amino acid and base sequence, molecular cloning, complementary DNA genetics, developmental gene expression regulation, in situ hybridization, molecular sequence data, reverse transcriptase PCR , amino acid sequence homology.

Liu, J., S.D. Fraser, P.W. Faloon, E.L. Rollins, J. Vom Berg, O. Starovic Subota, A.L. Laliberte, J.N. Chen, F.C. Serluca, and S.J. Childs (2007). **A betaPix Pak2a signaling pathway regulates cerebral vascular stability in zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 104(35): 13990-5. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: The vasculature tailors to the needs of different tissues and organs. Molecular, structural, and functional specializations are observed in different vascular beds, but few genetic models give insight into how these differences arise. We identify a unique cerebrovascular mutation in the zebrafish affecting the integrity of blood vessels supplying the brain. The zebrafish bubblehead (bbh) mutant exhibits hydrocephalus and severe cranial hemorrhage during early embryogenesis, whereas blood vessels in other regions of the embryo appear intact. Here we show that hemorrhages are associated with poor cerebral endothelial-mesenchymal contacts and an immature vascular pattern in the head. Positional cloning of bbh reveals a hypomorphic mutation in betaPix, a binding partner for the p21-activated kinase (Pak) and a guanine nucleotide exchange factor for Rac and Cdc42. betaPix is broadly expressed during embryonic development and is enriched in the brain and in large blood vessels. By knockdown of specific betaPix splice variants, we show that they play unique roles in embryonic vascular stabilization or hydrocephalus. Finally, we show that Pak2a signaling is downstream of betaPix. These data identify an essential in vivo role for betaPix and Pak2a during embryonic development and illuminate a previously unrecognized pathway specifically involved in cerebrovascular stabilization.
Descriptors: zebrafish, cell cycle cerebrovascular circulation physiology, guanine nucleotide exchange factors genetics, protein serine threonine kinases physiology, alternative splicing, blood vessels physiology, brain pathology, cerebral hemorrhage genetics, chromosome mapping, molecular cloning, vascular endothelium physiology, ethylnitrosourea, exons, guanine nucleotide exchange factors deficiency, molecular sequence data, variation genetics.

Liu, J., Y.H. Sun, N. Wang, Y.P. Wang, and Z.Y. Zhu (2006). **Upstream regulatory region of zebrafish lunatic fringe: isolation and promoter analysis.** *Marine Biotechnology* 8(4): 357-65. ISSN: 1436-2228.

NAL Call Number: TP248.27.M37M37

Abstract: Lunatic fringe (Lfng), one modulator of Notch signaling, plays an essential part in demarcation of tissues boundaries during animal early development, especially somitogenesis. To characterize the promoter of zebrafish lfng and generate somite-specific transgenic zebrafish, we isolated the upstream regulatory region of zebrafish lfng by blast search at the Ensembl genome database (http://www.ensembl.org) and analyzed the promoter activity using green fluorescent protein (GFP) as a reporter. Promoter activity assay in zebrafish shows that the 0.2-kb fragment containing GC-box, CAAT-box, and TATA-box can direct tissue-specific GFP expression, while the 0.4-kb and 1.2-kb fragments with further upstream sequence included drive GFP expression more efficiently. We produced lfngEGFP-transgenic founders showing somite-specific expression of GFP and consequently generated a hemizygous lfngEGFP-transgenic line. The eggs from lfngEGFP-transgenic female zebrafish show strong GFP expression, which is consistent to the reverse-transcription polymerase chain reaction PCR (RT-PCR) detection of lfng transcripts in the fertilized eggs. This reveals that zebrafish lfng is a maternal factor existing in matured eggs, suggesting that fish somitogenesis may be influenced by maternal factors.

Descriptors: zebrafish, glycosyltransferases genetics, promoter regions genetics, regulatory sequences, nucleic acid genetics, genetically modified, base sequence, Western blotting, molecular cloning, DNA primers chemistry, complementary DNA chemistry, developmental gene expression regulation physiology, gene transfer techniques, glycosyltransferases physiology, green fluorescent proteins analysis and biosynthesis, fluorescence microscopy , molecular sequence data, reverse transcriptase PCR, sequence alignment.

Liu Jing, H. Sun Yong, Wang Na, P. Wang Ya, and Z.Y. Zhu (2006). **Cloning, characterization and promoter analysis of common carp hairy/enhancer-of-split-related gene, her6**. *Journal of Genetics* 85(3): 171-178. ISSN: 0022 1333.

NAL Call Number: 442.8J823

Abstract: Some members of hairy/Enhancer-of-split-related gene (HES) family have important effects on axial mesoderm segmentation and the establishment and maintenance of the somite fringe. In fishes, the her6 gene, a member of the HES family, is the homologue of hes1 in mammals and chicken. In this study, the her6 gene and its full-length complementary DNA from the common carp (*Cyprinus carpio*) were isolated and characterized. The genomic sequence of common carp her6 is approximately 1.7 kb, with four exons and three introns, and the full-length cDNA of 1314 bp encodes a putative polypeptide of 271 amino acids. To analyse the promoter sequence of common carp her6, sequences of various lengths upstream from the transcription initiation site of her6 were fused to enhanced green fluorescent protein gene (eGFP) and introduced into zebrafish *(Danio rerio*) embryos by microinjection to generate transgenic embryos. Our results show that the upstream sequence of 500 bp can direct highly efficient and tissue-specific expression of eGFP in zebrafish embryos, whereas a fragment of 200 bp containing the TATA box and a partial suppressor of hairless paired site sequence (SPS) is not sufficient to drive eGFP expression in zebrafish embryos. Copyright © 2008 CABI

Descriptors: zebrafish, complementary DNA, DNA cloning, embryos, exons, introns, gene expression, genes, nucleotide sequences, PCR, promoters, TATA box, carp, *Danio rerio.*

Liu Lin and Ge Wei (2007). **Growth differentiation factor 9 and its spatiotemporal expression and regulation in the zebrafish ovary**. *Biology of Reproduction* 76(2): 294-302. ISSN: 0006 3363.

NAL Call Number: QD415.F4

Abstract: Growth differentiation factor 9 (GDF9) is a member of the transforming growth factor beta (TGFB) superfamily. As an oocyte-specific growth factor, GDF9 plays critical roles in controlling folliculogenesis in mammals. In the present study, we cloned a 2.1-kb cDNA of the zebrafish GDF9 homolog (Gdf9, gdf9), which shares ~60% homology with that of mammals in the mature region. RT-PCR analysis showed that zebrafish gdf9 expression was present only in the gonads and Northern blot analysis revealed a single transcript of about 2.0 kb in the ovary. Real-time RT-PCR analysis revealed that gdf9 expression was highest in primary growth (PG, stage I) follicles and gradually decreased during follicular development, with the lowest level being found in fully grown (FG) follicles. The expression of gdf9 was maintained through fertilization and early embryonic development until gastrulation, at which point the expression level dramatically decreased. Expression was barely detectable after the late gastrula stage. Within the follicle, gdf9 mRNA was localized exclusively in the oocytes, as demonstrated by RT-PCR of denuded oocytes and freshly isolated follicle layers as well as by in situ hybridization. Interestingly, when amplified for high numbers of cycles, the expression of gdf9 was detected in cultured zebrafish follicular cells that were free of oocytes. The expression of gdf9 was downregulated by hCG in both ovarian fragments and isolated follicles in dose- and time-dependent manners, and this inhibition appeared to be stage-dependent, with the strongest inhibition observed for the FG follicles and no effect seen for the PG follicles. This correlates well with the expression profile of the LH receptor (lhcgr) in zebrafish follicles. In conclusion, as an oocyte-derived growth factor, GDF9 is highly conserved across vertebrates. With its biological advantages, zebrafish provides an alternative model for studying gene function and regulation. Copyright © 2008 CABI
Descriptors: zebrafish embryos, embryonic development, fertilization, gene expression , genes, messenger RNA, oocytes, ovarian follicles, transforming growth factor, *Danio rerio.*

Liu, M.Y., Y.S. Yang, T. Sugahara, S. Yasuda, and M.C. Liu (2005). **Identification of a novel zebrafish SULT1 cytosolic sulfotransferase: cloning, expression, characterization, and developmental expression study.** *Archives of Biochemistry and Biophysics* 437(1): 10-9. ISSN: 0003 9861.

NAL Call Number: 381 Ar2

Abstract: By searching the zebrafish expressed sequence tag database, we had identified two partial cDNA clones encoding the 5'- and 3'-regions of a putative cytosolic sulfotransferase (SULT). Using the reverse transcription-polymerase chain reaction (RT-PCR) technique, a full-length cDNA encoding this zebrafish SULT was amplified, cloned, and sequenced. Analysis of the sequence data revealed that this novel zebrafish SULT displays 49, 46, and 45% amino acid sequence identity to human SULT1A1, mouse SULT1D1, and rat SULT1C1. This zebrafish SULT therefore appears to belong to the SULT1 cytosolic SULT gene family. Recombinant zebrafish SULT (designated SULT1 isoform 4), expressed using the pGEX-2TK prokaryotic expression vector and purified from transformed Escherichia coli cells, migrated as a 35kDa protein upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Among the endogenous compounds tested as substrates, the purified SULT1 isoform 4 displayed significant sulfating activities toward thyroid hormones, estrone, and dehydroepiandrosterone. The enzyme also showed activities toward a number of xenobiotics including some flavonoids, isoflavonoids, and other phenolic compounds, with a pH optimum at 7.0. A thermostability experiment revealed the enzyme to be relatively stable over a temperature range between 28 and 37 degrees C. Among 10 divalent metal cations tested, Fe2+, Hg2+, Co2+, Zn2+, Cu2+, and Cd2+ exhibited dramatic inhibitory effects on the activity of the enzyme. Developmental expression study using RT-PCR revealed that the zebrafish SULT1

isoform 4 showed a low level of expression in the segmentation period during the embryonic development, which gradually increased to a high level of expression throughout the larval stage onto maturity.
Descriptors: zebrafish, arylsulfotransferase genetics, amino acid sequence, arylsulfotransferase biosynthesis, molecular cloning, gene expression, isoenzymes biosynthesis and genetics, mice, molecular sequence data, phylogeny, amino acid sequence homology, proteins biosynthesis.

Liu, N.A., Q. Liu, K. Wawrowsky, Z. Yang, S. Lin, and S. Melmed (2006). **Prolactin receptor signaling mediates the osmotic response of embryonic zebrafish lactotrophs.** *Molecular Endocrinology* 20(4): 871-80. ISSN: 0888-8809.
NAL Call Number: QP187.3M64M6
Abstract: The pituitary hormone prolactin (PRL) regulates salt and water homeostasis by altering ion retention and water uptake through peripheral osmoregulatory organs. To understand the role of osmotic homeostasis in the development of PRL-secreting lactotrophs, we generated germline transgenic zebrafish coexpressing red fluorescent protein directed by Prolactin regulatory elements (PRL-RFP) and green fluorescent protein by the Pro-opiomelanocortin promoter (POMC-GFP). Transparent embryos expressing fluorescent markers specifically targeted to lactotrophs and corticotrophs, the two pituitary lineages involved in teleost osmotic adaptation, allowed in vivo dynamic tracing of pituitary ontogeny during altered environmental salinity. Physiological osmotic changes selectively regulate lactotroph but not corticotroph proliferation during early ontogeny. These changes are not suppressed by pharmacological dopamine receptor blockade but are completely abrogated by morpholino knockdown of the PRL receptor. PRL receptor signaling exerts robust effects on lactotroph development and plays a permissive role in lactotroph osmo-responsiveness, reflecting the dual peripheral and central interactions required for early pituitary development and embryonic homeostasis.
Descriptors: zebrafish, anterior pituitary gland physiology, prolactin receptors physiology, amino acid sequence, genetically modified, base sequence, antisense DNA genetics, molecular sequence data, phylogeny, anterior pituitary gland embryology, RNA interference, dopamine receptors deficiency, genetics and physiology, prolactin receptors deficiency and genetics, amino acid sequence homology, signal transduction , water electrolyte balance.

Liu, Q., E. Azodi, A.E. Kerstetter, and A.L. Wilson (2004). **Cadherin-2 and cadherin-4 in developing, adult and regenerating zebrafish cerebellum.** *Brain Research* 150(1): 63-71. ISSN: 0006 8993.
NAL Call Number: QP376
Abstract: Cadherins are cell adhesion molecules that regulate development of a variety of tissues and maintenance of adult structures. In this study, we examined expression of two zebrafish classical cadherins, cadherin-2 and cadherin-4, in the cerebellum of developing, normal adult, and regenerating adult zebrafish using in situ hybridization and immunohistochemical methods. Cadherin-2 was widely expressed by the cerebellum of embryonic (24-50-h post fertilization) and larval zebrafish (3-14 days). Cadherin-2 expression became much reduced in the adult cerebellum, but it was greatly up-regulated in the regenerating adult cerebellum. Cadherin-4 was not detected in the embryonic cerebellum, but it was expressed in the Purkinje cells of the larval and adult cerebellum. To gain insight into cadherin-2 role in the formation of the cerebellum, we analyzed embryos injected with a specific cadherin-2 antisense morpholino oligonucleotide (cdh2MO1), and found that the cerebellar development of the cdh2MO1-injected embryos was severely disrupted. This phenotype was

confirmed by examining a cadherin-2 mutant, glass onion. Our results suggest that cadherins are crucial for the normal development of the zebrafish cerebellum, and they may also be involved in the regeneration of injured fish cerebellum.

Descriptors: zebrafish, cadherins metabolism, cerebellum growth, development and metabolism, larva growth, development and metabolism, cadherins genetics, cerebellum cytology, down regulation genetics, larva cytology, nerve regeneration genetics, antisense oligonucleotides pharmacology, Purkinje cells cytology and metabolism, messenger RNA metabolism, up regulation genetics.

Liu, Q., R.J. Duff, B. Liu, A.L. Wilson, S.G. Babb Clendenon, J. Francl, and J.A. Marrs (2006). **Expression of cadherin10, a type II classic cadherin gene, in the nervous system of the embryonic zebrafish.** *Gene Expression Patterns* 6(7): 703-10. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Cadherins are cell surface adhesion molecules that play important roles in development of tissues and organs. In this study, we analyzed expression pattern of cadherin10, a member of the type II classic cadherin subfamily, in the embryonic zebrafish using in situ hybridization methods. cadherin10 message (cdh10) is first and transiently expressed by the notochord. In the developing nervous system, cdh10 was first detected in a subset of the cranial ganglia, then in restricted brain regions and neural retina. As development proceeds, cdh10 expression domain and/or expression levels increased in the embryonic nervous system. Our results show that cdh10 expression in the zebrafish developing nervous system is both spatially and temporally regulated.

Descriptors: zebrafish, cadherins genetics, central nervous system embryology, developmental gene expression regulation, amino acid sequence, cadherins chemistry, central nervous system metabolism, in situ hybridization, molecular sequence data, reverse transcriptase PCR, sequence alignment.

Liu, Q., R.D. Ensign, and E. Azodi (2003). **Cadherin-1, -2 and -4 expression in the cranial ganglia and lateral line system of developing zebrafish.** *Gene Expression Patterns* 3(5): 653-8. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Cadherins are cell adhesion molecules that play important roles in development of a variety of tissues and organs including the nervous system. In this study we analyzed expression patterns of three zebrafish classical (type I) cadherins (cadherin-1, -2, and -4) in the embryonic zebrafish cranial ganglia and lateral line system using in situ hybridization and immunohistochemical methods. All three cadherins exhibit distinct spatiotemporal patterns of expression during cranial ganglia and lateral line system development. cadherin-1 message was detected in the trigeminal and facial ganglia, in the lateral line ganglia, and in most of neuromasts in the lateral lines. Cadherin-2 mRNA and protein were expressed by the majority of the cranial ganglia and lateral line system. Both cadherins were found in embryos younger than 24 hours post fertilization as well as in 2-3-day old embryos and larvae. In contrast, cadherin-4 mRNA and protein expression was detected in embryos older than 30 hours post fertilization and limited to the trigeminal, statoacoustic, and vagal cranial ganglia, and the lateral line ganglia of older embryos and larvae.

Descriptors: zebrafish embryos, cadherins metabolism, cranial nerves metabolism, sensory ganglia metabolism, base sequence developmental gene expression regulation, molecular sequence data, nervous system physiology.

Liu, Q.Y., Z.L. Wu, W.J. Lv, Y.C. Yan, and Y.P. Li (2007). **Developmental expression of cyclin H and Cdk7 in zebrafish: the essential role of cyclin H during early embryo development.** *Cell Research* 17(2): 163-73. ISSN: 1001 0602.

NAL Call Number: QH573 .C4552

Abstract: Cyclin-dependent kinase 7 (Cdk7) is the catalytic subunit of the metazoan Cdk-activating kinase (CAK). Activation of Cdk7 requires its association with a regulatory subunit, Cyclin H. Although the Cdk7/Cyclin H complex has been implicated in the regulation of RNA polymerase in several species, the precise function of their orthologs in zebrafish has not been fully elucidated. In this study, we isolated from zebrafish blastula embryos two cDNAs encoding the orthologs of human Cyclin H and Cdk7, and examined the role of Cdk7/Cyclin H in zebrafish embryogenesis. Sequence analysis showed that the zebrafish Cyclin H and Cdk7 cDNAs encode proteins with 65% and 86% identity to the respective human orthologs. RT-PCR and whole-mount in situ hybridization analyses of their expression in unfertilized eggs, embryos and organs of adult fish suggested that Cyclin H and Cdk7 messages are maternally loaded. Our data also showed that their transcripts were detected throughout development. Distribution of Cyclin H transcripts was found to be ubiquitous during early stages of development and become restricted to the anterior neural tube, brain, eyes, procreate tissues, liver and heart by 5 days post-fertilization. Expression of a dominant-negative form of Cyclin H delayed the onset of zygotic transcription in the early embryo, resulting in apoptosis at 5 hours post-fertilization and leading to sever defects in tissues normally exhibiting high levels of Cyclin H expression. These results implicate Cyclin H in the regulation of the transcriptional machinery during midblastula transition and suggest that it is an essential gene in early zebrafish larval development.

Descriptors: zebrafish, blastula physiology, cyclin dependent kinases genetics, cyclins biosynthesis and genetics, developmental gene expression regulation genetics, amino acid sequence, blastula enzymology, molecular cloning, cyclin dependent kinases biosynthesis, complementary DNA, molecular sequence data, organ specificity genetics, ovum metabolism, phylogeny, DNA sequence analysis.

Liu, R.Z., E.M. Denovan Wright, A. Degrave, C. Thisse, B. Thisse, and J.M. Wright (2004). **Differential expression of duplicated genes for brain-type fatty acid-binding proteins (fabp7a and fabp7b) during early development of the CNS in zebrafish (*Danio rerio*).** *Gene Expression Patterns* 4(4): 379-87. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: A gene for the zebrafish brain-type fatty acid-binding protein (fabp7b) was identified and its structure defined. The zebrafish fabp7b gene spans 1479 bp and consists of four exons encoding 24, 58, 34 and 16 amino acids, respectively, which is identical to the structure of the fabp7a gene previously described. The complete fabp7b cDNA was isolated by 5' and 3' RACE and its nucleotide sequence determined. The deduced amino acid sequence of FABP7B encoded by the zebrafish fabp7b gene shares 82% identity with that of FABP7A encoded by the zebrafish fabp7a gene. A single transcription start site for the fabp7b gene was mapped by 5' RNA ligase-mediated RACE. Phylogenetic analysis indicated that the duplication of the fabp7 genes occurred in the fish lineage after their divergence from mammals. The zebrafish fabp7b gene was assigned to linkage group 20 by radiation hybrid mapping. Reverse transcription-polymerase chain reaction detected fabp7b transcripts in the same adult tissues as fabp7a transcripts. In the brain, levels of fabp7b transcripts were lower than fabp7a transcripts. Whole-mount in situ hybridization showed that the zebrafish fabp7a transcripts were distributed in the early developing central nervous system. In addition to being expressed in the developing brain and retina, zebrafish fabp7b mRNA was also detected in the swim

bladder and pharynx during the embryonic to larval transitory phase.
Descriptors: zebrafish, carrier proteins biosynthesis, central nervous system embryology, developmental gene expression regulation physiology, duplicate genes physiology, nerve tissue proteins biosynthesis, proteins biosynthesis, base sequence, carrier central nervous system metabolism, chromosome mapping, fatty acid binding proteins, developmental gene expression regulation genetics, duplicate molecular sequence data, nerve tissue organ specificity.

Liu, R.Z., E.M. Denovan Wright, A. Degrave, C. Thisse, B. Thisse, and J.M. Wright (2004). **Spatio-temporal distribution of cellular retinol-binding protein gene transcripts (CRBPI and CRBPII) in the developing and adult zebrafish (*Danio rerio*).** *Federation of European Biochemical Societies Journal* 271(2): 339-48. ISSN: 1742 464X.
NAL Call Number: QP501.E8
Abstract: We have cloned and determined the nucleotide sequence of the cDNA coding for a cellular retinol-binding protein type I (CRBPI) from zebrafish. The deduced amino acid sequence of the zebrafish CRBPI showed highest sequence identity (approximately 59%) to the mammalian CRBPIs of the intracellular lipid-binding protein (iLBP) multigene family. Phylogenetic analysis clustered the zebrafish CRBPI to the CRBPI clade. The zebrafish CRBPI gene (rbp1) and CRBPII gene (rbp2) both consist of four exons separated by three introns, identical to all other iLBP genes in vertebrates. Two transcription start sites were identified in the rbp1 promoter and a single transcription start site was identified for rbp2. Radiation hybrid mapping assigned the zebrafish rbp1 gene to linkage group 16 and conserved syntenic genes were found by comparative analysis of mammalian orthologous rbp1 genes. RT-PCR detected mRNA transcripts in the adult intestine, liver, brain, ovary and testis for rbp1 gene and in the intestine and liver for rbp2 gene. Whole mount in situ hybridization of zebrafish embryos revealed rbp1 mRNA expression in the developing zebrafish central nervous system at specific sites that are known to have abundant retinoic acid distribution and significant retinoic acid action. Whole mount in situ hybridization also showed that the zebrafish rbp2 mRNA was localized specifically in the embryonic intestinal bulb and the developing intestine during the larval stage, implying a novel function for the rbp2 gene product during organogenesis and development of the zebrafish intestine.
Descriptors: zebrafish, developmental gene expression regulation, retinol binding amino acid and base sequence, chromosome mapping, conserved sequence, complementary DNA chemistry and genetics, in situ hybridization, linkage genetics, lipid metabolism, molecular sequence data, messenger RNA metabolism, radiation hybrid mapping, retinol binding proteins metabolism, reverse transcriptase PCR, amino acid sequence homology, transcription initiation site, genetic transcription, tretinoin metabolism.

Liu, R.Z., E.M. Denovan Wright, and J.M. Wright (2003). **Structure, linkage mapping and expression of the heart-type fatty acid-binding protein gene (fabp3) from zebrafish (*Danio rerio*).** *Federation of European Biochemical Societies Journal* 270(15): 3223-34. ISSN: 1742 464X.
NAL Call Number: QP501.E8
Abstract: We have determined the cDNA nucleotide sequence, deduced the amino acid sequence and defined the gene structure for the cellular heart-type (H-FABP) or fatty acid-binding protein 3 (FABP3) from zebrafish. The zebrafish FABP3 exhibited the greatest amino acid sequence identity to fish and mammalian heart-type FABPs. 3' RACE and 5' RLM-RACE mapped two alternative polyadenylation sites and three transcription start sites, respectively. Southern blot and hybridization analysis indicated that a single fabp3 gene exists in the zebrafish genome. The zebrafish fabp3 gene consists of four exons interrupted by three

introns with identical exon/intron structure and coding capacity with that of orthologous mammalian H-FABP genes. Radiation hybrid mapping assigned the zebrafish fabp3 gene to linkage group 19 of the zebrafish genome. Comparative genomic analysis revealed conserved syntenies of the zebrafish fabp3 gene and the orthologous human and mouse fabp3 genes. Northern blot analysis detected an mRNA transcript of 780 nucleotides. In situ hybridization of the zebrafish fabp3-specific oligonucleotide probe to tissue sections of adult zebrafish revealed that the fabp3 mRNA was localized in the ovary and liver, but not in the heart, muscle or brain as reported for the mammalian fabp3 gene transcript. RT-PCR, however, detected zebrafish fabp3 mRNA in all the tissues examined. Emulsion autoradiography further revealed that the zebrafish fabp3 mRNA was most abundant in primary growth stage (stage I) oocytes and decreased during the oocyte growth phase. The fabp3 mRNA levels were reduced and restricted to the ooplasm of cortical alveolus stage (stage II) oocytes, and nearly undetectable in stage III and matured oocytes. Inspection of the 5' upstream sequence of the zebrafish fabp3 gene revealed a number of cis elements that may be involved in the expression of the zebrafish fabp3 gene in oocytes and liver.

Descriptors: zebrafish, carrier myocardium metabolism, neoplasm proteins, nerve tissue proteins, 5' flanking region, amino acid and base sequence, Southern blotting, carrier proteins biosynthesis and chemistry, chromosome mapping, molecular cloning, fatty acid binding proteins, gene components, molecular sequence data, regulatory sequences, nucleic acid, sequence alignment, tissue distribution, transcription initiation site , genetic transcription, metabolism, proteins biosynthesis.

Liu, R.Z., E.M. Denovan Wright, and J.M. Wright (2003). **Structure, mRNA expression and linkage mapping of the brain-type fatty acid-binding protein gene (FABP7) from zebrafish (*Danio rerio*).** *Federation of European Biochemical Societies Journal* 270(4): 715-25. ISSN: 1742 464X.

NAL Call Number: QP501.E8

Abstract: The brain fatty acid-binding protein (B-FABP) is involved in brain development and adult neurogenesis. We have determined the sequence of the gene encoding the B-FABP in zebrafish. The zebrafish B-FABP gene spans 2370 bp and contains four exons interrupted by three introns. The coding sequence of zebrafish B-FABP gene is identical to its cDNA sequence and the coding capacity of each exon is the same as that for the human and mouse B-FABP genes. A 1249 bp sequence 5' upstream of exon 1 of the zebrafish B-FABP gene was cloned and sequenced. Several brain development/growth-associated transcription factor binding elements, including POU-domain binding elements and the proposed lipogenic-associated transcription factor NF-Y elements, were found within the 5' region of the B-FABP gene. RT-PCR analysis using mRNA extracted from different tissues of adult zebrafish demonstrated that the zebrafish B-FABP mRNA was predominant in brain with lower levels in liver, testis and intestine, but not in ovary, skin, heart, kidney and muscle. Quantitative RT-PCR revealed a similar tissue-specific distribution for zebrafish B-FABP mRNA except that very low levels of B-FABP mRNA, normalized to beta-actin mRNA, were detected in the heart and muscle RNA, but not in liver RNA. Zebrafish B-FABP mRNA was detected by RT-PCR in embryos beyond 12 h postfertilization, suggesting a correlation of zebrafish B-FABP mRNA expression with early brain development. Radiation hybrid mapping assigned the zebrafish B-FABP gene to linkage group 17. Conserved syntenies of the zebrafish B-FABP gene and the human and mouse orthologous B-FABP genes were observed by comparative genomic analysis.

Descriptors: zebrafish, carrier chromosome mapping, neoplasm proteins, nerve tissue proteins, messenger RNA biosynthesis, tumor suppressor proteins, proteins, 5' untranslated

regions genetics, amino acid and base sequence, brain metabolism, carrier proteins metabolism, molecular cloning, DNA primers chemistry, fatty acid binding proteins, fatty acids genetics, fatty acids metabolism, developmental gene expression regulation, linkage genetics, mice, molecular sequence data, promoter regions genetics, radiation hybrid mapping, reverse transcriptase PCR, amino acid sequence homology, nucleic acid sequence homology.

Liu, R.Z., V. Saxena, M.K. Sharma, C. Thisse, B. Thisse, E.M. Denovan Wright, and J.M. Wright (2007). **The fabp4 gene of zebrafish (*Danio rerio*)--genomic homology with the mammalian FABP4 and divergence from the zebrafish fabp3 in developmental expression.** *Federation of European Biochemical Societies Journal* 274(6): 1621-33. ISSN: 1742 464X.
NAL Call Number: QP501.E8
Abstract: Teleost fishes differ from mammals in their fat deposition and distribution. The gene for adipocyte-type fatty acid-binding protein (A-FABP or FABP4) has not been identified thus far in fishes. We have determined the cDNA sequence and defined the structure of a fatty acid-binding protein gene (designated fabp4) from the zebrafish genome. The polypeptide sequence encoded by zebrafish fabp4 showed highest identity to the H(ad)-FABP or H6-FABP from Antarctic fishes and the putative orthologs from other teleost fishes (83-88%). Phylogenetic analysis clustered the zebrafish FABP4 with all Antarctic fish H6-FABPs and putative FABP4s from other fishes in a single clade, and then with the mammalian FABP4s in an extended clade. Zebrafish fabp4 was assigned to linkage group 19 at a distinct locus from fabp3. A number of closely linked syntenic genes surrounding the zebrafish fabp4 locus were found to be conserved with human FABP4. The zebrafish fabp4 transcripts showed sequential distribution in the developing eye, diencephalon and brain vascular system, from the middle somitogenesis stage to 48 h postfertilization, whereas fabp3 mRNA was located widely in the embryonic and/or larval central nervous system, retina, myotomes, pancreas and liver from middle somitogenesis to 5 days postfertilization. Differentiation in developmental regulation of zebrafish fabp4 and fabp3 gene transcription suggests distinct functions for these two paralogous genes in vertebrate development.
Descriptors: zebrafish, fatty acid binding developmental gene expression regulation, genome, amino acid and base sequence, complementary DNA, molecular sequence data, phylogeny, messenger RNA genetics, reverse transcriptase PCR, amino acid sequence homology.

Liu, R.Z., M.K. Sharma, Q. Sun, C. Thisse, B. Thisse, E.M. Denovan Wright, and J.M. Wright (2005). **Retention of the duplicated cellular retinoic acid-binding protein 1 genes (crabp1a and crabp1b) in the zebrafish genome by subfunctionalization of tissue-specific expression.** *Federation of European Biochemical Societies Journal* 272(14): 3561-71. ISSN: 1742 464X.
NAL Call Number: QP501.E8
Abstract: The cellular retinoic acid-binding protein type I (CRABPI) is encoded by a single gene in mammals. We have characterized two crabp1 genes in zebrafish, designated crabp1a and crabp1b. These two crabp1 genes share the same gene structure as the mammalian CRABP1 genes and encode proteins that show the highest amino acid sequence identity to mammalian CRABPIs. The zebrafish crabp1a and crabp1b were assigned to linkage groups 25 and 7, respectively. Both linkage groups show conserved syntenies to a segment of the human chromosome 15 harboring the CRABP1 locus. Phylogenetic analysis suggests that the zebrafish crabp1a and crabp1b are orthologs of the mammalian CRABP1 genes that likely arose from a teleost fish lineage-specific genome duplication. Embryonic whole mount in situ hybridization detected zebrafish crabp1b transcripts in the posterior hindbrain and

spinal cord from early stages of embryogenesis. crabp1a mRNA was detected in the forebrain and midbrain at later developmental stages. In adult zebrafish, crabp1a mRNA was localized to the optic tectum, whereas crabp1b mRNA was detected in several tissues by RT-PCR but not by tissue section in situ hybridization. The differential and complementary expression patterns of the zebrafish crabp1a and crabp1b genes imply that subfunctionalization may be the mechanism for the retention of both crabp1 duplicated genes in the zebrafish genome.
Descriptors: zebrafish, gene duplication, developmental gene expression regulation genetics, duplicate genome, retinoic acid receptors genetics, amino acid sequence, chromosomes genetics, complementary DNA genetics, larva genetics, growth and development, molecular sequence data, organ specificity, phylogeny, messenger RNA genetics and metabolism, retinoic acid receptors chemistry, sequence alignment, genetic transcription genetics.

Liu, R.Z., Q. Sun, C. Thisse, B. Thisse, J.M. Wright, and E.M. Denovan Wright (2005). **The cellular retinol-binding protein genes are duplicated and differentially transcribed in the developing and adult zebrafish (*Danio rerio*).** *Molecular Biology and Evolution* 22(3): 469-77. ISSN: 0707-4038.
NAL Call Number: QH506.M642
Abstract: There are single copies of the genes encoding the cellular retinol-binding protein type I and II (CRBPI and CRBPII) in the human and rodent genomes. We have identified duplicate genes for both CRBPI and CRBPII in the zebrafish (*Danio rerio*) genome (rbp1b and rbp2b). The zebrafish rbp1b and rbp2b have conserved gene structures, amino acid sequence similarities, gene phylogenies, and syntenic relationships with their mammalian orthologs and zebrafish paralogs, rbp1a and rbp2a. Like the mammalian genes for CRBPI and CRBPII, the zebrafish rbp1b and rbp2b genes are closely linked on a single linkage group. Comparative analysis suggests that the duplicate genes of rbp1 and rbp2 in the zebrafish genome may have arisen by chromosomal or whole-genome duplication. During embryonic development, rbp1b transcripts were detected in the gall bladder of 5-day postfertilization (5 dpf) larvae. The rbp2b mRNA was abundant in the developing liver through 48 hours postfertilization (48 hpf) to 5 dpf. Using reverse transcription-polymerase chain reaction (RT-PCR), rbp1b transcripts were detected in the ovary, and rbp2b mRNA was observed predominantly in the adult liver. Tissue section in situ hybridization and emulsion autoradiography localized rbp1b mRNA to primary oocytes within the zebrafish ovary. The differential mRNA distribution patterns of the rbp1a, rbp1b, rbp2a, and rbp2b genes in the developing and adult zebrafish suggest that shuffling of subfunctions among duplicate copies of paralogous genes may be a mechanism for the retention of duplicated genes in vertebrates.
Descriptors: zebrafish, gene duplication, developmental gene expression regulation physiology, retinol binding mice, organ specificity genetics and physiology, rats, genetic transcription physiology.
Notes: Comments:Erratum In: *Mol Biol Evol.* 2005Apr;22(4):1157
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Liu, S., B. Liu, S. He, Y. Zhao, and Z. Wang (2005). **Cloning and characterization of zebra fish SPATA4 gene and analysis of its gonad specific expression.** *Biochemistry* 70(6): 638-44. ISSN: 0006 2960.
NAL Call Number: 381 B523
Abstract: The spermatogenesis associated 4 gene (SPATA4, previously named TSARG2) was first cloned in human tissues and was reported to be a candidate spermatocyte apoptosis-related gene that is expressed specifically in testis. Analysis of SPATA4 expression and regulation in zebra fish may provide insight into the understanding of the complicated

process of gonadogenesis. In this study, we cloned and characterized the SPATA4 gene from zebra fish (Danio rerio), which is homologous to human and mouse SPATA4. Zebra fish SPATA4 consists of six exons separated by five introns, as all SPATA4 genes in vertebrates. A promoter region was predicted using homologous blast and cloned for further study, and possible transcription factors were analyzed in this region. The putative protein encoded by this gene was analyzed using bioinformatics methods. Multi-tissue RT-PCR results demonstrated that the zebra fish SPATA4 gene is expressed specifically in testis and slightly in ovary. Analysis of the SPATA4 sequence and its spatial expression pattern indicate that this gene is highly conserved and may play an important role in the process of zebra fish gonadogenesis.
Descriptors: zebrafish, gonads physiology, spermatogenesis genetics, amino acid and base sequence, molecular cloning, gene expression, mice, molecular sequence data, promoter regions genetics, reverse transcriptase PCR, spermatogenesis physiology.

Liu, S.h., B. Liu, S.h. He, Y. Zhao, and Z.h. Wang (2005). **Cloning and characterization of zebra fish SPATA4 gene and analysis of its gonad specific expression .** *Biokhimiya/Biochemistry* 70(6): 773-781. ISSN: 0320-9725.
Descriptors: zebrafish , chromosomes, cloning, computer programs, controlled conditions, DNA, experimental research , genes, gonads, proteins, spermatogenesis, *Danio rerio.*

Liu, W.Y., Y. Wang, Y. Qin, Y.P. Wang, and Z.Y. Zhu (2007). **Site-directed gene integration in transgenic zebrafish mediated by Cre recombinase using a combination of mutant lox sites.** *Marine Biotechnology* 9(4): 420-8. ISSN: 1436-2228.
NAL Call Number: TP248.27.M37M37
Abstract: With current gene-transfer techniques in fish, insertion of DNA into the genome occurs randomly and in many instances at multiple sites. Associated position effects, copy number differences, and multiple gene interactions make gene expression experiments difficult to interpret and fish phenotype less predictable. To meet different fish engineering needs, we describe here a gene targeting model in zebrafish. At first, four target zebrafish lines, each harboring a single genomic lox71 target site, were generated by zebrafish transgenesis. The zygotes of transgenic zebrafish lines were coinjected with capped Cre mRNA and a knockin vector pZklox66RFP. Site-specific integration event happened from one target zebrafish line. In this line two integrant zebrafish were obtained from more than 80,000 targeted embryos (integrating efficiency about 10(-4) to 10(-5)) and confirmed to have a sole copy of the integrating DNA at the target genome site. Genomic polymerase chain reaction analysis and DNA sequencing verified the correct gene target events where lox71 and lox66 have accurately recombined into double mutant lox72 and wild-type loxP. Each integrant zebrafish chosen for analysis harbored the transgene rfp at the designated egfp concatenates. Although the Cre-mediated recombination is site specific, it is dependent on a randomly placed target site. That is, a genomic target cannot be preselected for integration based solely on its sequence. Conclusively, an rfp reporter gene was successfully inserted into the egfp target locus of zebrafish genome by Cre-lox-mediated recombination. This site-directed knockin system using the lox71/lox66 combination should be a promising gene-targeting platform serving various purposes in fish genetic engineering.
Descriptors: zebrafish, gene targeting methods and transfer techniques, integrases metabolism, insertional mutagenesis methods, genetically modified genetics, base sequence, DNA primers chemistry, gene order, gene-targeting models, genetic recombination, transgenes genetics gene-targeting .

Liu, X., H. Wang, and Z. Gong (2006). **Tandem-repeated zebrafish zp3 genes possess oocyte-specific promoters and are insensitive to estrogen induction.** *Biology of Reproduction* 74(6): 1016-25. ISSN: 0006 3363.

NAL Call Number: QD415.F4

Abstract: Zona pellucida (Zp) proteins are glycoproteins in fish chorion and are encoded by multiple gene families, including zp1, zp2, zp3, and potentially other zp genes. Expression of zp genes in teleosts is either in the liver under the induction of estrogen or in the ovaries. In the present study, we isolated and characterized a zebrafish zp3 genomic clone and found three tandem-repeated zp3 genes with high sequence identities. We estimated that there were 10-15 zp2 and 17-21 zp3 genes in a haploid genome. We also found some variant zp2 and zp3 subfamilies, and each subfamily may have multiple members. These zp2 and zp3 genes are distributed in several different chromosomes. Ontogenetic expression of zp2 and zp3 mRNAs was first detected at 3 wk postfertilization, which was about 5 wk earlier than initial vtg1 expression, indicating that ovary development was earlier than vitellogenesis. Both zp2 and zp3 mRNAs were expressed specifically in early-growing oocytes and are insensitive to estrogen induction. Because zp3 genes are organized in tandem repeats, to investigate whether an individual zp3 promoter is capable of driving oocyte-specific expression, green fluorescence protein (gfp)-transgenic zebrafish were developed by using a 3.8-kb zp3 5' upstream region, and we found that the gfp reporter gene was faithfully expressed in developing oocytes in zp3:gfp transgenic females. Thus, the new transgenic line not only provided a convenient living marker for monitoring female gonad development, but also demonstrated that a single zp3 gene promoter is sufficient for oocyte-specific transcription.

Descriptors: zebrafish, egG estrogens pharmacology, gene expression regulation drug effects, membrane glycopromoter regions genetics, cell surface receptors genetics, tandem repeat sequences genetics, amino acid sequence, genetically modified, base sequence, egG proteins metabolism, gene expression regulation genetics and physiology, genome genetics and physiology, green fluorescent proteins analysis and genetics, membrane glycoproteins metabolism, molecular sequence data, oocytes chemistry and physiology, promoter regions genetics physiology, messenger RNA analysis and genetics, cell surface receptors metabolism, tandem repeat sequences physiology, genetic transcription genetics and physiology.

Liu, X.F., Y.X. Wang, Q. Jiang, L.X. Qian, G.W. Gao , Y.X. Dong, T. Zhong, and H.Y. Song (2006). **Mef2c gene expression profile during zebrafish embryogenesis**. *Fudan Xuebao (Yixuekexueban)* 33(1): 89-91. ISSN: 0257 8131.

Abstract: Purpose To analyze the expression pattern of Mef2c during zebrafish embryogenesis. Methods We collected all the phases of zebrafish embryos to detect the expression pattern of Mef2c in the somite and the heart during zebrafish embryogenesis by whole mount in situ hybridization. Results We first detected Mef2c expression at 13 hours post-fertilization in the somite that is the latest expression in the somite of Mef2c family. Expression of Mef2c in heart was detected as early as in somite and continued for 48 hours post-fertilization. Conclusions We made clear the expression pattern of Mef2c during zebrafish embryogenesis in order to make sure that Mef2c plays important roles in the regulation of cardiac and skeletal muscle development. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, development, molecular genetics, biochemistry and molecular biophysics, embryogenesis.

Language of Text: Chinese.

Liu, Y.W., W. Gao, H.L. Teh, J.H. Tan, and W.K. Chan (2003). **Prox1 is a novel coregulator of Ff1b and is involved in the embryonic development of the zebrafish interrenal primordium.** *Molecular and Cellular Biology* 23(20): 7243-55. ISSN: 0270-7306.

NAL Call Number: QH506.M664

Abstract: Steroidogenic factor 1 (SF-1) plays an essential role in adrenal development, although the exact molecular mechanisms are unclear. Our previous work established that Ff1b is the zebra fish homologue of SF-1 and that its disruption by antisense morpholinos leads to a complete ablation of the interrenal organ. In this study, results of biochemical analyses suggest that Ff1b and other Ff1 members interact with Prox1, a homeodomain protein. Fine mapping using site-directed mutants showed that this interaction requires an intact Ff1b heptad 9 and AF2, as well as Prox1 NR Box I. In vivo, this physical interaction led to the inhibition of Ff1-mediated transactivation of pLuc3XFRE, indicating that Prox1 acts to repress the transcriptional activity of Ff1b. In situ hybridization demonstrates that prox1 colocalizes with ff1a and ff1b in the liver and interrenal primordia, respectively. Embryos microinjected with prox1 morpholino displayed a consistent partial reduction of 3 eta-Hsd activity in the interrenal organ, while ff1b morpholino led to a disappearance of prox1. Based on these results, we propose that during the course of interrenal organogenesis, Prox1 functions as a tissue-specific coregulator of Ff1b and that the subsequent inhibition of Ff1b activity, after its initial roles in the specification of interrenal primordium, is critical for the maturation of the interrenal organ.

Descriptors: zebrafish, DNA binding proteins genetics, proteins metabolism, homeodomain, kidney embryology, transcription factors metabolism, amino acid sequence, Western blotting, drug dose response relationship, down regulation, polyacrylamide gel electrophoresis, fushi tarazu transcription factors, homeodomain proteins metabolism, in situ hybridization, liver metabolism, genetic models, molecular sequence data, site directed mutagenesis mutation, plasmids metabolism, precipitin tests, protein binding and isoforms, tertiary protein structure, cytoplasmic receptors and nuclear, genetic transcription, two hybrid system techniques, beta galactosidase metabolism.

Liu, Z.J. and J.F. Cordes (2004). **DNA marker technologies and their applications in aquaculture genetics** . *Aquaculture* 238(1/4): 1-37. ISSN: 0044 8486.

NAL Call Number: SH1.A6

Abstract: The development of DNA-based genetic markers has had a revolutionary impact on animal genetics. With DNA markers, it is theoretically possible to observe and exploit genetic variation in the entire genome. Popular genetic markers in the aquaculture community include allozymes, mitochondrial DNA, RFLP, RAPD, AFLP, microsatellite, SNP, and EST markers. The application of DNA markers has allowed rapid progress in aquaculture investigations of genetic variability and inbreeding, parentage assignments, species and strain identification, and the construction of high-resolution genetic linkage maps for aquaculture species. Well-designed studies using these genetic markers will undoubtedly accelerate identification of genes involved in quantitative trait loci (QTL) for marker-assisted selection. In this review, the principles, potential power, requirements, advantages, and disadvantages of the various marker types are discussed, along with their applications in a variety of aquaculture studies. Included are discussions on how to efficiently exploit research progress made from the Human Genome Project and from other model species such as zebrafish for the benefit of aquaculture genomics and aquaculture genetics research. Copyright © 2008 CABI

Descriptors: zebrafish, aquaculture, DNA amplification, genetic diversity, mapping, markers and polymorphism, genomes, identification, microsatellites, mitochondrial DNA,

nucleotide sequences, quantitative trait loci, random amplified polymorphic DNA, restriction fragment length polymorphism, *Danio rerio.*

Lo, J., S. Lee, M. Xu, F. Liu, H. Ruan, A. Eun, Y. He, W. Ma, W. Wang, Z. Wen, and J. Peng (2003). **15000 unique zebrafish EST clusters and their future use in microarray for profiling gene expression patterns during embryogenesis.** *Genome Research* 13(3): 455-66. ISSN: 1088-9051.

NAL Call Number: QP606.D 46P34

Abstract: A total of 15590 unique zebrafish EST clusters from two cDNA libraries have been identified. Most significantly, only 22% (3437) of the 15590 unique clusters matched 2805 (of 15200) clusters in the *Danio rerio* UniGene database, indicating that our EST set is complementary to the existing ESTs in the public database and will be invaluable in assisting the annotation of genes based on the upcoming zebrafish genome sequence. Blast search showed that 7824 of our unique clusters matched 6710 known or predicted proteins in the nonredundant database. A cDNA microarray representing approximately 3100 unique zebrafish cDNA clusters has been generated and used to profile the gene expression patterns across six different embryonic stages (cleavage, blastula, gastrula, segmentation, pharyngula, and hatching). Analysis of expression data using K-means clustering revealed that genes coding for muscle-specific proteins displayed similar expression patterns, confirming that the coordinate gene expression is important for myogenesis. Our results demonstrate that the combination of microarray technology with the zebrafish model system can provide useful information on how genes are coordinated in a genetic network to control zebrafish embryogenesis and can help to identify novel genes that are important for organogenesis.

Descriptors: zebrafish, cluster analysis, expressed sequence tags, gene expression profiling trends, developmental gene expression regulation genetics, oligonucleotide array sequence analysis trends , gene expression profiling statistics and numerical data, gene library and physiology, molecular sequence data, muscles chemistry and metabolism, normal distribution, oligonucleotide array sequence analysis statistics and numerical data, predictive value of tests.

Loeb Hennard, C., X. Cousin, I. Prengel, and E. Kremmer (2004). **Cloning and expression pattern of vat-1 homolog gene in zebrafish.** *Gene Expression Patterns* 5(1): 91-6. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: The VAT-1 protein is present in the electric organ of marine rays where it is suggested to play a central role in nerve signal transmission. VAT-1 homolog protein was also identified in mouse and human but its function remains to be determined. We have investigated VAT-1 homolog in zebrafish Danio rerio since it is an excellent model amenable to the combination of genetic, molecular and embryological studies. Amino acid sequence analysis shows that the zebrafish VAT-1 homolog shares approximately 51-61% identity with the electric ray, mouse, and human counterparts. By in situ hybridization, vat-1 homolog mRNA is first observed in the trigeminal nuclei at the 8-somite stage. At 20-somite stage, vat-1 homolog is detected in the brain, namely in primary clusters of neurons, in the epiphysis and in the hindbrain. vat-1 homolog is also present in the neural tube but this expression disappears after 72 h post-fertilization. At 24 h post-fertilization, vat-1 homolog starts to be expressed in the developing gut. At later stages, vat-1 homolog is present throughout the brain, appears in the maturing retina and the pharyngeal cavity.

Descriptors: zebrafish, adenosine triphosphatases biosynthesis and amino acid and base sequence, brain embryology and metabolism, branchial region embryology and metabolism,

gene expression profiling, mice, molecular sequence data, retina embryology and metabolism, sequence alignment.

Loeb Hennard, C., E. Kremmer, and L. Bally Cuif (2005). **Prominent transcription of zebrafish N-myc (nmyc1) in tectal and retinal growth zones during embryonic and early larval development.** *Gene Expression Patterns* 5(3): 341-7. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Because of its oncogenic capacity and expression restricted to embryonic and newborn tissues, the N-myc proto-oncogene is suggested to play a key role in vertebrate organogenesis as well as in the control of cell proliferation and differentiation. To further approach the developmental function of N-myc, we cloned full-length zebrafish N-myc (nmyc1) and analyzed its expression in the embryo and early larva. nmyc1 transcription is initiated at the mid-blastula stage. At somitogenesis stages, its expression was detected in the retina, midbrain, posterior hindbrain and presumptive spinal cord. nmyc1 was also transcribed in the endoderm and its derivatives as well as in branchial arches. At later developmental stages, posterior neural expression of nmyc1 was switched off, but expression remained intense in the brain, mainly in the optic tectum, cerebellar plate and dorsal rhombomere 2. Comparison of nmyc1 transcription with proliferation zones using a M phase mitotic marker revealed that nmyc1 expression is specifically associated with mitosis in the optic tectum and the retina. This result contrasts with previous studies in other vertebrates where N-myc expression can persist in differentiating cells.
Descriptors: zebrafish, genes, myc, proto oncogene proteins biosynthesis, retina growth and development, superior colliculus growth and development, genetic transcription, proteins biosynthesis, amino acid and base sequence, larva growth and development and metabolism, mitosis, molecular sequence data, proto oncogene proteins chemistry and retina embryology and metabolism, superior colliculus embryology and metabolism.

Londin, E.R., L. Mentzer, K.P. Gates, and H.I. Sirotkin (2007). **Expression and regulation of the zinc finger transcription factor Churchill during zebrafish development.** *Gene Expression Patterns* 7(6): 645-50. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: During gastrulation dynamic cell movements establish the germ layers and shape the body axis of the vertebrate embryo. The zinc finger protein Churchill (chch) has been proposed to be a key regulator of these movements. We examined the expression pattern of chch in zebrafish and studied the regulation of chch by FGF signaling. We observed zygotic expression of chch during early cleavage stages. Two lines of evidence demonstrate that chch is zygotically expressed prior to the mid-blastula transition. First, blocking transcription during early cleavage stages represses chch expression. Second, endogenous levels of chch transcripts increase between 1-cell and 16-cell embryos. chch remains widely expressed during blastula and gastrula stages but scattered cells express higher levels of chch. By somitogenesis, chch is expressed in the ventral-most cells of the embryo adjacent to the yolk. In addition, transcripts are also observed in superficial cells on the surface of the yolk, in presumptive mucous cells and keratinocytes. By 30 hpf transcripts are observed in anterior neural tissue and ventral cells adjacent to the yolk. Over the next three days chch expression is indistinct until 4 dpf when we observe expression in the pharynx and gut. We show that activation of FGF signaling during gastrulation is sufficient to induce chch expression. In addition, we demonstrate that blocking FGF signaling between the 4-cell and shield stages represses chch expression.

Descriptors: zebrafish, zinc finger transcription factor, Churchill, chch, development, FGF signaling, gastrulation.

Loosli, F., W. Staub, K.C. Finger Baier, E.A. Ober, H. Verkade, J. Wittbrodt, and H. Baier (2003). **Loss of eyes in zebrafish caused by mutation of chokh/rx3.** *European Molecular Biology Organization Journal Reports* 4(9): 894-9. ISSN: 1469 221X.
NAL Call Number: QH506 .E463
Abstract: The vertebrate eye forms by specification of the retina anlage and subsequent morphogenesis of the optic vesicles, from which the neural retina differentiates. chokh (chk) mutant zebrafish lack eyes from the earliest stages in development. Marker gene analysis indicates that retinal fate is specified normally, but optic vesicle evagination and neuronal differentiation are blocked. We show that the chk gene encodes the homeodomain-containing transcription factor, Rx3. Loss of Rx3 function in another teleost,medaka, has also been shown to result in an eyeless phenotype.The medaka rx3 locus can fully rescue the zebrafish mutant phenotype. We provide evidence that the regulation of rx3 is evolutionarily conserved, whereas the downstream cascade contains significant differences in gene regulation. Thus, these mutations in orthologous genes allow us to study the evolution of vertebrate eye development at the molecular level.
Descriptors: zebrafish, eye abnormalities genetics, fish homeodomain proteins genetics, eye proteins and biosynthesis, nerve tissue proteins biosynthesis genetics, paired box transcription factors, point mutation, repressor proteins, retina embryology and metabolism.

Loots, G. and I. Ovcharenko (2007). **ECRbase: database of evolutionary conserved regions, promoters, and transcription factor binding sites in vertebrate genomes**. *Bioinformatics* 23(1): 122-124. ISSN: 1367 4803.
NAL Call Number: QH313.C65
Abstract: Motivation: Evolutionary conservation of DNA sequences provides a tool for the identification of functional elements in genomes. We have created a database of evolutionary conserved regions (ECRs) in vertebrate genomes, entitled ECRbase, which is constructed from a collection of whole-genome alignments produced by the ECR Browser. ECRbase features a database of syntenic blocks that recapitulate the evolution of rearrangements in vertebrates and a comprehensive collection of promoters in all vertebrate genomes generated using multiple sources of gene annotation. The database also contains a collection of annotated transcription factor binding sites (TFBSs) in evolutionary conserved and promoter elements. ECRbase currently includes human, rhesus macaque, dog, opossum, rat, mouse, chicken, frog, zebrafish and fugu genomes. It is freely accessible at http://ecrbase.dcode.org.
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Descriptors: zebrafish, binding sites, databases, evolution, genome analysis, genomes, nucleotide sequences, poultry, promoters, transcription factors, *Danio rerio*, Didelphidae, dogs, fowls , frogs, *Macaca mulatta*, human, mice, rats, Tetraodontidae.

Lopez, M., G. Nica, P. Motte, J.A. Martial, M. Hammerschmidt, and M. Muller (2006). **Expression of the somatolactin beta gene during zebrafish embryonic development.** *Gene Expression Patterns* 6(2): 156-61. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Somatolactin (Sl) is a pituitary hormone closely related to prolactin (Prl) and growth hormone that was until now only found in various fish species. We isolated the cDNA coding for zebrafish Slbeta and we identified the gene encoding this hormone. We also obtained a 1kb genomic fragment corresponding to the slbeta upstream pro-

moter region. Furthermore, the slbeta expression pattern was examined during zebrafish embryogenesis using whole-mount in situ hybridization. Slbeta mRNA is first detected in a single cell at the anterior border of the neural plate starting at 23h post fertilization (hpf). Slbeta-expressing cells also express the transcription factor pit1 and are located close to prl-expressing cells. Using combined fluorescent in situ hybridization, we show that slbeta- and prl-expressing cells are clearly distinct at 29 hpf. Starting at 30 hpf, the number of slbeta positive cells increases and their location becomes more clearly distinct from lactotrope cells, in a more posterior position. At later stages (48 hpf), slbeta expression was observed posterior to growth hormone expression, again in a distinct cell type. We show that zebrafish mutants aal, as well as mutants in the pit1 gene, are deficient in slbeta expression. In conclusion, slbeta expression defines a new, additional cell type in zebrafish pituitary that depends on pit1 and aal for its differentiation.

Descriptors: zebrafish, fish glycopituitary hormones genetics, base sequence, complementary DNA genetics, developmental gene expression regulation, growth hormone genetics, in situ hybridization, fluorescence , mutation, pituitary gland embryology, pituitary gland metabolism, prolactin genetics, messenger RNA genetics and metabolism.

Lu, G., S. Ren, P. Korge, J. Choi, Y. Dong, J. Weiss, C. Koehler, J. Chen, and Y. Wang (2007). **A novel mitochondrial matrix serine/threonine protein phosphatase regulates the mitochondria permeability transition pore and is essential for cellular survival and development**. *Genes & Development* 21(7): 784-796. ISSN: 0890-9369.

NAL Call Number: QH426.G46

Descriptors: zebrafish, apoptosis, biological membranes, brain, cell survival, embryogenesis, embryonic development, heart, hepatocytes, MpTp, membrane permeability and potential, mitochondria, oxidative phosphorylation, phosphoprotein phosphatase, pores, proteins, RNA, serine , signal transduction, threonine, protein phosphatase, *Danio rerio*, development and cell cycle, physiology, biochemistry, biophysics.

Lu, X., F. le Noble, L.i. Yuan, Q. Jiang, B. deLafarge, D. Sugiyama, C. Breant, F. Claes , F. Desmet, J.-L. Thomas, M. Autiero, P. Carmeliet, M. Tessier-Lavigne, and A. Eichmann (2004). **The netrin receptor UNC5B mediates guidance events controlling morphogenesis of the vascular system**. *Nature* 432(7014): 179-186. ISSN: 0028-0836.

NAL Call Number: 472 N21

Descriptors: zebrafish, blood vessels, circulatory system, embryonic development, endothelial cells, genes, morphogenesis, pseudopodia, receptors, vascular system, *Danio rerio*, morphogenesis, UNC5B gene, netrin receptors, netrin-1a gene.

Ludwig, T.E., V. Bergendahl, M.E. Levenstein, J. Yu, M.D. Probasco, and J.A. Thomson (2006). **Feeder-independent culture of human embryonic stem cells**. *Nature Methods* 3(8): 637-646. ISSN: 1548 7091.

NAL Call Number: QH315

Abstract: We recently reported the development of TeSR1, a serum-free, animal product-free medium that supports the derivation and long-term feeder-independent culture of human embryonic stem cells(1). Although the derivation of new human embryonic stem cell lines in those defined conditions offered an important proof of principle, the costs of some of the defined components in that culture system made it impractical for everyday research use. Here we describe modifications to the medium (mTeSR1) that include the use of animal-sourced proteins (bovine serum albumin (BSA) and Matrigel) and cloned zebrafish basic fibroblast growth factor (zbFGF). We include a simple protocol that allows purification of

up to 100 mg zbFGF in less than three days (Fig. 1), an amount sufficient to make 1,000 l of mTeSR1 medium. The modifications presented here make mTeSR1 practical for routine research use, and the protocols presented are those currently used in our laboratory for standard human embryonic stem cell culture. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, methods and techniques, cell biology, cell culture and culturing techniques.

Lukowski, C.M., R.G. Ritzel, and A.J. Waskiewicz (2006). **Expression of two insm1-like genes in the developing zebrafish nervous system.** *Gene Expression Patterns* 6(7): 711-8. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Insulinoma associated-1 (INSM1, formerly IA-1) is a Cys(2)-His(2) zinc finger transcription factor sharing conserved regions with *Caenorhabditis elegans* EGL-46 and *Drosophila* Nerfin-1. INSM, EGL-46, and Nerfin proteins comprise the EIN family of zinc finger transcription factors. egl-46 and nerfin-1 have been implicated in various aspects of neuronal differentiation including cell fate specification, axon guidance decisions and cell migration. Murine Insm1 has a restricted expression pattern in the developing CNS. We have characterized two zebrafish *(Danio rerio*) Insm1-like genes, insm1a and insm1b, and analyzed their expression patterns during embryonic development. Zebrafish insm1a and insm1b share an embryonic expression pattern comparable to the proneural deltaA as well as overlapping the neuronal marker elavl3. The expression pattern observed for zebrafish insm1a and insm1b is similar to other EIN homologues. Both zebrafish insm1-like transcripts are also present in a region of the embryo where pancreatic progenitors originate. The expression data along with functional characterization of invertebrate homologues suggest a conserved pathway involving the EIN transcription factors in early neurogenesis.
Descriptors: zebrafish, developmental gene expression regulation, nervous system embryology, transcription factors genetics, amino acid sequence, molecular cloning, in situ hybridization, molecular sequence data, nervous system metabolism , neurons metabolism, phylogeny, reverse transcriptase PCR, sequence alignment.

Lunde, K., H.G. Belting, and W. Driever (2004). **Zebrafish pou5f1/pou2, homolog of mammalian Oct4, functions in the endoderm specification cascade.** *Current Biology* 14(1): 48-55. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Abstract: pou5f1, also known as Oct4, is required to establish the pluripotent cell population necessary for embryogenesis in mouse. Additional roles during development, including endoderm formation, have been proposed. In zebrafish, the zygotic pou5f1/pou2 mutant spiel ohne grenzen (spg) shows neural plate patterning defects and reduced endoderm at the tailbud stage. To investigate the function of maternal and early zygotic pou5f1 expression, we rescued zygotic spg(m793) mutants by injecting pou5f1 mRNA at the one-cell stage and raised them into fertile homozygous spg(m793) adults that mate to produce maternal-zygotic spg (MZspg) mutant embryos. Although neurectoderm, mesoderm, and germ cells develop in MZspg mutants, gastrulation is delayed and proceeds abnormally. Further, MZspg mutants do not maintain expression of sox32/casanova, express little or no sox17, and fail to develop endodermal tissue. Constitutively active Nodal receptor TARAM-A or sox32 overexpression induces ubiquitous sox17 expression in wild-type embryos, but not in MZspg mutants. Overexpression of a Pou5f1-VP16 activator fusion protein can rescue gastrulation and endodermal tissues in MZspg mutants. We propose that pou5f1 plays an activating role in zebrafish endodermal development, where it maintains sox32 expression during gastrula-

tion and acts with sox32 to induce sox17 expression in endodermal precursor cells.
Descriptors: zebrafish, endoderm metabolism, gastrula physiology, signal transduction, transcription factors metabolism, DNA binding proteins genetics, proteins metabolism, high mobility group proteins metabolism, histological techniques, in situ hybridization, mutation genetics, octamer transcription factor 3.

Lunde, K., G. Belting-Heinz, and W. Driever (2005). **Overexpression of dominant activator and repressor variants of maternally and zygotically expressed zebrafish pou5f1/pou2, homolog of mammalian Oct4, affects dorsoventral patterning**. *Federation of American Societies for Experimental Biology Journal* 19(5, Suppl. S, Part 2): A1338. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: Pou5f1/Oct-4 in mice is required for embryonic pluripotent cell populations. In zebrafish, pou5f1/pou2 has both maternal and zygotic expression. The zygoticpou5f1 mutant spiel ohne grenzen (spg) develops neural plate patterning defects and reduced endoderm. To study the function of maternal and early zygotic pou5f1, we rescued spg mutants by pou5f1 mRNA injection. These fish mate to produce maternal-zygotic spg (MZspg) mutants. Although neurectoderm, mesoderm, and germ cells develop in MZspg mutants, endoderm is absent while gastrulation is delayed and abnormal. We have shown that pou5f1 plays an activating role in endodermal development, where it maintains sox32 expression during gastrulation and acts with sox32 to induce sox17 in endodermal precursor cells (Lunde et al., 2004, Curt. Biol. 14, 48-55). Low-level overexpression of a Pou5f1-VP16 activator fusion protein can rescue gastrulation and endodermal tissues in MZspg mutants, indicating that pou5f1 acts as a transcriptional activator during development. In contrast, a Pou5f1-En repressor acts as a dominant negative, inducing aspects of the spg phenotype in wild-type embryos. Further, overexpression of larger quantities of pou5f1-En dorsalize wildtype embryos, while pou5f1-VP16 can ventralize. Thus, pou5f1 may also contribute to the control of genesactive in dorsoventral patterning. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics, development, dorsoventral patterning.

Luo, T., T.F. Schilling, and T.D. Sargent (2005). **A novel factor in neural crest development**. *Birth Defects Research* 73(5): 315. ISSN: 1542 0752.
NAL Call Number: QL991.T4
Descriptors: zebrafish, nervous system, neural coordination, development, tunel staining, histology and cytology techniques, microarry analysis, genetic techniques.

Luo, W., J. Williams, P.M. Smallwood, J.W. Touchman, L.M. Roman, and J. Nathans (2004). **Proximal and distal sequences control UV cone pigment gene expression in transgenic zebrafish.** *Journal of Biological Chemistry* 279(18): 19286-93. ISSN: 0021-9258.
NAL Call Number: 381J824
Abstract: The molecular basis of cone photoreceptor-specific gene expression is largely unknown. In this study, we define cis-acting DNA sequences that control the cell type-specific expression of the zebrafish UV cone pigment gene by transient expression of green fluorescent protein transgenes following their injection into zebrafish embryos. These experiments show that 4.8 kb of 5'-flanking sequences from the zebrafish UV pigment gene direct expression specifically to UV cones and that this activity requires both distal and proximal sequences. In addition, we demonstrate that a proximal region located between -215 and -110 bp (with respect to the initiator methionine codon) can function in the context of a zebrafish rhodopsin promotor to convert its specificity from rod-only expression to rod and UV cone expression. These experiments demonstrate the power of transient transgenesis in

zebrafish to efficiently define cis-acting regulatory sequences in an intact vertebrate.
Descriptors: zebrafish, retina cones chemistry, gene expression regulation, opsin genetics, regulatory sequences, nucleic acid, 5' untranslated regions, genetically modified, base sequence, green fluorescent proteins, luminescent molecular sequence data, promoter regions genetics , rhodopsin genetics, ultraviolet rays.

Lutfalla, G., H.R. Crollius, N. Stange-thomann, O. Jaillon, K. Mogensen, and D. Monneron (2003). **Comparative genomic analysis reveals independent expansion of a lineage- specific gene family in vertebrates: the class II cytokine receptors and their ligands in mammals and fish**. *Bio Med Central Genomics* 4 ISSN: 1471-2164.
NAL Call Number: QH447.B63
Descriptors: zebrafish, amino acid sequence, conserved sequence, cytokine receptors, freshwater fish, gene expression, gene families, genomes, genomic analysis, immune system, infection, interleukin 10, 19, 20, 22 and 24, kidney, ligands, nucleotide sequence, evolution phylogeny, receptors, recombinants, tissue factor, *Danio rerio*, *Takifugu rubripes*, *Tetraodon*, *Tetraodon nigroviridis.*

Lv, D., P. Song, Y. Chen, W. Gong, and S. Mo (2005). **Cloning and characterization of full length of a novel zebrafish gene Zsrg abundantly expressed in the germline stem cells.** *Biochemical and Biophysical Research Communications* 329(2): 632-7. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: Using the digital differential display program of the National Center for Biotechnology Information, we identified a contig of expression sequence tags (ESTs) (Accession No. BM316936), which came from zebrafish ovary and testis libraries. The full-length cDNA of this transcript was cloned and further confirmed by polymerase chain reaction and sequencing. The full-length cDNA of the novel gene is 807bp and encodes a novel protein of 187 amino acids, which shares no significant homology with any other known proteins. Characterization of genomic sequences of the gene revealed that it spans 6kb on the linkage group 3 and is composed of five exons and four introns. RT-PCR analysis showed that it was expressed in mature oocytes and one-cell stage, and persisted until 24h of development. RT-PCR also revealed that it is expressed in gonad and kidney, with the highest level of expression in the testis. The expression sites of the novel gene in adult gonad were further localized by in situ hybridization to oogonia and growing oocytes in ovary and to spermatogonia, spermatocytes but not to spermatids in testis. Based on its abundance in testis and the germline stem cell-spermatogonia and oogonia, we hypothesize that it may function as a testicular development and gametogenesis related gene that plays important roles in spermatogenesis, and named it Zsrg (zebrafish testis spermatogenesis related gene, Zsrg).
Descriptors: zebrafish, germ cells metabolism, stem cells metabolism, proteins chemistry, amino acid sequence, cultured cells, molecular cloning, developmental gene expression regulation physiology, molecular sequence data, organ specificity, recombinant proteins chemistry and metabolism, amino acid sequence homology, tissue distribution.

Lyons, D.A., H.M. Pogoda, M.G. Voas, I.G. Woods, B. Diamond, R. Nix, N. Arana, J. Jacobs, and W.S. Talbot (2005). **Erbb3 and Erbb2 are essential for schwann cell migration and myelination in zebrafish.** *Current Biology* 15(6): 513-24. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Abstract: BACKGROUND: Myelin is critical for efficient axonal conduction in the vertebrate nervous system. Neuregulin (Nrg) ligands and their ErbB receptors are required for the development of Schwann cells, the glial cells that form myelin in the peripheral nervous

system. Previous studies have not determined whether Nrg-ErbB signaling is essential in vivo for Schwann cell fate specification, proliferation, survival, migration, or the onset of myelination. RESULTS: In genetic screens for mutants with disruptions in myelinated nerves, we identified mutations in erbb3 and erbb2, which together encode a heteromeric tyrosine kinase receptor for Neuregulin ligands. Phenotypic analysis shows that both genes are essential for development of Schwann cells. BrdU-incorporation studies and time-lapse analysis reveal that Schwann cell proliferation and migration, but not survival, are disrupted in erbb3 mutants. We show that Schwann cells can migrate in the absence of DNA replication. This uncoupling of proliferation and migration indicates that erbb gene function is required independently for these two processes. Pharmacological inhibition of ErbB signaling at different stages reveals a continuing requirement for ErbB function during migration and also provides evidence that ErbB signaling is required after migration for proliferation and the terminal differentiation of myelinating Schwann cells. CONCLUSIONS: These results provide in vivo evidence that Neuregulin-ErbB signaling is essential for directed Schwann cell migration and demonstrate that this pathway is also required for the onset of myelination in postmigratory Schwann cells.

Descriptors: zebrafish, cell movement physiology, genes, erbb genetics, ErbB 2 genes genetics, myelin sheath metabolism, Schwann cells metabolism, signal transduction physiology, aphidicolin pharmacology, base sequence, bromodeoxyuridine, cell division drug effects, chromosome mapping, complementary DNA genetics, immunohistochemistry, in situ hybridization, molecular sequence data, mutation genetics, neuregulin 1 metabolism, Schwann cells physiology, DNA sequence analysis.

Ma, M. and Y.J. Jiang (2007). **Jagged2a-notch signaling mediates cell fate choice in the zebrafish pronephric duct.** *Public Library of Science Genetics* 3(1): E18. ISSN: 1553-7390.

Abstract: Pronephros, a developmental model for adult mammalian kidneys (metanephros) and a functional kidney in early teleosts, consists of glomerulus, tubule, and duct. These structural and functional elements are responsible for different kidney functions, e.g., blood filtration, waste extraction, salt recovery, and water balance. During pronephros organogenesis, cell differentiation is a key step in generating different cell types in specific locations to accomplish designated functions. However, it is poorly understood what molecules regulate the differentiation of different cell types in different parts of the kidney. Two types of epithelial cells, multi-cilia cells and principal cells, are found in the epithelia of the zebrafish distal pronephric duct. While the former is characterized by at least 15 apically localized cilia and expresses centrin2 and rfx2, the latter is characterized by a single primary cilium and sodium pumps. Multi-cilia cells and principal cells differentiate from 17.5 hours post-fertilization onwards in a mosaic pattern. Jagged2a-Notch1a/Notch3-Her9 is responsible for specification and patterning of these two cell types through a lateral inhibition mechanism. Furthermore, multi-cilia cell hyperplasia was observed in mind bomb mutants and Mind bomb was shown to interact with Jagged2a and facilitate its internalization. Taken together, our findings add a new paradigm of Notch signaling in kidney development, namely, that Jagged2a-Notch signaling modulates cell fate choice in a nephric segment, the distal pronephric duct.

Descriptors: zebrafish, calcium binding proteins metabolism, cell lineage, kidney embryology, Notch receptors metabolism, signal transduction, calcium binding cell differentiation, developmental gene expression regulation, kidney ultrastructure, electron transmission microscopy, molecular sequence data, Notch receptors genetics.

Maak, S., K. Neumann, and H.H. Swalve (2006). **Identification and analysis of putative regulatory sequences for the MYF5/MYF6 locus in different vertebrate species**. *Gene* 379: 141-147 . ISSN: 0378 1119.

NAL Call Number: QH442.A1G4

Abstract: The myogenic factors (MYF) 5 and 6 are integral to the initiation and development of skeletal muscle and to the maintenance of its phenotype. Thus, they are candidate genes for growth- and meat quality-related traits. We performed a comparative sequence analysis of the MYF5/MYF6 locus in swine, cattle, dog, chicken and zebrafish on the basis of structural and functional information from human and mouse. Beside the characterization of upstream regulatory elements recently identified in mice, we demonstrate the existence of further highly conserved elements (E1 to E4) which may play a role in the regulation of MYF5 and MYF6 expression. Comparative sequence analysis of putative regulatory sequences in swine revealed a total of 21 single nucleotide polymorphisms (SNP) including 1 and 6 SNPs new for the promoters of MYF5 and MYF6, respectively. The conserved organization of the locus in vertebrates indicates a common basic mechanism of muscle development. However, the existence of numerous regulatory elements at large distances to MYF5 and MYF6 points to a very complex pattern of the gene regulation with significant differences between species. Copyright © 2008 CABI

Descriptors: zebrafish, genes, loci , nucleotide sequences, phenotypes, poultry , promoters, regulatory sequences, single nucleotide polymorphism, cattle, *Danio rerio*, dogs, fowls, pigs.

Maaswinkel, H., B. Mason, and L. Li (2003). **ENU-induced late-onset night blindness associated with rod photoreceptor cell degeneration in zebrafish.** *Mechanisms of Ageing and Development* 124(10-12): 1065-71. ISSN: 0047-6374.

NAL Call Number: QP84.M4

Abstract: We describe a dominant mutation, night blindness d (nbd), that causes late-onset rod photoreceptor cell degeneration in zebrafish. The mutation was induced by treating male zebrafish with N-ethyl-N-nitrosourea (ENU). Visual sensitivity was tested using a behavioral assay based on a visually mediated escape response. At a young age, the heterozygous (nbd+/-) fish did not show any signs of night blindness or retinal degeneration. At 2 years, their behaviorally assessed visual sensitivity was decreased, albeit no alterations in the electroretinogram (ERG) were detected. Histology revealed that in the mutant retinas the rod photoreceptor cell outer segments (ROS) were thinned out. In homozygous larvae (nbd-/-), mass neural degeneration was detectable at about 2 days post fertilization (dpf). They died at an early age, usually no later than 8 dpf. In conclusion, nbd is a dominant mutation that causes late-onset night blindness with slow progression. However, nbd is not photoreceptor cell-specific, as becomes clear from the systemic dysfunctions of the homozygous larvae.

Descriptors: zebrafish, dominant genes drug effects, mutation, night blindness genetics, rods retina, behavior, electroretinography, escape reaction, ethylnitrosourea pharmacology, homozygote, retina pathology, retina physiopathology, retinal degeneration pathology, physiopathology and genetics, retinal degeneration psychology, vision.

Mabee, P.M., G. Arratia, M. Coburn, M. Haendel, E.J. Hilton, J.G. Lundberg, R.L. Mayden, N. Rios, and M. Westerfield (2007). **Connecting evolutionary morphology to genomics using ontologies: a case study from Cypriniformes including zebrafish.** *Journal of Experimental Zoology. Part B. Molecular and Developmental Evolution* 308(5): 655-68. ISSN: 1552-5007.

NAL Call Number: QL1.J87

Descriptors: zebrafish, body patterning physiology, Cypriniformes embryology and

genetics, evolution, genomics, body patterning genetics, computational biology methods, embryonic development, models, mutation physiology, phenotype.

Mably, J.D., M.A. Mohideen, C.G. Burns, J.N. Chen, and M.C. Fishman (2003). **Heart of glass regulates the concentric growth of the heart in zebrafish.** *Current Biology* 13(24): 2138-47. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: BACKGROUND: Patterned growth of vertebrate organs is essential for normal physiological function, but the underlying pathways that govern organotypic growth are not clearly understood. Heart function is critically dependent upon the concentric thickening of the ventricular wall generated by the addition of cells to the myocardium along the axis from the endocardium (inside) to the outside of the chamber. In heart of glass mutant embryos, the number of cells in the myocardium is normal, but they are not added in the concentric direction. As a consequence, the chambers are huge and dysfunctional, and the myocardium remains a single layer. RESULTS: To begin to define the factors controlling the concentric growth of cells in the myocardium, we used positional cloning to identify the heart of glass (heg) gene. heg encodes a protein of previously undescribed function, expressed in the endocardial layer of the heart. By alternative splicing, three distinct isoforms are generated, one of which is predicted to be transmembrane and two other secreted. By selective morpholino perturbation, we demonstrate that the transmembrane form is critical for the normal pattern of growth. CONCLUSIONS: heart of glass encodes a previously uncharacterized endocardial signal that is vital for patterning concentric growth of the heart. Growth of the heart requires addition of myocardial cells along the endocardial-to-myocardial axis. This axis of patterning is driven by heg, a novel transmembrane protein expressed in the endocardium.

Descriptors: zebrafish, developmental gene expression regulation, heart embryology, membrane glycomyocardium cytology, amino acid sequence, chromatography, DNA primers, gene transfer techniques, histological techniques, immunohistochemistry, in situ hybridization, electron microscopy, molecular sequence data, myocardium ultrastructure, organogenesis genetics, protein conformation, reverse transcriptase PCR, DNA sequence analysis, signal transduction.

Mackenzie, N.C., M. Brito, A.E. Reyes, and M.L. Allende (2004). **Cloning, expression pattern and essentiality of the high-affinity copper transporter 1 (ctr1) gene in zebrafish.** *Gene* 328: 113-20. ISSN: 0378-1119.

NAL Call Number: QH442.A1G4

Abstract: The high-affinity copper transporter 1 (Ctr1) is a highly conserved transmembrane protein that mediates the internalization of copper ions from the extracellular medium. In this study, we have isolated the zebrafish ctr1 gene. The zebrafish ctr1 cDNA encodes a protein with 69% identity to the human orthologue and shows conservation of specific amino acid residues involved in copper transport. We find only a single ctr1 gene in the zebrafish genome which maps to linkage group 5. The genomic structure of the zebrafish gene shows that it consists of five exons and that exon-intron boundaries are absolutely conserved with the mammalian ctr1 genes. Expression in embryos was analyzed by reverse transcription-polymerase chain reaction (RT-PCR) and by in situ hybridization. Zebrafish ctr1 is maternally loaded, and transcripts can be detected throughout development and in adult fish. Distribution of ctr1 message appears ubiquitous during early stages becoming restricted to the brain and ventral tissues by 24 h post fertilization (hpf). Beginning at 3 days post fertilization (dpf), expression is found mainly in the developing intestine. Specific knockdown of ctr1 by antisense morpholino oligonucleotides (MOs) causes early larval

lethality. Defects include cell death in tissues where ctr1 is most heavily expressed, a finding similar to that described for a mouse knockout of mCtr1. Despite the existence of at least one other copper transport mechanism in the fish, our studies show that zebrafish ctr1 is an essential gene for development.
Descriptors: zebrafish, cation transport gene expression profiling, membrane transport proteins genetics, amino acid and base sequence, binding sites genetics, cation transport proteins metabolism, molecular cloning, complementary DNA chemistry and genetics, drug effects, metabolism, embryonic development, developmental gene expression regulation drug effects, essential in situ hybridization, introns, exons, microinjections, molecular sequence data, antisense oligonucleotides pharmacology, messenger RNA genetics and metabolism, reverse transcriptase PCR, sequence alignment, DNA sequence analysis, amino acid sequence homology.

Macqueen, D.J. and I.A. Johnston (2008). **Evolution of follistatin in teleosts revealed through phylogenetic, genomic and expression analyses**. *Development Genes and Evolution* 218(1): 1-14. ISSN: 0949-944X.
NAL Call Number: QL951.R68
Descriptors: zebrafish, evolution, follistatin, teleost, analyses, phylogeny.

MacRae, C.A. and R.T. Peterson (2003). **Zebrafish-based small molecule discovery.** *Chemistry and Biology* 10(10): 901-8. ISSN: 1074 5521.
NAL Call Number: QP501
Descriptors: zebrafish, preclinical drug evaluation, models, cardiovascular system embryology, central nervous system embryology, cloning, disease models, ear embryology, drug effects, genetic screening , genetically modified, phenotype, skin embryology.

Maldonado, E., F. Hernandez, C. Lozano, M.E. Castro, and R.E. Navarro (2006). **The zebrafish mutant vps18 as a model for vesicle-traffic related hypopigmentation diseases.** *Pigment Cell Research* 19(4): 315-26. ISSN: 0893-5785.
Abstract: Hypopigmentation is a characteristic of several diseases associated with vesicle traffic defects, like the Hermansky-Pudlak, Chediak-Higashi, and Griscelli syndromes. Hypopigmentation is also a characteristic of the zebrafish mutant vps18(hi2499A), which is affected in the gene vps18, a component of the homotypic fusion and protein sorting complex that is involved in tethering during vesicular traffic. Vps18, as part of this complex, participates in the formation of early endosomes, late endosomes, and lysosomes. Here, we show that Vps18 is also involved in the formation of melanosomes. In the zebrafish mutant vps18(hi2499A) the retroviral insertion located at exon 4 of vps18, leads to the formation of two abnormal splicing variants lacking the coding sequence for the clathrin repeat and the RING finger conserved domains. A deficiency of Vps18 in zebrafish larvae results in hepatomegaly and skin hypopigmentation. We also observed a drastic reduction in the number of melanosomes in the eye's retinal pigmented epithelium along with the accumulation of immature melanosomes. A significant reduction in the vps18(hi2499A) larvae visual system capacity was found using the optokinetic response assay. We propose that the insertional mutant vps18(hi2499A) can be used as a model for studying hypopigmentation diseases in which vesicle traffic problems exist.
Descriptors: zebrafish, hypopigmentation genetics, mutant transport vesicles pathology, ubiquitin protein ligases genetics, biological adaptation, alternative splicing genetics, amino acid sequence, biological transport genetics hepatomegaly etiology, hypopigmentation etiology, molecular sequence data, optokinetic nystagmus, phenotype, phylogeny, pigment

epithelium of eye abnormalities, point mutation, protein isoforms genetics, amino acid sequence homology, transport vesicles genetics.

Mangos, S., Y. Liu, and I.A. Drummond (2007). **Dynamic expression of the osmosensory channel trpv4 in multiple developing organs in zebrafish.** *Gene Expression Patterns* 7(4): 480-4. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Transient receptor potential channels function in a wide spectrum of tissues and transduce sensory stimuli. The vanilloid (capsaicin) channel TRPV4 is sensitive to osmotic changes and plays a central role in osmoregulatory responses in a variety of organisms. We cloned a zebrafish trpv4 cDNA and assayed its expression during embryogenesis. trpv4 is expressed as maternal mRNA in 4-cell embryos and later zygotic expression is first observed in the forming notochord at the one somite stage. Notochord expression persists to 24 hpf when broad expression in the brain is observed. At 32 hpf trpv4 expression is observed in the endocardium, restricted primarily to the ventricular endothelium. Low level expression of trpv4 is also seen from 32-48 hpf in the pronephric kidney with strongest expression in the most distal nephron segment and in the cloaca. Expression is also observed in lateral line organs starting at 32 hpf, primarily in the hair cells. At 72 hpf, expression of trpv4 in heart, kidney, brain, and lateral line organs persists while expression in the notochord is down-regulated.

Descriptors: zebrafish, TRPV cation channels genetics, amino acid sequence, complementary DNA, metabolism, gene expression, lateral line system, molecular sequence data, notochord metabolism, phylogeny, messenger RNA, stored genetics, amino acid sequence homology.

Mann, C.J., Y. Hinits, and S.M. Hughes (2006). **Comparison of neurolin (ALCAM) and neurolin-like cell adhesion molecule (NLCAM) expression in zebrafish.** *Gene Expression Patterns* 6(8): 952-63. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Many immunoglobulin (Ig)-superfamily cell adhesion molecules influence skeletal muscle formation. In *Drosophila*, dumbfounded (duf/kirre), irreC, sticks and stones and hibris encode related Ig-family proteins expressed in subsets of neurons and muscle precursor cells. The family mediates cell migration, axon guidance and fusion of myoblasts. Despite the importance of these genes in invertebrate myogenesis, no obvious functional parallels are known in vertebrate myogenesis. Here we investigate the gene expression pattern and phylogenetic and protein-structural relationships of the duf-related molecules neurolin and neurolin-like cell adhesion molecule (NLCAM), members of the activated leukocyte cell adhesion molecule (ALCAM) sub-family of Ig-molecules. These proteins are among the closest to Duf/Kirre by sequence. During zebrafish development, neurolin is expressed in subsets of somite and muscle cells, heart and numerous sites of neuronal maturation. The new ALCAM-family member, NLCAM, appears to have arisen by duplication of neurolin/ALCAM. NLCAM is expressed widely during gastrulation, particularly in the nascent neural plate, but later becomes predominantly expressed in sites of muscle and nerve maturation and in the fin fold. The expression of each gene is often in groups of cells in similar parts of the embryo; for example, in the region of Rohon Beard neurons, trigeminal ganglion and fusing fast and migrating slow muscle fibres. However, expression can also be distinct and dynamic; for example, muscle pioneer fibres express neurolin but not NLCAM at high level. Both molecules are expressed in subsets of muscle precursors at times prior to fusion.

Descriptors: zebrafish, activated leukocyte cell adhesion molecule metabolism, neuronal

metabolism, activated leukocyte cell adhesion molecule genetics, amino acid sequence, cell adhesion molecules, neuronal genetics, central nervous system embryology, central nervous system metabolism gastrula metabolism, gene duplication, developmental gene expression regulation, molecular sequence data, phylogeny, amino acid sequence homology, somites metabolism, tissue distribution.

Mann, C.J., D.P. Osborn, and S.M. Hughes (2007). **Vestigial-like-2b (VITO-1b) and Tead-3a (Tef-5a) expression in zebrafish skeletal muscle, brain and notochord.** *Gene Expression Patterns* 7(8): 827-36. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: The vestigial gene has been shown to control skeletal muscle formation in *Drosophila* and the related Vestigial-like 2 (Vgl-2) protein plays a similar role in mice. Vgl-family proteins are thought to regulate tissue-specific gene expression by binding to members of the broadly expressed Scalloped/Tef/TEAD transcription factor family. Zebrafish have at least four Vgl genes, including two Vgl-2s, and at least three TEAD genes, including two Tead3s. We describe the cloning and expression of one member from each family in the zebrafish. A novel gene, vgl-2b, with closest homology to mouse and human vgl-2, is expressed transiently in nascent notochord and in muscle fibres as they undergo terminal differentiation during somitogenesis. Muscle cells also express a TEAD-3 homologue, a possible partner of Vgl-2b, during myoblast differentiation and early fibre assembly. Tead-3a is also expressed in rhombomeres, eye and epiphysis regions.
Descriptors: zebrafish , brain , Vestigial-like 2, Vgl-2, protein, TEAD-3a, skeletal muscle, notochord.

Manning, A.G., B.D. Crawford, A.J. Waskiewicz, and D.B. Pilgrim (2004). **UNC-119 homolog required for normal development of the zebrafish nervous system.** *Genesis* 40(4): 223-30. ISSN: 1526-954X.
NAL Call Number: QH426.D32
Abstract: The UNC-119 proteins, found in all metazoans examined, are highly conserved at both the sequence and functional levels. In the invertebrates *Caenorhabditis elegans* and *Drosophila melanogaster*, unc-119 genes are expressed pan-neurally. Loss of function of the unc-119 gene in *C. elegans* results in a disorganized neural architecture and paralysis. The function of UNC-119 proteins has been conserved throughout evolution, as transgenic expression of the human UNC119 gene in *C. elegans* unc-119 mutants restores a wild-type phenotype. However, the nature of the conserved molecular function of UNC-119 proteins is poorly understood. Although unc-119 genes are expressed throughout the nervous system of the worm and fly, the analysis of these genes in vertebrates has focused on their function in the photoreceptor cells of the retina. Here we report the characterization of an unc-119 homolog in the zebrafish. The Unc119 protein is expressed in various neural tissues in the developing zebrafish embryo and larva. Morpholino oligonucleotide (MO)-mediated knockdown of Unc119 protein results in a "curly tail down" phenotype. Examination of neural patterning demonstrates that these "curly tail down" zebrafish experience a constellation of neuronal defects similar to those seen in *C. elegans* unc-119 mutants: missing or misplaced cell bodies, process defasciculation, axon pathfinding errors, and aberrant axonal branching. These findings suggest that UNC-119 proteins may play an important role in the development and/or function of the vertebrate nervous system.
Descriptors: zebrafish embryos, nerve tissue nervous system embryology, amino acid sequence, body patterning, immunoblotting, molecular sequence data, nervous system

metabolism, radiation hybrid mapping, recombinant fusion proteins genetics and metabolism, amino acid sequence homology.

Manzoni, M., P. Colombi, N. Papini, L. Rubaga, N. Tiso, A. Preti, B. Venerando, G. Tettamanti, R. Bresciani, F. Argenton, G. Borsani, and E. Monti (2007). **Molecular cloning and biochemical characterization of sialidases from zebrafish (*Danio rerio*).** *Biochemical Journal, The* 408(3): 395-406. ISSN: 0264 6021.
NAL Call Number: 382B52
Abstract: Sialidases remove sialic acid residues from various sialo-derivatives. To gain further insights into the biological roles of sialidases in vertebrates, we exploited zebrafish (*Danio rerio*) as an animal model. A zebrafish transcriptome- and genome-wide search using the sequences of the human NEU polypeptides as templates revealed the presence of seven different genes related to human sialidases. neu1 and neu4 are the putative orthologues of the mammalian sialidases NEU1 and NEU4 respectively. Interestingly, the remaining genes are organized in clusters located on chromosome 21 and are all more closely related to mammalian sialidase NEU3. They were thus named neu3.1, neu3.2, neu3.3, neu3.4 and neu3.5. Using RT-PCR (reverse transcription-PCR) we detected transcripts for all genes, apart from neu3.4, and whole-mount in situ hybridization experiments show a localized expression pattern in gut and lens for neu3.1 and neu4 respectively. Transfection experiments in COS7 (monkey kidney) cells demonstrate that Neu3.1, Neu3.2, Neu3.3 and Neu4 zebrafish proteins are sialidase enzymes. Neu3.1, Neu3.3 and Neu4 are membrane-associated and show a very acidic pH optimum below 3.0, whereas Neu3.2 is a soluble sialidase with a pH optimum of 5.6. These results were further confirmed by subcellular localization studies carried out using immunofluorescence. Moreover, expression in COS7 cells of these novel zebrafish sialidases (with the exception of Neu3.2) induces a significant modification of the ganglioside pattern, consistent with the results obtained with membrane-associated mammalian sialidases. Overall, the redundancy of sialidases together with their expression profile and their activity exerted on gangliosides of living cells indicate the biological relevance of this class of enzymes in zebrafish.
Descriptors: zebrafish, molecular cloning, sialidases, neu1, neu4, gangliosides.

Mao, L., A.L. Bryantsev, M.B. Chechenova, and E.A. Shelden (2005). **Cloning, characterization, and heat stress-induced redistribution of a protein homologous to human hsp27 in the zebrafish *Danio rerio*.** *Experimental Cell Research* 306(1): 230-41. ISSN: 0014 4827.
NAL Call Number: 442.8EX7
Abstract: Hsp27 is a small heat shock protein (shsp) regulating stress tolerance and increasingly thought to play roles in tissue homeostasis and differentiation. The zebrafish *Danio rerio* is an important model for the study of developmental processes, but little is known regarding shsps in this animal. Here, we report the sequence, expression, regulation, and function of a zebrafish protein (zfHsp27) homologous to human Hsp27. zfHsp27 contains three conserved phosphorylatable serines and a cysteine important for regulation of apoptosis, but it lacks much of a C-terminal tail domain and shows low homology in two putative actin interacting domains that are features of mammalian Hsp27. zfHsp27 mRNA is most abundant in adult skeletal muscle and heart and is upregulated during early embryogenesis. zfHsp27 expressed in mammalian fibroblasts was phosphorylated in response to heat stress and anisomycin, and this phosphorylation was prevented by treatment with SB202190, an inhibitor of p38 MAPK. Expression of zfHsp27 and human Hsp27 in mammalian fibroblasts promoted a similar degree of tolerance to heat stress. zfHsp27 fusion proteins entered the nucleus and associated with the cytoskeleton of heat stressed cells in vitro and in zebrafish

embryos. These results reveal conservation in regulation and function of mammalian and teleost Hsp27 proteins and define zebrafish as a new model for the study of Hsp27 function.
Descriptors: zebrafish, amino acid and base sequence, cell nucleus metabolism, molecular cloning, cytoplasm metabolism, epithelial cells metabolism, gene expression profiling, developmental gene expression regulation, heat shock proteins immunology, genetics and metabolism, heat shock response physiology, mice, molecular sequence data, muscle cells metabolism, myofibrils metabolism, NIH 3t3 cells, neoplasm phosphorylation, protein transport, messenger RNA genetics and metabolism, reverse transcriptase PCR, amino acid sequence homology, transfection.

Mao, L. and E.A. Shelden (2006). **Developmentally regulated gene expression of the small heat shock protein Hsp27 in zebrafish embryos.** *Gene Expression Patterns* 6(2): 127-33. ISSN: 1567 133X.
NAL Call Number: QH450.B73
Abstract: Mammalian small heat shock proteins including Hsp27 and alpha-B crystallin are constitutively expressed in various adult and embryonic tissues including skeletal and cardiac muscle. However, the function of these proteins during embryonic development is not understood, and information on their expression during the earliest stages of development is limited. We have recently demonstrated constitutive and stress inducible expression of a homologue of human Hsp27 in adult zebrafish, an important experimental model of vertebrate developmental processes. Here, we assessed the temporospatial dynamics of zebrafish Hsp27 (hsp27) and alpha-B crystallin (cryab) gene expression using reverse-transcriptase PCR (RT-PCR) and hsp27 expression by in situ hybridization. Our results reveal that initial upregulation of hsp27 expression occurs during early gastrulation. Expression of hsp27 is detected transiently in developing myotomes, lens and brain, and more persistently in developing heart. The constitutive expression level of hsp27 in embryos at some stages of development is considerably greater than that observed in unstressed adult tissues. Expression of hsp27 was also observed in all tissues examined in embryos recovering from heat stress. The pattern of expression observed for hsp27 overlaps partially, but not completely, with that reported for other heat shock proteins.
Descriptors: zebrafish, heat shock base sequence, developmental gene expression regulation, in situ hybridization, messenger RNA genetics and metabolism, alpha crystallin B chain genetics.

Mara, A. and S.A. Holley (2007). **Oscillators and the emergence of tissue organization during zebrafish somitogenesis.** *Trends in Cell Biology* 17(12): 593-9. ISSN: 0962-8924.
NAL Call Number: QH573.T73
Abstract: Genetic networks that include positive and negative feedback can exhibit oscillations. These oscillations are a form of emergence, which is when novel patterns or properties arise during self organization of complex systems. Within the extending trunk and tail of the developing vertebrate embryo, the somitogenesis oscillator governs the periodic formation of segments that ultimately become the vertebral column and musculature. These oscillations occur within the context of noise created by cell movement, mitosis, and stochastic gene expression. Here, we review recent progress in our understanding of the role of the Notch signaling pathway in the zebrafish segmentation oscillator and our appreciation of how the oscillator interfaces with different sources of noise.
Descriptors: zebrafish, somitogenesis, genetic networks, tissue organization, oscillator.

Marlow, F., B. Holloway, D. Wagner, R. Dosch, W. Mei, T. Gupta, and M. Mullins (2006). **Maternal control of vertebrate development: molecular-genetics from the zebrafish**. *Genes and Genetic Systems* 81(2): 150. ISSN: 1341 7568.
NAL Call Number: QH426.G462
Descriptors: zebrafish, development, genetics, cloning, genetic techniques, development, maternal factors.

Marshall, W.F. (2004). **Human cilia proteome contains homolog of zebrafish polycystic kidney disease gene qilin.** *Current Biology* 14(21): R913-4 . ISSN: 0960 9822.
NAL Call Number: QH301.C85
Descriptors: zebrafish, *Chlamydomonas* genetics, cilia genetics, polycystic kidney diseases genetics, proteome genetics, computational biology.
Notes: Comment On: Curr Biol. 2004 Jul 27; 14(14):R575-7.

Martin, E.D. and M. Grealy (2004). **Plakoglobin expression and localization in zebrafish embryo development.** *Biochemical Society Transactions* 32(Pt 5): 797-8. ISSN: 0300 5127.
NAL Call Number: QD415.A1B58
Abstract: Plakoglobin (gamma-catenin) and beta-catenin are major components of the adherens junctions and can be localized to the nucleus by activation of the Wnt signalling pathway. In addition, plakoglobin is also found in desmosomes, a vertebrate-specific cell-cell adhesion structure. Plakoglobin expression and localization were examined at the protein level during zebrafish embryonic development by Western blotting and confocal microscopy. Plakoglobin was expressed throughout embryo development at the protein level. Western blotting revealed that embryonic plakoglobin protein content increased between 12- and 24-h post-fertilization (hpf). Confocal microscopy showed that at stages up to 12 hpf, plakoglobin and beta-catenin were co-localized and expressed in both the nucleus and in cell-cell junctions. At 24- and 72-hpf, separate patterns were seen for plakoglobin and beta-catenin. These data indicate that plakoglobin localization in the heart region shifts from adherens junctions to desmosomes during heart chamber development.
Descriptors: zebrafish, adherens junctions metabolism, cytoskeletal proteins biosynthesis, physiology and metabolism, cytoskeletal, desmosomes metabolism, physiology, developmental gene expression regulation, heart embryology, Western blotting, cell adhesion , desmoplakins, confocal microscopy, time factors, trans activators metabolism, proteins, beta and gamma catenin.

Mathavan, S., S.G.P. Lee, A. Mak, L.D. Miller, K.R. Krishna-Murthy, K.R. Govindarajan, Y. Tong, Y.L. Wu, S.H. Lam, H. Yang, Y. Ruan, V. Korzh, Z. Gong, E.T. Liu, and T. Lufkin (2005). **Transcriptome analysis of zebrafish embryogenesis using microarrays**. *Public Library of Science: Genetics* 1(2): 260-276. ISSN: 1553 7390.
Abstract: Zebrafish *(Danio rerio*) is a well-recognized model for the study of vertebrate developmental genetics, yet at the same time little is known about the transcriptional events that underlie zebrafish embryogenesis. Here we have employed microarray analysis to study the temporal activity of developmentally regulated genes during zebrafish embryogenesis. Transcriptome analysis at 12 different embryonic time points covering five different developmental stages (maternal, blastula, gastrula, segmentation, and pharyngula) revealed a highly dynamic transcriptional profile. Hierarchical clustering, stage-specific clustering, and algorithms to detect onset and peak of gene expression revealed clearly demarcated transcript clusters with maximum gene activity at distinct developmental stages as well as coregulated expression of gene groups involved in dedicated functions such as organogenesis. Our study

also revealed a previously unidentified cohort of genes that are transcribed prior to the mid-blastula transition, a time point earlier than when the zygotic genome was traditionally thought to become active. Here we provide, for the first time to our knowledge, a comprehensive list of developmentally regulated zebrafish genes and their expression profiles during embryogenesis, including novel information on the temporal expression of several thousand previously uncharacterized genes. The expression data generated from this study are accessible to all interested scientists from our institute resource database (http://giscompute.gis.a-star.edu.sg/similar to govind/zebrafish/data_download.html). Copyright©Thomson Reuters 2009
Descriptors: zebrafish, methods and techniques, development, molecular genetics, microarray analysis, genetic techniques, algorithm, mathematical and computer techniques, transcriptome analysis , genetic techniques, hierarchical clustering, embryogenesis, genbank sequence data, developmental stage, mid blastula transition, transcriptional event.

Mathieu, M., S. Trombino, F. Argenton, D. Larhammar, and M. Vallarino (2005). **Developmental expression of NPY/PYY receptors zYb and zYc in zebrafish.** *Annals of the New York Academy of Sciences* 1040: 399-401. ISSN: 0077 8923.
NAL Call Number: 500 N484
Abstract: This study describes the developmental expression of zYb and zYc receptors in zebrafish. RT-PCR demonstrated that both mRNAs are present from 24-hour postfertilization (hpf) and that their expression increased during larval development. Whole-mount in situ hybridization showed zYb mRNA expression in the epithalamus (24 hpf), telencephalon, hypothalamus, rhombencephalon (24-96 hpf), mesencephalon (48-96 hpf), hatching gland, and otic vesicle (48-64 hpf). zYc mRNA is expressed in the epithalamus (24 and 96 hpf), telencephalon (48-96 hpf), spinal cord, notochord (64 hpf), and hatching gland (96 hpf). These results show that zYb and zYc receptors are expressed during ontogeny, suggesting a role for neuropeptide Y (NPY) and/or peptide YY (PYY) in organogenesis.
Descriptors: zebrafish, developmental gene expression regulation physiology, neuropeptide Y biosynthesis, gastrointestinal hormone receptors biosynthesis, metabolism, neuropeptide Y genetics, gastrointestinal hormone receptors genetics.

Matsui, T., A. Raya, Y. Kawakami, C. Callol Massot, J. Capdevila, C. Rodriguez Esteban, and J.C. Izpisua Belmonte (2005). **Noncanonical Wnt signaling regulates midline convergence of organ primordia during zebrafish development.** *Genes and Development* 19(1): 164-75. ISSN: 0890-9369.
NAL Call Number: QH426.G46
Abstract: Several components of noncanonical Wnt signaling pathways are involved in the control of convergence and extension (CE) movements during zebrafish and *Xenopus* gastrulation. However, the complexity of these pathways and the wide patterns of expression and activity displayed by some of their components immediately suggest additional morphogenetic roles beyond the control of CE. Here we show that the key modular intracellular mediator Dishevelled, through a specific activation of RhoA GTPase, controls the process of convergence of endoderm and organ precursors toward the embryonic midline in the zebrafish embryo. We also show that three Wnt noncanonical ligands wnt4a, silberblick/wnt11, and wnt11-related regulate this process by acting in a largely redundant way. The same ligands are also required, nonredundantly, to control specific aspects of CE that involve interaction of Dishevelled with mediators different from that of RhoA GTPase. Overall, our results uncover a late, previously unexpected role of noncanonical Wnt signaling in the control of midline assembly of organ precursors during vertebrate embryo development.
Descriptors: zebrafish, animal structures growth and development, intercellular signaling

peptides and, morphogenesis, signal transduction physiology, signal transducing adaptor proteins, animal structures embryology, embryo, endoderm, gastrula, glyco, growth and development, phospho, proto oncogene, Wnt proteins, *Xenopus* proteins, proteins, Rho GTP binding proteins metabolism.

Matthews, R.P., K. Lorent, and M. Pack (2005). **The zebrafish ortholog of onecut-3 functions upstream of hnf-6 during biliary development**. *Hepatology* 42(4, Suppl. 1): 741A-742A. ISSN: 0270 9139.
Descriptors: zebrafish, development, molecular genetics, digestive system, ingestion and assimilation, ortholog, biliary development, upstream function.

McClintock, J.M., C. Jozefowicz, S. Assimacopoulos, E.A. Grove, A. Louvi, and V.E. Prince (2003). **Conserved expression of Hoxa1 in neurons at the ventral forebrain/midbrain boundary of vertebrates**. *Development Genes and Evolution* 213(8): 399-406. ISSN: 0949-944X.
NAL Call Number: QL951.R68
Descriptors: zebrafish, amphibiotic species, central nervous system, conserved sequence, evolution , forebrain, freshwater fish, gene expression, mesencephalon, neurons, spinal cord, *Danio rerio*, *Oryzias latipes*, *Xenopus*, Hoxa1 gene.

McCormick, L.J., J.A. Hutt, J. Hazan, C. Houart, and J. Cohen (2007). **The homeodomain transcription factor drg11 is expressed in primary sensory neurons and their putative CNS targets during embryonic development of the zebrafish.** *Gene Expression Patterns* 7(3): 289-96. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: The drg11 gene is a member of the vertebrate aristaless-related gene family and encodes a paired homeodomain transcription factor. Its expression is largely restricted to PNS neurons subserving somatosensory functions and their CNS targets in rodents. The phenotype of drg11 null mice suggests that it is crucial for the proper development in the embryo of nociceptive circuits. To allow functional studies in the zebrafish, a simple vertebrate model organism, we have cloned the homologous gene and studied its expression throughout embryonic development. drg11 transcripts are first detected at neurula stage in the developing trigeminal ganglion, where it persists throughout development. This is followed by transient expression in spinal cord mechanosensory Rohon-Beard neurons shortly before axogenesis. Expression is later evident in neuronal populations of the dorsal spinal cord and in the dorsal root ganglia. In the developing brain, drg11 expression is mainly restricted to sensory neuron populations of the midbrain and hindbrain, in cranial sensory ganglia and in the habenula. Unlike rodents, however, trochlear motor neurons transiently express drg11. Our results suggest that drg11 expression in the developing zebrafish is, in common with its mammalian homologous gene, predominantly localised to neurons in sensory processing areas of the embryonic nervous system and is both spatially and temporally regulated.
Descriptors: zebrafish, developmental gene expression regulation, afferent neurons metabolism, transcription factors genetics, amino acid sequence, central nervous system, molecular cloning, metabolism, embryonic development, sensory ganglia embryology and metabolism , mesencephalon embryology and metabolism, molecular sequence data, organ specificity, prosencephalon embryology and metabolism, rhombencephalon embryology and metabolism, sequence alignment, spinal cord embryology, spinal cord metabolism, transcription factors metabolism.

McDermott, B.M.J., J.M. Baucom, and A.J. Hudspeth (2007). **Analysis and functional evaluation of the hair-cell transcriptome**. *Proceedings of the National Academy of Sciences, USA* 104(28): 11820-11825. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, adhesion, cytoskeleton, DNA microarrays , deafness, freshwater fish, gene expression, hair cells, morphogenesis, mutations, oligonucleotides, phylogenetics, RNA, signal transduction, synaptic transmission, transcription, *Danio rerio.*

Mcloughlin, S., R.T. Bree, S. O'boyle, M. Grealy, and L. Byrnes (2004). **Gene expression at the midblastula transition in zebrafish**. *Biochemical Society Transactions* 32(Part 4): 135A. ISSN: 0300 5127.
NAL Call Number: QD415.A1B58
Descriptors: zebrafish, development, molecular genetics, semiquantitative reverse transcriptase PCR, genetic techniques, suppression subtractive hybridization, whole mount in situ hybridization, histology and cytology techniques, cell division, gastrulation, midblastula transition, sequence similarity, zygotic transcription.

McNeill, M.S., J. Paulsen, G. Bonde, E. Burnight, M.Y. Hsu, and R.A. Cornell (2007). **Cell death of melanophores in zebrafish trpm7 mutant embryos depends on melanin synthesis.** *Journal of Investigative Dermatology* 127(8): 2020-30. ISSN: 0022-202X.
Abstract: Transient receptor potential melastatin 7 (TRPM7) is a broadly expressed, nonselective cation channel. Studies in cultured cells implicate TRPM7 in regulation of cell growth, spreading, and survival. However, zebrafish trpm7 homozygous mutants display death of melanophores and temporary paralysis, but no gross morphological defects during embryonic stages. This phenotype implies that melanophores are unusually sensitive to decreases in Trpm7 levels, a hypothesis we investigate here. We find that pharmacological inhibition of caspases does not rescue melanophore viability in trpm7 mutants, implying that melanophores die by a mechanism other than apoptosis. Consistent with this possibility, ultrastructural analysis of dying melanophores in trpm7 mutants reveals abnormal melanosomes and evidence of a ruptured plasma membrane, indicating that cell death occurs by necrosis. Interestingly, inhibition of melanin synthesis largely prevents melanophore cell death in trpm7 mutants. These results suggest that melanophores require Trpm7 in order to detoxify intermediates of melanin synthesis. We find that unlike TRPM1, TRPM7 is expressed in human melanoma cell lines, indicating that these cells may also be sensitized to reduction of TRPM7 levels.
Descriptors: zebrafish embryos, melanins biosynthesis, melanophores cytology, TRPMcation channels physiology, apoptosis, caspases antagonists and inhibitors, tumor cell line, cell survival, cytology, magnesium pharmacology, melanoma chemistry, mutation, proto oncogene proteins C kit physiology, TRPMcation channels analysisand genetics, tumor suppressor protein p53 antagonists, inhibitors and physiology.

McPartland, J.M., M. Glass, I. Matias, R.W. Norris, and C.W. Kilpatrick (2007). **A shifted repertoire of endocannabinoid genes in the zebrafish (*Danio rerio*).** *Molecular Genetics and Genomics* 277(5): 555-70. ISSN: 1617-4615.
NAL Call Number: Q431.M552
Abstract: The zebrafish has served as a model organism for developmental biology. Sequencing its genome has expanded zebrafish research into physiology and drug-development testing. Several cannabinoid pharmaceuticals are in development, but expression of endocannabinoid receptors and enzymes remains unknown in this species. We conducted a

bioinformatics analysis of the zebrafish genome using 17 human endocannabinoid genes as a reference set. Putative zebrafish orthologs were identified in filtered BLAST searches as reciprocal best hits. Orthology was confirmed by three in silico methods: phylogenetic testing, synteny analysis, and functional mapping. Zebrafish expressed orthologs of cannabinoid receptor 1, transient receptor potential channel vanilloid receptor 4, GPR55 receptor, fatty acid amide hydrolase 1, monoacylglycerol lipase, NAPE-selective phospholipase D, abhydrolase domain-containing protein 4, and diacylglycerol lipase alpha and beta; and paired paralogs of cannabinoid receptor 2, fatty acid amide hydrolase 2, peroxisome proliferator-activated receptor alpha, prostaglandin-endoperoxide synthase 2, and transient receptor potential cation channel subtype A1. Functional mapping suggested the orthologs of transient receptor potential vanilloid receptor 1 and peroxisome proliferator-activated receptor gamma lack specific amino acids critical for cannabinoid ligand binding. No orthologs of N-acylethanolamine acid amidase or protein tyrosine phosphatase, non-receptor type 22 were identified. In conclusion, the zebrafish genome expresses a shifted repertoire of endocannabinoid genes. In vitro analyses are warranted before using zebrafish for cannabinoid development testing.

Descriptors: zebrafish, endocannabinoids genetics, algorithms, amidohydrolases genetics, amino acid sequence, calcium channels, chromosome mapping methods, cyclooxygenase 2 genetics, genome, lipoprotein lipase genetics, membrane molecular sequence data, nerve tissue proteins, ppar alpha and ppar gamma genetics, phospholipase D genetics, phylogeny, protein tyrosine phosphatase, cannabinoid receptor CB1 and CB2 genetics, TRPV cation channels.

McWhorter, M.L., U.R. Monani, A.H. Burghes, and C.E. Beattie (2003). **Knockdown of the survival motor neuron (Smn) protein in zebrafish causes defects in motor axon outgrowth and pathfinding.** *Journal of Cell Biology* 162(5): 919-31. ISSN: 0021-9525.

NAL Call Number: 442.8J828

Abstract: Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by a loss of alpha motoneurons in the spinal cord. SMA is caused by low levels of the ubiquitously expressed survival motor neuron (Smn) protein. As it is unclear how low levels of Smn specifically affect motoneurons, we have modeled SMA in zebrafish, a vertebrate model organism with well-characterized motoneuron development. Using antisense morpholinos to reduce Smn levels throughout the entire embryo, we found motor axon-specific pathfinding defects. Reduction of Smn in individual motoneurons revealed that smn is acting cell autonomously. These results show for the first time, in vivo, that Smn functions in motor axon development and suggest that these early developmental defects may lead to subsequent motoneuron loss.

Descriptors: zebrafish, axons metabolism, cell movement physiology, motor neurons metabolism, nerve tissue proteins metabolism, cell death physiology, cyclic AMP response element binding protein, in situ hybridization, motor neurons cytology, muscular atrophy, spinal metabolism, nerve tissue neuromuscular junction, antisense oligonucleotides metabolism, RNA binding proteins, cholinergic receptors metabolism.

Mei, W., D. Wagner, R. Dosch, and M.C. Mullins (2004). **The maternal-effect gene, brom bones, regulates the cytoskeleton in egg activation and establishment of the dorsal-ventral axis**. *Molecular Biology of the Cell* 15(Suppl. S): 336A. ISSN: 1059 1524.

NAL Call Number: QH604.C452

Descriptors: zebrafish, biochemistry and molecular biophysics, egg activation, dorsal ventral axis.

Mellgren, E.M. and S.L. Johnson (2005). **Kitb, a second zebrafish ortholog of mouse Kit.** *Development Genes and Evolution* 215(9): 470-77. ISSN: 0949 944X.

NAL Call Number: QL951.R68

Abstract: The large numbers of duplicated pairs of genes in zebrafish compared to their mammalian counterparts has lead to the notion that expression of zebrafish co-orthologous pairs in some cases can together describe the expression of their mammalian counterpart. Here, we explore this notion by identification and analysis of a second zebrafish ortholog of the mammalian Kit receptor tyrosine kinase (kitb). We show that in embryos, kitb is expressed in a non-overlapping pattern to that of kita, in the anterior ventral mesoderm, Rohon-beardRohon-Beard neurons, the otic vesicle, and trigeminal ganglia. The expression pattern of kita and kitb in zebrafish together approximates that of Kit in mouse, with the exception that neither zebrafish kit gene is expressed in primordial germ cells, a site of kit expression in the mouse embryo. In addition, zebrafish kita is expressed in a site of zebrafish primitive hematopoiesis but not required for blood development, and we fail to detect kitb expression in sites of zebrafish hematopoiesis. Thus, the expression and function of zebrafish kit genes cannot be described as a simple partition of the expression and function of mouse Kit. We discuss the possibility that these unaccounted for expression domains and functions are derived from more ancestral gene duplications and partitioning instead of the relatively recent teleost teleost-specific duplication.

Descriptors: zebrafish embryo, mice genetics, proto oncogene proteins C kit genetics, embryo metabolism, gene duplication, in situ hybridization, phylogeny, trigeminal ganglion embryology.

Mellgren, E.M. and S.L. Johnson (2006). **Pyewacket, a new zebrafish fin pigment pattern mutant.** *Pigment Cell Research* 19(3): 232-8. ISSN: 0893-5785.

Abstract: Many mutants that disrupt zebrafish embryonic pigment pattern have been isolated, and subsequent cloning of the mutated genes causing these phenotypes has contributed to our understanding of pigment cell development. However, few mutants have been identified that specifically affect development of the adult pigment pattern. Through a mutant screen for adult pigment pattern phenotypes, we identified pyewacket (pye), a novel zebrafish mutant in which development of the adult caudal fin pigment pattern is aberrant. Specifically, pye mutants have fin melanocyte pigment pattern defects and fewer xanthophores than wild-type fins. We mapped pye to an interval where a single gene, the zebrafish ortholog of the human gene DHRSX, is present. pye will be an informative mutant for understanding how xanthophores and melanocytes interact to form the pigment pattern of the adult zebrafish fin.

Descriptors: zebrafish, mutation, pigmentation genetics, chromatophores physiology, melanocytes physiology, phenotype.

Mellgren, E.M. and S.L. Johnson (2004). **A requirement for kit in embryonic zebrafish melanocyte differentiation is revealed by melanoblast delay.** *Development Genes and Evolution* 214(10): 493-502. ISSN: 0949 944X.

NAL Call Number: QL951.R68

Abstract: Exploring differences in gene requirements between species can allow us to delineate basic developmental mechanisms, provide insight into patterns of evolution, and explain heterochronic differences in developmental processes. One example of differences in gene requirements between zebrafish and mammals is the requirement of the kit receptor tyrosine kinase in melanocyte development. kit is required for migration, survival and differentiation of all neural crest-derived melanocytes in mammals. In contrast, zebrafish kit is not required

for differentiation of embryonic melanocytes during normal development. When melanoblast development in zebrafish embryos is delayed by injecting morpholinos targeted to the mitfa gene, we show that these delayed melanoblasts fail to differentiate in kit mutants. Thus, we show that there is a kit requirement for melanocyte differentiation in zebrafish when melanoblast development is delayed. Furthermore, we show that kit is not involved in maintaining melanocyte precursors through the developmental delay, but instead is required for differentiation of melanocytes after the block on their development is removed. Finally, we suggest there is a heterochronic shift in the onset of melanocyte differentiation between fish and mouse, and developmental delay of melanoblast development in zebrafish removes this heterochronic difference.

Descriptors: zebrafish, cell differentiation physiology, developmental gene expression regulation, melanocytes metabolism, proto oncogene proteins C kit metabolism, in situ hybridization, melanocytes physiology, morphogenesis, oligonucleotides, species specificity, temperature, time factors.

Mendelsohn, B.A., C. Yin, S.L. Johnson, T.P. Wilm, L. Solnica-Krezel, and J.D. Gitlin (2006). **Atp7a determines a hierarchy of copper metabolism essential for notochord development**. *Cell Metabolism* 4(2): 155-162. ISSN: 1550 4131.

Abstract: The critical developmental and genetic requirements of copper metabolism during embryogenesis are unknown. Utilizing a chemical genetic screen in zebrafish, we identified small molecules that perturb copper homeostasis. Our findings reveal a role for copper in notochord formation and demonstrate a hierarchy of copper metabolism within the embryo. To elucidate these observations, we interrogated a genetic screen for embryos phenocopied by copper deficiency, identifying calamity, a mutant defective in the zebrafish ortholog of the Menkes disease gene (atp7a). Copper metabolism in calamity is restored by human ATP7A, and transplantation experiments reveal that atp7a functions cell autonomously, findings with important therapeutic implications. The gene dosage of atp7a determines the sensitivity to copper deprivation, revealing that the observed developmental hierarchy of copper metabolism is informed by specific genetic factors. Our data provide insight into the developmental pathophysiology of copper metabolism and suggest that suboptimal copper metabolism may contribute to birth defects. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, nutrition, metabolism, molecular genetics, development, copper deficiency, nutritional disease, genetic screening, techniques, embryogenesis, copper deprivation.

Mendenhall, E.M., C.E. Eckfeldt, C.M. Flynn, T. Wang, S.C. Ekker, and C.M. Verfaillie (2005). **Identification of novel regulators of hematopoiesis by combining human hsc expression profiling and genetic screening in zebrafish**. *Experimental Hematology* 33(7, Suppl. 1): 56. ISSN: 0301 472X.

NAL Call Number: RB145.E97

Descriptors: zebrafish, blood and lymphatics, transport and circulation, molecular genetics, genetic screening, genetic techniques, transplantation therapy, therapeutic and prophylactic techniques, quantitative reverse transcriptase PCR, hematopoiesis.

Menuet, A. (2003). ***Expression et regulation des recepteurs aux oestrogenes et de la forme cerebrale de l'aromatase chez les poissons teleosteens [Expression and regulation of estrogen receptors and brain aromatase in teleost fish.]***. Dissertation, Universite Rennes 1, Rennes, France: 169 p.

Descriptors: zebrafish, fish, gene expression, hormones, receptors, sex hormones, Cyprini-

dae, *Danio rerio*, *Oncorhynchus mykiss*, Pisces, Salmonidae, carps, rainbow trout, salmonids, physiology, biochemistry, biophysics.
Notes: Author's degree:Thesis(Biologie);Order number: 2845 Includes a bibliography: 313refs.

Menuet, A., Y.l. Page, O. Torres, L. Kern, O. Kah, and F. Pakdel (2004). **Analysis of estrogen regulation of zebrafish estrogen receptor reveals distinct effect of ERa, ER beta 1 and ER beta 2**. *Journal of Molecular Endocrinology* 32(3): 975-986. ISSN: 0952 5041.
NAL Call Number: QP187.3.M64J68
Abstract: We have previously cloned and characterized three estrogen receptors in zebrafish (zfER alpha , zfER beta 1 and zfER beta 2). We have also shown that they are functional in vitro and exhibit a distinct expression pattern although partial overlapping in the brain of zebrafish. In this paper, we show that the hepatic expression of these zfER genes responds differently to estradiol (E2). In fact, a 48-h direct exposure of zebrafish to E2 results in a strong stimulation of the zfER alpha gene expression while zfER beta 1 gene expression is markedly reduced and zfER beta 2 remains virtually unchanged. To establish the potential implication of each zfER in the E2-upregulation of the zfER alpha gene, the promoter region of this gene was isolated and characterized. Transfection experiments with promoter-luciferase reporter constructs together with different zfER expression vectors were carried out in different cell-contexts. These data showed that in vivo E2-upregulation of zfER alpha gene requires ER alpha itself and a conserved transcription unit sequence including at least an imperfect ERE and an AP1/ERE half site at the proximal transcription initiation site. Interestingly, although in the presence of E2, zfER alpha is the most potent to induce the expression of its own gene, the effect of E2 mediated by zfER beta 2 represents 50% of the zfER alpha activity. In contrast, zfER beta 1 is unable to upregulate the zfER alpha gene whereas this receptor form is able to tightly bind E2 and activate a reporter plasmid containing a consensus ERE. Altogether these results indicate that the two ER beta forms recently characterized in teleost fish could have partially distinct and not redundant functions. Copyright © 2008 CABI
Descriptors: zebrafish, estradiol, gene expression , genes, hormonal control, liver, estrogen receptors, plasmids, promoters, reporter genes, transcription, *Danio rerio.*

Meyer, M.P., J.S. Trimmer, J.D. Gilthorpe, and S.J. Smith (2005). **Characterization of zebrafish PSD-95 gene family members.** *Journal of Neurobiology* 63(2): 91-105. ISSN: 0022-3034.
NAL Call Number: QP351.J55
Descriptors: zebrafish embryo, nerve tissue nucleoside phosphate kinase genetics, Western blotting methods, COS cells, *Cercopithecus aethiops*, molecular cloning methods, Cricetinae, gene expression physiology and developmental regulation physiology, guanylate kinase, immunohistochemistry methods, in situ hybridization methods, intracellular signaling peptides and proteins, membrane proteins, mice, nerve tissue proteins classification and physiology, nervous system growth, development and metabolism, nucleoside phosphate kinase metabolism, phylogeny, rats, saposins metabolism, sequence alignment methods.

Mhanni, A.A. and R.A. McGowan (2004). **Global changes in genomic methylation levels during early development of the zebrafish embryo.** *Development Genes and Evolution* 214(8): 412-7. ISSN: 0949 944X.
NAL Call Number: QL951.R68
Abstract: We have examined the methylation status of the zebrafish genome during early embryogenesis and we find evidence that methylation fluxes do occur in that organism. The parental genetic contributions to the zygote are, initially, differently methylated with

the genome of the sperm being hypermethylated relative to the genome of the oocyte. Post-fertilization there is an immediate decrease in methylation of the embryonic genome but the methylation begins to increase rapidly and is re-established by the gastrulation stage. These results are consistent with the results of Santos et al. (Dev Biol 241:172-182, 2002), who examined the methylation of early mouse embryos, and this conservation argues that demethylation/re-methylation is an important part of vertebrate development.
Descriptors: zebrafish, DNA methylation, epigenesis, genetic, genome, autoradiography, Southern blotting, DNA primers and probes, embryology, oocytes metabolism, spermatozoa metabolism, staining and labeling.

Miao, M., A.E. Bruce, T. Bhanji, E.C. Davis, and F.W. Keeley (2007). **Differential expression of two tropoelastin genes in zebrafish.** *Matrix Biology* 26(2): 115-24. ISSN: 0945-053X.
NAL Call Number: QP552.C6
Abstract: Elastin is the extracellular matrix protein responsible for properties of extensibility and elastic recoil in large blood vessels, lung and skin of most vertebrates. Elastin is synthesized as a monomer, tropoelastin, but is rapidly transformed into its final polymeric form in the extracellular matrix. Until recently information on sequence and developmental expression of tropoelastins was limited to mammalian and avian species. We have recently identified and characterized two expressed tropoelastin genes in zebrafish. This was the first example of a species with multiple tropoelastin genes, raising the possibility of differential expression and function of these tropoelastins in elastic tissues of the zebrafish. Here we have investigated the temporal expression and tissue distribution of the two tropoelastin genes in developing and adult zebrafish. Expression was detected early in skeletal cartilage structures of the head, in the developing outflow tract of the heart, including the bulbus arteriosus and the ventral aorta, and in the wall of the swim bladder. While the temporal pattern of expression was similar for both genes, the upregulation of eln2 was much stronger than that of eln1. In general, both genes were expressed and their gene products deposited in most of the elastic tissues examined, with the notable exception of the bulbus arteriosus in which eln2 expression and its gene product was predominant. This finding may represent a sub-specialization of eln2 to provide the unique architecture of elastin and the specific mechanical properties required by this organ.
Descriptors: zebrafish, developmental gene expression regulation, myocardium metabolism, tropoelastin metabolism, amino acid sequence, Northern blotting, DNA primers, immunohistochemistry, in situ hybridization, molecular sequence data, peptides genetics, reverse transcriptase PCR, tropoelastin genetics, metabolism.

Miguel Queralt, S., G.V. Avvakumov, M. Blazquez, F. Piferrer, and G.L. Hammond (2005). **Sea bass (*Dicentrarchus labrax*) sexhormone binding globulin: molecular and biochemical properties and phylogenetic comparison of its orthologues in multiple fish species**. *Molecular and Cellular Endocrinology* 229(1/2): 21-29. ISSN: 0303 7207.
NAL Call Number: QP187.A1M6
Abstract: Sex hormone binding globulin (SHBG) binds and transports androgens and oestrogens in the blood of vertebrate species including fish. We have used oligonucleotide primers corresponding to highly conserved regions of the SHBG coding sequences within the zebrafish and fugufish genomes to obtain a 1528 bp cDNA encoding SHBG from tissue RNA extracts from the European sea bass. Amino-terminal sequence analysis of recombinant sea bass SHBG indicated that its deduced precursor polypeptide includes a 35-residue secretion signal polypeptide, and the 361-residue mature sea bass SHBG sequence exhibits 45-67% sequence identity with SHBGs from other fish species that have been determined

directly (for zebrafish) or deduced (for rainbow trout, medaka and fugufish) from sequences within public databases. The sea bass SHBG (39,894 Da) comprises a tandem repeat of laminin G-like domains typical of SHBG sequences; contains three N-glycosylation sites, and exists as a 118,300+or-11,500 Da homodimer. Sea bass SHBG exhibits a high affinity (Kd=8.8 nM for 17 beta -estradiol) and specificity for gonadal steroids and their precursors (e.g., 17 beta -estradiol > testosterone > dehydroepiandrosterone > 5 alpha -dihydrotestosterone > androstenedione > 11-ketotesterone). Interestingly, the affinity of sea bass SHBG for the synthetic oestrogen, 17 alpha -ethynylestradiol was found to be essentially identical to that for 17 beta -estradiol. The availability of SHBG sequences in sea bass and other fish set the stage for detailed studies of SHBG function in fish reproductive physiology, as well as its potential role as a target of endocrine disruptors. Copyright © 2008 CABI
Descriptors: zebrafish, estradiol, gene expression, genes, globulins, laminins, nucleotide sequences, phylogeny, recombinant proteins, repetitive DNA, sex hormones, steroids, synthetic estrogens, *Dicentrarchus labrax*.

Mikami, Y., T. Yoshida, N. Matsuda, and M. Mishina (2004). **Expression of zebrafish glutamate receptor delta2 in neurons with cerebellum-like wiring.** *Biochemical and Biophysical Research Communications* 322(1): 168-76. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: Mammalian glutamate receptor (GluR) delta2 is selectively expressed in cerebellar Purkinje cells and plays key roles in cerebellar plasticity, motor learning, and neural wiring. Here, we isolated cDNA encoding the zebrafish ortholog of mammalian GluRdelta2. We found that in adult zebrafish brain, glurdelta2 mRNA was expressed not only in cerebellar Purkinje cells, but also in the crest cells of the medial octavolateral nucleus (MON) and the type I neurons of the optic tectum. Immunohistochemical analysis revealed that zebrafish GluRdelta2 proteins were selectively localized in the apical dendrites of these neurons. Interestingly, the crest cells of the MON and the type I neurons of the optic tectum receive large numbers of parallel fiber inputs at the apical dendrites and sensory inputs at the proximal or basal dendrites. These results suggest that the expression of zebrafish GluRdelta2 is selective for cerebellum-like neural wiring with large numbers of parallel fiber inputs.
Descriptors: zebrafish, cerebellum cytology, cerebellum metabolism, neural pathways cytology, neurons cytology, neurons metabolism, glutamate receptors chemistry, glutamate receptors metabolism, amino acid sequence, computer simulation, dendrites metabolism, dendrites ultrastructure, chemical models, molecular sequence data, neural pathways metabolism, protein conformation, Purkinje cells cytology, Purkinje cells metabolism, glutamate receptors genetics, glutamate receptors ultrastructure, recombinant proteins chemistry and metabolism , recombinant proteins ultrastructure, amino acid sequence homology, tissue distribution, zebrafish.

Miles, C.G., L. Rankin, S.I. Smith, M. Niksic, G. Elgar, and N.D. Hastie (2003). **Faithful expression of a tagged Fugu WT1 protein from a genomic transgene in zebrafish: efficient splicing of pufferfish genes in zebrafish but not mice.** *Nucleic Acids Research* 31(11): 2795-802. ISSN: 0305-1048.
NAL Call Number: QD341.A2N8
Abstract: The teleost fish are widely used as model organisms in vertebrate biology. The compact genome of the pufferfish, *Fugu rubripes*, has proven a valuable tool in comparative genome analyses, aiding the annotation of mammalian genomes and the identification of conserved regulatory elements, whilst the zebrafish is particularly suited to genetic and developmental studies. We demonstrate that a pufferfish WT1 transgene can be expressed

and spliced appropriately in transgenic zebrafish, contrasting with the situation in transgenic mice. By creating both transgenic mice and transgenic zebrafish with the same construct, we show that Fugu RNA is processed correctly in zebrafish but not in mice. Furthermore, we show for the first time that a Fugu genomic construct can produce protein in transgenic zebrafish: a full-length Fugu WT1 transgene with a C-terminal beta-galactosidase fusion is spliced and translated correctly in zebrafish, mimicking the expression of the endogenous WT1 gene. These data demonstrate that the zebrafish:Fugu system is a powerful and convenient tool for dissecting both vertebrate gene regulation and gene function in vivo.
Descriptors: transgenic zebrafish, alternative splicing, genetically modified, *Takifugu* genetics, Wt1 proteins genetics, gene expression, genome, introns, c57bl inbred mice, CBA inbred mice, transgenic mice, species specificity, transgenes, Wt1 proteins biosynthesis.

Ming Li, W., S.E. Webb, K.W. Lee, and A.L. Miller (2006). **Recruitment and SNare-mediated fusion of vesicles in furrow membrane remodeling during cytokinesis in zebrafish embryos.** *Experimental Cell Research* 312(17): 3260-75. ISSN: 0014 4827.
NAL Call Number: 442.8EX7
Abstract: Cytokinesis is the final stage in cell division that serves to partition cytoplasm and daughter nuclei into separate cells. Membrane remodeling at the cleavage plane is a required feature of cytokinesis in many species. In animal cells, however, the precise mechanisms and molecular interactions that mediate this process are not yet fully understood. Using real-time imaging in live, early stage zebrafish embryos, we demonstrate that vesicles labeled with the v-SNARE, VAMP-2, are recruited to the cleavage furrow during deepening in a microtubule-dependent manner. These vesicles then fuse with, and transfer their VAMP-2 fluorescent label to, the plasma membrane during both furrow deepening and subsequent apposition. This observation indicates that new membrane is being inserted during these stages of cytokinesis. Inhibition of SNAP-25 (a cognate t-SNARE of VAMP-2), using a monoclonal antibody, blocked VAMP-2 vesicle fusion and furrow apposition. Transient expression of mutant forms of SNAP-25 also produced defects in furrow apposition. SNAP-25 inhibition by either method, however, did not have any significant effect on furrow deepening. Thus, our data clearly indicate that VAMP-2 and SNAP-25 play an essential role in daughter blastomere apposition, possibly via the delivery of components that promote the cell-to-cell adhesion required for the successful completion of cytokinesis. Our results also support the idea that new membrane addition, which occurs during late stage cytokinesis, is not required for furrow deepening that results from contractile band constriction.
Descriptors: zebrafish, cell membrane metabolism, cytokinesis physiology, cytology, synaptosomal associated protein 25 physiology, vesicle associated membrane protein 2 physiology, metabolism, exocytosis, green fluorescent microtubules metabolism.

Mishima, Y., A.J. Giraldez, Y. Takeda, T. Fujiwara, H. Sakamoto, A.F. Schier, and K. Inoue (2006). **Differential regulation of germline mRNAs in soma and germ cells by zebrafish miR-430.** *Current Biology* 16(21): 2135-42. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Abstract: Early in development, primordial germ cells (PGCs) are set aside from somatic cells and acquire a unique gene-expression program . The mechanisms underlying germline-specific gene expression are largely unknown. Nanos expression is required during germline development and is posttranscriptionally restricted to PGCs . Here we report that the microRNA miR-430 targets the 3' untranslated region (UTR) of nanos1 during zebrafish embryogenesis. A miR-430 target site within the nanos1 3' UTR reduces poly(A) tail length, mRNA stability, and translation. Repression is disrupted in maternal-zygotic dicer mutants

(MZdicer), which lack mature miRNAs , and is restored by injection of processed miR-430. Although miR-430 represses other genes equally in germline and soma, specific regions in the nanos1 3' UTR compensate for microRNA-mediated repression in PGCs and allow germline-specific expression. We show that the 3' UTR of an additional PGC-specific gene, TDRD7, is also targeted by miR-430. These results indicate that miR-430 targets the 3' UTRs of germline genes and suggest that differential susceptibility to microRNAs contributes to tissue-specific gene expression.
Descriptors: zebrafish, developmental gene expression regulation, germ cells metabolism, microRNAs genetics, messenger RNA metabolism, 3' untranslated regions, base sequence, molecular sequence data, polyadenylation, RNA interference and binding proteins, ribonuclease III genetics.

Mishiro, E., M.Y. Liu, Y. Sakakibara, M. Suiko, and M.C. Liu (2004). **Zebrafish tyrosylprotein sulfotransferase: molecular cloning, expression, and functional characterization.** *Biochemistry and Cell Biology* 82(2): 295-303. ISSN: 0829 8211.
NAL Call Number: QP501.B42
Abstract: By employing the reverse transcriptase-polymerase chain reaction technique in conjunction with 3' rapid amplification of cDNA ends, a full-length cDNA encoding a zebrafish (Danio rerio) tyrosylprotein sulfotransferase (TPST) was cloned and sequenced. Sequence analysis revealed that this zebrafish TPST is, at the amino acid sequence level, 66% and 60% identical to the human and mouse TPST-1 and TPST-2, respectively. The recombinant form of the zebrafish TPST, expressed in COS-7 cells, exhibited a pH optimum at 5.75. Manganese appeared to exert a stimulatory effect on the zebrafish TPST. The activity of the enzyme determined in the presence of 20 mM MnCl2 was more than 2.5 times that determined in the absence of MnCl2. Of the other nine divalent metal cations tested at a 10 mM concentration, Co2+ also showed a considerable stimulatory effect, while Ca2+, Pb2+, and Cd2+ exerted some inhibitory effects. The other four divalent cations, Fe2+, Cu2+, Zn2+, and Hg2+, inhibited completely the sulfating activity of the zebrafish TPST. Using the wild-type and mutated P-selectin glycoprotein ligand-1 N-terminal peptides as substrates, the zebrafish TPST was shown to exhibit a high degree of substrate specificity for the tyrosine residue on the C-terminal side of the peptide. These results constitute a first study on the cloning, expression, and characterization of a zebrafish cytosolic TPST.
Descriptors: zebrafish, sulfotransferases genetics, metabolism, amino acid and base sequence, COS cells, divalent cations pharmacology, cercopithecus aethiops, molecular cloning, complementary DNA, hydrogen ion concentration, membrane glycomembrane glycoproteins metabolism, molecular sequence data, substrate specificity, sulfotransferases chemistry, sulfotransferases metabolism, temperature, proteins chemistry.

Mitani, H., A. Shima, K. Naruse and M. Tanaka (2004). ***Medaka* genome mapping for functional genomics**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd.: Singapore, Singapore, p. 612-636. ISBN: 9812388214.
NAL Call Number: QL638.C94 F57
Abstract: Large-scale genome sequencing using several model organisms has provided a new pathway to identify new genes, the function of which can be discovered through comparative approaches. Pufferfish (*Fugu rubripes and Tetraodon nigroviridis*) has been sequenced by whole genome shotgun (WGS) assembly, and the zebrafish (*Danio rerio*) genome sequencing project, including whole genome shotgun sequencing and selective BAC shotgun sequencing, is now on going. Still, the number of model organisms is too small to understand fully how

fish genomes evolved. Now, medaka (*Oryzias latipes*) is emerging as another important model fish that is phylogenically distant from zebrafish but closer to pufferfish. Recent additions to genetic toolkit of medaka, such as BAC resources, WGS sequences, and highly dense genome markers based on polymorphic inbred strains, can facilitate genome assembly with high quality (http://medaka.dsp.jst.go.jp/MGI/). About 1400 markers including 800 randomly selected EST markers were mapped, and all of them successfully assembled into 24 linkage groups that correspond to the medaka chromosome number. This genomic map is a powerful tool for positional cloning of mutated genes in medaka, and conserved syntenies of medaka, pufferfish and zebrafish genes to the human genome provide evidence for a whole genome duplication event that occurred after divergence of fish and tetrapods and before divergence of medaka and zebrafish. For gene regulation studies, polyploidy in model fish species might be advantageous, because regulatory elements and functional domains in each of the fish duplicates may have unique functional roles. In this review we summarize the current status of medaka genome studies for functional genomics. Copyright © 2008 CABI
Descriptors: zebrafish, models, bacterial artificial chromosomes, chromosomes, gene mapping, genes , genomes, polyploidy, *Danio rerio*, *Oryzias latipes*, Tetraodontidae.
Notes: A review.

Miyasaka, K.Y., Y.S. Kida, T. Sato, M. Minami, and T. Ogura (2007). **Csrp1 regulates dynamic cell movements of the mesendoderm and cardiac mesoderm through interactions with Dishevelled and Diversin**. *Proceedings of the National Academy of Sciences, USA* 104(27): 11274-11279. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, cell morphology, cytology, metabolites, *Danio rerio*, physiology, biochemistry, biophysics.

Mizgireuv, I.V. and S.Y. Revskoy (2006). **Transplantable tumor lines generated in clonal zebrafish.** *Cancer Research* 66(6): 3120-5. ISSN: 0008 5472.
Abstract: Transplantable zebrafish tumors are a novel and very promising model in cancer research. However, further progress in this field has been contained by a lack of true inbred lines in zebrafish. To overcome this problem, we generated two lines of homozygous diploid clonal zebrafish lines (i.e., CB1 and CW1), which allowed us to carry out transplantation of any tissue, including tumors, from one fish to another within a line without rejection of the graft. The primary tumors in CB1 fish were induced by N-nitrosodiethylamine (DEN). The histologic analysis of these tumors revealed different types of hepatocellular carcinomas, hepatoblastomas, hepatoma, cholangiocarcinoma, and pancreatic carcinoma. Four spontaneous acinar cell carcinomas of pancreas were also found in 10- to 18-month-old CB1 fish. Small pieces of tissue or cell suspensions of either DEN-induced or spontaneous tumors were serially transplanted into the peritoneal cavity of syngeneic fish at different stages of development from 5-day-old larvae to adult fish. The development of grossly visible tumors occurred from 2 weeks to 3 months after tumor grafting and grew either as solitary smooth nodules or as an amorphous jelly-like mass infiltrating abdominal organs. The majority of tumors were also successfully transplanted to isogeneic (F1 generation from crossing CB1 x CW1) fish. At the present time, 19 transplantable zebrafish tumor lines have been generated and maintained for as long as 3 to 25 passages. This model provides a novel tool for studying experimental tumor biology and therapy and will become a cost effective system for high throughput screening of anticancer drugs.
Descriptors: zebrafish, neoplasms, experimental pathology, tumor cell line, diethylnit-

rosamine, diploidy, disease models, homozygote, neoplasm transplantation, neoplasms, experimental chemically induced, neoplasms, experimental genetics.

Mo, S., P. Song, D. Lv, Y. Chen, W. Zhou, W. Gong, and Z. Zhu (2005). **Zebrafish z-Otu, a novel Otu and Tudor domain-containing gene, is expressed in early stages of oogenesis and embryogenesis.** *Biochimica Et Biophysica Acta* 1732(1-3): 1-7. ISSN: 0006 3002.
NAL Call Number: 381B522
Abstract: Several studies have suggested that Otu domain had de-ubiquitinating activity and Tudor domain was important for the formation of germ cells. Here, we reported a novel zebrafish ovary-specific gene containing Otu and Tudor domain, z-otu, which was expressed at stages I-III oocytes and embryonic stages from zygotes to early blastula during embryonic cells maintained their totipotency. Therefore, z-otu might link the ubiquitin signaling pathway to early oogenesis and maintaining the totipotency of embryonic cell.
Descriptors: zebrafish, cysteine endopeptidases genetics, embryonic development genetics, developmental gene expression regulation, oogenesis genetics, amino acid sequence, cysteine endopeptidases chemistry, cytology, exons genetics, gene expression profiling , introns genetics, molecular sequence data, ovary cytology, tertiary protein structure, messenger RNA genetics and metabolism, sequence alignment, proteins chemistry.

Moens, P.B. (2006). **Zebrafish: chiasmata and interference.** *Genome* 49(3): 205-8. ISSN: 0831-2796.
NAL Call Number: QH431.G452
Abstract: With immunofluorescence microscopy, the positions of centromeres and MLH1 (MutL homolog) foci representing the sites of presumptive chiasmata are shown for zebrafish (*Danio rerio* Hamilton 1822) synaptonemal complexes (SCs) in spermatocyte nuclei at meiotic prophase. Most SCs have a single focus and a few (7 of 140) have 2 chiasmata. MLH1 foci tend to be in the distal regions of SCs, with progressively fewer occurring towards the middle of the SCs. This non-random distribution suggests chiasma interference. Synaptic initiation, as well as replication protein A (RPA) foci at the chromosome ends, correlates with the distal localization of MLH1 foci. These observations may provide the physical basis for the reported limited genetic recombination in the centromeric region of androgenetic offspring of a male.
Descriptors: zebrafish , chromosomes physiology, crossing over, genetic, cell nucleus metabolism and ultrastructure, centromere genetics and metabolism, chromosome pairing genetics and physiology, karyotyping, meiosis, genetic recombination, spermatocytes metabolism and ultrastructure, spermatogenesis, synaptonemal complex genetics and ultrastructure.

Moore, J.L., L.M. Rush, C. Breneman, M.A. Mohideen, and K.C. Cheng (2006). **Zebrafish genomic instability mutants and cancer susceptibility.** *Genetics* 174(2): 585-600. ISSN: 0016-6731.
NAL Call Number: 442.8G28
Abstract: Somatic loss of tumor suppressor gene function comprising the second hit of Knudson's two-hit hypothesis is important in human cancer. A genetic screen was performed in zebrafish (*Danio rerio*) to find mutations that cause genomic instability (gin), as scored by Streisinger's mosaic-eye assay that models this second hit. The assay, based on a visible test for loss of wild-type gene function at a single locus, golden, is representative of genomewide events. Twelve ENU-induced genomic instability (gin) mutations were isolated. Most mutations showed weak dominance in heterozygotes and all showed a stronger phenotype in homozygotes. Trans-heterozygosity for 7 of these mutations showed greatly enhanced insta-

bility. A variety of spontaneous tumors were found in heterozygous adults from all gin lines, consistent with the expectation that genomic instability (mutator) mutations can accelerate carcinogenesis. The incidence of spontaneous cancer at 30-34 months was increased 9.6-fold in heterozygotes for the mutant with the strongest phenotype, gin-10. Tumors were seen in skin, colon, kidney, liver, pancreas, ovary, testis, and neuronal tissues, with multiple tumors in some fish. The study of these mutants will add to our understanding of the mechanisms of somatic loss of gene function and how those mechanisms contribute to cancer susceptibility.
Descriptors: zebrafish, genetic predisposition to disease, genomic instability, mutagenesis, neoplasms genetics, phenotype.

Moore, J.L., E.E. Gestl, and K.C. Cheng (2004). **Mosaic eyes, genomic instability mutants, and cancer susceptibility**. *Methods in Cell Biology* 76: 555-568. ISSN: 0091 679X.
NAL Call Number: 442P92
Abstract: The zebrafish mosaic eye assay developed by George Streisinger (1984) takes advantage of the organism's transparency to provide an excellent assay for detecting somatic mutation. We now know that genomic instability contributes to cancer. We therefore took advantage of this assay to identify zebrafish mutants with increased frequencies of somatic mutation and spontaneous cancer. This chapter describes the details of mutagenesis and half-tetrad screening, the basis and practical use of the mosaic eye assay, and the histological methods used to study genomic instability mutants and cancer susceptibility. These techniques should prove useful to other zebrafish researchers, since they are broadly applicable to other biological investigations of zebrafish embryos, larvae and adults. Copyright©Thomson Reuters 2009
Descriptors: mutant zebrafish, tumor biology, molecular genetics and biophysics, sense organs, cancer, neoplastic disease, pathology, transgenic analysis, zebrafish mosaic eye assay, disease susceptibility, mutagenesis, genomic instability.

Moose, H.E., L.E. Kelly, A.J. Fischer, A. Otoshi, B.A. Link, and H.M. El Hodiri (2005). **Identification of Rx promoter elements in vertebrates**. *Investigative Ophthalmology & Visual Science* 46(Suppl. S): 3132. ISSN: 0146 0404.
Abstract: Purpose: The retinal homeobox (Rx) gene product is necessary for specificationof the presumptive eye fields from the anterior neural plate of developing vertebrates. We are investigating Rx regulation by comparing the regulatory regions of Rx1A and Rx2A in *Xenopus laevis*. The previously characterized Rx1A promoter is being utilized as a model to study the Rx2A promoter. We are characterizing a 2.8 KB fragment of the Rx2A promoter to identify cis- and trans-actingelements involved in Rx regulation. Elucidation of the molecular events underlying the initiation of Rx expression will provide a basis for understanding vertebrate eye specification. Methods: Rx2A/GFP transgene constructs were introduced into X. laevis embryos via intracytosolic sperm injections (ICSI). Candidate trans-acting factors were tested for binding specificity using electrophoretic mobility shift assays (EMSA). The Rx2A/GFP transgene was introduced into zebrafish embryos by microinjection, cultures of embryonic chick retina by transfection, and mouse embryos by pronuclear injection. GFP expression was visualized by fluorescent microscopy. Results: Deletion analysis reveals that Rx2A promoter contains a proximal region necessary for late expression, and a distal region necessary for early expression. This organization is shared with the Rx1A promoter. The Rx2A sequence contains a putative forkhead binding site necessary for late promoter activity. Rx and FoxN4, a forkhead transcription factor, are expressed in developing *X. laevis* retinas. We tested and confirmed that FoxN4 binds the putative Rx2A forkhead binding element. This data is consistent with a role for FoxN4 in the regulation of Rx promoter activ-

ity. In primary culture of embryonic chick retina, the full-length Rx2A/GFP transgene is expressed in retinal progenitor cells (RPCs). The transgene is expressed in retinas of zebrafish, frogs and mice. These data demonstrate that the Rx2A regulatory region is active in four vertebrate species, suggesting a common mechanism for Rx regulation among vertebrates. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, molecular genetics, sense organs, sensory reception, intracytosolic sperm injection, eye specification.

Morgan, K. and R.P. Millar (2004). **Evolution of GnRH ligand precursors and GnRH receptors in protochordate and vertebrate species**. *General and Comparative Endocrinology* 139(3): 191-197 . ISSN: 0016 6480.
NAL Call Number: 444.8G28
Abstract: Primary structure relationships between GnRH precursors or GnRH receptors have received significant attention recently due to rapid DNA sequence determination of gene fragments and cDNAs from diverse species. Concepts concerning the evolutionary history of the GnRH system and its function in mammals, including humans, are likely to be modified as more complete sequence information becomes available. Current evidence suggests occurrence of fewer GnRH ligand and GnRH receptor genes in mammals compared to protochordates, fish and amphibians. Whilst several sequence-related GnRH decapeptide precursors and 2 or 3 separate GnRH receptors are encoded within the genomes of protochordates, fish and amphibians, only two types of GnRH (GnRH-I and GnRH-II) and two GnRH receptors occur in mammals. In addition, fish and mammalian genomes both retain inactive remnants of GnRH ligand or GnRH receptor genes. The number of distinct GnRH receptor genes in teleosts (at least five complete genes in pufferfish and three in zebrafish) partly reflects whole genome duplication during the evolution of this order of animals. Three GnRH receptor genes occur in certain frog species, consistent with the occurrence of up to three types of prepro-GnRH in amphibians. In contrast, only one functional GnRH receptor gene (the type I GnRH receptor) has been identified in humans and chimpanzees and a gene encoding a second receptor, homologous to a functional monkey receptor (the type II GnRH receptor), is either partially or completely silenced in a range of mammalian species (human, chimpanzee, sheep, cow, rat, and mouse). Further work is required to determine the significance of species-specific differences in the GnRH system to reproductive biology. For instance, recent data show that even species as closely related as humans and chimpanzees exhibit important organizational changes in the genes comprising the GnRH system. Copyright © 2008 CABI
Descriptors: zebrafish, evolution, genes, GnRH, hormone receptors, phylogenetics, amphibia, fishes, mammals, Urochordata.
Notes: A review.

Moriarty, M.A., E.D. Martin, L. Byrnes, and M. Grealy (2008). **Molecular cloning and developmental expression of plakophilin 2 in zebrafish**. *Biochemical and Biophysical Research Communications* 367(1): 124-129. ISSN: 0006-291X.
NAL Call Number: 442.8B5236
Descriptors: zebrafish, 2448bp, 815 amino acid protein, armadillo, p120-catenin, N-glycosylation.

Morris, A.C., E.H. Schroeter, J. Bilotta, R.O. Wong, and J.M. Fadool (2005). **Cone survival despite rod degeneration in XOPS-mCFP transgenic zebrafish.** *Investigative Ophthalmology and Visual Science* 46(12): 4762-71. ISSN: 0146-0404.

Abstract: PURPOSE: In animal models of retinitis pigmentosa, rod photoreceptor degeneration eventually leads to loss of cone photoreceptors. The purpose of this study was to characterize a transgenic model of rod degeneration in zebrafish. METHODS: Zebrafish transgenic for XOPS-mCFP, a membrane-targeted form of cyan fluorescent protein driven by the Xenopus rhodopsin promoter, were generated by plasmid injection. Immunohistochemistry was used to detect cell type, proliferation, and TUNEL markers in larval and adult retinas. Rod- and cone-specific transcripts were detected by RT-PCR. Visual responses in transgenic adults were measured by electroretinogram. RESULTS: The XOPS promoter directed specific expression of mCFP in rods by 55 hours post fertilization (hpf). Rods in XOPS-mCFP heterozygotes began dying at 3.5 days post fertilization (dpf) and were almost completely absent by 5 dpf. A few rods were observed at the retinal margin, and numerous immature rods were observed in the outer nuclear layer (ONL) of transgenic adults. Apoptosis was increased in the ONL of larval and adult transgenic animals, and an elevation of rod precursor proliferation in adults was observed. ERG analysis confirmed that rod responses were absent in this line. Cone morphology and electrophysiology appeared normal in transgenic animals up to 7 months of age. CONCLUSIONS: The XOPS-mCFP transgene causes selective degeneration of rods without secondary loss of cones in animals up to 7 months of age. This raises important questions about the significance of rod-cone interactions in zebrafish and their potential as a model of human inherited retinal degenerations.
Descriptors: zebrafish, retina cones cytology, green fluorescent recombinant fusion retinal degeneration genetics, rhodopsin genetics, retinal rodspathology, genetically modified, apoptosis, cell survival physiology, retina cones physiology, disease models, electroretinography, fluorescent antibody technique, indirect, in situ nick end labeling, confocal microscopy, plasmids, retinal degeneration metabolism, retinal degeneration pathology, reverse transcriptase PCR, retinal rods metabolism, transgenes.

Morvan Dubois, G., D. Le Guellec, R. Garrone, L. Zylberberg, and L. Bonnaud (2003). **Phylogenetic analysis of vertebrate fibrillar collagen locates the position of zebrafish alpha3(I) and suggests an evolutionary link between collagen alpha chains and hox clusters.** *Journal of Molecular Evolution* 57(5): 501-14. ISSN: 0022-2844.
NAL Call Number: QH359.J6
Abstract: Type I collagen in tetrapods is usually a heterotrimeric molecule composed of two alpha1 and one alpha2 chains. In some teleosts, a third alpha chain has been identified by chromatography, suggesting that type I collagen should also exist as an alpha1(I)alpha2(I) alpha3(I) heterotrimer. We prepared, from zebrafish, three distinct cDNAs identified to be those of the collagen alpha1(I), alpha2(I), and alpha3(I) chains. In this study on the evolution of fibrillar collagen alpha chains and their relationships, an exhaustive phylogenetic analysis, using vertebrate fibrillar collagen sequences, showed that each alpha chain constitutes a monophyletic cluster. Results obtained with the newly isolated sequences of the zebrafish showed that the alpha3(I) chain is phylogenetically close to the alpha1(I) chain and support the hypothesis that the alpha3(I) chain arose from a duplication of the alpha1(I) gene. The duplication might occur during the duplication of the actinopterygian genome, soon after the divergence of actinopterygians and sarcopterygians, a hypothesis supported by the demonstration of a syntenic evolution between a set of fibrillar collagen genes and Hox clusters in mammals. An evolutionary scenario is proposed in which phylogenetic relationships of the alpha chains of fibrillar collagens of vertebrates could be related to Hox cluster history.
Descriptors: zebrafish, collagen Type I genetics, molecular evolution, amino acid sequence, homeobox genes, molecular sequence data, multigene family, phylogeny, sequence alignment.

Moser, M., Q. Yu, C. Bode, J.W. Xiong, and C. Patterson (2007). **BMPER is a conserved regulator of hematopoietic and vascular development in zebrafish.** *Journal of Molecular and Cellular Cardiology* 43(3): 243-53. ISSN: 0009 7322.

NAL Call Number: RC681.A1C8

Abstract: For the proper development of vertebrate embryos as well as for survival of the adult organism, it is essential to form a functional vascular system. Molecules involved in this process are members of highly conserved families of proteins that exert conserved functions across species. Bone morphogenetic proteins (BMP) are extracellular factors that are regulated by extracellular modulators and bind to BMP receptors, which in turn activate intracellular signaling cascades. BMPs are necessary not only for induction of endothelial and hematopoietic lineages but also for further endothelial and hematopoietic cell differentiation. Previously, we identified BMPER (BMP endothelial cell precursor derived regulator) and demonstrated its spatiotemporal expression at sites of vasculogenesis and direct modulation of BMP activity. To directly investigate the role of BMPER in vascular development, we cloned the BMPER ortholog in zebrafish (zbmper). It is expressed at sites of high BMP activity, including vascular precursor cells located in the aortic arches and the intermediate cell mass during zebrafish embryonic development. Knockdown of zbmper results in a dorsalized phenotype, a reduced number of gata1 expressing hematopoietic precursor cells and of circulating blood cells as well as in a vascular phenotype. The generation of the caudal vein is compromised and the pattern guiding of the intersomitic vessels is disturbed, indicating that zbmper is required for early steps in vascular pattern formation and hematopoiesis in zebrafish.

Descriptors: zebrafish, blood vessels embryology, carrier GTPase activatinG, developmental gene expression regulation, hematopoiesis genetics, amino acid sequence, genetically modified, carrier, molecular cloning GTPase activatinG green fluorescent proteins metabolism, hematopoiesis physiology , in situ hybridization, molecular sequence data, phylogeny, tertiary protein structure, messenger RNA genetics and metabolism, amino acid sequence homology, transgenes, proteins genetics.

Mould, A.P., J.A. McLeish, J. Huxley Jones, A.C. Goonesinghe, A.F. Hurlstone, R.P. Boot Handford, and M.J. Humphries (2006). **Identification of multiple integrin beta1 homologs in zebrafish (*Danio rerio*).** *BioMed Central Cell Biology* 7: 24. ISSN: 1471-2121.

NAL Call Number: QH573.B53

Abstract: BACKGROUND: Integrins comprise a large family of alpha,beta heterodimeric, transmembrane cell adhesion receptors that mediate diverse essential biological functions. Higher vertebrates possess a single beta1 gene, and the beta1 subunit associates with a large number of alpha subunits to form the major class of extracellular matrix (ECM) receptors. Despite the fact that the zebrafish *(Danio rerio*) is a rapidly emerging model organism of choice for developmental biology and for models of human disease, little is currently known about beta1 integrin sequences and functions in this organism. RESULTS: Using RT-PCR, complete coding sequences of zebrafish beta1 paralogs were obtained from zebrafish embryos or adult tissues. The results show that zebrafish possess two beta1 paralogs (beta1-1 and beta1-2) that have a high degree of identity to other vertebrate beta1 subunits. In addition, a third, more divergent, beta1 paralog is present (beta1-3), which may have altered ligand-binding properties. Zebrafish also have other divergent beta1-like transcripts, which are C-terminally truncated forms lacking the transmembrane and cytoplasmic domains. Together with beta1-3 these truncated forms comprise a novel group of beta1 paralogs, all of which have a mutation in the ADMIDAS cation-binding site. Phylogenetic and genomic analyses indicate that the duplication that gave rise to beta1-1 and beta1-2 occurred after the

divergence of the tetrapod and fish lineages, while a subsequent duplication of the ancestor of beta1-2 may have given rise to beta1-3 and an ancestral truncated paralog. A very recent tandem duplication of the truncated beta1 paralogs appears to have taken place. The different zebrafish beta1 paralogs have varied patterns of temporal expression during development. Beta1-1 and beta1-2 are ubiquitously expressed in adult tissues, whereas the other beta1 paralogs generally show more restricted patterns of expression. CONCLUSION: Zebrafish have a large set of integrin beta1 paralogs. beta1-1 and beta1-2 may share the roles of the solitary beta1 subunit found in other vertebrates, whereas beta1-3 and the truncated beta1 paralogs may have acquired novel functions.

Descriptors: zebrafish, antigens, cD29 genetics, multigene family genetics, amino acid sequence, D29 antigens chemistry and physiology, molecular evolution, expressed sequence tags, gene duplication, developmental gene expression regulation, molecular models, molecular sequence data, organ specificity, phylogeny, protein isoforms chemistry and genetics, protein isoforms physiology, tertiary protein structure, reverse transcriptase PCR, sequence alignment, amino acid sequence homology, species specificity, vertebrates genetics.

Muncan, V., A. Faro, A.P. Haramis, A.F. Hurlstone, E. Wienholds, J. van Es, J. Korving, H. Begthel, D. Zivkovic, and H. Clevers (2007). **T-cell factor 4 (Tcf7l2) maintains proliferative compartments in zebrafish intestine.** *European Molecular Biology Organization Reports* 8(10): 966-73. ISSN: 1469 221X.

NAL Call Number: QH506 .E463

Abstract: Previous studies have shown that Wnt signals, relayed through beta-catenin and T-cell factor 4 (Tcf4), are essential for the induction and maintenance of crypts in mice. We have now generated a tcf4 (tcf7l2) mutant zebrafish by reverse genetics. We first observe a phenotypic defect at 4 weeks post-fertilization (wpf), leading to death at about 6 wpf. The phenotype comprises a loss of proliferation at the base of the intestinal folds of the middle and distal parts of the intestine. The proximal intestine represents an independent compartment, as it expresses sox2 in the epithelium and barx1 in the surrounding mesenchyme, which are early stomach markers in higher vertebrates. Zebrafish are functionally stomachless, but the proximal intestine might share its ontogeny with the mammalian stomach. Rare adult homozygous tcf4(-/-) 'escapers' show proliferation defects in the gut epithelium, but have no other obvious abnormalities. This study underscores the involvement of Tcf4 in maintaining proliferative self-renewal in the intestine throughout life.

Descriptors: zebrafish, T-cell factor 4, Tcf7l2, Wnt, sox2, intestine.

Murakami, S.L., L.L. Cunningham, L.A. Werner, E. Bauer, R. Pujol, D.W. Raible, and E.W. Rubel (2003). **Developmental differences in susceptibility to neomycin-induced hair cell death in the lateral line neuromasts of zebrafish (*Danio rerio*).** *Hearing Research* 186(1-2): 47-56. ISSN: 0378-5955.

Abstract: Mechanosensory hair cells of the inner ear are susceptible to death when exposed to a variety of drugs including aminoglycoside antibiotics. During avian and mammalian development, there is a period of relative insensitivity to aminoglycoside-induced hair cell death. This study was designed to test the hypothesis that zebrafish (Danio rerio) have developmental differences in sensitivity to aminoglycoside-induced hair cell death in the lateral line neuromasts. Larval zebrafish of various ages were exposed to several concentrations of neomycin, and their hair cells were examined using the potentiometric vital dye, DASPEI. Results indicate that zebrafish larvae aged 4 days post-fertilization are relatively insensitive to aminoglycoside-induced hair cell death compared to older fish. Thus zebrafish hair cells show developmental differences in sensitivity to aminoglycoside-induced death similar to those

reported for inner ear hair cells of birds and mammals.
Descriptors: zebrafish, anti bacterial agents toxicity, hair cells drug effects, neomycin toxicity, protein synthesis inhibitors toxicity, analysis of variance, cell death, critical period psychology, drug dose response relationship, hair cells physiology and ultrastructure, chemically induced hearing loss, immunohistochemistry, electron microscopy.

Muraoka, O., T. Shimizu, T. Yabe, H. Nojima, Y.K. Bae, H. Hashimoto, and M. Hibi (2006). **Sizzled controls dorso-ventral polarity by repressing cleavage of the chordin protein**. *Nature Cell Biology* 8(4): 329. ISSN: 1465 7392.
NAL Call Number: QH573
Abstract: The Bone morphogenetic protein (Bmp) signalling gradient has a major function in the formation of the dorso-ventral axis. The zebrafish ventralized mutant, ogon, encodes Secreted Frizzled (Sizzled). sizzled is ventrally expressed in a Bmp-dependent manner and is required for the suppression of Bmp signalling on the ventral side of zebrafish embryos. However, it remains unclear how Sizzled inhibits Bmp signalling and controls ventro-lateral cell fate. We found that Sizzled stabilizes Chordin, a Bmp antagonist, by binding and inhibiting the Tolloid-family metalloproteinase, Bmp1a, which cleaves and inactivates Chordin. The cysteine-rich domain of Sizzled is required for inhibition of Bmp1a activity. Loss of both Bmp1a and Tolloid-like1 (Tll1; another Tolloid-family metalloproteinase) function leads to a complete suppression and reversal of the ogon mutant phenotype. These results indicate that Sizzled represses the activities of Tolloid-family proteins, thereby creating the Chordin - Bmp activity gradient along the dorso-ventral axis. Here, we describe a previously unrecognized role for a secreted Frizzled-related protein. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, nervous system, neural coordination, development, molecular genetics, phenotype, cell fate.

Murphey, R.D., H.M. Stern, C.T. Straub, and L.I. Zon (2006). **A chemical genetic screen for cell cycle inhibitors in zebrafish embryos.** *Chemical Biology and Drug Design* 68(4): 213-19. ISSN: 1747 0277.
NAL Call Number: QD431.A1I5
Abstract: Chemical genetic screening is an effective strategy to identify compounds that alter a specific biological phenotype. As a complement to cell line screens, multicellular organism screens may reveal additional compounds. The zebrafish embryo is ideal for small molecule studies because of its small size and the ease of waterborne treatment. We first examined a broad range of known cell cycle compounds in embryos using the mitotic marker phosphohistone H3. The majority of the known compounds exhibited the predicted cell cycle effect in embryos. To determine whether we could identify novel compounds, we screened a 16 320-compound library for alterations of pH3. This screen revealed 14 compounds that had not been previously identified as having cell cycle activity despite numerous mitotic screens of the same library with mammalian cell lines. With six of the novel compounds, sensitivity was greater in embryos than cell lines, but activity was still detected in cell lines at higher doses. One compound had activity in zebrafish embryos and cell lines but not in mammalian cell lines. The remaining compounds exhibited activity only in embryos. These findings demonstrate that small molecule screens in zebrafish can identify compounds with novel activity and thus may be useful tools for chemical genetics and drug discovery.
Descriptors: zebrafish, cell cycle drug effects, preclinical drug evaluation methods, drug effects, pharmaceutical preparations administration and dosage, aphidicolin administration and dosage, cell cycle genetics, cell cycle physiology, cell line, DNA metabolism, dimethyl sulfoxide administration and dosage, cytology, metabolism, flow cytometry, histones metabo-

lism, immunohistochemistry, mice , mitosis drug effects, genetics and physiology, NIH 3t3 cells, nocodazole administration and dosage, pharmaceutical preparations classification, phenotype, phosphorylation drug effects, tubulin metabolism.

Murtha, J.M. and E.T. Keller (2003). **Characterization of the heat shock response in mature zebrafish (*Danio rerio*).** *Experimental Gerontology* 38(6): 683-91. ISSN: 0531 5565.
NAL Call Number: QP86.E85
Abstract: Heat shock proteins (Hsps) are involved in many physiological and pathological processes and are diminished with age in a variety of species. As zebrafish embryos have proven to be excellent models for studying Hsp response during development, we sought to characterize the response in mature zebrafish to demonstrate the utility of the zebrafish model in studying late-life diseases and the biology of aging. Accordingly, mature zebrafish were exposed to a 37 degrees C heat stress and mRNA was isolated from various tissues and subjected to analysis by RT-PCR. We found that Hsp70 was upregulated in brain, liver, and muscle, while Hsp47 was upregulated in brain, but not liver or muscle. Hsp90alpha, Hsp90beta, and heat shock factor 1a (Hsf1a) were expressed in all three tissues, but were not upregulated in response to heat stress. A comparison of Hsp expression in young versus mature zebrafish revealed decreased basal levels of Hsp70 and increased levels of Hsf1a in mature fish. These results indicate that the heat shock response is detectable in mature zebrafish and that there are age differences in their heat shock response, suggesting that mature zebrafish may be a useful model for studying Hsp response during the aging process.
Descriptors: zebrafish, aging metabolism, heat shock proteins biosynthesis and physiology, DNA binding proteins genetics, proteins biosynthesis and genetics, electrophoretic mobility shift assay, Hsp47 heat shock proteins, HSP70 heat shock proteins biosynthesis and models, messenger RNA genetics, reverse transcriptase PCR, transcription factors, up regulation.

Muto, A., M.B. Orger, A.M. Wehman, M.C. Smear, J.N. Kay, P.S. Page McCaw, E. Gahtan, T. Xiao, L.M. Nevin, N.J. Gosse, W. Staub, K. Finger Baier, and H. Baier (2005). **Forward genetic analysis of visual behavior in zebrafish.** *Public Library of Science Genetics* 1(5): E66. ISSN: 1553-7390.
Abstract: The visual system converts the distribution and wavelengths of photons entering the eye into patterns of neuronal activity, which then drive motor and endocrine behavioral responses. The gene products important for visual processing by a living and behaving vertebrate animal have not been identified in an unbiased fashion. Likewise, the genes that affect development of the nervous system to shape visual function later in life are largely unknown. Here we have set out to close this gap in our understanding by using a forward genetic approach in zebrafish. Moving stimuli evoke two innate reflexes in zebrafish larvae, the optomotor and the optokinetic response, providing two rapid and quantitative tests to assess visual function in wild-type (WT) and mutant animals. These behavioral assays were used in a high-throughput screen, encompassing over half a million fish. In almost 2,000 F2 families mutagenized with ethylnitrosourea, we discovered 53 recessive mutations in 41 genes. These new mutations have generated a broad spectrum of phenotypes, which vary in specificity and severity, but can be placed into only a handful of classes. Developmental phenotypes include complete absence or abnormal morphogenesis of photoreceptors, and deficits in ganglion cell differentiation or axon targeting. Other mutations evidently leave neuronal circuits intact, but disrupt phototransduction, light adaptation, or behavior-specific responses. Almost all of the mutants are morphologically indistinguishable from WT, and many survive to adulthood. Genetic linkage mapping and initial molecular analyses show that our approach was effective in identifying genes with functions specific to the visual system. This collection of

zebrafish behavioral mutants provides a novel resource for the study of normal vision and its genetic disorders.

Descriptors: zebrafish, behavior, vision, axons, ethylnitrosourea pharmacology , gene expression regulation, genetic techniques, linkage genetics, mutagenesis, ocular physiology, phenotype, photoreceptors.

Muto, E., Y. Tabata, T. Taneda, Y. Aoki, A. Muto, K. Arai, and S. Watanabe (2004). **Identification and characterization of Veph, a novel gene encoding a pH domain-containing protein expressed in the developing central nervous system of vertebrates**. *Biochimie* 86(8): 523-531. ISSN: 0300 9084.

NAL Call Number: 383S01

Abstract: We isolated Veph, a novel gene encoding a pleckstrin homology (PH) domain-containing protein from a mouse. Veph was strongly expressed in the embryonic brain, and its expression level gradually decreased in later stages. In situ hybridization analysis of sectioned embryo brains revealed that Veph was expressed exclusively in the ventricular zone. We then isolated a zebrafish orthologue of Veph (zVeph). As observed in the mouse gene, zVeph was expressed in the ventricular zone of developing brain and spinal cord. Blockage of zVeph expression by injection of zVeph-specific morpholino antisense oligo into zebrafish fertilized eggs resulted in a defect in the midbrain-hindbrain boundary and otic vesicle formation, suggesting the important function of zVeph in central nervous system (CNS) development. On the other hand, homozygous knockout mice of Veph showed no significant defect in the CNS, pointing to possible different functions of Veph between the zebrafish and mouse. Copyright 2004 Elsevier SAS. All rights reserved. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, development, molecular genetics, nervous system, neural coordination.

Nadauld, L.D., I.T. Sandoval, S. Chidester, H.J. Yost, and D.A. Jones (2004). **Adenomatous polyposis coli control of retinoic acid biosynthesis is critical for zebrafish intestinal development and differentiation.** *Journal of Biological Chemistry* 279(49): 51581-9. ISSN: 0021 9258.

NAL Call Number: 381J824

Abstract: Mutations in the APC (adenomatous polyposis coli) tumor suppressor gene cause uncontrolled proliferation and impaired differentiation of intestinal epithelial cells. Recent studies indicate that human colon adenomas and carcinomas lack retinol dehydrogenases (RDHs) and that APC regulates the expression of human RDHL. These data suggest a model wherein APC controls enterocyte differentiation by controlling retinoic acid production. However, the importance of APC and retinoic acid in mediating control of normal enterocyte development and differentiation remains unclear. To examine the relationship between APC and retinoic acid biosynthesis in normal enterocytes, we have identified two novel zebrafish retinol dehydrogenases, termed zRDHA and zRDHB, that show strong expression within the gut of developing zebrafish embryos. Morpholino knockdown of either APC or zRDHB in zebrafish embryos resulted in defects in structures known to require retinoic acid. These defects included cardiac abnormalities, pericardial edema, failed jaw and pectoral fin development, and the absence of differentiated endocrine and exocrine pancreas. In addition, APC or zRDHB morphant fish developed intestines that lacked columnar epithelial cells and failed to express the differentiation marker intestinal fatty acid-binding protein. Treatment of either APC or zRDHB morphant embryos with retinoic acid rescued the defective phenotypes. Downstream of retinoic acid production, we identified hoxc8 as a

retinoic acid-induced gene that, when ectopically expressed, rescued phenotypes of APC- and zRDHB-deficient zebrafish. Our data establish a genetic link supporting a critical role for retinoic acid downstream of APC and confirm the importance of retinoic acid in enterocyte differentiation.

Descriptors: zebrafish, adenomatous polyposis coli protein physiology, alcohol oxidoreductases genetics, intestines embryology, growth and development, tretinoin physiology, alcohol oxidoreductases chemistry, amino acid sequence, genetically modified, cell differentiation, high pressure liquid chromatography, complementary DNA metabolism, enterocytes metabolism, developmental gene expression regulation, homeodomain proteins metabolism, molecular sequence data, phenotype, phylogeny, RNA metabolism, messenger RNA metabolism, reverse transcriptase PCR, amino acid sequence homology, tretinoin metabolism.

Nagai, T., S. Otani, T. Saito, S. Maegawa, K. Inoue, K. Arai, and E. Yamaha (2005). **Germ-line chimera produced by blastoderm transplantation in zebrafish**. *Nippon Suisan Gakkaishi* 71(1): 1-9. ISSN: 0021 5392.

Abstract: To elucidate the effects of blastoderm graft for chimeric-fish production on further development, transplantations of upper, lower or entire part of blastoderm were performed onto the animal part of host embryos during the blastula stage in zebrafish. In the case of upper, all resultant chimeric embryos developed normally, while in the lower part, many acephalic embryos appeared. When the entire blastoderm was transplanted, many resultant embryos showed normal phenotype with extra cell-mass, while a few had double axis. vas mRNA, one of the markers of PGCs, also kept the expressions in both donor and recipient blastomeres in transplantation of the entire blastoderm. Donor PGCs were frequently detected at germinal anlagen in histological sections of 6-day larvae developed from transplantations of lower and entire blastoderm, but seldom in those of the upper part. These results might suggest that graft transplantation was effective for production of germ-line chimera, but sometimes caused un-regulatory differentiation of graft cells under the community effect. Copyright © 2008 CABI

Descriptors: zebrafish, blastocyst, chimaeras, embryonic development, embryos, grafts, messenger RNA, phenotypes, *Danio rerio.*

Language of Text: Japanese.

Nakada, C., S. Satoh, Y. Tabata, K. Arai, and S. Watanabe (2006). **Transcriptional repressor foxl1 regulates central nervous system development by suppressing shh expression in zebrafish.** *Molecular and Cellular Biology* 26(19): 7246-57. ISSN: 0270-7306.

NAL Call Number: QH506.M664

Abstract: We identified zebra fish forkhead transcription factor l1 (zfoxl1) as a gene strongly expressed in neural tissues such as midbrain, hindbrain, and the otic vesicle at the early embryonic stage. Loss of the function of zfoxl1 effected by morpholino antisense oligonucleotide resulted in defects in midbrain and eye development, and in that of formation of the pectoral fins. Interestingly, ectopic expression of shh in the midbrain and elevated pax2a expression in the optic stalk were observed in foxl1 MO-injected embryos. In contrast, expression of pax6a, which is negatively regulated by shh, was suppressed in the thalamus and pretectum regions, supporting the idea of augmentation of the shh signaling pathway by suppression of foxl1. Expression of zfoxl1-EnR (repressing) rather than zfoxl1-VP16 (activating) resulted in a phenotype similar to that induced by foxl1-mRNA, suggesting that foxl1 may act as a transcriptional repressor of shh in zebra fish embryos. Supporting this notion, foxl1 suppressed isolated 2.7-kb shh promoter activity in PC12 cells, and the minimal region of foxl1 required for its transcriptional repressor activity showed strong homology with the

groucho binding motif, which is found in genes encoding various homeodomain proteins. In view of all of our data taken together, we propose zfoxl1 to be a novel regulator of neural development that acts by suppressing shh expression.
Descriptors: zebrafish, brain embryology, forkhead transcription factors metabolism, developmental gene expression regulation, repressor proteins metabolism, trans activators metabolism, genetic transcription, metabolism, amino acid sequence, biological markers, brain cytology, brain metabolism, cultured cells, cytology, embryology, fibroblasts metabolism, forkhead transcription factors chemistry, isolation and purification, gastrula metabolism, Hedgehog proteins, mice, molecular sequence data, NIH 3t3 cells, antisense oligonucleotides metabolism, pc12 cells, promoter regions genetics, protein binding, tertiary protein structure, messenger RNA genetics and metabolism, rats, repressor proteins chemistry, isolation and purification, trans activators genetics, proteins chemistry, isolation and purification.

Nakamura, T., K. Amanuma, and Y. Aoki (2005). **Frameshift mutations induced by the acridine mustard ICR-191 in embryos and in the adult gill and hepatopancreas of rpsL transgenic zebrafish.** *Mutation Research* 578(1-2): 272-83. ISSN: 0027-5107.
NAL Call Number: QH431.M8
Abstract: To determine whether frameshift mutations can be detected in rpsL transgenic zebrafish (Brachydanio rerio), embryos, and adult fish were treated with 6-chloro-9-[3-(2-chloroethylamino)-propylamino]-2-methoxyacridine (ICR-191). Embryos exposed to 0, 10, or 20 microM ICR-191 in a water bath for 18 h exhibited induced mutant frequencies (MFs) of 14 x 10(-5), 16 x 10(-5), and 25 x 10(-5), respectively. Only embryos exposed to 20 microM ICR-191 showed a significant increase in MF. The mutational spectra differed between the control and ICR-191-treated groups and single G:C pair insertions, which are a marked characteristic of ICR-191 mutagenesis, were observed in both 10 and 20 microM-treated embryos. In adult fish treated with 1 microM ICR-191 in a water bath for 18 h, a significant increase in MFs was observed in both gill (12 x 10(-5) and 44 x 10(-5) in control and treated fish, respectively), and hepatopancreas (5 x 10(-5) and 29 x 10(-5), respectively) 2 weeks after exposure. Sequence analysis showed that 58% of mutations in gill and 94% of mutations in hepatopancreas were single G:C pair insertions, which is typical of mutations induced by ICR-191. Additionally, these mutations occurred predominantly at a single site (CC sequence at bps 140-141) in the rpsL gene. Three weeks after exposure, however, the increased MFs and prominent mutational spectra of ICR-treated fish were undetectable. These findings suggest that using our protocols the rpsL transgenic zebrafish mutation assay is more effective for adult fish than for embryos, but that frameshift mutations can be detected in both embryos and adults at appropriate sampling times after treatment with ICR-191.
Descriptors: zebrafish, aminacrine analogs and derivatives, frameshift mutation drug effects, gills drug effects, hepatopancreas drug effects, mutagens toxicity, nitrogen mustard compounds toxicity, aminacrine chemistry and toxicity, genetically modified, dose response relationship, drug gills metabolism , hepatopancreas metabolism, molecular structure, mutagens chemistry, nitrogen mustard compounds chemistry.

Nakaya, N. and S. Tomarev (2007). **Expression patterns of alternative transcripts of the zebrafish olfactomedin 1 genes.** *Gene Expression Patterns* 7(7): 723-9. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Olfactomedin 1 (Olfm1) is a founding member of the family of olfactomedin domain-containing proteins. It is a secreted protein that performs different roles in different

species. Although the molecular mechanisms of Olfm1 action are not known, its possible roles include the regulation of neural crest cell production, neuronal differentiation, and ischemic neuronal death in adult. Two zebrafish olfm1 genes (olfm1a and olfm1b) located on chromosomes 5 and 21 were identified in zebrafish genome. Four different transcripts are produced from each olfm1 gene. The distribution of these transcripts in the course of zebrafish early development was studied by in situ hybridization and quantitative RT-PCR. Different variants of olfm1 mRNA were present mainly in neurogenic tissues and demonstrated overlapping expression patterns.

Descriptors: zebrafish, extracellular matrix proteins biosynthesis, gene expression profiling , glycoproteins biosynthesis, neurons metabolism, alternative splicing, amino acid sequence, gene expression regulation, developmental gene expression regulation, mice, molecular sequence data, neural crest metabolism, amino acid sequence homology, trigeminal ganglion metabolism, *Xenopus.*

Naruse, K., M. Tanaka, K. Mita, A. Shima, J. Postlethwait, and H. Mitani (2004). **A medaka gene map: the trace of ancestral vertebrate proto-chromosomes revealed by comparative gene mapping**. *Genome Research* 14(5): 820-828. ISSN: 1088 9051.

NAL Call Number: QP606.D46P34

Abstract: The mapping of Hox clusters and many duplicated genes in zebrafish indicated an extra whole-genome duplication in ray-fined fish. However, to reconstruct the preduplication chromosomes (proto-chromosomes), the comparative genomic studies of more distantly related teleosts are essential. Medaka and zebrafish are ideal for this purpose, because their lineages separated from their last common ancestor ~140 million years ago. To reconstruct ancient vertebrate chromosomes, including the chromosomes of the vertebrate ancestor of humans from 450 million years ago, we mapped 818 genes and expressed sequence tags (ESTs) on a single meiotic backcross panel obtained from inbred strains of the medaka, Oryzias latipes. Comparisons of linkage relationships of orthologous genes among three species of vertebrates (medaka, zebrafish, and human) indicate the number and content of the chromosomes of the last common ancestor of ray-fined fish and lobe-fined fish (including humans), and the extra whole genome duplication event in the ray-fin lineage occurred in the common ancestor of perhaps all teleosts. Copyright © 2008 CABI

Descriptors: zebrafish, chromosome maps, chromosomes, duplication, gene mapping, genes, linkage, phylogeny, *Danio rerio*, fishes, *Oryzias latipes.*

Neuhauss, S.C. (2003). **Behavioral genetic approaches to visual system development and function in zebrafish.** *Journal of Neurobiology* 54(1): 148-60. ISSN: 0022-3034.

NAL Call Number: QP351.J55

Descriptors: zebrafish larvae, behavioral genetics methods, vision physiology, electrophysiology, escape reaction, eye growth and development, genetic screening, larva genetics, larva growth and development, larva physiology, light, mutation, optokinetic nystagmus physiology, photic stimulation, retina anatomy, histology, cytology, growth, development and physiology, vision genetics.

Neuhauss, S.C., M.W. Seeliger, C.P. Schepp, and O. Biehlmaier (2003). **Retinal defects in the zebrafish bleached mutant.** *Documenta Ophthalmologica* 107(1): 71-8. ISSN: 0012 4486.

Abstract: The recessive zebrafish mutant bleached has, apart from its defects in pigmentation, a heritable defect leading to larval blindness. Here, we analyze the retina of homozygous bleached larvae, employing morphological and electrophysiological methods. Electroretinography revealed a complete lack of electrical signals in response to light. Histological analysis

of mutant retinae showed a severely affected outer retina with a hypopigmented pigment epithelium and a disorganized outer nuclear layer containing few or no intact photoreceptors. Using the TUNEL assay for cell death detection, we noticed a strong increase of apoptotic cells in all retinal cell layers, starting in young larvae even before retinal support of visual function. At later stages cell death is most pronounced at the marginal zone, where new cells are constantly added to the retina. At early stages increased apoptosis is mainly confined to the retina, while at later stages elevated cell death is al so apparent in extra-retinal tissues, particularly in the brain. Hence, the lack of visual responses in homozygous bleached larvae can be attributed to a severe defect of the outer retina, preceded by increased levels of apoptotic cell death in all retinal cell layers.
Descriptors: zebrafish, mutation, retinal degeneration genetics, apoptosis, blindness etiology, electroretinography, eye recessive genes, larva, retina growth and development and pathology, retina physiopathology, retinal degeneration pathology, retinal degeneration physiopathology.

Neve, E.P.A., K. Svensson, J. Fuxe, and R.F. Pettersson (2003). **VIPL, a VIP36-like membrane protein with a putative function in the export of glycoproteins from the endoplasmic reticulum**. *Experimental Cell Research* 288(1): 70-83. ISSN: 0014-4827.
NAL Call Number: 442.8EX7
Descriptors: zebrafish, biological membranes, cell organelles, endoplasmic reticulum, freshwater fish, glycoproteins, golgi apparatus, lectins, membrane proteins, phylogeny, plasma membranes, protein transport, receptors, secretion, *Danio rerio*, VIPL protein, physiology, biochemistry, biophysics.

Ng, D., N. Thakker, C.M. Corcoran, D. Donnai, R. Perveen, A. Schneider, D.W. Hadley, C. Tifft, L. Zhang, A.O.M. Wilkie, J.J. Van der Smagt, R.J. Gorlin, S.M. Burgess, V.J. Bardwell, G.C.M. Black, and L.G. Biesecker (2004). **Oculofaciocardiodental and Lenz microphthalmia syndromes result from distinct classes of mutations in BCOR**. *Nature Genetics* 36(4): 411-416. ISSN: 1061-4036.
NAL Call Number: QH426.N37
Descriptors: zebrafish, abnormalities, biological development, craniofacial syndromes, diseases, eyes, genes, hereditary diseases, marine fish, microphthalmia, mutation, mutations, nervous system, Skeleton, teeth, *Danio rerio*, BCOR , lenz microphthalmia syndrome, oculofaciocardiodental syndrome, genetic diseases, reproduction and development.

Nica, G., W. Herzog, C. Sonntag, and M. Hammerschmidt (2004). **Zebrafish pit1 mutants lack three pituitary cell types and develop severe dwarfism.** *Molecular Endocrinology* 18(5): 1196-209. ISSN: 0888-8809.
NAL Call Number: QP187.3M64M6
Abstract: The Pou domain transcription factor Pit-1 is required for lineage determination and cellular commitment processes during mammalian adenohypophysis development. Here we report the cloning and mutational analysis of a pit1 homolog from zebrafish. Compared with mouse, zebrafish pit1 starts to be expressed at a much earlier stage of adenohypophysis development. However, as in the mouse, expression is restricted to a subset of pituitary cell types, excluding proopiomelanocortin (pomc)-expressing cells (corticotropes, melanotropes) and possibly gonadotropes. We could identify two N-ethyl-N-nitrosourea-induced zebrafish pit1 null mutants. Most mutants die during larval stages, whereas survivors develop severe dwarfism. Mutant larvae lack lactotropes, somatotropes, and thyrotropes, although the adenohypophysis is of normal size, without any sign of increased apoptosis rates. Instead,

mutant embryos initiate ectopic expression of pomc in pit1-positive cells, leading to an expansion of the Pomc lineage. Similarly, the number of gonadotropes seems increased, as indicated by the expression of gsualpha, a marker for thyrotropes and gonadotropes. In pit1 mutants, the total number of gsualpha-positive cells is normal despite the loss of gsualpha and tshbeta coexpressing cells. Together, these data suggest a transfating of the Pit1 lineage to the Pomc and possibly the gonadotroph lineages in the mutant, and a pomc- and gonadotropin-repressive role of Pit1 during normal zebrafish development. This is different from mouse, for which a repressive role of Pit-1 has only been reported for the gonadotropin Lhbeta, but not for Pomc. In sum, our data point to both conserved and class-specific aspects of Pit1 function during pituitary development in different vertebrate species.
Descriptors: zebrafish, cell differentiation genetics, DNA binding proteins genetics, proteins metabolism, dwarfism genetics, anterior pituitary gland embryology, transcription factors metabolism, cell lineage genetics, cytology, developmental gene expression regulation physiology, genetic techniques, homeodomain proteins metabolism, mutation genetics, transcription factor pit 1.

Nichane, M., C. Van Campenhout, H. Pendeville, M.L. Voz, and E.J. Bellefroid (2006). **The Na+/PO4 cotransporter SLC20A1 gene labels distinct restricted subdomains of the developing pronephros in *Xenopus* and zebrafish embryos.** *Gene Expression Patterns* 6(7): 667-72. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: The embryonic pronephric kidneys of *Xenopus* and zebrafish serve as models to study vertebrate nephrogenesis. Recently, multiple subdomains within the *Xenopus pronephros* have been defined based on the expression of several transport proteins. In contrast, very few studies on the expression of renal transporters have been conducted in zebrafish. We have recently shown that the anterior and posterior segments of the zebrafish pronephric duct may correspond to the proximal tubule and distal tubule/duct compartments of the Xenopus and higher vertebrate pronephros, respectively. Here, we report the embryonic expression pattern of the Na(+)/PO(4) cotransporter SLC20A1 (PiT1/Glvr-1) gene encoding a type III sodium-dependent phosphate cotransporter in *Xenopus* and zebrafish. In *Xenopus*, SLC20A1 mRNA is expressed in the somitic mesoderm and lower level of expression is detected in the neural tube, eye, and neural crest cells. From stage 25, SLC20A1 is also detectable in the developing pronephros where expression is restricted to the late portion of the distal pronephric tubules. In zebrafish, SLC20A1 is transcribed from mid-somitogenesis in the anterior part of the pronephros where its expression corresponds to the rostral portion of the expression of other proximal tubule-specific markers. Outside the pronephros, lower level of SLC20A1 expression is also observed in the posterior cardinal and caudal veins. Based on the SLC20A1 expression domain and that of other transporters, four segments have been defined within the zebrafish pronephros. Together, our data reveal that the zebrafish and *Xenopus* pronephros have non-identical proximo-distal organizations.
Descriptors: zebrafish, kidney embryology, sodium phosphate cotransporter proteins, Type III genetics, *Xenopus laevis* embryology, developmental gene expression regulation, in situ hybridization, kidney metabolism, organ specificity, organogenesis, *Xenopus laevis* genetics and metabolism.

Nicoli, S., C.N. Gilardelli, O. Pozzoli, M. Presta, and F. Cotelli (2005). **Regulated expression pattern of gremlin during zebrafish development.** *Gene Expression Patterns* 5(4): 539-44. ISSN: 1567-133X.
NAL Call Number: QH450.B73

Abstract: Xenopus laevis Gremlin has been isolated as a novel dorsalizing factor, belonging to a family of secreted proteins with axial patterning activity . In a search for genes that control development in zebrafish (*Danio rerio*), we have identified a sequence homologous to *Xenopus* gremlin. This paper describes the cloning of zebrafish gremlin (grm) and its expression pattern during development. Our results show that grm encodes a maternal transcript, and the zygotic transcription is turned on at the mid-blastula transition (MBT), when grm is detected in the entire blastoderm. In the gastrula grm becomes restricted to the dorsolateral region of the embryo, and during somitogenesis it is strongly expressed in the presomitic mesoderm and developing somites, and in the ventral neural tube. From 24 hpf to 48 hpf, we show that grm transcription is downregulated in the whole embryo, even though Grm protein is still present and localized into the entire myotome at 48-72 hpf. Finally, grm transcript is strongly downregulated in fibroblast growth factor-8 (fgf8) and sonic hedgehog (shh) mutants, thus implicating a putative role of Fgf/Shh signalling loop in grm expression regulation.
Descriptors: zebrafish, carrier developmental gene expression regulation, amino acid and base sequence, blastula physiology, body patterning, conserved sequence, molecular sequence data, morphogenesis, sequence alignment, amino acid sequence homology, genetic transcription.

Nicoli, S., D. Ribatti, F. Cotelli, and M. Presta (2007). **Mammalian tumor xenografts induce neovascularization in zebrafish embryos.** *Cancer Research* 67(7): 2927-31. ISSN: 0008 5472.
Abstract: The zebrafish (*Danio rerio*)/tumor xenograft model represents a powerful new model system in cancer. Here, we describe a novel exploitation of the zebrafish model to investigate tumor angiogenesis, a pivotal step in cancer progression and target for antitumor therapies. Human and murine tumor cell lines that express the angiogenic fibroblast growth factor (FGF) 2 and/or vascular endothelial growth factor (VEGF) induce the rapid formation of a new microvasculature when grafted close to the developing subintestinal vessels of zebrafish embryos at 48 h postfertilization. Instead, no angiogenic response was exerted by related cell clones defective in the production of these angiogenic growth factors. The newly formed blood vessels sprout from the subintestinal plexus of the zebrafish embryo, penetrate the tumor graft, and express the transcripts for the zebrafish orthologues of the early endothelial markers Fli-1, VEGF receptor-2 (VEGFR2/KDR), and VE-cadherin. Accordingly, green fluorescent protein-positive neovessels infiltrate the graft when tumor cells are injected in transgenic VEGFR2:G-RCFP zebrafish embryos that express green fluorescent protein under the control of the VEGFR2/KDR promoter. Systemic exposure of zebrafish embryos immediately after tumor cell injection to prototypic antiangiogenic inhibitors, including the FGF receptor tyrosine kinase inhibitor SU5402 and the VEGFR2/KDR tyrosine kinase inhibitor SU5416, suppresses tumor-induced angiogenesis without affecting normal blood vessel development. Accordingly, VE-cadherin gene inactivation by antisense morpholino oligonucleotide injection inhibits tumor neovascularization without affecting the development of intersegmental and subintestinal vessels. These data show that the zebrafish/tumor xenograft model represents a novel tool for investigating the neovascularization process exploitable for drug discovery and gene targeting in tumor angiogenesis.
Descriptors: zebrafish, angiogenesis inhibitors pharmacology, embryo blood supply, neoplasms blood supply and drug therapy, xenograft model antitumor assays methods, genetically modified, tumor cell line, embryo pathology, metabolism, pathology, fibroblast growth factor 2 biosynthesis, mice, neoplasm transplantation, pathologic neovascularization drug therapy, pathologic neovascularization genetics, heterologous transplantation.

Nicolson, T. (2005). **The genetics of hearing and balance in zebrafish**. *Annual Review of Genetics* 39: 9-22. ISSN: 0066 4197.

NAL Call Number: QH431.A1A5

Abstract: The zebrafish (*Danio rerio*) is an excellent model system for studying the molecular basis of inner ear development and function. The eggs develop ex utero and the ear is transparent for the first few weeks of life. Forward genetic screens and antisense technology have helped to elucidate the signalling pathways and molecules required for inner ear development and function. This review addresses the most recent advances in our understanding of how the ear forms and discusses the molecules in hair cells that are essential for sensing sound and movement in the zebrafish. Copyright © 2008 CABI

Descriptors: zebrafish, biochemical pathways, ears, genes, genetics, hearing, histology, molecular genetics, morphology, *Danio rerio*.

Notes: A review.

Niehrs, C. (2004). **Regionally specific induction by the Spemann-Mangold Organizer**. *Nature Reviews: Genetics* 5(6): 425-434. ISSN: 1471-0056.

NAL Call Number: QH426

Descriptors: zebrafish embryos, amphibiotic species, bone morphogenetic proteins, cell differentiation, developmental genetics, embryogenesis, embryonic development, genes, growth factors, intercellular signalling, Nodal protein, orientation, pattern formation, Wnt protein, amphibia, amphibians, Spemann-Mangold organizer.

Notes: A review.

Nielsen, A.L. and A.L. Jorgensen (2003). **Structural and functional characterization of the zebrafish gene for glial fibrillary acidic protein, GFAP.** *Gene* 310: 123-32. ISSN: 0378-1119.

NAL Call Number: QH442.A1G4

Abstract: Glial fibrillary acidic protein, GFAP, is an astrocyte-specific member of the family of intermediate filament proteins which are involved in formation of the cytoskeletal structure. We here present a characterization of the zebrafish GFAP gene and corresponding protein. The zebrafish GFAP gene have the same exon-intron organization as the mammalian orthologoue genes. Comparison of the protein with mammalian GFAP shows that the amino acid sequence is highly conserved in the rod and tail domains whereas the head domain has diverged. Zebrafish GFAP exhibits functional characteristics of an intermediate filament protein such as dimerization potential, capacity to assembly into filaments, and cytoskeletal localization. Mutations in human GFAP have been associated with a severe childhood brain disorder called Alexander disease. Interestingly, the mutations affect preferentially amino acid residues of GFAP that are evolutionarily conserved. This indicates that a change of functionally core residues in GFAP is a prerequisite for the disease phenotype to develop and the initial steps in the pathogenesis may thus be modeled in zebrafish.

Descriptors: zebrafish, glial fibrillary acidic protein genetics, Alexander disease genetics, amino acid and base sequence, conserved sequence genetics, DNA chemistry and genetics, dimerization, molecular evolution, exons, glial fibrillary acidic protein chemistry and physiology, green fluorescent proteins, 16th century history, intermediate filaments chemistry, genetics and metabolism, introns, luminescent proteins genetics and metabolism, fluorescence microscopy, molecular sequence data, mutation, sequence alignment, DNA sequence analysis, amino acid sequence homology, nucleic acid sequence homology, tumor cultured cells.

Niimura, Y. and M. Nei (2005). **Evolutionary dynamics of olfactory receptor genes in fishes and tetrapods** . *Proceedings of the National Academy of Sciences, USA* 102(17): 6039-6044. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, amphibiotic species, environmental changes, evolution, freshwater fish , genomes, odorant receptors, olfaction, phylogenetics, phylogeny, receptors, survival, anura, *Danio rerio*, pisces, Tetrapoda, frogs, chickens, human, mice.

Ninkovic, J., A. Folchert, Y.V. Makhankov, S.C. Neuhauss, I. Sillaber, U. Straehle, and L. Bally Cuif (2006). **Genetic identification of AChE as a positive modulator of addiction to the psychostimulant D-amphetamine in zebrafish.** *Journal of Neurobiology* 66(5): 463-75. ISSN: 0022-3034.
NAL Call Number: QP351.J55
Abstract: Addiction is a complex maladaptive behavior involving alterations in several neurotransmitter networks. In mammals, psychostimulants trigger elevated extracellular levels of dopamine, which can be modulated by central cholinergic transmission. Which elements of the cholinergic system might be targeted for drug addiction therapies remains unknown. The rewarding properties of drugs of abuse are central for the development of addictive behavior and are most commonly measured by means of the conditioned place preference (CPP) paradigm. We demonstrate here that adult zebrafish show robust CPP induced by the psychostimulant D-amphetamine. We further show that this behavior is dramatically reduced upon genetic impairment of acetylcholinesterase (AChE) function in ache/+ mutants, without involvement of concomitant defects in exploratory activity, learning, and visual performance. Our observations demonstrate that the cholinergic system modulates drug-induced reward in zebrafish, and identify genetically AChE as a promising target for systemic therapies against addiction to psychostimulants. More generally, they validate the zebrafish model to study the effect of developmental mutations on the molecular neurobiology of addiction in vertebrates.
Descriptors: zebrafish, acetylcholine metabolism, acetylcholinesterase metabolism, amphetamine related disorders metabolism, brain drug effects and metabolism, dextroamphetamine adverse effects, acetylcholinesterase genetics, amphetamine related disorders genetics and physiopathology, brain physiopathology, brain chemistry drug effects and genetics, central nervous system stimulants adverse effects, cholinergic fibers drug effects and metabolism, cholinesterase inhibitors pharmacology and therapeutic use, conditioning psychology drug effects and physiology, disease models, dopamine metabolism, evolution, mutation genetics, reward.

Nishihara, H., A.F.A. Smit, and N. Okada (2006). **Functional noncoding sequences derived from SINEs in the mammalian genome**. *Genome Research* 16(7): 864-874. ISSN: 1088 9051.
NAL Call Number: QP606.D46P34
Abstract: Recent comparative analyses of mammalian sequences have revealed that a large number of nonprotein-coding genomic regions are under strong selective constraint. Here, we report that some of these loci have been derived from a newly defined family of ancient SINEs (short interspersed repetitive elements). This is a surprising result, as SINEs and other transposable elements are commonly thought to be genomic parasites. We named the ancient SINE family AmnSINE1, for Amniota SINE1, because we found it to be present in mammals as well as in birds, and some copies predate the mammalian-bird split 310 million years ago (Mya). AmnSINE1 has a chimeric structure of a 5S rRNA and a tRNA-derived SINE, and is related to five tRNA-derived SINE families that we characterized here in the

coelacanth, dogfish shark, hagfish, and amphioxus genomes. All of the newly described SINE families have a common central domain that is also shared by zebrafish SINE3, and we collectively name them the DeuSINE (Deuterostomia SINE) superfamily. Notably, of the ~1000 still identifiable copies of AmnSINE1 in the human genome, 105 correspond to loci phylogenetically highly conserved among mammalian orthologs. The conservation is strongest over the central domain. Thus, AmnSINE1 appears to be the best example of a transposable element of which a significant fraction of the copies have acquired genomic functionality. Copyright © 2008 CABI

Descriptors: zebrafish, characterization, genome analysis, genomes, loci, nucleotide sequences, phylogeny, poultry, promoters, RNA, Atlantic salmon, brown trout, dogfishes, fowls, mammals, *Oncorhynchus tshawytscha*, rainbow trout, *Salmo trutta*, salmon, *Squalus acanthias*, trout.

Nishikawa, K., M. Kobayashi, A. Masumi, S.E. Lyons, B.M. Weinstein, P.P. Liu, and M. Yamamoto (2003). **Self-association of Gata1 enhances transcriptional activity in vivo in zebra fish embryos.** *Molecular and Cellular Biology* 23(22): 8295-305. ISSN: 0270-7306.

NAL Call Number: QH506.M664

Abstract: Gata1 is a prototype transcription factor that regulates hematopoiesis, yet the molecular mechanisms by which Gata1 transactivates its target genes in vivo remain unclear. We previously showed, in transgenic zebra fish, that Gata1 autoregulates its own expression. In this study, we characterized the molecular mechanisms for this autoregulation by using mutations in the Gata1 protein which impair autoregulation. Of the tested mutations, replacement of six lysine residues with alanine (Gata1KA6), which inhibited self-association activity of Gata1, reduced the Gata1-dependent induction of reporter gene expression driven by the zebra fish gata1 hematopoietic regulatory domain (gata1 HRD). Furthermore, overexpression of wild-type Gata1 but not Gata1KA6 rescued the expression of Gata1 downstream genes in vlad tepes, a germ line gata1 mutant fish. Interestingly, both GATA sites in the double GATA motif in gata1 HRD were critical for the promoter activity and for binding of the self-associated Gata1 complex, whereas only the 3'-GATA site was required for Gata1 monomer binding. These results thus provide the first in vivo evidence that the ability of Gata1 to self-associate critically contributes to the autoregulation of the gata1 gene.

Descriptors: zebrafish, DNA binding proteins genetics, proteins metabolism, transcription factors metabolism, acetylation, amino acid sequence, genetically modified, base sequence, binding sites genetics, complementary DNA genetics, DNA binding proteins genetics, proteins chemistry and gentics, erythroid specific DNA binding proteins genetics, factors, GATA1 transcription factor, lysine chemistry , molecular sequence data, site directed mutagenesis recombinant proteins chemistry, recombinant proteins genetics and metabolism, amino acid sequence homology, trans activation genetics, transcription factors chemistry, transcription factors genetics, zinc fingers genetics.

Nolan, C.M., K. McCarthy, E. Eivers, R.L. Jirtle, and L. Byrnes (2006). **Mannose 6-phosphate receptors in an ancient vertebrate, zebrafish.** *Development Genes and Evolution* 216(3): 144-51. ISSN: 0949 944X.

NAL Call Number: QL951.R68

Abstract: The endosome/lysosome system plays key roles in embryonic development, but difficulties posed by inaccessible mammalian embryos have hampered detailed studies. The accessible, transparent embryos of *Danio rerio*, together with the genetic and experimental approaches possible with this organism, provide many advantages over rodents. In mammals, mannose 6-phosphate receptors (MPRs) target acid hydrolases to endosomes and lysosomes,

but nothing is known of acid hydrolase targeting in zebrafish. Here, we describe the sequence of the zebrafish cation-dependent MPR (CD-MPR) and cation-independent MPR (CI-MPR), and compare them with their mammalian orthologs. We show that all residues critical for mannose 6-phosphate (M6P) recognition are present in the extracellular domains of the zebrafish receptors, and that trafficking signals in the cytoplasmic tails are also conserved. This suggests that the teleost receptors possess M6P binding sites with properties similar to those of mammalian MPRs, and that targeting of lysosomal enzymes by MPRs represents an ancient pathway in vertebrate cell biology. We also determined the expression patterns of the CD-MPR and CI-MPR during embryonic development in zebrafish. Both genes are expressed from the one-cell stage through to the hatching period. In early embryos, expression is ubiquitous, but in later stages, expression of both receptors is restricted to the anterior region of the embryo, covering the forebrain, midbrain and hindbrain. The expression patterns suggest time- and tissue-specific functions for the receptors, with particular evidence for roles in neural development. Our study establishes zebrafish as a novel, genetically tractable model for in vivo studies of MPR function and lysosome biogenesis.
Descriptors: zebrafish, IGF type 2 receptor genetics, amino acid sequence, binding sites, carbohydrate metabolism, cations metabolism, cattle, chickens genetics, molecular cloning, conserved sequence, cytoplasmic structures metabolism embryonic development, gene expression, insulin like growth factor II metabolism, mice, molecular sequence data , neurons metabolism, opossums genetics, phylogeny, platypus genetics, amino acid sequence homology, signal transduction, tissue distribution.

Nornes, S., C. Groth, E. Camp, P. Ey, and M. Lardelli (2003). **Developmental control of Presenilin1 expression, endoproteolysis, and interaction in zebrafish embryos.** *Experimental Cell Research* 289(1): 124-32. ISSN: 0014 4827.
NAL Call Number: 442.8EX7
Abstract: Dominant mutations in presenilin1 (PS1) and presenilin2 (PS2) are a major cause of early-onset Alzheimer's disease. In this report we analyze the expression of the zebrafish presenilin1 (Psen1) and presenilin2 (Psen2) proteins during embryogenesis. We demonstrate that Psen1 and Psen2 holoproteins are relatively abundant in zebrafish embryos and are proteolytically processed. Psen1 is maternally expressed, whereas Psen2 is expressed at later stages during development. The Psen1 C-terminal proteolytic fragment (CTF) is present at varying levels during embryogenesis, indicating the existence of developmental control mechanisms regulating its production. We examine the codependency of Psen1 and Psen2 expression during early embryogenesis. Forced overexpression of psen2 increases expression of Psen2 holoprotein, but not the N-terminal fragment (NTF), indicating that levels of Psen2 NTF are strictly controlled. Overexpression of psen2 did not alter levels of Psen1 holoprotein, CTF, or higher molecular weight complexes. Reduction of Psen1 activity in zebrafish embryos produces similar developmental defects to those seen for loss of PS1 activity in knockout mice. The relevance of these results to previous work on presenilin protein regulation and function are discussed. Our work shows that zebrafish embryos are a valid and valuable system in which to study presenilin interactions, regulation, and function.
Descriptors: zebrafish, embryology, developmental gene expression regulation drug effects and genetics, membrane proteins metabolism, Alzheimer disease genetics and metabolism, antisense elements genetics pharmacology, disease models, drug effects, metabolism, membrane proteins antagonists, inhibitors and genetics, peptide fragments genetics, peptide hydrolases genetics, peptide hydrolases metabolism, presenilin 1, presenilin 2, tertiary protein structure genetics, somites cytology, drug effects and metabolism.

Nousch, M., V. Reed, R.J. Bryson-Richardson, P.D. Currie, and T. Preiss (2007). **The eIF4G-homolog p97 can activate translation independent of caspase cleavage**. *RNA* 13(3): 374-384. ISSN: 1355-8382.

Descriptors: zebrafish, animal morphology, apoptosis, caspase, conservation, embryogenesis, embryonic development, embryos, freshwater fish, gene expression, genetic diversity, granules, initiation factor eIf-4g, initiation factors, Lethality, mesoderm , polyribosomes, population genetics, RNA, repressors, ribosomes, translation, translation initiation, *Danio rerio*, protein-nucleic acids association, genetics and evolution.

Novince, Z.M., E. Azodi, J.A. Marrs, P.A. Raymond, and Q. Liu (2003). **Cadherin expression in the inner ear of developing zebrafish.** *Gene Expression Patterns* 3(3): 337-9. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Cadherins are cell adhesion molecules that have been implicated in development of a variety of organs including the ear. In this study we analyzed expression patterns of three zebrafish cadherins (Cadherin-2, -4, and -11) in the embryonic and larval zebrafish inner ear using both in situ hybridization and immunocytochemical methods. All three Cadherins exhibit distinct spatiotemporal patterns of expression during otic vesicle morphogenesis. Cadherin-2 and Cadherin-4 proteins and their respective mRNAs were detected mainly in the sensory patches and the statoacoustic ganglion (SAg), respectively. In contrast, cadherin-11mRNA was widely expressed earlier in the otic placode, and later became restricted to a subset of cells in the inner ear, including hair cells.

Descriptors: zebrafish, cadherins genetics, inner ear genetics and metabolism, cadherins biosynthesis, inner ear embryology, gene expression profiling, in situ hybridization, messenger RNA metabolism.

Nowak, M. and M. Hammerschmidt (2006). **Ubc9 regulates mitosis and cell survival during zebrafish development.** *Molecular Biology of the Cell* 17(12): 5324-36. ISSN: 1059-1524.

NAL Call Number: QH604.C452

Abstract: Many proteins are modified by conjugation with Sumo, a gene-encoded, ubiquitin-related peptide, which is transferred to its target proteins via an enzymatic cascade. A central component of this cascade is the E2-conjugating enzyme Ubc9, which is highly conserved across species. Loss-of-function studies in yeast, nematode, fruit fly, and mouse blastocystes point to multiple roles of Ubc9 during cell cycle regulation, maintenance of nuclear architecture, chromosome segregation, and viability. Here we show that in zebrafish embryos, reduction of Ubc9 activity by expression of a dominant negative version causes widespread apoptosis, similar to the effect described in Ubc9-deficient mice. However, antisense-based knock down of zygotic ubc9 leads to much more specific defects in late proliferating tissues, such as cranial cartilage and eyes. Affected cartilaginous elements are of relatively normal size and shape, but consist of fewer and larger cells. Stainings with mitotic markers and 5-Bromo-2'-deoxyuridine incorporation studies indicate that fewer chondrocyte precursors are in mitosis, whereas the proportion of cells in S-phase is unaltered. Consistently, FACS analyses reveal an increase in the number of cells with a DNA content of 4n or even 8n. Our data indicate an in vivo requirement of Ubc9 for G2/M transition and/or progression through mitosis during vertebrate organogenesis. Failed mitosis in the absence of Ubc9 is not necessarily coupled with cell death. Rather, cells can continue to replicate their DNA, grow to a larger size, and finish their normal developmental program.

Descriptors: zebrafish, cytology, embryonic development, mitosis, ubiquitin conjugating enzymes metabolism, body patterning physiology, branchial region cytology and embryology, cartilage abnormalities, cell count, death, proliferation and survival, chondrocytes

cytology, DNA metabolism, abnormalities, developmental gene expression regulation, messenger RNA genetics and metabolism, ubiquitin conjugating enzymes deficiency and genetics, zygote cytology.

Nowak, M., C. Koster, and M. Hammerschmidt (2005). **Perp is required for tissue-specific cell survival during zebrafish development.** *Cell Death and Differentiation* 12(1): 52-64. ISSN: 1350 9047.

NAL Call Number: QH671

Abstract: The tumor suppressor p53 has two alternative effects, causing either cell cycle arrest or apoptosis. These different effects are supposed to be mediated by the transcriptional activation of different target genes. perp, encoding a transmembrane protein of the Pmp22 family, is a transcriptional p53 target exclusively upregulated in apoptotic cells. However, its role during normal development had remained largely unclear. Here, we report the isolation and characterization of a zebrafish perp homolog. Upon overexpression in early zebrafish embryos, perp induces apoptosis. In addition, it contributes to p53-dependent and UV-induced cell death. However, during normal zebrafish development, perp displays a p53-independent and spatially restricted expression in specific cell types and tissues. Antisense-mediated loss of Perp function leads to increased apoptosis in perp-expressing cells of the developing skin and notochord. We conclude that, in contrast to its proapoptotic function in stressed cells, Perp plays an antiapoptotic role during normal zebrafish development to regulate tissue-specific cell survival.

Descriptors: zebrafish, membrane apoptosis physiology, apoptosis radiation effects, caspases antagonists and inhibitors, caspases metabolism, cell survival radiation effects, molecular cloning, metabolism, radiation effects, developmental gene expression regulation radiation effects, membrane, notochord embryology and metabolism, phosphomessenger RNAadministration and dosage, skin embryology and metabolism, trans activators genetics, transcription factors genetics, tumor suppressor protein p53 genetics, ultraviolet rays.

O'Boyle, S., R.T. Bree, S. McLoughlin, M. Grealy, and L. Byrnes (2007). **Identification of zygotic genes expressed at the midblastula transition in zebrafish.** *Biochemical and Biophysical Research Communications* 358(2): 462-8. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: Early development of the embryo is directed by maternal gene products and characterised by limited zygotic gene activity, cell division synchrony and no cell motility in several vertebrates including fish and frogs. At the midblastula transition (MBT), zygotic transcription is grossly activated, cells become motile and cell divisions become asynchronous. The aim of this study was to identify genes whose expression is up-regulated at the MBT in zebrafish. Suppression subtractive hybridisation (SSH) was employed to isolate 48 unique cDNAs, 28 of which show significant similarity to known genes and 20 represent novel cDNAs. Twenty one of these genes, with potential roles in transcriptional regulation, cell cycle control, and embryonic patterning showed increased expression at the MBT. Our results demonstrate the value of SSH as a tool to clone novel, zygotic, developmentally regulated genes that may be important in the progression of the MBT and embryonic patterning.

Descriptors: zebrafish, cell cycle physiology, cell cycle proteins metabolism, developmental gene expression regulation physiology, metabolism, zygote cytology and metabolism, cultured cells.

O'Sullivan, L.A., N.S. Sprigg, R.S. Lewis, S.E. Nicholson, and A.C. Ward (2005). **The zebrafish socs1 gene plays a role in hematopoiesis and brain development**. *Federation of European Biochemical Societies Journal* 272(Suppl. 1): 447-448. ISSN: 1742 464X.

NAL Call Number: QP501.E8

Descriptors: zebrafish, immune system, chemical coordination and homeostasis, enzymology, development, asthma, respiratory system disease, allergy, immune system disease, autoimmune disorders, immune system disease, cell proliferation, hematopoiesis, brain development.

Ober, E.A., B. Olofsson, T. Makinen, S.W. Jin, W. Shoji, G.Y. Koh, K. Alitalo, and D.Y. Stainier (2004). **Vegfc is required for vascular development and endoderm morphogenesis in zebrafish.** *European Molecular Biology Organization Journal Reports* 5(1): 78-84. ISSN: 1469 221X.

NAL Call Number: QH506 .E463

Abstract: During embryogenesis, complex morphogenetic events lead endodermal cells to coalesce at the midline and form the primitive gut tube and associated organs. While several genes have recently been implicated in endoderm differentiation, we know little about the genes that regulate endodermal morphogenesis. Here, we show that vascular endothelial growth factor C (Vegfc), an angiogenic as well as a lymphangiogenic factor, is unexpectedly involved in this process in zebrafish. Reducing Vegfc levels using morpholino antisense oligonucleotides, or through overexpression of a soluble form of the VEGFC receptor, VEGFR-3, affects the coalescence of endodermal cells in the anterior midline, leading to the formation of a forked gut tube and the duplication of the liver and pancreatic buds. Further analyses indicate that Vegfc is additionally required for the initial formation of the dorsal endoderm. We also demonstrate that Vegfc is required for vasculogenesis as well as angiogenesis in the zebrafish embryo. These data argue for a requirement of Vegfc in the developing vasculature and, more surprisingly, implicate Vegfc signalling in two distinct steps during endoderm development, first during the initial differentiation of the dorsal endoderm, and second in the coalescence of the anterior endoderm to the midline.

Descriptors: zebrafish, endoderm physiology, vascular endothelial growth factor C physiology, cell differentiation, metabolism, embryonic development, endoderm cytology, developmental gene expression regulation, morphogenesis, signal transduction.

Ochocinska, M. and P.F. Hitchcock (2004). **Cellular expression of neuroD in the retina of the embryonic zebrafish**. *Investigative Ophthalmology & Visual Science* 45(Suppl. 2): U636. ISSN: 0146 0404.

Abstract: Purpose: NeuroD is a basic helix-loop-helix (bHLH) transcription factor that in the adult teleost retina is expressed in cells of the rod photoreceptor lineage (Hitchcock and Ochocinska, ARVO Abstract 2003). The purpose of this investigation is to characterize the spatial and temporal expression pattern of neuroD in the developing retina of the zebrafish. Methods: In situ hybridization using DIG-labeled riboprobes was performed in zebrafish embryos between 25 and 76 hours post fertilization (hpf), Bromodeoxyuridine (BrdU) was used to label mitotically active cells in these animals by briefly soaking them in 5mM BrdU and 15%DMSO in embryo rearing solution. Results: The expression of neuroD begins at 31hpf in a cluster of cells in the precocious ventronasal patch. Between31hpf and 48hpf, neuroD expression expands from the vetronasal patch through the developing layers of the retina. At 48hpf and beyond, when the three nuclear layers are formed, neuroD is expressed in the inner and the outer nuclear layers. In the INL, neuroD is expressed in putative amacrine cells and INL progenitors, characterized by their neuroepithelial morphology. In the

ONL, neuroDis expressed in developing cone photoreceptors, and rod precursors, identified by double labeling with neuroD and BrdU. Conclusions: In early retinal development neuroD defines the cellular origin of the precocious ventral patch of rod photoreceptors. At later embryonic stages the cellular pattern of neuroD expression appears similar to that observed in the adult teleost retina. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, sense organs, sensory reception, neuroD, retina, BrdU, bHLH.

Ochocinska, M. and P.F. Hitchcock (2005). **Overexpression of neurod in the developing retina of the zebrafish**. *Investigative Ophthalmology & Visual Science* 46(Suppl. S): 5330. ISSN: 0146 0404.
Abstract: Purpose: In the retinas of larval and adult teleosts, the bHLH transcription factor, neuroD, is expressed in proliferating cells of the rod lineage and differentiating cone photoreceptors. The purpose of this study is to investigate the function of neuroD in these cells. Methods: The open reading frame (ORF) ofthe zebrafish neuroD cDNA was subcloned into the Clontech promoterless enhanced green fluorescent protein (EGFP), pEGFP-1, expression vector containing thezebrafish heat shock promoter (HSP) 70/4. The neuroD ORF was inserted into the modified pHSP70/4-EGFP vector between the heat shock promoter and sequences encoding the enhanced green fluorescent protein. The functionality of this construct was tested in vitro in HEK293 cells and in vivo in transient transgenicembryos injected at the 2-4 cell stage and treated at 24 hrs post fertilization by heat shock (37 degrees C for 60 min). Several hundred embryos were also injected and raised to sexual maturity to establish stable transgenic lines. Progeny of injected embryos were screened by heat shock to identify those displaying germline transmission. Cells and embryos were evaluated using fluorescence microscopy and in situ hybridization with probes to neuroD and islet1. Results: Examining EGFP expression in transfected HEK293 cells showed that the HSPpromoter drives transcription, and the neuroD(EGFP) fusion protein is trafficked to the nucleus. In situ hybridization on sections from transient transgenic animals shows that heat shock can induce ectopic expression of neuroD mRNA in vivo, including in the nervous system and retina. In these animals,ectopic mRNA was present by 24hrs after heat shock, and was maintained for atleast 72hrs. A line of animals transgenic for HSP70/4-neuroD(EGFP) was identified. Heat shock treatment of these animals resulted in robust EGFP fluorescence throughout the embryo, including the retina, and ectopic expression of islet1, a downstream target of neuroD. Retinas from transgenic animals heat shocked at 24hpf and sacrificed at 48hpf showed a developmental delay compared to heat shocked, age-matched controls. Conclusions: The HSP70/4-neuroDEGFPconstruct gives rise to functional protein in vitro and in vivo, and a line of transgenic animals has been established. Conditional gain-of-function experiments will be used to investigate the role of neuroD in cells of the rod lineage and newly differentiated cone photoreceptors. Copyright©Thomson Reuters 2009
Descriptors: zebrafish larvae, development, molecular genetics, sensory reception, heat shock, retina development , rod lineage, cone photoreceptor.

Oehlmann, V.D., S. Berger, C. Sterner, and S.I. Korsching (2004). **Zebrafish beta tubulin 1 expression is limited to the nervous system throughout development, and in the adult brain is restricted to a subset of proliferative regions.** *Gene Expression Patterns* 4(2): 191-8. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Tubulin, the building block of microtubules, consists of an alpha and beta subunit, each in itself a family of several highly homologous isotypes. Abundance, tissue specificity, developmental regulation, and possibly function vary between isotypes. Six iso-

types of beta tubulin (class I to class VI) have been cloned from several vertebrate species. Class I beta tubulin is believed to be widely expressed, but has not been studied by in situ hybridization in any vertebrate species so far. We have cloned a beta tubulin from zebrafish that appears most similar to other vertebrate class I tubulins and name it zbeta1 tubulin, accordingly. We report a distinct expression pattern of zbeta1 tubulin in the zebrafish embryo in restricted regions of the peripheral and central nervous system that comprise early-differentiating neurons. The expression pattern changes during development and in the adult zebrafish expression mostly is confined to a subset of proliferative zones that include the subependymal zone around the telencephalic ventricle, zones in the preoptic and hypothalamic area and in the olfactory epithelium. Thus, zbeta1 tubulin is expressed with remarkable selectivity during neuronal differentiation and neurogenesis in the embryonic and adult nervous system, respectively.

Descriptors: zebrafish, brain embryology, brain metabolism, gene expression physiology, tubulin genetics and metabolism, base sequence, cell division physiology, molecular evolution, gene expression profiling, molecular sequence data, olfactory bulb metabolism, phylogeny, metabolism.

Ogoshi, M., K. Inoue, and Y. Takei (2003). **Identification of a novel adrenomedullin gene family in teleost fish**. *Biochemical and Biophysical Research Communications* 311(4): 1072-1077. ISSN: 0006-291X.

NAL Call Number: 442.8B5236

Descriptors: zebrafish, adrenomedullin, brain, classification, freshwater fish, gene families, genes, hormones, kidneys, nucleotide sequence, peptides, phylogenetics, phylogeny, synteny, *Danio rerio*, *Takifugu rubripes*, Teleostei, *Tetraodon nigroviridis*, taxonomy and morphology.

Oishi, I., Y. Kawakami, A. Raya, C. Callol Massot, and J.C. Izpisua Belmonte (2006). **Regulation of primary cilia formation and left-right patterning in zebrafish by a noncanonical Wnt signaling mediator, duboraya.** *Nature Genetics* 38(11): 1316-22. ISSN: 1061-4036.

NAL Call Number: QH426.N37

Abstract: Primary cilia are microtubule-based organelles that project from the surface of nearly every animal cell. Although important functions of primary cilia in morphogenesis and tissue homeostasis have been identified, the mechanisms that control the formation of primary cilia are not understood. Here we characterize a zebrafish gene, termed duboraya (dub), that is essential for ciliogenesis. Knockdown of dub in zebrafish embryos results in both defects in primary cilia formation in Kupffer's vesicle and randomization of left-right organ asymmetries. We show that, at the molecular level, the function of dub in ciliogenesis is regulated by phosphorylation, which in turn depends on Frizzled-2-mediated noncanonical Wnt signaling. We also provide evidence that, at the cellular level, dub function is essential for actin organization in the cells lining Kupffer's vesicle. Taken together, our findings identify a molecular factor that links noncanonical Wnt signaling with the control of left-right axis specification, and provide an entry point for analyzing the mechanisms that regulate primary cilia formation.

Descriptors: zebrafish, body patterning genetics, cilia genetics, intracellular signaling peptides proteins genetics and physiology, Wnt proteins metabolism, genetically modified, body patterning physiology, molecular cloning developmental gene expression regulation, organogenesis genetics, signal transduction.

Okabe, M. and A. Graham (2004). **The origin of the parathyroid gland**. *Proceedings of the National Academy of Sciences, USA* 101(51): 17716-17719. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, aquatic environment, calcium, calcium homeostasis, calcium-sensing receptors, chemoreceptors, endoderm, evolution, gills, glands, hormones, phylogenetics, phylogeny, receptors, thyroid, *Danio rerio*, *Scyliorhinus canicula*, gcm2 protein, small-spotted catshark parathyroid and parathyroid hormones, taxonomy, systematics and evolutionary genetics.

Okamoto, H., H. Segawa and S.I. Higashijima (2003). **Toward genetic dissection of motor neuron differentiation.** *Aquatic Genomics: Steps Toward a Great Future,* Springer-Verlag: New York Inc., 175 Fifth Avenue, New York, NY, 10010-7858, USA, p. 139-152. ISBN: 4431703314.
NAL Call Number: QL638.99 .A76
Descriptors: zebrafish, nervous system, neural coordination, genetic dissection, motor neuron differentiation.

Olsvik, P.A., K.K. Lie, A.K. Stavrum, and S. Meier (2007). **Gene-expression profiling in gill and liver of zebrafish exposed to produced water**. *International Journal of Environmental Analytical Chemistry* 87(3): 195-210. ISSN: 0306 7319.
Abstract: The effects of produced water (a by-product of oil and gas extraction) on gene expression were studied in gills and liver tissues of zebrafish. Adult, non-breeding zebrafish were exposed to control (freshwater mixed with 5% seawater) or produced water (freshwater mixed with 5% of produced water from the Oceberg C Oil Platform in the North Sea). A zebrafish library was used to make a microarray that consisted of 15 806 unique genes. The results indicate that 27 genes in the gills and 55 genes in the liver show significantly altered expression (greater than two-fold change). More than 70% of these gene sequences have not been annotated in the Gene Ontology (GO) database, making it difficult to characterize the affected genes. CYPIA displayed the greatest upregulation in the gills (eightfold, verified with quantitative real-time PCR). This study illustrates the utility of microarray approaches in investigations of environmental effects of toxicants. Copyright © 2008 CABI
Descriptors: zebrafish, biochemical markers, DNA libraries, DNA microarrays, environmental impact, product of oil and gas extraction, produced water, fish livers, gene expression, genes, gills, toxic substances, toxicity, toxicogenomics, *Danio rerio.*

Omori, Y. and J.A. Malicki (2006). **Oko meduzy and related crumbs genes are determinants of apical cell features in the vertebrate embryo**. *Current Biology* 16(10): 945-957. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Abstract: Background: Polarity is an essential attribute of most eukaryotic cells. One of the most prominent features of cell polarity in many tissues is the subdivision of cell membrane into apical and basolateral compartments by a belt of cell junctions. The proper formation of this subdivision is of key importance. In sensory cells, for example, the apical membrane compartment differentiates specialized structures responsible for the detection of visual, auditory, and olfactory stimuli. In other tissues, apical specializations are responsible for,the propagation of fluid flow. Despite its importance, the role of genetic determinants of apico-basal polarity in vertebrate embryogenesis remains poorly investigated.Results: We show that zebrafish oko meduzy (ome) locus encodes a crumbs gene homolog essential for the proper apico-basal polarity of neural tube epithelia. Two ome paralogs, crb2b and crb3a, promote the formation of apical cell features: photoreceptor inner segments and cilia in renal

and auditory systems. The motility of cilia is defective following the impairment of crb2b function. Apical surface defects in ome- and crb2b-deficient animals are associated with profound disorganization of neuronal architecture and with the formation of pronephric cysts, respectively. Unexpectedly, despite differences in their structure and expression patterns, crumbs genes are, at least partially, functionally interchangeable.Conclusions: ome and related crumbs genes are necessary for the formation of gross morphological features in several organs, including the CNS and the renal system. On the cellular level, crumbs genes regulate the formation of both ciliary and nonciliary apical membrane compartment. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, development, membranes : cell biology, molecular genetics, biochemistry, polarity.

Onnebo, S.M.N. and A.C. Ward (2004). **Tel/etv6 is important for angiogenesis, gut development and neuronal cell survival**. *Biochemical Society Transactions* 32(Part 4): 94A. ISSN: 0300 5127.

NAL Call Number: QD415.A1B58

Descriptors: zebrafish, development, ingestion and assimilation, molecular genetics, nervous system, neural coordination, cancer, neoplastic disease, angiogenesis, vasculature.

Ott, E.B., A.J. Te Velthuis, and C.P. Bagowski (2007). **Comparative analysis of splice form-specific expression of LIM Kinases during zebrafish development.** *Gene Expression Patterns* 7(5): 620-9. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: LIM Kinases (LIMK) are genes encoding multi-domain proteins that can contain up to two LIM domains, a single PDZ domain, and a tyrosine kinase domain. Alternative splicing is a source for different combinations of these domains. Two family members, LIMK1 and LIMK2 have been described in mammals and are important for organization of the actin cytoskeleton. We have cloned LIMK1 and LIMK2 from zebrafish and characterized their domain specific expression patterns during embryogenesis. The results on temporal and spatial expression of the LIM Kinases during embryogenesis indicate overlapping and distinct expression domains for LMK1 and LIMK2. Differences in expression during embryogenesis were observed for PDZ and LIM encoding splice forms for both LIM Kinases. To better understand the transcriptional regulation of LIM Kinases, we searched for conserved regulatory elements. We identified evolutionary conserved smad binding sites for LIMK2. In summary, we present here the splice-form specific temporal and spatial expression patterns for both LIMK1 and LIMK2 during zebrafish embryogenesis.

Descriptors: zebrafish embryos, alternative splicing, developmental gene expression regulation, protein kinases genetics and metabolism, binding sites, molecular cloning, protein kinases metabolism, transcriptional regulatory elements.

Otteson, D.C., M. Tsujikawa, T. Gunatilaka, J. Malicki, and D.J. Zack (2005). **Genomic organization of zebrafish cone-rod homeobox gene and exclusion as a candidate gene for retinal degeneration in niezerka and mikre oko.** *Molecular Vision* 11: 986-95. ISSN: 1090-0535.

NAL Call Number: QP475 .M65

Abstract: PURPOSE: To determine the genomic organization of the zebrafish crx gene and to evaluate if mutations in crx are responsible for the retinal degeneration phenotype in the zebrafish *(Danio rerio*) mutants niezerka (nie(m743)) and mikre oko (mok(m632)). METHODS: Overlapping fragments were PCR amplified from genomic DNA isolated from homozygous mutant embryos and wild-type siblings (sibs). Amplicons were sequenced and

sequence data assembled into contigs. Genomic organization was determined by alignment of contigs with published cDNA sequences and zebrafish genomic sequence from Sanger and Ensembl databases. Linkage analysis used DNA from mapping panels of single homozygous mutant animals with mixed genetic backgrounds. RESULTS: The analysis indicated that the zebrafish crx gene consisted of three exons and 2 introns, and spans 3.8 kb of genomic DNA. The splice junctions were all located within the coding region. Highly repetitive sequences present in non-coding regions of crx and extended tetra-nucleotide repeats in intronic regions were associated with sequence variation between different strains. Homozygous mok(m632) or nie(m743) mutants and their respective wild-type sibs, showed identical patterns of heterozygosity and sequence variations within each line. No mutation in crx were identified in homozygous mok(m632) or nie(m743). Consistent with the absence of identified mutations, linkage analysis excluded linkage of the mutant phenotypes to crx. CONCLUSIONS: Despite the presence of sequence variations in their respective genetic backgrounds, within each line the sequence of crx was identical. Consistent with the absence of mutations, further analysis excluded linkage of the mutant phenotypes to crx. Analysis is in progress to map these loci and identify the genes responsible for the retinal degeneration phenotype in these mutant lines.

Descriptors: mutant zebrafish, genetically modified, homeodomain retinal degeneration genetics, trans activators genetics, base sequence, genetic crosses, DNA mutational analysis, genotype, introns and exons genetics, linkage genetics, molecular sequence data, phenotype, PCR.

Paddock, M.N., K. Graef, H. Clay, C.L. Cosma, and L. Ramakrishnan (2006). **Mycobacterium 19 kda lipoprotein is not required for apoptosis in vivo**. *Journal of Investigative Medicine* 54(1, Suppl. S): S88. ISSN: 1081 5589.

Descriptors: zebrafish, infection, immune system, chemical coordination and homeostasis, tuberculosis, bacterial disease, Tunel method, genetic techniques, flow cytometry, histology and cytology, fluorescent microscopy, imaging and microscopy techniques, macrophage apoptosis.

Pan, H., H.N. Dung, H.M. Hsu, K.M. Hsiao, and L.Y. Chen (2003). **Cloning and developmental expression of p73 cDNA in zebrafish.** *Biochemical and Biophysical Research Communications* 307(2): 395-400. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: p73 is one of the p53 family members which are transcription factors involved in the regulation of cell proliferation, apoptosis, and differentiation. In this study, we cloned the p73 cDNA from zebrafish ovary RNA. The consensus open reading frame (1923bp) encodes a polypeptide of 640 amino acids which shares 70-95% identity to the p73 of other vertebrates. Expression of zebrafish p73 mRNA is restricted to tissues such as skin, fin, brain, ovary, and testis, in contrast to the ubiquitous expression of zebrafish p53 and p63. During embryonic development, p73 transcripts are detected from the zygote period to the early larva stage. Whole-mount in situ hybridization reveals that p73 expression is in the brain, including olfactory bulbs, telencephalon, and hypothalamus, as well as in the pharyngeal arches and the nose. Moreover, p73 protein is found in the ovary and testis sections by immunohistochemical staining.

Descriptors: zebrafish, developmental gene expression regulation, transcription factors genetics and metabolism, proteins metabolism and genetics, amino acid and base sequence, molecular cloning, tumor suppressor genes, in situ hybridization, molecular sequence data, tissue distribution, transcription factors chemistry.

Pan, X., H. Wan, W. Chia, Y. Tong, and Z. Gong (2005). **Demonstration of site-directed recombination in transgenic zebrafish using the Cre/loxP system.** *Transgenic Research* 14(2): 217-23. ISSN: 0962-8819.
NAL Call Number: QH442.6T74
Abstract: To test the Cre/loxP recombination system in zebrafish, a stable transgenic zebrafish line was developed by using a floxed (loxP flanked) gfp (green fluorescent protein) gene construct under the muscle-specific mylz2 promoter. Like our previous non-floxed gfp transgenic line under the same promoter, the new transgenic line expresses GFP reporter faithfully in fast skeletal muscles to the same intensity. To demonstrate the excision of floxed gfp transgene, in vitro synthesized Cre RNA was injected into embryos of floxed gfp transgenic zebrafish and we found a dramatic reduction of GFP expression. To confirm the excision, PCR was performed and a DNA fragment of correct size was amplified as predicted from the Cre/loxP mediated excision. Finally, we cloned the fragment and sequence information confirmed that the excision occurred at the precise site as predicted. Our experiments demonstrated that the Cre/loxP system can function efficiently and accurately in the zebrafish system.
Descriptors: mutant zebrafish, integrases genetics, transgenes, viral proteins genetics, base sequence, DNA analysis, gene expression regulation, genetic markers, green fluorescent molecular sequence data, skeletal muscle, plasmids, PCR, promoter regions genetics.

Panagos, P.G., K.P. Dobrinski, X. Chen, A.W. Grant, D. Traver, J.Y. Djeu, S. Wei, and J.A. Yoder (2006). **Immune-related, lectin-like receptors are differentially expressed in the myeloid and lymphoid lineages of zebrafish.** *ImmunoGenetics* 58(1): 31-40. ISSN: 0093-7711.
NAL Call Number: QR184.I4
Abstract: The identification of C-type lectin (Group V) natural killer (NK) cell receptors in bony fish has remained elusive. Analyses of the Fugu rubripes genome database failed to identify Group V C-type lectin domains (Zelensky and Gready, BMC Genomics 5:51, 2004) suggesting that bony fish, in general, may lack such receptors. Numerous Group II C-type lectin receptors, which are structurally similar to Group V (NK) receptors, have been characterized in bony fish. By searching the zebrafish genome database we have identified a multi-gene family of Group II immune-related, lectin-like receptors (illrs) whose members possess inhibiting and/or activating signaling motifs typical of Group V NK receptors. Illr genes are differentially expressed in the myeloid and lymphoid lineages, suggesting that they may play important roles in the immune functions of multiple hematopoietic cell lineages.
Descriptors: zebrafish , chromosomes, C type lectins genetics, lymphocytes metabolism, myeloid cells metabolism , proteins metabolism and genetics, amino acid sequence, cell survival physiology, artificial bacterial chromosomes, natural killer cells metabolism, C type lectins chemistry, metabolism and physiology, molecular models, molecular sequence data, phylogeny, immunology.

Pandian, T.J. (2004). **Transgenesis and androgenesis: Challenges and opportunities**. *Fisheries Biotechnology,* Narendra Publishing House: Delhi, India, p. 1-36. ISBN: 8185375860.
Descriptors: zebrafish, androgenesis, biomarkers, biotechnology, DNA, fisheries, genes, genetics, India.

Papasani, M.R., R.C. Gensure, Y.L. Yan, Y. Gunes, J.H. Postlethwait, B. Ponugoti, M.R. John, H. Juppner, and D.A. Rubin (2004). **Identification and characterization of the zebrafish and fugu genes encoding tuberoinfundibular peptide 39.** *Endocrinology* 145(11): 5294-304. ISSN: 0013 7227.

NAL Call Number: 448.8EN2
Abstract: Although the PTH type 2 receptor (PTH2R) has been isolated from mammals and zebrafish, only its mammalian agonist, tuberoinfundibular peptide 39 (TIP39), has been characterized thus far. To determine whether zebrafish TIP39 (zTIP39) functions similarly with the zebrafish PTHR (zPTH2R) and human PTH2Rs and to determine its tissue-specific expression, fugu (*Takifugu rubripes*) and zebrafish (*Danio rerio*) genomic databases were screened with human TIP39 (hTIP39) sequences. A single TIP39 gene was identified for each fish species, which showed significant homology to mammalian TIP39. Using standard molecular techniques, we isolated cDNA sequences encoding zTIP39. The fugu TIP39 precursor was encoded by a gene comprising at least three exons. It contained a hydrophobic signal sequence and a predicted prosequence with a dibasic cleavage site, similar to that found in mammalian TIP39 ligands. Phylogenetic analyses suggested that TIP39 forms the basal group from which PTH and PTHrP have been derived. Functionally, subtle differences in potency could be discerned between hTIP39 and zTIP39. The human PTH2R and zPTH2R were stimulated slightly better by both hTIP39 and zTIP39, whereas zTIP39 had a higher potency at a previously isolated zPTH2R splice variant. Whole-mount in situ hybridization of zebrafish revealed strong zTIP39 expression in the region of the hypothalamus and in the heart of 24- and 48-h-old embryos. Similarly, zPTH2R expression was highly expressed throughout the brain of 48- and 72-h-old embryos. Because the mammalian PTH2R was also most abundantly expressed in these tissues, the TIP39-PTH2R system may serve conserved physiological roles in mammals and fishes.
Descriptors: zebrafish, neuropeptides genetics, *Takifugu* genetics, amino acid and base sequence, COS cells, molecular cloning, cyclic AMP metabolism, complementary DNA, developmental gene expression regulation, molecular sequence data, neuropeptides metabolism, parathyroid hormone related protein genetics, phylogeny.

Parichy, D.M. (2006). **Evolution of *danio* pigment pattern development**. *Heredity* 97(3): 200-210. ISSN: 0018 067X.
NAL Call Number: 443.8H42
Abstract: Pigment patterns of danio fishes are emerging as a useful system for studying the evolution of developmental mechanisms underlying adult form. Different closely related species within the genera *Danio* and *Devario* exhibit a range of pigment patterns including horizontal stripes, vertical bars, and others. In this review, I summarize recent work identifying the genetic and cellular bases for adult pigment pattern formation in the zebrafish *Danio rerio*, as well as studies of how these mechanisms have evolved in other danios. Together, these analyses highlight the importance of latent precursors at post-embrynoic stages, as well as interactions within and among pigment cell classes, for both pigment pattern development and evolution. Copyright © 2008 CABI
Descriptors: zebrafish embryos, embryonic development, evolution, genetic analysis, pigments, *Danio rerio*.
Notes: Special issue:Evolutionary developmental biology.(A review).

Park, S.H., S.Y. Yeo, K.W. Yoo, S.K. Hong, S. Lee, M. Rhee, A.B. Chitnis, and C.H. Kim (2003). **Zath3, a neural basic helix-loop-helix gene, regulates early neurogenesis in the zebrafish.** *Biochemical and Biophysical Research Communications* 308(1): 184-90. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: We have isolated a basic helix-loop-helix (bHLH) gene homologous to the *Drosophila* proneural gene atonal, termed zath3, from zebrafish. zath3 is expressed in neurons of the central nervous system and in subsets of cranial ganglia. Zebrafish mindbomb (mib)

mutants have a higher density of zath3 expressing cells and narrowminded (nrd) mutants lack zath3 expression in a domain corresponding to primary sensory neurons showing that the expression of zath3 is regulated by both mib and nrd. Injection of synthetic zath3 RNA into zebrafish embryos expands the neural plate size, promotes ectopic expression of neuronal markers, and partially rescues the deficit of sensory neurons seen in nrd mutants. Interfering with zath3 function using antisense morpholino oligonucleotides (MO) has no significant effect on early neurogenesis. However, a double knock down of zath3 and neurogenin1 (ngn1), another atonal homologue, with morpholinos (MOs) leads to more severe defects in neurogenesis than are seen with ngn1 MO alone: a subtle reduction of motor and interneurons, and an almost complete loss all cranial ganglia. This study suggests that zath3 and ngn1 have partially overlapping roles in early neurogenesis.
Descriptors: zebrafish, DNA binding proteins genetics, helix loop helix motifs, nerve tissue nervous system embryology, amino acid and base sequence, basic helix loop helix transcription factors, molecular cloning, DNA primers and binding proteins chemistry, molecular sequence data, nerve tissue proteins chemistry, messenger RNA genetics, amino acid sequence homology.

Park, S.W., J. Lin, B. Ghosh, and S.D. Leach (2004). **Lambda red-mediated BAC recombineering allows real-time visualization of pancreas development in living zebrafish embryos.** *Pancreas* 29(4): 341. ISSN: 0885 3177.
Descriptors: zebrafish, development, molecular genetics, biochemistry and molecular biophysics, digestive system, ingestion and assimilation.

Parker, L.H., M. Schmidt, S.W. Jin, A.M. Gray, D. Beis, T. Pham, G. Frantz, S. Palmieri, K. Hillan, D.Y.R. Stainier, F.J. DeSauvage, and W. Ye (2004). **The endothelial-cell-derived secreted factor Egfl7 regulates vascular tube formation**. *Nature* 428(6984): 754-758. ISSN: 0028-0836.
NAL Call Number: 472N21
Abstract: Vascular development is a complex but orderly process that is tightly regulated. A number of secreted factors produced by surrounding cells regulate endothelial cell (EC) differentiation, proliferation, migration and coalescence into cord-like structures1,2. Vascular cords then undergo tubulogenesis to form vessels with a central lumen3,4. But little is known about how tubulogenesis is regulated in vivo. Here we report the identification and characterization of a new EC-derived secreted factor, EGF-like domain 7 (Egfl7). Egfl7 is expressed at high levels in the vasculature associated with tissue proliferation, and is downregulated in most of the mature vessels in normal adult tissues. Loss of Egfl7 function in zebrafish embryos specifically blocks vascular tubulogenesis. We uncover a dynamic process during which gradual separation and proper spatial arrangement of the angioblasts allow subsequent assembly of vascular tubes. This process fails to take place in Egfl7 knockdown embryos, leading to the failure of vascular tube formation. Our study defines a regulator that controls a specific and important step in vasculogenesis. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, blood and lymphatics, transport and circulation, development, Egfl7, Egf like domain, endothelial cell derived secreted factor, angioblasts, downregulation, endothelial cell differentiation, migration and proliferation, spatial arrangement, tissue proliferation, vascular tube formation, vascular tubulogenesis, vasculogenesis.

Pathak, N., T. Obara, S. Mangos, Y. Liu, and I.A. Drummond (2007). **The zebrafish fleer gene encodes an essential regulator of cilia tubulin polyglutamylation.** *Molecular Biology of the Cell* 18(11): 4353-64. ISSN: 1059-1524.

NAL Call Number: QH604.C452
Abstract: Cilia and basal bodies are essential organelles for a broad spectrum of functions, including the development of left-right asymmetry, kidney function, cerebrospinal fluid transport, generation of photoreceptor outer segments, and Hedgehog signaling. Zebrafish fleer (flr) mutants exhibit kidney cysts, randomized left-right asymmetry, hydrocephalus, and rod outer segment defects, suggesting a pleiotropic defect in ciliogenesis. Positional cloning flr identified a tetratricopeptide repeat protein homologous to the *Caenorhabditis elegans* protein DYF1 that was highly expressed in ciliated cells. flr pronephric cilia were shortened and showed a reduced beat amplitude, and olfactory cilia were absent in mutants. flr cilia exhibited ultrastructural defects in microtubule B-tubules, similar to axonemes that lack tubulin posttranslational modifications (polyglutamylation or polyglycylation). flr cilia showed a dramatic reduction in cilia polyglutamylated tubulin, indicating that flr encodes a novel modulator of tubulin polyglutamylation. We also found that the *C. elegans* flr homologue, dyf-1, is also required for tubulin polyglutamylation in sensory neuron cilia. Knockdown of zebrafish Ttll6, a tubulin polyglutamylase, specifically eliminated tubulin polyglutamylation and cilia formation in olfactory placodes, similar to flr mutants. These results are the first in vivo evidence that tubulin polyglutamylation is required for vertebrate cilia motility and structure, and, when compromised, results in failed ciliogenesis.
Descriptors: zebrafish mutants, fleer gene, encode, kidney cysts, regulator, cilia, tubulin, polyglutamylation.

Patten, S.A., R.K. Sihra, K.S. Dhami, C.A. Coutts, and D.W. Ali (2007). **Differential expression of PKC isoforms in developing zebrafish.** *International Journal of Developmental Neuroscience* 25(3): 155-64. ISSN: 0736-5748.
NAL Call Number: QP363.5
Abstract: Protein kinase C isozymes are a biologically diverse group of enzymes known to be involved in a wide variety of cellular processes. They fall into three families (conventional, novel and atypical) depending upon their mode of activation. Several classes of zebrafish neurons have been shown to express PKCalpha during development, but the expression of other isoforms remains unknown. In this study we performed immunohistochemistry to determine if zebrafish express various isoforms of PKC. We used antibodies to test for the presence of enzymes that are thought to be preferentially expressed in the nervous system (PKCgamma, betaII, delta, epsilon, theta and zeta). Here, we show that PKCgamma, epsilon, theta and zeta are expressed in the zebrafish CNS. Anti-PKCgamma labels Rohon-Beard sensory neurons and Mauthner cells. PKCepsilon and zeta staining is widespread in the CNS, and PKCtheta and betaII are expressed in skeletal muscle, especially at intersegmental boundaries. Immunoblot experiments confirm the specificity of the antibodies in zebrafish and indicate that the fish isoforms of PKCgamma, betaII, epsilon and zeta are similar to the mammalian isoforms. Interestingly, PKCtheta appears to be similar to PKCthetaII, which, to date, has been found exclusively in mouse testis, but not in the mammalian CNS. Overall, our findings indicate that several different PKC isoforms are expressed in zebrafish, and that Rohon-Beard, Mauthner cells and muscle fibers preferentially express some isoforms over others.
Descriptors: zebrafish, developmental gene expression regulation physiology, protein kinase C metabolism and genetics, genetically modified, Western blotting methods, embryo, immunohistochemistry methods, isoenzymes genetics and metabolism, mutation, neurons metabolism, zebrafish anatomy and histology.

Paukert, M., S. Sidi, C. Russell, M. Siba, S.W. Wilson, T. Nicolson, and S. Grunder (2004). **A family of acid-sensing ion channels from the zebrafish: widespread expression in the central nervous system suggests a conserved role in neuronal communication.** *Journal of Biological Chemistry* 279(18): 18783-91. ISSN: 0021-9258.

NAL Call Number: 381J824

Abstract: Acid-sensing ion channels (ASICs) are excitatory receptors for extracellular H(+). Proposed functions include synaptic transmission, peripheral perception of pain, and mechanosensation. Despite the physiological importance of these functions, the precise role of ASICs has not yet been established. In order to increase our understanding of the physiological role and basic structure-function relationships of ASICs, we report here the cloning of six new ASICs from the zebrafish (zASICs). zASICs possess the basic functional properties of mammalian ASICs: activation by extracellular H(+), Na(+) selectivity, and block by micromolar concentrations of amiloride. The zasic genes are broadly expressed in the central nervous system, whereas expression in the peripheral nervous system is scarce. This pattern suggests a predominant role for zASICs in neuronal communication. Our results suggest a conserved function for receptors of extracellular H(+) in the central nervous system of vertebrates.

Descriptors: zebrafish embryos and larvae, central nervous system chemistry, membrane nerve tissue neurons chemistry, sodium channels genetics, base sequence, cell communication, electrophysiology, gene expression profiling, developmental gene expression regulation, hydrogen ion concentration, membrane, molecular sequence data, multigene family, nerve tissue, messenger RNA analysis, sodium channels physiology.

Peatman, E. and Z.J. Liu (2006). **CC chemokines in zebrafish: evidence for extensive intrachromosomal gene duplications**. *Genomics* 88(3): 381-385. ISSN: 0888 7543.

NAL Call Number: QH445.2.G45

Abstract: Chemokines are a family of structurally related chemotactic cytokines that regulate the migration of leukocytes. CC chemokines represent the largest subfamily of chemokines, with 28 genes in mammals. In recent studies in channel catfish, Ictalurus punctatus, we identified 26 distinct CC chemokine transcripts and obtained the genomic sequences and structures of 23 CC chemokine genes. However, without the availability of similar sets of CC chemokines in closely related species or a sequenced genome in catfish, it was difficult to make inferences as to the origins and modes of duplication of these molecules or to analyse conserved synteny between teleost and mammalian CC chemokines. Here, we have identified as many as 46 loci in the zebrafish genome that encode putative CC chemokines. The zebrafish CC chemokines are highly clustered on several chromosomes and show evidence of extensive, species-specific intrachromosomal duplications. Copyright © 2008 CABI

Descriptors: zebrafish, chemokines, chromosomes, cytokines, genes, genome analysis, genomes, loci, *Danio rerio.*

Pei, D.S., Y.H. Sun, S.P. Chen, Y.P. Wang, W. Hu, and Z.Y. Zhu (2007). **Identification of differentially expressed genes from the cross-subfamily cloned embryos derived from zebrafish nuclei and rare minnow enucleated eggs.** *Theriogenology* 68(9): 1282-91. ISSN: 0093-691X.

NAL Call Number: QP251.A1T5

Abstract: Cross-species nuclear transfer (NT) has been used to retain the genetic viability of a species near extinction. However, unlike intra-species NT, most embryos produced by cross-species NT were unable to develop to later stages due to incompatible nucleo-cytoplasmic interactions between the donor nuclei and the recipient cytoplasm from different species.

To study the early nucleo-cytoplasmic interaction in cross-species NT, two laboratory fish species (zebrafish and rare minnow) from different subfamilies were used to generate cross-subfamily NT embryos in the present study. Suppression subtractive hybridization (SSH) was performed to screen out differentially expressed genes from the forward and reverse subtractive cDNA libraries. After dot blot and real-time PCR analysis, 80 of 500 randomly selective sequences were proven to be differentially expressed in the cloned embryos. Among them, 45 sequences shared high homology with 28 zebrafish known genes, and 35 sequences were corresponding to 22 novel expressed sequence tags (ESTs). Based on functional clustering and literature mining analysis, up- and down-regulated genes in the cross-subfamily cloned embryos were mostly relevant to transcription and translation initiation, cell cycle regulation, protein binding, etc. To our knowledge, this is the first report on the determination of genes involved in the early development of cross-species NT embryos of fish.
Descriptors: zebrafish, identification, differentially expressed genes, cross-subfamily, clone, embryos, nuclei, minnow, enucleated eggs.

Pei, D.S., Y.H. Sun, S.P. Chen, Y.P. Wang, W. Hu, and Z.Y. Zhu (2007). **Zebrafish GAPDH can be used as a reference gene for expression analysis in cross-subfamily cloned embryos.** *Analytical Biochemistry* 363(2): 291-3. ISSN: 0003 2697.
NAL Call Number: 381 An13
Descriptors: zebrafish, metabolism, developmental gene expression regulation, glyceraldehyde 3 phosphate dehydrogenases genetics, Cyprinidae embryology and genetics, reverse transcriptase PCR.

Pei, D.S., Y.H. Sun, S.P. Chen, Y.P. Wang, and Z.Y. Zhu (2007). **Cloning and characterization of cytochrome C oxidase subunit I (COXI) in *Gobiocypris rarus*.** *DNA Sequence* 18(1): 1-8. ISSN: 1042-5179.
NAL Call Number: QH435.D57
Descriptors: zebrafish, 3' untranslated regions, amino acid sequence, amino acids, cytochrome-c oxidase, cytochromes, DNA, eggs, embryos, freshwater fish, gene expression, nuclei, nucleotide sequence, open reading frames, phylogenetics, RNA editing, taxonomy, *Danio rerio*, *Gobiocypris rarus*, Gobioninae.

Pelegri, F. (2004). **The role of maternal factors in early zebrafish development**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd.: Singapore, Singapore: p. 1-38. ISBN: 9812388214.
NAL Call Number: QL638.C94 F57
Abstract: The earliest events in zebrafish development are driven by maternal factors deposited in the egg during oogenesis that become activated upon fertilization and initiate cascades of events that drive early development. This review summarizes the forward and reverse genetic methods used to identify and analyze genes coding for such maternal factors. I also discuss current knowledge on the cellular processes involved in two important developmental transitions: the redistribution and activation of maternal factors at fertilization, and the transition from maternal to zygotic genetic programs. In addition, I summarize current knowledge on the function of maternal factors, both before and after zygotic gene activation, in embryonic processes involved in general cellular functions, axis formation, and cell fate specification. Copyright © 2008 CABI
Descriptors: zebrafish , embryonic development, fertilization, fish eggs, genes, genetic effects, maternal effects, messenger RNA, oogenesis, RNA, zygotes, *Danio rerio*, fishes.

Pelegri, F.J. (2005). **Analysis of maternal-effect mutations that affect zebrafish embryogenesis**. *Federation of American Societies for Experimental Biology Journal* 19(5, Suppl. S, Part 2): A1337. ISSN: 0892 6638.

NAL Call Number: QH301.F3

Abstract: In animal species, early developmental processes are driven by maternally-derived factors. We previously used a gynogenesis-based approach to identify recessive maternal-effect mutations in the zebrafish. Homozygosity for these mutations in adult females leads to the inviability of their offspring. Confocal microscopy of embryos labeled with a DNA dye and a membrane marker allowed us to further analyze mutant embryos for defects in the nuclear and cellular divisions. The mutations result in a range of defects in processes including egg activation, early nuclear events, mitosis, cytokinesis, axial patterning and gastrulation. The sample of mutations that we have identified reflects the diversity of maternally-driven functions in early development and underscores the importance of maternal factors in this process. We are currently studying in more detail several of these maternal-effect mutations. Two mutations, in the genesnebel and aura, affect early cytoskeletal rearrangements required for the secretion of internal membrane during cytokinesis and the segregation of the zebrafish germ plasm. Another mutation, in the gene hecate, results in defects in Wnt-based signaling pathways important for the induction of the dorsal axis inearly embryos. Another identified gene, mission impossible, is required for aprocess that occurs during gastrulation, which is important for morphogenesisand the induction of the mesendoderm. We are currently carrying out positional cloning projects to determine the molecular identity of these genes.
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Descriptors: zebrafish embryos, molecular genetics, biochemistry and molecular biophysics, development, embryogenesis, cytoskeletal rearrangement.

Pellegrini, E., A. Menuet, C. Lethimonier, F. Adrio, M.M. Gueguen, C. Tascon, I. Anglade, F. Pakdel, and O. Kah (2005). **Relationships between aromatase and estrogen receptors in the brain of teleost fish**. *General and Comparative Endocrinology* 142(1/2): 60-66. ISSN: 0016 6480.

NAL Call Number: 444.8G28

Abstract: Teleost fish are known for exhibiting a high aromatase activity mainly due to the expression of the cyp19b gene, encoding aromatase B (AroB). Recent studies based on both in situ hybridization and immunohistochemistry have demonstrated in three different species that this activity is restricted to radial glial cells. In agreement with measurements of aromatase activity, such aromatase-expressing cells are more abundant in the telencephalon, preoptic area, and mediobasal hypothalamus, although positive cells are also found in the midbrain and hindbrain. Comparative distribution of AroB and oestrogen receptor (ER alpha , ER beta 1, and ER beta 2) expression indicates that the preoptic region and hypothalamus are major target for locally produced estradiol (E2) which is likely involved in controlling expression of genes implicated in neuroendocrine regulations. However, AroB and ER have never been reported to be co-expressed in the same cells which is intriguing given that, at least in some species, AroB is strongly up-regulated by E2 itself in agreement with the presence of an estrogen-responsive element (ERE) in the proximal promoter of the cyp19b gene. In vivo data in zebrafish have shown that E2 up-regulates AroB only in radial glial cells. This is in agreement with in vitro transfection experiments indicating that this ERE is functional, but not sufficient, as the E2 regulation of AroB only occurs in glial cell contexts, suggesting a cooperation between ER and so far unidentified glial-specific factors. These data also suggest that radial glial cells may express low amounts of ER that escaped detection until now. The expression of AroB in radial cells, well known for their roles in neurogenesis and now considered as progenitor cells, suggests that local E2 production within

these cells could influence the well-documented capacity of the brain of teleosts to grow during adulthood. Copyright © 2008 CABI

Descriptors: zebrafish, antibodies, brain, enzyme activity, estradiol, estrogens, gene expression, genes, hypothalamus, neuroglia, estrogen receptors, preoptic area, promoters, reviews, sex determination, transfection.

Notes: A review.

Pendeville, H., B. Peers, K. Kas, and M.L. Voz (2006). **Cloning and embryonic expression of zebrafish PLAG genes.** *Gene Expression Patterns* 6(3): 267-76. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: PLAG transcription factors play important roles in oncogenesis. To date three members of this subfamily of zinc finger proteins have been identified in humans and mice: PLAG1, PLAGL1 and PLAGL2. In this study, we identified zebrafish orthologs of PLAG1 and PLAGL2 and a novel member of this family, PLAGX. We examined the temporal expression of these three genes by quantitative real time RT-PCR and found that all three genes are maternally provided, expressed at low level during early somitogenesis and, during late somitogenesis and beyond, PLAG expression increases to reach a plateau level around 5 dpf. Whole mount in situ experiments revealed that PLAG1, PLAGL2 and PLAGX display a similar pattern of expression characterized by a low ubiquitous expression overcame by high expression in some restricted compartments such as the ventricular zone of the brain, the pectoral fin buds, the developing pharyngeal arches and the axial vasculature. We show that this pattern resembles the one observed for the proliferative marker PCNA, suggesting that the PLAG genes are expressed more strongly in zones of active proliferation. This hypothesis was proven for the ventricular zone shown to be a highly proliferative zone using the anti-phosphohistone H3 antibody that detects cells in mitosis.

Descriptors: zebrafish, molecular cloning, developmental gene expression regulation, amino acid motifs, amino acid sequence, brain embryology and metabolism, conserved sequence, embryology, immunohistochemistry, in situ hybridization, molecular sequence data, phylogeny, tertiary protein structure, reverse transcriptase PCR, amino acid sequence homology, synteny, transcription factors genetics.

Perrino, M.A. and P.C. Yelick (2004). **Immunolocalization of Alk8 during replacement tooth development in zebrafish.** *Cells, Tissues, Organs* 176(1): 17-27. ISSN: 1422 6405.

Abstract: The novel type I transforming growth factor-beta (TGF-beta) family member receptor Alk8 was previously identified in a degenerate RT-PCR screen for zebrafish type I and II TGF-beta family member receptors. Functional analyses revealed that Alk8 acts through Bmp signaling pathways in early embryonic dorsoventral patterning, in neural crest cell specification, and in patterning and differentiation of neural crest cell-derived pharyngeal arch cartilages. In addition, Alk8 forms active signaling complexes with TGF-beta1 and the TGF-beta RII receptor, suggesting that Alk8 mediates cross talk between Bmp and TGF-beta subfamily members. In this study, immunohistochemical analysis was performed on zebrafish aged 2 days postfertilization to 1 year, revealing immunolocalization of Alk8 to tissues of the tooth-bearing ceratobranchial 5 (cb5) arch including dental epithelial and mesenchymal tooth tissues of developing primary and replacement teeth, mucous-producing crypt epithelium, keratinized bite plate, and developing taste buds. These results suggest roles for Alk8 in patterning tooth-bearing pharyngeal epithelium, in the initiation of tooth development, in odontoblast and ameloblast differentiation, and in osteoblast maturation. The ability for zebrafish to continuously form teeth throughout their lives allows for the comparison of Alk8 expression in both primary and replacement tooth development, revealing identical Alk8

expression profiles. This study advances our current understanding of the functions of Alk8, particularly with respect to primary and replacement tooth formation, reveals additional roles for Alk8 in dental epithelial patterning and in odontoblast, ameloblast and osteoblast differentiation, and demonstrates the utility of the zebrafish as a model for primary and replacement tooth development.
Descriptors: zebrafish, Type I activin receptors analysis, tooth chemistry, metabolism, proteins analysis, Type I activin receptors physiology, ameloblasts chemistry and cytology, vascular endothelium chemistry, epithelium chemistry, goblet cells chemistry and cytology, immunohistochemistry, larva chemistry, mouth chemistry, odontoblasts chemistry and cytology, odontogenesis physiology , osteoblasts chemistry and cytology, pharynx anatomy, histology and chemistry, skull base anatomy and histology and chemistry, tooth growth and development.

Peterkin, T., A. Gibson, and R. Patient (2003). **GATA-6 maintains BMP-4 and Nkx2 expression during cardiomyocyte precursor maturation**. *European Molecular Biology Organization Journal Journal* 22(16): 4260-4273. ISSN: 0261-4189.
NAL Call Number: QH506.E46
Descriptors: zebrafish, antisense, embryonic development, gene expression, heart, mesoderm, myocytes, oligonucleotides, organogenesis, *Danio rerio*, *Xenopus*, BMp-4 gene, clawed frogs, GATA-6 protein, Nkx2 gene, developmental genetics, genetics and evolution, reproduction and development.

Peterson, R.T., S.Y. Shaw, T.A. Peterson, D.J. Milan, T.P. Zhong, S.L. Schreiber, C.A. MacRae, and M.C. Fishman (2004). **Chemical suppression of a genetic mutation in a zebrafish model of aortic coarctation.** *Nature Biotechnology* 22(5): 595-9. ISSN: 1087-0156.
NAL Call Number: QH442.B5
Abstract: Conventional drug discovery approaches require a priori selection of an appropriate molecular target, but it is often not obvious which biological pathways must be targeted to reverse a disease phenotype. Phenotype-based screens offer the potential to identify pathways and potential therapies that influence disease processes. The zebrafish mutation gridlock (grl, affecting the gene hey2) disrupts aortic blood flow in a region and physiological manner akin to aortic coarctation in humans. Here we use a whole-organism, phenotype-based, small-molecule screen to discover a class of compounds that suppress the coarctation phenotype and permit survival to adulthood. These compounds function during the specification and migration of angioblasts. They act to upregulate expression of vascular endothelial growth factor (VEGF), and the activation of the VEGF pathway is sufficient to suppress the gridlock phenotype. Thus, organism-based screens allow the discovery of small molecules that ameliorate complex dysmorphic syndromes even without targeting the affected gene directly.
Descriptors: zebrafish mutation gridlock, aortic coarctation prevention, control and genetics, base sequence, DNA primers, disease models.

Peterson, R.T. (2006). **A noncanonical path to mechanism of action**. *Chemistry and Biology* 13(9): 924-926. ISSN: 1074 5521.
NAL Call Number: QP501
Abstract: Improved methods for discovering small-molecule mechanisms of action are needed. In this issue of Chemistry & Biology, Zhang et al. [1] make clever use of the zebrafish to study the mechanism of the angiogenesis inhibitor fumagillin and reveal that it targets the noncanonical Wnt pathway. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, pharmacology, molecular genetics , fumagillin, angiogenesis inhibi-

tor, enzymology, phenotype, Wnt pathway.
Notes: A review.

Petit, H. (2005). **A fluorescent transgenic fish.** *Biofutur* 254: 38-40. ISSN: 0294-3506.
Descriptors: zebrafish, bioluminescence, fluorescence, genomes, karyotypes, phylogenetics, transgenic fish, *Aequorea victoria*, *Danio rerio*, *Oryzias latipes*, medaka, water jellyfish, genetics and evolution.
Language of Text: French.

Pezeron, G., I. Anselme, M. Laplante, S. Ellingsen, T.S. Becker, F.M. Rosa, P. Charnay, S. Schneider Maunoury, P. Mourrain, and J. Ghislain (2006). **Duplicate sfrp1 genes in zebrafish: sfrp1a is dynamically expressed in the developing central nervous system, gut and lateral line.** *Gene Expression Patterns* 6(8): 835-42. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: The secreted frizzled-related proteins (Sfrp) are a family of soluble proteins with diverse biological functions having the capacity to bind Wnt ligands, to modulate Wnt signalling, and to signal directly via the Wnt receptor, Frizzled. In an enhancer trap screen for embryonic expression in zebrafish we identified an sfrp1 gene. Previous studies suggest an important role for sfrp1 in eye development, however, no data have been reported using the zebrafish model. In this paper, we describe duplicate sfrp1 genes in zebrafish and present a detailed analysis of the expression profile of both genes. Whole mount in situ hybridisation analyses of sfrp1a during embryonic and larval development revealed a dynamic expression profile, including: the central nervous system, where sfrp1a was regionally expressed throughout the brain and developing eye; the posterior gut, from the time of endodermal cell condensation; the lateral line, where sfrp1a was expressed in the migrating primordia and interneuromast cells that give rise to the sensory organs. Other sites included the blastoderm, segmenting mesoderm, olfactory placode, developing ear, pronephros and fin-bud. We have also analysed sfrp1b expression during embryonic development. Surprisingly this gene exhibited a divergent expression profile being limited to the yolk syncytium under the elongating tail-bud, which later covered the distal yolk extension, and transiently in the tail-bud mesenchyme. Overall, our studies provide a basis for future analyses of these developmentally important factors using the zebrafish model.
Descriptors: zebrafish, central nervous system embryology, central nervous system metabolism, gastrula metabolism, proteins metabolism and genetics, amino acid sequence, cleavage stage, ovum metabolism eye embryology and metabolism, gene duplication, developmental gene expression regulation, mesoderm metabolism, molecular sequence data, phylogeny, amino acid sequence homology, metabolism.

Pfaff, K.L., C.T. Straub, K. Chiang, D.M. Bear, Y. Zhou, and L.I. Zon (2007). **The zebra fish cassiopeia mutant reveals that SIL is required for mitotic spindle organization.** *Molecular and Cellular Biology* 27(16): 5887-97. ISSN: 0270-7306.
NAL Call Number: QH506.M664
Abstract: A critical step in cell division is formation of the mitotic spindle, which is a bipolar array of microtubules that mediates chromosome separation. Here, we report that the SCL-interrupting locus (SIL), a vertebrate-specific cytosolic protein, is necessary for proper mitotic spindle organization in zebrafish and human cells. A homozygous lethal zebrafish mutant, cassiopeia (csp), was identified by a genetic screen for mitotic mutant. csp mutant embryos have an increased mitotic index, have highly disorganized mitotic spindles, and often lack one or both centrosomes. These phenotypes are caused by a loss-of-function

mutation in zebrafish sil. To determine if the requirement for SIL in mitotic spindle organization is conserved in mammals, we generated an antibody against human SIL, which revealed that SIL localizes to the poles of the mitotic spindle during metaphase. Furthermore, short hairpin RNA knockdown of SIL in human cells recapitulates the zebrafish csp mitotic spindle defects. These data, taken together, identify SIL as a novel, vertebrate-specific regulator of mitotic spindle assembly.

Descriptors: zebrafish, cell cycle proteins metabolism, intracellular signaling peptides and proteins metabolism, mitotic spindle apparatus metabolism, mutation genetics, metabolism, body patterning, cytology, HeLa cells, microtubule associated proteins metabolism, mitosis, phenotype, protein transport, small interfering RNA metabolism.

Pfister, P. and I. Rodriguez (2005). **Olfactory expression of a single and highly variable V1r pheromone receptor-like gene in fish species**. *Proceedings of the National Academy of Sciences, USA* 102(15): 5489-5494. ISSN: 0027-8424.

NAL Call Number: 500N21P

Descriptors: zebrafish, biological membranes, chemoreception, freshwater fish, gene expression, homology, neurons, odorant receptors, olfaction, olfactory system, pheromone receptors, sensory neurons, taste, taste receptors, vomeronasal organ, *Danio rerio*, taste, smell and chemical senses, pheromones and other infochemicals.

Phelan, P.E., M.E. Pressley, P.E. Witten, M.T. Mellon, S. Blake, and C.H. Kim (2005). **Characterization of snakehead rhabdovirus infection in zebrafish (*Danio rerio*).** *Journal of Virology* 79(3): 1842-52. ISSN: 0022-538X.

NAL Call Number: QR360.J6

Abstract: The zebrafish, *Danio rerio*, has become recognized as a valuable model for the study of development, genetics, and toxicology. Recently, the zebrafish has been recognized as a useful model for infectious disease and immunity. In this study, the pathogenesis and antiviral immune response of zebrafish to experimental snakehead rhabdovirus (SHRV) infection was characterized. Zebrafish 24 h postfertilization to 30 days postfertilization were susceptible to infection by immersion in 10(6) 50% tissue culture infective doses (TCID50) of SHRV/ml, and adult zebrafish were susceptible to infection by intraperitoneal (i.p.) injection of 10(5) TCID50 of SHRV/ml. Mortalities exceeded 40% in infected fish, and clinical presentation of infection included petechial hemorrhaging, redness of the abdomen, and erratic swim behavior. Virus reisolation and reverse transcription-PCR analysis of the viral nucleocapsid gene confirmed the presence of SHRV. Histological sections of moribund embryonic and juvenile fish revealed necrosis of the pharyngeal epithelium and liver, in addition to congestion of the swim bladder by cell debris. Histopathology in adult fish injected i.p. was confined to the site of injection. The antiviral response in zebrafish was monitored by quantitative real-time PCR analysis of zebrafish interferon (IFN) and Mx expression. IFN and Mx levels were elevated in zebrafish exposed to SHRV, although expression and intensity differed with age and route of infection. This study is the first to examine the pathogenesis of SHRV infection in zebrafish. Furthermore, this study is the first to describe experimental infection of zebrafish embryos with a viral pathogen, which will be important for future experiments involving targeted gene disruption and forward genetic screens.

Descriptors: zebrafish, disease models, novirhabdovirus pathogenicity, Rhabdoviridae infections physiopathology, zebrafish virology, cultured cells, GTP binding proteins metabolism, interferons metabolism, novirhabdovirus genetics, isolation and purification, Rhabdoviridae infections pathology and virology.

Phillips, R.B., A. Amores, M.R. Morasch, C. Wilson, and J.H. Postlethwait (2006). **Assignment of zebrafish genetic linkage groups to chromosomes.** *Cytogenetic and Genome Research* 114(2): 155-62. ISSN: 1424 8581.

NAL Call Number: QH431.C95

Abstract: In this report the zebrafish genetic linkage groups are assigned to specific chromosomes using fluorescence in situ hybridization (FISH) with BAC probes containing genes mapped to each linkage group (LG). Chromosomes were identified using a combination of relative size and arm ratios. The largest genetic maps generally corresponded to the largest chromosomes, but genetic recombination tended to be elevated in the smaller chromosomes and near telomeres. Large insert clones containing genes near telomeres often hybridized to telomeres of multiple chromosome pairs, suggesting the presence of shared subtelomeric repetitive DNAs near telomeres. Evidence from comparative gene mapping in medaka, zebrafish, pufferfish, and humans suggests that the linkage groups of these species have the content of duplicate proto-chromosomes. However, these duplicate linkage groups are not associated with chromosomes of similar size or morphology. This suggests that considerable chromosome restructuring occurred subsequent to the genome duplication in teleosts.

Descriptors: zebrafish, chromosomes genetics, linkage genetics, cell line, chromosome mapping, karyotyping.

Pi, W., Z. Yang, J. Wang, L. Ruan, X. Yu, J. Ling, S. Krantz, C. Isales, S.J. Conway, S. Lin , and D. Tuan (2004). **The LTR enhancer of ERV-9 human endogenous retrovirus is active in oocytes and progenitor cells in transgenic zebrafish and humans.** *Proceedings of the National Academy of Sciences of the United States of America* 101(3): 805-10. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: The solitary LTRs of ERV-9 human endogenous retrovirus are middle repetitive DNAs associated with 3,000-4,000 human gene loci including the beta-globin gene locus where the ERV-9 LTR is juxtaposed to the locus control region (beta-LCR) far upstream of the globin genes. The ERV-9 LTRs are conserved during primate evolution, but their function in the primate genomes is unknown. Here, we show that in transgenic zebrafish harboring the beta-globin ERV-9 LTR coupled to the GFP gene, the LTR enhancer was active and initiated synthesis of GFP mRNA in oocytes but not in spermatozoa, and GFP expression in the embryos was maternally inherited. The LTR enhancer was active also in stem/progenitor cell regions of adult tissues of transgenic zebrafish. In human tissues, ERV-9 LTR enhancer was active also in oocytes and stem/progenitor cells but not in spermatozoa and a number of differentiated, adult somatic cells. Transcriptional analyses of the human beta-globin gene locus showed that the beta-globin ERV-9 LTR enhancer initiated RNA synthesis from the LTR in the direction of the downstream beta locus control region and globin genes in ovary and erythroid progenitor cells. The findings suggest that, during oogenesis, ERV-9 LTR enhancers in the human genome could activate the cis-linked gene loci to synthesize maternal mRNAs required for early embryogenesis. Alternatively, the ERV-9 LTR enhancers, in initiating RNA syntheses into the downstream genomic DNAs, could transcriptionally potentiate and preset chromatin structure of the cis-linked gene loci in oocytes and adult stem/progenitor cells.

Descriptors: zebrafish, endogenous retroviruses genetics, enhancer elements genetics, oocytes virology, stem cells virology, terminal repeat sequences, genetically modified, cell differentiation , globins genetics, green fluorescent proteins, locus control region, luminescent oocytes metabolism and genetics, recombinant spermatozoa virology, stem cells metabolism.

Pichler, F.B., S. Laurenson, L.C. Williams, A. Dodd, B.R. Copp, and D.R. Love (2003). **Chemical discovery and global gene expression analysis in zebrafish.** *Nature Biotechnology* 21(8): 879-83. ISSN: 1087-0156.

NAL Call Number: QH442.B5

Abstract: The zebrafish (*Danio rerio*) provides an excellent model for studying vertebrate development and human disease because of its ex utero, optically transparent embryogenesis and amenability to in vivo manipulation. The rapid embryonic developmental cycle, large clutch sizes and ease of maintenance at large numbers also add to the appeal of this species. Considerable genomic data has recently become publicly available that is aiding the construction of zebrafish microarrays, thus permitting global gene expression analysis. The zebrafish is also suitable for chemical genomics, in part as a result of the permeability of its embryos to small molecules and consequent avoidance of external confounding maternal effects. Finally, there is increasing characterization and analysis of zebrafish models of human disease. Thus, the zebrafish offers a high-quality, high-throughput bioassay tool for determining the biological effect of small molecules as well as for dissecting biological pathways.

Descriptors: zebrafish, biological assay methods, disease models, drug design, gene expression regulation physiology, oligonucleotide array sequence analysis methods, pharmaceutical preparations metabolism, developmental gene expression regulation physiology.

Pickart, M.A., S. Sivasubbu, A.L. Nielsen, S. Shriram, R.A. King, and S.C. Ekker (2004). **Functional genomics tools for the analysis of zebrafish pigment.** *Pigment Cell Research* 17(5): 461-70. ISSN: 0893-5785.

Abstract: Genetic model organisms are increasingly valuable in the post-genomics era to provide a basis for comparative analysis of the human genome. For higher order processes of vertebrate pigment cell biology and development, the mouse has historically been the model of choice. A complementary organism, the zebrafish (*Danio rerio*), shares many of the signaling and biological processes of vertebrates, e.g. neural crest development. The zebrafish has a number of characteristics that make it an especially valuable model for the study of pigment cell biology and disease. Large-scale genetic screens have identified a collection of pigmentation mutants that have already made valuable contributions to pigment research. An increasing repertoire of genomic resources such as an expressed sequence tag-based Gene Index (The Institute for Genomic Research) and improving methods of mutagenesis, transgenesis, and gene targeting make zebrafish a particularly attractive model. Morpholino phosphorodiamidate oligonucleotide (MO) 'knockdown' of pigment gene expression provides a non-conventional antisense tool for the analysis of genes involved in pigment cell biology and disease. In addition, an ongoing, reverse-genetic, MO-based screen for the rapid identification of gene function promises to be a valuable complement to other high-throughput microarray and proteomic approaches for understanding pigment cell biology. Novel reagents for zebrafish transgenesis, such as the Sleeping Beauty transposon system, continue to improve the capacity for genetic analysis in this system and ensure that the zebrafish will be a valuable genetic model for understanding a variety of biological processes and human diseases for years to come.

Descriptors: zebrafish, genomics, mutagenesis, pigmentation genetics, biological pigments genetics, developmental gene expression regulation genetics, gene targeting methods, antisense oligonucleotides genetics , biological pigments metabolism, proteins metabolism, research animal model.

Pinal Seoane, N., I.R. Martin, V. Gonzalez Nunez , E.M. de Velasco, F.A. Alvarez, R.G. Sarmiento, and R.E. Rodriguez (2006). **Characterization of a new duplicate delta-opioid receptor from zebrafish.** *Journal of Molecular Endocrinology* 37(3): 391-403. ISSN: 0952-5041.
NAL Call Number: QP187.3.M64J68
Abstract: A new full-length cDNA (ZFOR4) that encodes an opioid receptor has been isolated from the teleost zebrafish. The encoded polypeptide is 375 amino acids long and shows high sequence similarity to other delta-opioid receptors, including ZFOR1, the other delta-opioid receptor from zebrafish previously characterized by us. In situ hybridization studies have revealed that ZFOR4 mRNA is highly expressed in particular brain areas that coincide with the expression of the delta-opioid receptor in other species. Pharmacological analysis of ZFOR4 shows specific and saturable binding with [(3)H] diprenorphine, displaying one binding site with K(D) = 3.42 +/- 0.38 nM and a receptor density of 6231 +/- 335 fmol/mg protein. Competition-binding experiments were performed using [(3)H]diprenorphine and several unlabelled ligands (peptidic and non-peptidic). The order of affinity obtained is Met-enkephalin>Naloxone>Leu-enkephalin>Dynorphin A>>BW373U86>Morphine>>>> [D-Pen(2),D-Pen(5)]-Enkephalin, U69,593. [(35)S]GTPgammaS stimulation studies show that the endogenous ligands Met- and Leu-enkephalin and the non-peptidic delta agonist BW373U86 were able to fully activate ZFOR4. Our results prove the existence of two functional duplicate genes of the delta-opioid receptor in the teleost zebrafish.
Descriptors: zebrafish, delta opioid receptors chemistry and metabolism, amino acid and base sequence, cell line, cell membrane metabolism, molecular cloning, conserved sequence, complementary DNA genetics, gene expression, in situ hybridization, molecular sequence data, phylogeny, protein binding, protein isoforms chemistry, protein isoforms genetics and metabolism, messenger RNA genetics, delta opioid receptors genetics, sequence alignment.

Pinheiro, P., M. Gering, and R. Patient (2004). **The basic helix-loop-helix transcription factor, Tal2, marks the lateral floor plate of the spinal cord in zebrafish.** *Gene Expression Patterns* 4(1): 85-92. ISSN: 1567 133X.
NAL Call Number: QH450.B73
Abstract: Basic helix-loop-helix (bHLH) transcription factors play key roles in the development of the central nervous system. Here we report the isolation of a zebrafish gene that encodes a homologue of the mammalian bHLH transcription factor, Tal2. In zebrafish embryos, tal2, like its mammalian homologue, is strongly expressed in the diencephalon and the mesencephalon, with the latter expression located in post-mitotic cells of the tectum. However, in addition to this conserved brain expression, we also detect expression in the floor plate of the spinal cord. By the location of this expression relative to other genes expressed in the floor plate and by analysing expression in a selection of midline mutants, we reveal that tal2 is expressed within the lateral floor plate as opposed to the medial floor plate, and also in more dorsal cells which are distinct from motorneurons and depend on either sonic hedgehog signalling or a signal coming from the lateral floor plate. This is to our knowledge the first report of a gene expressed specifically in lateral cells of the floor plate in the spinal cord.
Descriptors: zebrafish, spinal cord metabolism, transcription factors genetics, amino acid sequence, basic helix loop helix transcription factors, metabolism, embryonic development, gene expression profiling, developmental gene expression regulation, genetic markers, in situ hybridization, molecular sequence data, amino acid sequence homology, spinal cord embryology, time factors.

Pini, B., T. Grosser, J.A. Lawson, T.S. Price, M.A. Pack, and G.A. FitzGerald (2005). **Prostaglandin E synthases in zebrafish.** *Arteriosclerosis Thrombosis and Vascular Biology* 25(2): 315-20. ISSN: 1079 5642.

Abstract: OBJECTIVE: Prostaglandin E synthases (PGESs) are being explored as antiinflammatory drug targets as alternatives to cyclooxygenase (COX)-2. Located downstream of the cyclooxygenases, PGESs catalyze PGE(2) formation, and deletion of microsomal (m)-PGES-1 abrogates inflammation. We sought to characterize the developmental expression of COX and PGES in zebrafish. METHODS AND RESULTS: We cloned zebrafish cytosolic (c) and m-PGES orthologs and mapped them to syntenic regions of chromosomes 23 and 5. cPGES was widely expressed during development and was coordinately regulated with zCOX-1 in the inner ear, the pronephros, and intestine. COX-2 and mPGES-1 exhibited restricted expression, dominantly in the vasculature of the aortic arch. However, the enzymes were anatomically segregated within the vessel wall. Experiments with antisense morpholinos and with nonsteroidal antiinflammatory drugs suggest that these genes may not be critical for development. CONCLUSIONS: mPGES-1 is developmentally coregulated with COX-2 in vasculature. Given the high fecundidity and translucency of the zebrafish, this model may afford a high throughput system for characterization of novel PGES inhibitors. Microsomal prostaglandin E synthase (mPGES)-1, located downstream of COX-2, may represent a novel antiinflammatory drug target. Zebrafish cytosolic (c) PGES-1 and COX-1 were coordinately expressed; mPGES-1 and COX-2 were expressed particularly in the vasculature. Zebrafish may afford a high throughput system for detection of novel PGES inhibitors.

Descriptors: zebrafish, intramolecular oxidoreductases genetics, prostaglandin E biosynthesis, blood vessels embryology, blood vessels enzymology, growth and development, chromosome mapping, cyclooxygenase 1, cyclooxygenase 2 developmental gene expression regulation, enzymologic gene expression regulation, intramolecular oxidoreductases biosynthesis, isoenzymes biosynthesis and genetics, kidney embryology, enzymology, growth and development, larva, microsomes enzymology, molecular sequence data, organ specificity, phylogeny, prostaglandin endoperoxide synthases biosynthesis and genetics, semicircular canals embryology, enzymology, growth and development.

Pinto, J.P., N.M. Conceicao, C.S. Viegas, R.B. Leite, L.D. Hurst, R.N. Kelsh, and M.L. Cancela (2005). **Identification of a new pebp2alphaA2 isoform from zebrafish runx2 capable of inducing osteocalcin gene expression in vitro.** *Journal of Bone and Mineral Research* 20(8): 1440-53. ISSN: 0884-0431.

Abstract: The zebrafish runx2b transcription factor is an ortholog of RUNX2 and is highly conserved at the structural level. The runx2b pebp2alphaA2 isoform induces osteocalcin gene expression by binding to a specific region of the promoter and seems to have been selectively conserved in the teleost lineage. INTRODUCTION: RUNX2 (also known as CBFA1/Osf2/AML3/PEBP2alphaA) is a transcription factor essential for bone formation in mammals, as well as for osteoblast and chondrocyte differentiation, through regulation of expression of several bone- and cartilage-related genes. Since its discovery, Runx2 has been the subject of intense studies, mainly focused in unveiling regulatory targets of this transcription factor in high vertebrates. However, no single study has been published addressing the role of Runx2 in bone metabolism of low vertebrates. While analyzing the zebrafish (*Danio rerio*) runx2 gene, we identified the presence of two orthologs of RUNX2, which we named runx2a and runx2b and cloned a pebp2alphaA-like transcript of the runx2b gene, which we named pebp2alphaA2. MATERIALS AND METHODS: Zebrafish runx2b gene and cDNA were isolated by RT-PCR and sequence data mining. The 3D structure of runx2b runt domain was modeled using mouse Runx1 runt as template. The regulatory effect of pebp2alphaA2

on osteocalcin expression was analyzed by transient co-transfection experiments using a luciferase reporter gene. Phylogenetic analysis of available Runx sequences was performed with TREE_PUZZLE 5.2. and MrBayes. RESULTS AND CONCLUSIONS: We showed that the runx2b gene structure is highly conserved between mammals and fish. Zebrafish runx2b has two promoter regions separated by a large intron. Sequence analysis suggested that the runx2b gene encodes three distinct isoforms, by a combination of alternative splicing and differential promoter activation, as described for the human gene. We have cloned a pebp2alphaA-like transcript of the runx2b gene, which we named pebp2alphaA2, and showed its high degree of sequence similarity with the mammalian pebp2alphaA. The cloned zebrafish osteocalcin promoter was found to contain three putative runx2-binding elements, and one of them, located at -221 from the ATG, was capable of mediating pebp2alphaA2 transactivation. In addition, cross-species transactivation was also confirmed because the mouse Cbfa1 was able to induce the zebrafish osteocalcin promoter, whereas the zebrafish pebp2alphaA2 activated the murine osteocalcin promoter. These results are consistent with the high degree of evolutionary conservation of these proteins. The 3D structure of the runx2b runt domain was modeled based on the runt domain of mouse Runx1. Results show a high degree of similarity in the 3D configuration of the DNA binding regions from both domains, with significant differences only observed in non-DNA binding regions or in DNA-binding regions known to accommodate considerable structure flexibility. Phylogenetic analysis was used to clarify the relationship between the isoforms of each of the two zebrafish Runx2 orthologs and other Runx proteins. Both zebrafish runx2 genes clustered with other Runx2 sequences. The duplication event seemed, however, to be so old that, whereas Runx2b clearly clusters with the other fish sequences, it is unclear whether Runx2a clusters with Runx2 from higher vertebrates or from other fish.

Descriptors: zebrafish, gene expression regulation, osteocalcin genetics, transcription factors metabolism, amino acid sequence, conserved sequence, molecular evolution, reporter molecular sequence data, phylogeny, promoter regions genetics, protein conformation, isoforms classification, metabolism and genetics, transcription factors classification and genetics, up regulation.

Piotrowski, T., K. Grant, and D.W. Raible (2005). **Glia inhibit differentiation of neuromast precursors in the zebrafish posterior lateral line**. *Federation of American Societies for Experimental Biology Journal* 19(4, Suppl. S, Part 1): A790. ISSN: 0892 6638.

NAL Call Number: QH301.F3

Abstract: The lateral line system of anammotes consists of hair cells, which enable the animals to orient themselves in their environment. The sensory organs (neuromasts) develop from cranial ectodermal placodes (primordia). These primordia migrate from the head into the trunk and along the way deposit clusters of cells fated to become sensory organs. To identify the molecular mechanisms underlying lateral line development we have performed a screen in which 20 mutants affecting lateral line development have been identified. Three of these mutants, cls/sox10, hypersensitive and rowgain, possess 'extra' neuromasts on their trunk, and lack glia along the posterior lateral line nerve. We have shown that the 'extra' neuromasts are precociously forming secondary neuromasts, which arise from a pool of latent precursors that are deposited by the primordium between the primary neuromasts. In wildtype embryos, these interneuromast cells lie adjacent to the nerve and associated glia and remain quiescent while they are juxtaposed with the glia; however, when they move away from the nerve they begin to differentiate into neuromasts. If glia are absent, as in the mutants, the interneuromast cells precociously differentiate. Transplantation of wt glia into mutants can rescue the appropriate temporal differentiation of the interneuromast cells. We

have made two fundamentally new observations relevant to zebrafish lateral line development: 1) we discovered a new embryonic source of neuromasts, and 2) identified a key role for glia in controlling their differentiation. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, molecular genetics, nervous system, glia, neural coordination.

Piotrowski, T. and J. Sasine (2006). **Cell migration and neurogenesis in the zebrafish lateral line**. *Federation of American Societies for Experimental Biology Journal* 20(4, Part 1): A438. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: All components of the lateral line system (hair cells, neurons and ganglion cells) are derived from ectodermal thickenings called placodes. The posterior lateral line placode migrates from the head towards the tail tip where it deposits sensory organ precursor cells at regular intervals. Once developed, the functional unit of the lateral line (the neuromast) consists of relatively few cells. Throughout their embryonic and postembryonic development, the primordia and the neuromasts are accessible to both experimental manipulations and live observations. Such relative simplicity and experimental accessibility make the lateral line system a unique model in which to study the complex cell-cell interactions that occur in the vertebrate nervous system. Yet, our understanding of the molecular mechanisms that regulate primordium migration and sensory organ deposition remains incomplete. We performed genetic screens and isolated approximately 20 mutants with different lateral line defects. Here we describe the phenotypic and molecular analysis of one of these mutants in which the primordium ceases to migrate prematurely and cell deposition is severely disrupted. Cloning of some of the other mutations has revealed that genes involved in lateral line development often participate in a variety of developmental processes in the embryo, such as cell migration, cell fate specification and patterning. These characteristics underscore the value of lateral line development as a tractable process, likely to inform fundamental aspects of vertebrate nervous system development. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, molecular genetics, nervous system, neural coordination, cloning, genetic screening, phenotypic analysis, genetic techniques, molecular mechanism, cell migration, neurogenesis, cell-cell interaction, cell patterning and fate specification, cell deposition.

Plaster, N., C. Sonntag, C.E. Busse, and M. Hammerschmidt (2006). **P-53 deficiency rescues apoptosis and differentiation of multiple cell types in zebrafish flathead mutants deficient for zygotic DNA polymerase delta1.** *Cell Death and Differentiation* 13(2): 223-35. ISSN: 1350 9047.
NAL Call Number: QH671
Abstract: Cell culture work has identified the tumor suppressor p53 as a component of the S-phase checkpoint control system, while in vivo studies of this role of p53 in whole-vertebrate systems were limited. Here, we describe zebrafish mutants in the DNA polymerase delta catalytic subunit 1, based on the positional cloning of the flathead (fla) gene. fla mutants display specific defects in late proliferative zones, such as eyes, brain and cartilaginous elements of the visceral head skeleton, where cells display compromised DNA replication, followed by apoptosis, and partial or complete loss of affected tissues. Antisense-mediated knockdown of p53 in fla mutants leads to a striking rescue of all phenotypic traits, including completion of replication, survival of cells, and normal differentiation and tissue formation. This indicates that under replication-compromised conditions, the p53 branch of the S-phase checkpoint is responsible for eliminating stalled cells that, given more time,

would have otherwise finished their normal developmental program.
Descriptors: mutant zebrafish, apoptosis, cell differentiation, tumor suppressor protein p53 physiology, amino acid sequence, brain cytology, brain embryology, cell proliferation, DNA polymerase III deficiency, metabolism and genetics, DNA replication, eye cytology and embryology, developmental gene expression regulation, molecular sequence data , phenotype, reverse transcriptase PCR, S phase, skull cytology and embryology, tumor suppressor protein p53 deficiency znd genetics, up regulation genetics and physiology, zygote cytology, enzymology and physiology.

Plata-Ramos, C., C.R. Sussman, Z.M. Josephs, M.H. Chang, J. Liang, and M.F. Romero (2006). **Localization and functional characterization of two Na+/monocarboxylate transporters in zebrafish (zSMCT): electrogenic (zSMCTe/slc5a8) and electroneutral (zSMCTn/slc5a12).** *Federation of American Societies for Experimental Biology Journal* 20(5, Part 2): A1234. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: We identified and characterized the function of two different Na+/monocarboxylate cotransporters (SMCTs) from zebrafish (*Danio rerio*): electrogenic zSMCTe and electroneutral zSMCTn. Both sequences have similar to 50% homology to SLC5A8 (hSMCT). We characterized the physiology of both zSMCTs by functional expression in *Xenopus* oocytes. We recorded intracellular Na+ activity ([Na+](i)) simultaneously with membrane potential. Both zSMCTs expressed in oocytes respond to monocarboxylate (MC) addition by increasing [Na+](i) as does hSMCT. Using two electrode voltage clamp, we measured current elicited by MCs. The MC-currents for zSMCTe are similar to hSMCT currents, yet no significant MC-elicited current was present in zSMCTn or controls. MC-dose response data show that zSMCTe has a lower Km for both lactate and nicotinate than zSMCTn. In situ hybridization showed that both zSMCT mRNAs are expressed in zebrafish embryos at 1, 3 and 5 days post fertilization. Both zSMCTs are expressed in the brain, eyes, swim bladder, and pronephros (kidney). However, zSMCTn is expressed in pronephric tubules (proximal) while zSMCTe is in the pronephric ducts (distal). Our data demonstrate that zSMCTe is the zebrafish ortholog of hSMCT/SLC5A8 and that zSMCTn is a novel, electroneutral form of SMCT (zSlc5a12). Our kinetic data and pronephric localization of these SMCTs suggest that zSMCTn is a low affinity, high capacity SMCT that reabsorbs the major [MCs] in proximal pronephros. zSMCTe is a high affinity SMCT implying it may used for absorbing the remaining MCs. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, biochemistry, reproductive system, in situ hybridization, genetic techniques, electrogenic and electroneutral zebrafish, Na+monocarboxylate co-transporters.

Poggi, L., M. Vitorino, I. Masai, and W.A. Harris (2005). **Influences on neural lineage and mode of division in the zebrafish retina in vivo.** *Journal of Cell Biology* 171(6): 991-9. ISSN: 0021-9525.
NAL Call Number: 442.8J828
Abstract: Cell determination in the retina has been under intense investigation since the discovery that retinal progenitors generate clones of apparently random composition (Price, J., D. Turner, and C. Cepko. 1987. Proc. Natl. Acad. Sci. USA. 84:156-160; Holt, C.E., T.W. Bertsch, H.M. Ellis, and W.A. Harris. 1988. Neuron. 1:15-26; Wetts, R., and S.E. Fraser. 1988. Science. 239:1142-1145). Examination of fixed tissue, however, sheds little light on lineage patterns or on the relationship between the orientation of division and cell fate. In this study, three-dimensional time-lapse analyses were used to trace lineages of retinal progenitors expressing green fluorescent protein under the control of the ath5 promoter.

Surprisingly, these cells divide just once along the circumferential axis to produce two post-mitotic daughters, one of which becomes a retinal ganglion cell (RGC). Interestingly, when these same progenitors are transplanted into a mutant environment lacking RGCs, they often divide along the central-peripheral axis and produce two RGCs. This study provides the first insight into reproducible lineage patterns of retinal progenitors in vivo and the first evidence that environmental signals influence the orientation of cell division and the lineage of neural progenitors.

Descriptors: zebrafish, cell lineage, retina embryology, genetically modified, cell differentiation , cell division genetics, DNA binding proteins genetics, and metabolism, green fluorescent green fluorescent proteins metabolism and genetics, video microscopy, mitosis, nervous system embryology, nervous system metabolism, retina metabolism, retinal ganglion cells metabolism.

Poon, K.L., M. Richardson, C.S. Lam, H.E. Khoo, and V. Korzh (2003). **Expression pattern of neuronal nitric oxide synthase in embryonic zebrafish.** *Gene Expression Patterns* 3(4): 463-6. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Nitric oxide synthase catalyzes the production of nitric oxide, a multifunctional signaling molecule that affects diverse aspects of animal physiology such as cell proliferation, differentiation, neurotransmission and apoptosis. Here, we report the cloning and expression pattern of the zebrafish nnos. This gene was mapped to zebrafish linkage group 5. The spatial and temporal expression pattern of nnos in embryonic zebrafish was analyzed by whole mount in situ hybridization. nnos is widely expressed in the embryonic nervous system. Expression of zebrafish nnos appeared at 16 hours post-fertilization in the hypothalamus and by 3 days post-fertilization was present in discrete locations in the central nervous system as well as the enteric nervous system. Some nnos-positive cells were mapped to specific locations in the central nervous system using tyrosine hydroxylase as a specific marker indicating that nnos transcripts were present in the olfactory bulb, anterior diencephalon, posterior hypothalamus and anterior hindbrain.

Descriptors: zebrafish, enteric nervous system embryology, enzymologic gene expression regulation, hypothalamus embryology, nitric oxide synthase metabolism, chromosome mapping, molecular cloning, enteric nervous system enzymology, hypothalamus enzymology, in situ hybridization, molecular sequence data, nitric oxide synthase Type I, tyrosine 3 monooxygenase metabolism.

Poss, K.D., A. Nechiporuk, K.F. Stringer, C. Lee, and M.T. Keating (2004). **Germ cell aneuploidy in zebrafish with mutations in the mitotic checkpoint gene mps1.** *Genes and Development* 18(13): 1527-32. ISSN: 0890-9369.

NAL Call Number: QH426.G46

Abstract: Aneuploidy, resulting from chromosome missegregation during meiosis, is a major cause of human infertility and birth defects. However, its molecular basis remains incompletely understood. Here we have identified a spectrum of chromosome anomalies in embryos of zebrafish homozygous for a hypomorphic mutation in Mps1, a kinase required for the mitotic checkpoint. These aneuploidies are caused by meiotic error and result in severe developmental defects. Our results reveal Mps1 as a critical regulator of chromosome number in zebrafish, and demonstrate how slight genetic perturbation of a mitotic checkpoint factor can dramatically reduce the fidelity of chromosome segregation during vertebrate meiosis.

Descriptors: zebrafish embryos, aneuploidy, cell cycle proteins, oocytes physiology, protein

kinases, protein serine threonine kinases and protein tyrosine kinases genetics, spermatozoa physiology, chromosome aberrations, pathology, fetal death genetics, mitosis, mutation, protein serine threonine kinases and protein tyrosine kinases metabolism, testis physiology.

Postlethwait, J., V. Ruotti, M.J. Carvan, and P.J. Tonellato (2004). **Automated analysis of conserved syntenies for the zebrafish genome.** *Methods in Cell Biology* 77: 255-71. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, chromosome mapping methods, computational biology methods, genome, synteny genetics, genetic markers.

Postlethwait, J.H. (2004). **Evolution of the zebrafish genome**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd.: Singapore, Singapore , p. 581-611. ISBN: 9812388214.
NAL Call Number: QL638.C94 F57
Abstract: Because the zebrafish lineage is basally diverging among Euteleost fish, analysis of its genome can complement information from other key species to understand the origin of genomes of teleost fish, the most species-rich group of vertebrates, and to infer some history of the human genome. Analysis of gene maps shows that zebrafish chromosomes have conserved syntenies with large segments of human chromosomes, suggesting that translocations have been rather infrequent during vertebrate evolution. Nevertheless, zebrafish chromosomes consist of mosaics of segments orthologous to several human chromosomes, in part, probably because ancestral chromosomes experienced fission in the human lineage. Because the zebrafish orthologs of small regions of human chromosomes appear to have exploded over much of the zebrafish chromosome, inversions have been fixed in populations much more frequently than translocations. Genetic maps show that segments of human chromosomes are generally present in zebrafish as two orthologous copies. These data suggest a genome duplication event in the zebrafish lineage. Comparative mapping of zebrafish, pufferfish and medaka genomes shows that this event occurred before the divergence of Euteleosts, suggesting the hypothesis that this genome duplication may have contributed to the evolutionary success of teleosts. The complete sequencing of the zebrafish genome will reveal the answer to many questions, such as the fraction of genes derived from this event that are retained in duplicate copy, and the evolutionary principles that cause gene duplicates to be retained. Because of the elegant functional analyses possible with zebrafish, the sequence of the zebrafish genome will facilitate exploration of conserved gene function in vertebrate development and physiology. In addition, functional analysis of duplicated zebrafish genes can reveal ancestral gene functions sometimes obscured in mammals by pleiotropy. Copyright © 2008 CABI
Descriptors: zebrafish, chromosomes, evolution, gene mapping, genes, genomes, reviews, *Danio rerio*, humans, *Oryzias latipes*, *Tetraodontidae.*
Notes: A review.

Postlethwait, J.H. (2007). **The zebrafish genome in context: ohnologs gone missing.** *Journal of Experimental Zoology. Part B. Molecular and Developmental Evolution* 308(5): 563-77. ISSN: 1552-5007.
NAL Call Number: QL1.J87
Descriptors: zebrafish, molecular evolution, genome, chordata genetics, gene duplication.
Notes: A review.

Pozzoli, O., C.N. Gilardelli, P. Sordino, S. Doniselli, C.L. Lamia, and F. Cotelli (2004). **Identification and expression pattern of mago nashi during zebrafish development.** *Gene Expression Patterns* 5(2): 265-72. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: In a search for zebrafish genes expressed during early stages of development, we have identified two ESTs encoding proteins related to *Drosophila* mago nashi. Zebrafish mago nashi codes for a small protein with no clearly identified functional domains, and which is highly conserved during evolution. This paper describes the identification and a detailed gene expression analysis of zebrafish mago nashi during development. Our results demonstrate that mago nashi encodes a maternal transcript detected in both blastomeres and yolk cell at the 1-2 cell stages, and in the blastoderm during segmentation. We show that a putative microtubule-mediated transport of mago nashi mRNA from the vegetal hemisphere into animal blastomeres determines the localization of the transcript in the animal pole, immediately after fertilization. Furthermore, the microtubule array contained into the yolk cell seems to be responsible for the high level of mago nashi transcript detected in the central blastomeres at the 8-16 cell stages. Zygotic mago nashi is expressed into the dorsal-marginal region during gastrulation, and starting from somitogenesis to 24 hpf, the expression domain becomes progressively restricted to the developing neural tube and paraxial structures, and ventrally to the pronephric ducts.
Descriptors: zebrafish, *Drosophila* nuclear proteins genetics, metabolism, amino acid and base sequence, biological transport drug effects and physiology, blastoderm metabolism, expressed sequence tags, gastrula metabolism, developmental gene expression regulation, in situ hybridization, microtubules drug effects and metabolism, molecular sequence data, messenger RNA biosynthesis, yolk sac embryology and metabolism.

Premzl, M., L. Sangiorgio, B. Strumbo, J.A.M. Graves, T. Simonic, and J.E. Gready (2003). **Shadoo, a new protein highly conserved from fish to mammals and with similarity to prion protein.** *Gene* 314: 89-102. ISSN: 0378 1119.
NAL Call Number: QH442.A1G4
Descriptors: zebrafish, evolution and adaptation, molecular genetics, embryonic development, shadoo, conserved protein.

Prohaska, S.J., C. Fried, C. Flamm, G.P. Wagner, and P.F. Stadler (2004). **Surveying phylogenetic footprints in large gene clusters: applications to Hox cluster duplications**. *Molecular PhyloGenetics and Evolution* 31(2): 581-604. ISSN: 1055-7903.
NAL Call Number: QH367.5.M56
Descriptors: zebrafish, Binding, DNA, duplication, evolution, evolutionary conservation, gene clusters, gene expression, gene regulation, genes, HOXA gene, HoxB gene, phylogeny, statistical analysis, *Danio rerio*, *Selachii*, *Takifugu*, gene regulation, HOX genes, cis-regulatory element evolution, taxonomy, systematics and evolutionary genetics.

Provost, E., J. Rhee, and S.D. Leach (2007). **Viral 2A peptides allow expression of multiple proteins from a single ORF in transgenic zebrafish embryos.** *Genesis* 45(10): 625-9. ISSN: 1526-954X.
NAL Call Number: QH426.D32
Descriptors: zebrafish, developmental gene expression regulation, open reading frames, peptides genetics, genetically modified, complementary DNA reporter genes, green fluorescent luminescent proteins metabolism , transgenes.

Prufert, K., C. Winkler, M. Paulin Levasseur, and G. Krohne (2004). **The lamina-associated polypeptide 2 (LAP2) genes of zebrafish and chicken: no LAP2alpha isoform is synThesised by non-mammalian vertebrates.** *European Journal of Cell Biology* 83(8): 403-11. ISSN: 0171 9335.

NAL Call Number: QH573.C83

Abstract: The mammalian lamina-associated polypeptide 2 (LAP2) gene encodes six isoforms (LAP2alpha, beta, delta, epsilon, gamma, zeta) that are synthesised from alternatively spliced mRNAs. The mammalian LAP2alpha is one of the predominant isoforms and localised in the nucleoplasm whereas LAP2beta, delta, epsilon, and gamma are integral membrane proteins of the inner nuclear membrane. We have analysed the LAP2 gene structure of the zebrafish Danio rerio as an attractive lower vertebrate model organism. The zebrafish LAP2 (ZLAP2) gene without regulatory sequences spans approximately 19 kb of genomic DNA. It contains 15 exons that encode the isoforms ZLAP2beta, gamma, and omega which are localised in the inner nuclear membrane. By radiation hybrid mapping, we have located the gene onto linkage group 4 between EST markers fc01g04 (213.97cR) and fb49f01 (215.69cR). The identification of a chicken genomic clone comprising the complete coding region of the avian LAP2 gene enabled us to compare the LAP2 gene structure amongst vertebrates. In contrast to the mammalian LAP2 gene, the zebrafish and the chicken sequences do not encode for an alpha-isoform. In parallel we searched for an alpha-isoform in birds using polyclonal and monoclonal LAP2 antibodies specific for the common evolutionary conserved aminoterminal domain present in all isoforms. We detected LAP2beta as the predominant isoform but no LAP2alpha in tissues of 10-day-old chicken embryos and cultured chicken fibroblasts thus confirming the genomic analysis. The comparison of each zebrafish and chicken LAP2 exon with the corresponding exons of the human LAP2 gene demonstrates that the degree of identity at the amino acid level is much higher between the human and chicken than between the human and zebrafish sequences. By Blast search with the nucleotide and amino acid sequences of the human LAP2alpha, we did not find any significant homologies in databases of the zebrafish and chicken sequences. Our data suggest that LAP2alpha is a novelty of mammals.

Descriptors: zebrafish, DNA binding proteins genetics, exons genetics, membrane thymopoietins genetics, amino acid sequence, chick embryo, DNA binding proteins genetics, and metabolism, membrane proteins metabolism, molecular sequence data, nuclear envelope metabolism, protein isoforms genetics and metabolism, amino acid sequence homology, species specificity, thymopoietins metabolism.

Pugacheva, E.M., Y.W. Kwon, N.A. Hukriede, S. Pack, P.T. Flanagan, J.C. Ahn, J.A. Park, K.S. Choi, K.W. Kim, D. Loukinov, I.B. Dawid, and V.V. Lobanenkov (2006). **Cloning and characterization of zebrafish CTCF: developmental expression patterns, regulation of the promoter region, and evolutionary aspects of gene organization.** *Gene* 375: 26-36. ISSN: 0378-1119.

NAL Call Number: QH442.A1G4

Abstract: CTCF is a nuclear phosphoprotein capable of using different subsets of its 11 Zn fingers (ZF) for sequence-specific binding to many dissimilar DNA CTCF-target sites. Such sites were identified in the genomic DNA of various multicellular organisms, in which the CTCF gene was cloned, including insects, birds, rodents, and primates. CTCF/DNA-complexes formed in vivo with different 50-bp-long sequences mediate diverse functions such as positive and negative regulation of promoters, and organization of all known enhancer-blocking elements ("chromatin insulators") including constitutive and epigenetically regulated elements. Abnormal functions of certain CTCF sites are implicated in cancer

and in epigenetic syndromes such as BWS and skewed X-inactivation. We describe here the cloning and characterization of the CTCF cDNA and promoter region from zebrafish, a valuable vertebrate model organism. The full-length zebrafish CTCF cDNA clone is 4244 bp in length with an open reading frame (ORF) of 2391 bp that encodes 797 amino acids. The zebrafish CTCF amino acid sequence shows high identity (up to 98% in the zinc finger region) with human CTCF, and perfect conservation of exon-intron organization. Southern blot analyses indicated that the zebrafish genome contains a single copy of the CTCF gene. In situ hybridization revealed the presence of zebrafish CTCF transcripts in all early stages of embryogenesis. Transfection assays with luciferase reporter-constructs identified a core promoter region within 146 bp immediately upstream of the transcriptional start site of zebrafish CTCF that is located at a highly conserved YY1/Initiator element.
Descriptors: zebrafish, DNA binding proteins genetics , molecular evolution, promoter regions genetics, repressor proteins genetics, amino acid and base sequence, molecular cloning , DNA primers, in situ hybridization, molecular sequence data, plasmids, amino acid sequence homology, genetic transcription.

Pujic, Z., Y. Omori, M. Tsujikawa, B. Thisse, C. Thisse, and J. Malicki (2006). **Reverse genetic analysis of neurogenesis in the zebrafish retina.** *Developmental Biology* 293(2): 330-47 . ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: To gain an understanding of molecular events that underlie pattern formation in the retina, we evaluated the expression profiles of over 8000 transcripts randomly selected from an embryonic zebrafish library. Detailed analysis of cDNAs that display restricted expression patterns revealed factors that are specifically expressed in single cell classes and are potential regulators of neurogenesis. These cDNAs belong to numerous molecular categories and include cell surface receptors, cytoplasmic enzymes, and transcription factors. To test whether expression patterns that we have uncovered using this approach are indicative of function in neurogenesis, we used morpholino-mediated knockdown approach. The knockdown of soxp, a transcript expressed in the vicinity of the inner plexiform layer, revealed its role in cell type composition of amacrine and ganglion cell layers. Blocking the function of cxcr4b, a chemokine receptor specifically expressed in ganglion cells, suggests a role in ganglion cell survival. These experiments demonstrate that in situ hybridization-based reverse genetic screens can be applied to isolate genetic regulators of neurogenesis. This approach very well complements forward genetic mutagenesis studies previously used to study retinal neurogenesis in zebrafish.
Descriptors: zebrafish, retina embryology, base sequence, complementary DNA genetics, eye gene expression profiling, in situ hybridization, oligodeoxyribonucleotides, antisense administration and dosage, oligodeoxyribonucleotides, antisense genetics, phenotype, vertebrate photoreceptors metabolism, pigment epithelium of eye metabolism, retinal ganglion cells metabolism, retinal horizontal cells metabolism.

Qi, F., S. Lin, and Q.C. Fan (2004). **Development of retrovirus-mediated insertional mutagenesis in zebrafish and its application in saturation mutagenesis and gene screening**. *Acta Genetica Sinica* 31(7): 750-7. ISSN: 0379-4172.
Abstract: The strategies of large-scale mutagenesis and gene screening include chemical mutagenesis, insertional mutagenesis and gene trap. Insertional mutagenesis is a method for identifying genes by using the integration of DNA as the mutagen, thereby facilitating the cloning of the mutated gene. The use of retrovirus-mediated insertional mutagenesis in zebrafish has led to the mutation and rapid identification of hundreds of genes required for

embryonic development and cell growth. Gene trap elements are also used in the construction of retrovirus vectors. The whole system is likely to make zebrafish the first vertebrate to achieve saturation mutagenesis and gene screening.
Descriptors: zebrafish, insertional mutagenesis methods, Retroviridae genetics, chromosome mapping, genetic vectors.
Language of Text: Chinese.

Qian, F., F. Zhen, J. Xu, M. Huang, W. Li, and Z. Wen (2007). **Distinct functions for different scl isoforms in zebrafish primitive and definitive hematopoiesis.** *Public Library of Science Biology* 5(5): E132. ISSN: 1544-9173.
NAL Call Number: QH301.P56
Abstract: The stem-cell leukemia (SCL, also known as TAL1) gene encodes a basic helix-loop-helix transcription factor that is essential for the initiation of primitive and definitive hematopoiesis, erythrocyte and megakarocyte differentiation, angiogenesis, and astrocyte development. Here we report that the zebrafish produces, through an alternative promoter site, a novel truncated scl (tal1) isoform, scl-beta, which manifests a temporal and spatial expression distinct from the previously described full-length scl-alpha. Functional analysis reveals that while scl-alpha and -beta are redundant for the initiation of primitive hematopoiesis, these two isoforms exert distinct functions in the regulation of primitive erythroid differentiation and definitive hematopoietic stem cell specification. We further demonstrate that differences in the protein expression levels of scl-alpha and -beta, by regulating their protein stability, are likely to give rise to their distinct functions. Our findings suggest that hematopoietic cells at different levels of hierarchy are likely governed by a gradient of the Scl protein established through temporal and spatial patterns of expression of the different isoforms.
Descriptors: zebrafish, basic helix loop helix transcription factors metabolism, gene expression, hematopoietic stem cells cytology, proto oncogene proteins metabolism, base sequence, basic helix loop helix transcription factors genetics, Northern blotting, cell differentiation physiology, molecular cloning, DNA primers, hematopoietic stem cells physiology, immunohistochemistry, in situ hybridization, molecular sequence data, protein isoforms metabolism, proto oncogene sequence alignment, DNA sequence analysis, proteins genetics.

Qiu, A., M. Shayeghi, and C. Hogstrand (2005). **Molecular cloning and functional characterization of a high-affinity zinc importer (DrZIP1) from zebrafish (*Danio rerio*).** *Biochemical Journal, The* 388(Pt 3): 745-54. ISSN: 0264 6021.
NAL Call Number: 382B52
Abstract: Zinc is a vital micronutrient to all organisms and a potential toxicant to aquatic animals. It is therefore of importance to understand the mechanism of zinc regulation. In the present study, we molecularly cloned and functionally characterized a zinc transporter of the SLC39A family [commonly referred to as the ZIP (Zrt- and Irt-related protein) family] from the gill of zebrafish (*Danio rerio*) (DrZIP1). DrZIP1 protein was found to localize at the plasma membrane and to function as a zinc uptake transporter when being expressed in either chinook salmon (*Oncorhynchus tshawytscha*) embryonic 214 cells or *Xenopus laevis* oocytes. In comparison with pufferfish transporter proteins (FrZIP2 and FrECaC) that are known to facilitate cellular zinc uptake, DrZIP1 appears to have high affinity to bind and transport zinc, suggesting that it maybe a high-affinity zinc uptake transporter (Km < 0.5 microM) in fish. Orthologues of DrZIP1 were also identified in both freshwater and seawater pufferfish (*Tetraodon nigroviridis and Takifugu rubripes*), indicating that these proteins may be functionally conserved among different fish species. DrZIP1 mRNA is expressed in all the

tissues examined in the present study and thus DrZIP1 may be a constitutive zinc uptake transporter in many cell types of zebrafish.
Descriptors: zebrafish, cation transport proteins genetics, chemistry and metabolism, zinc metabolism, amino acid sequence, cell line, molecular cloning, conserved sequence, gene expression profiling, molecular sequence data, oocytes metabolism , phylogeny, protein transport, messenger RNA genetics, and metabolism, salmon, sequence alignment, amino acid sequence homology, tetraodontiformes, *Xenopus laevis.*

Quan, Y.c., L.q. Liang, X.w. Sun, K. Yao, and Q.q. Lei (2006). **Identification of genetic polymorphism between three species of common carp using zebrafish *Danio rerio* microsatellite molecular markers.** *Journal of Fishery Sciences of China* 13(2): 300-305. ISSN: 1005 8737.
Abstract: Using 6 072 pairs of zebrafish (*Danio rerio*) microsatellite primers to analyze the genetic variances between three species of common carp collected from the Heilongjiang River (*Cyprinus carpio haematopterus* Temminck et Schlegel, *Cyprinus carpio* var. wuyuanensis) and Yunnan province (*Cyprinus pellegrini* Tchang), totally 563 zebrafish microsatellites reveal inheritance polymorphism, which could be used in genotyping of these common carp. 25 pairs of zebrafish microsatellite primers were randomly selected from these 563 primers to identify the genetic diversities. The Nei genetic distances were computed by the software package of Phylip3. 63, and the evolutionary tree based on Neighbor-Joining method of Saitou and Nei (1987) were drawn by the software of MEGA3.0. Also 1 000 times of bootstrapping were tested using the maximum likelihood method, the UPGMA method and NJ method. The results show that *Cyprinus carpio haematopterus* Temminck et Schlegel and *Cyprinus carpio* var. wuyuanensis cluster first and then *Cyprinus pellegrini* Tchang and *Danio rerio*. The polymorphic microsatellites can be used as genetic markers of these species, so as to increase the molecular markers of the carp genetic linkage map. Screening interspecies microsatellite primers, especially from a model organism can advance the relative re-search rapidly. Then we can compare genetic data conveniently, in order to breed carps at a molecular level more quickly.
Descriptors: zebrafish , *Cyprinus carpio haematopterus, Cyprinus carpio var. wuyuanensis, Cyprinus pellegrini*, nucleic acids, DNA variation, molecular genetics, biochemical variation, China.
Language of Text: Chinese.

Rai, K., S. Chidester, C.V. Zavala, E.J. Manos, S.R. James, A.R. Karpf, D.A. Jones, and B.R. Cairns (2007). **Dnmt2 functions in the cytoplasm to promote liver, brain, and retina development in zebrafish.** *Genes and Development* 21(3): 261-6. ISSN: 0890-9369.
NAL Call Number: QH426.G46
Abstract: The roles of DNA methyltransferase-2 (DNMT2) enzymes are controversial; whether DNMT2 functions primarily as a nuclear DNA methyltransferase or as a cytoplasmic tRNA methyltransferase, and whether DNMT2 activity impacts development, as dnmt2 mutant mice or *Drosophila* lack phenotypes. Here we show that morpholino knockdown of Dnmt2 protein in zebrafish embryos confers differentiation defects in particular organs, including the retina, liver, and brain. Importantly, proper organ differentiation required Dnmt2 activity in the cytoplasm, not in the nucleus. Furthermore, zebrafish Dnmt2 methylates an RNA species of approximately 80 bases, consistent with tRNA methylation. Thus, Dnmt2 promotes zebrafish development, likely through cytoplasmic RNA methylation.
Descriptors: zebrafish, brain embryology, cytoplasm enzymology, DNA cytosine 5 methyltransferase physiology, liver embryology, retina embryology, genetically modified, brain enzymology, cultured cells, DNA cytosine 5 methyltransferase metabolism developmental

gene expression regulation, enzymologic gene expression regulation, liver enzymology, retina enzymology.

Rai, K., L.D. Nadauld, S. Chidester, E.J. Manos, S.R. James, A.R. Karpf, B.R. Cairns, and D.A. Jones (2006). **Zebrafish Dnmt1 and Suv39h1 regulate organ-specific terminal differentiation during development.** *Molecular and Cellular Biology* 26(19): 7077-85. ISSN: 0270-7306.

NAL Call Number: QH506.M664

Abstract: DNA methylation and histone methylation are two key epigenetic modifications that help govern heterochromatin dynamics. The roles for these chromatin-modifying activities in directing tissue-specific development remain largely unknown. To address this issue, we examined the roles of DNA methyltransferase 1 (Dnmt1) and the H3K9 histone methyltransferase Suv39h1 in zebra fish development. Knockdown of Dnmt1 in zebra fish embryos caused defects in terminal differentiation of the intestine, exocrine pancreas, and retina. Interestingly, not all tissues required Dnmt1, as differentiation of the liver and endocrine pancreas appeared normal. Proper differentiation depended on Dnmt1 catalytic activity, as Dnmt1 morphants could be rescued by active zebra fish or human DNMT1 but not by catalytically inactive derivatives. Dnmt1 morphants exhibited dramatic reductions of both genomic cytosine methylation and genome-wide H3K9 trimethyl levels, leading us to investigate the overlap of in vivo functions of Dnmt1 and Suv39h1. Embryos lacking Suv39h1 had organ-specific terminal differentiation defects that produced largely phenocopies of Dnmt1 morphants but retained wild-type levels of DNA methylation. Remarkably, suv39h1 overexpression rescued markers of terminal differentiation in Dnmt1 morphants. Our results suggest that Dnmt1 activity helps direct histone methylation by Suv39h1 and that, together, Dnmt1 and Suv39h1 help guide the terminal differentiation of particular tissues.

Descriptors: zebrafish, cell differentiation physiology, DNA cytosine 5 methyltransferase metabolism, cytology, methyltransferases metabolism, DNA cytosine 5 methyltransferase genetics, embryonic development genetics, gene expression profiling, developmental gene expression regulation, histones metabolism, intestines abnormalities and pathology, methyltransferases genetics, organ specificity, pancreas abnormalitiesand pathology, phenotype, messenger RNA genetics and metabolism, retina abnormalities and pathology.

Raible, D.W. and A. Nechiporuk (2005). **Endoderm-derived Fgf3 is necessary and sufficient to induce neurogenesis in the epibranchial placodes in zebrafish**. *Federation of American Societies for Experimental Biology Journal* 19(4, Suppl. S, Part 1): A764. ISSN: 0892 6638.

NAL Call Number: QH301.F3

Abstract: In vertebrates, epibranchial placodes are transient ectodermal thickenings that exclusively contribute sensory neurons to the epibranchial ganglia. These ganglia innervate internal organs and transmit information on heart rate, blood pressure, and visceral distension from the periphery to the central nervous system. Despite their importance, very little is known about molecular mechanisms that govern the induction and neurogenesis of the epibranchial placodes. Using genetic analysis in zebrafish, we demonstrated that endoderm is required for neurogenesis of the epibranchial placodes. Mosaic analyses confirmed that endoderm is the source of the inductive signal. Using a morpholino knockdown approach, we found that Fgf3 is required for the majority of EB placode cells to undergo neurogenesis. Tissue transplants demonstrated that fgf3 activity is specifically required in the endodermal pouches. Furthermore, ectopic fgf3 expression was sufficient to induce phox2a-positive neurons in wildtype. embryos and endoderm-deficient morphants. Surprisingly, ectodermal foxi1 expression, a marker for the EB placode precursors, was present in both endoderm-defi-

cient embryos and fgf3-morphants, indicating that neither endoderm nor Fgf3 was required for initial placode induction. Based on these findings, we propose a novel model for the epibranchial placode development where Fgf3 is a major endodermal determinant required for the EB placode neurogenesis. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development model, nervous system, neural coordination, neurogenesis, epibrachial placodes.

Rambabu, K.M., S.H. Rao, and N.M. Rao (2005). **Efficient expression of transgenes in adult zebrafish by electroporation.** *BioMed Central Biotechnology* 5: 29. ISSN: 1472 6750.
NAL Call Number: TP248.13.B532
Abstract: BACKGROUND: Expression of transgenes in muscle by injection of naked DNA is widely practiced. Application of electrical pulses at the site of injection was demonstrated to improve transgene expression in muscle tissue. Zebrafish is a precious model to investigate developmental biology in vertebrates. In this study we investigated the effect of electroporation on expression of transgenes in 3-6 month old adult zebrafish. RESULTS: Electroporation parameters such as number of pulses, voltage and amount of plasmid DNA were optimized and it was found that 6 pulses of 40 V.cm(-1) at 15 microg of plasmid DNA per fish increased the luciferase expression 10-fold compared to controls. Similar enhancement in transgene expression was also observed in Indian carp (*Labeo rohita*). To establish the utility of adult zebrafish as a system for transient transfections, the strength of the promoters was compared in A2 cells and adult zebrafish after electroporation. The relative strengths of the promoters were found to be similar in cell lines and in adult zebrafish. GFP fluorescence in tissues after electroporation was also studied by fluorescence microscopy. CONCLUSION: Electroporation after DNA injection enhances gene expression 10-fold in adult zebrafish. Electroporation parameters for optimum transfection of adult zebrafish with tweezer type electrode were presented. Enhanced reporter gene expression upon electroporation allowed comparison of strengths of the promoters in vivo in zebrafish.
Descriptors: zebrafish, biotechnology methods, electroporation methods, genetic techniques, transgenes, molecular cloning, gene expression, reporter genes, green fluorescent proteins metabolism, luciferases, muscles metabolism, plasmids metabolism, transfection.

Ransom, D.G., N. Bahary, K. Niss, D. Traver, C. Burns, N.S. Trede, N. Paffett Lugassy, W.J. Saganic, C.A. Lim, C. Hersey, Y. Zhou, B.A. Barut, S. Lin, P.D. Kingsley, J. Palis, S.H. Orkin, and L.I. Zon (2004). **The zebrafish moonshine gene encodes transcriptional intermediary factor 1gamma, an essential regulator of hematopoiesis.** *Public Library of Science Biology* 2(8): E237. ISSN: 1544-9173.
NAL Call Number: QH301.P56
Abstract: Hematopoiesis is precisely orchestrated by lineage-specific DNA-binding proteins that regulate transcription in concert with coactivators and corepressors. Mutations in the zebrafish moonshine (mon) gene specifically disrupt both embryonic and adult hematopoiesis, resulting in severe red blood cell aplasia. We report that mon encodes the zebrafish ortholog of mammalian transcriptional intermediary factor 1gamma (TIF1gamma) (or TRIM33), a member of the TIF1 family of coactivators and corepressors. During development, hematopoietic progenitor cells in mon mutants fail to express normal levels of hematopoietic transcription factors, including gata1, and undergo apoptosis. Three different mon mutant alleles each encode premature stop codons, and enforced expression of wild-type tif1gamma mRNA rescues embryonic hematopoiesis in homozygous mon mutants. Surprisingly, a high level of zygotic tif1gamma mRNA expression delineates ventral mesoderm during hematopoietic stem cell and progenitor formation prior to gata1 expression.

Transplantation studies reveal that tif1gamma functions in a cell-autonomous manner during the differentiation of erythroid precursors. Studies in murine erythroid cell lines demonstrate that Tif1gamma protein is localized within novel nuclear foci, and expression decreases during erythroid cell maturation. Our results establish a major role for this transcriptional intermediary factor in the differentiation of hematopoietic cells in vertebrates.
Descriptors: mutant zebrafish, erythrocytes pathology, transcription factors genetics and physiology, alleles, apoptosis, bone marrow transplantation, cell differentiation, cell line, cell nucleus metabolism, cell survival, cell transplantation, molecular cloning, terminator codon, DNA chemistry, DNA binding proteins genetics and chemistry, erythrocytes cytology, gene expression regulation, hematopoietic stem cells cytology, heterochromatin metabolism, homozygote, immunoblotting, mice, molecular sequence data, phenotype, protein binding, messenger RNA metabolism, transcription factors metabolism.

Rawls, J.F., M.R. Frieda, A.R. McAdow, J.P. Gross, C.M. Clayton, C.K. Heyen, and S.L. Johnson (2003). **Coupled mutagenesis screens and genetic mapping in zebrafish.** *Genetics* 163(3): 997-1009. ISSN: 0016-6731.
NAL Call Number: 442.8G28
Abstract: Forward genetic analysis is one of the principal advantages of the zebrafish model system. However, managing zebrafish mutant lines derived from mutagenesis screens and mapping the corresponding mutations and integrating them into the larger collection of mutations remain arduous tasks. To simplify and focus these endeavors, we developed an approach that facilitates the rapid mapping of new zebrafish mutations as they are generated through mutagenesis screens. We selected a minimal panel of 149 simple sequence length polymorphism markers for a first-pass genome scan in crosses involving C32 and SJD inbred lines. We also conducted a small chemical mutagenesis screen that identified several new mutations affecting zebrafish embryonic melanocyte development. Using our first-pass marker panel in bulked-segregant analysis, we were able to identify the genetic map positions of these mutations as they were isolated in our screen. Rapid mapping of the mutations facilitated stock management, helped direct allelism tests, and should accelerate identification of the affected genes. These results demonstrate the efficacy of coupling mutagenesis screens with genetic mapping.
Descriptors: zebrafish, chromosome mapping, mutagenesis genetics , inbred strains genetics, base sequence, genetic crosses, DNA primers, genetic markers, melanocytes physiology, genetic models.

Rawls, J.F., M.A. Mahowald, A.L. Goodman, C.M. Trent, and J.I. Gordon (2007). **In vivo imaging and genetic analysis link bacterial motility and symbiosis in the zebrafish gut.** *Proceedings of the National Academy of Sciences of the United States of America* 104(18): 7622-7. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: Complex microbial communities reside within the intestines of humans and other vertebrates. Remarkably little is known about how these microbial consortia are established in various locations within the gut, how members of these consortia behave within their dynamic ecosystems, or what microbial factors mediate mutually beneficial host-microbial interactions. Using a gnotobiotic zebrafish-Pseudomonas aeruginosa model, we show that the transparency of this vertebrate species, coupled with methods for raising these animals under germ-free conditions can be used to monitor microbial movement and localization within the intestine in vivo and in real time. Germ-free zebrafish colonized with isogenic P. aeruginosa strains containing deletions of genes related to motility and pathogenesis revealed

that loss of flagellar function results in attenuation of evolutionarily conserved host innate immune responses but not conserved nutrient responses. These results demonstrate the utility of gnotobiotic zebrafish in defining the behavior and localization of bacteria within the living vertebrate gut, identifying bacterial genes that affect these processes, and assessing the impact of these genes on host-microbial interactions.

Descriptors: zebrafish, intestines microbiology, movement, pseudomonas aeruginosa genetics, *Pseudomonas aeruginosa* physiology, symbiosis genetics, zebrafish microbiology, flagella genetics and physiology, germ free life, intestines immunology and ultrastructure, electron transmission microscopy, pseudomonas aeruginosa immunology and ultrastructure, time factors.

Rawls, J.F., M.A. Mahowald, R.E. Ley, and J.I. Gordon (2006). **Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection.** *Cell* 127(2): 423-33. ISSN: 0092 8674.

NAL Call Number: QH573.C42

Abstract: The gut microbiotas of zebrafish and mice share six bacterial divisions, although the specific bacteria within these divisions differ. To test how factors specific to host gut habitat shape microbial community structure, we performed reciprocal transplantations of these microbiotas into germ-free zebrafish and mouse recipients. The results reveal that communities are assembled in predictable ways. The transplanted community resembles its community of origin in terms of the lineages present, but the relative abundance of the lineages changes to resemble the normal gut microbial community composition of the recipient host. Thus, differences in community structure between zebrafish and mice arise in part from distinct selective pressures imposed within the gut habitat of each host. Nonetheless, vertebrate responses to microbial colonization of the gut are ancient: Functional genomic studies disclosed shared host responses to their compositionally distinct microbial communities and distinct microbial species that elicit conserved responses.

Descriptors: zebrafish, bacteria classification, gastrointestinal tract microbiology, germ free life, RNA, bacterial genetics, ribosomal RNA 16s genetics, selection genetics, zebrafish microbiology, bacteria genetics, ecosystem, gastrointestinal tract metabolism, gene expression profiling, ileum metabolism and microbiology, intercellular signaling peptides and intercellular signaling peptides genetics and metabolism, metabolism genetics, mice, molecular sequence data, phylogeny, ribotyping, sequence alignment, sequence analysis, RNA, symbiosis, genetic transcription.

Raya, A., Y. Kawakami, C. Rodriguez-Esteban, D. Buescher, C.M. Koth, T. Itoh, M. Morita, R.M. Raya, I. Dubova, J.G. Bessa, J.L. de la Pompa , and J.C.I. Belmonte (2003). **Notch activity induces Nodal expression and mediates the establishment of left-right asymmetry in vertebrate embryos**. *Genes & Development* 17(10): 1213-1218. ISSN: 0890-9369.

NAL Call Number: QH426.G46

Descriptors: zebrafish, embryogenesis, embryonic development, embryos, freshwater fish, gene expression, pattern formation, promoters, *Danio rerio*, Nodal gene, Notch gene, left-right asymmetry, mice, developmental genetics, genetics and evolution.

Raz, E. (2003). **Primordial germ-cell development: the zebrafish perspective**. *Nature Reviews Genetics* 4(9): 690-700. ISSN: 1471 0056.

NAL Call Number: QH426

Abstract: Primordial germ cells follow a characteristic developmental path that is manifested in the specialized regulation of basic cell functions and behaviour. Recent studies in zebrafish

have greatly enhanced our understanding of the mode of specification of primordial germ cells, cell-fate maintenance and the migration of these cells towards their target, the gonad, where they differentiate into gametes. Copyright © 2008 CABI
Descriptors: zebrafish, animal models, cell differentiation, gametes, germ cells, gonads, *Danio rerio*.

Raz, E. and M. Reichman Fried (2006). **Attraction rules: germ cell migration in zebrafish.** *Current Opinion in Genetics and Development* 16(4): 355-9. ISSN: 0959 437X.
NAL Call Number: QH426.C88
Abstract: The migration of zebrafish primordial germ cell towards the region where the gonad develops is guided by the chemokine SDF-1a. Recent studies show that soon after their specification, the cells undergo a series of morphological alterations before they become motile and are able to respond to attractive cues. As migratory cells, primordial germ cells move towards their target while correcting their path upon exiting a cyclic phase in which morphological cell polarity is lost. In the following stages, the cells gather at specific locations and move as cell clusters towards their final target. In all of these stages, zebrafish germ cells respond as individual cells to alterations in the shape of the sdf-1a expression domain, by directed migration towards their target - the position where the gonad develops.
Descriptors: zebrafish, cell movement, Cxc chemokines physiology and genetics, germ cells physiology.

Rehbein, H. and J. Bogerd (2007). **Identification of genetically modified zebrafish *(Danio rerio)* by protein- and DNA-analysis**. *Journal Fur Verbraucherschutz Und Lebensmittelsicherheit* 2(2): 122-125. ISSN: 1661 5751.
NAL Call Number: TX531.J68
Abstract: The import, sale and possession of fluorescent transgenic zebrafish, offered under the name "GloFish" in U.S. aquarium shops, are not permitted in the European Union. A polymerase chain reaction (PCR)-based method has been developed to detect transgenic zebrafish harbouring the gene (dsRed) coding for the red fluorescent protein, originally isolated from the marine sponge *Discosoma striata*. Two types of PCR have been performed: (i) PCR to detect amplifiable genomic zebrafish DNA was checked using primers specific for the zebrafish parvalbumin gene; (ii) PCR with primers to specifically amplify the dsRed gene. In both PCR systems, genomic DNA isolated from wild type zebrafish was used as control template, in the second PCR system, the plasmid dsRed2-N1 was used as a positive control. Applying this method to several specimens of presumed GloFish from traders in the Netherlands and Germany revealed the presence of transgenic fish. In addition, a rapid method for screening zebrafish suspected to be genetically modified has been developed by measuring the fluorescence of water-soluble protein. Copyright © 2008 CABI
Descriptors: zebrafish, DNA, genes, methodology, PCR, proteins, *Danio rerio.*
Language of Text: German.

Rembold, M., K. Lahiri, N.S. Foulkes, and J. Wittbrodt (2006). **Transgenesis in fish: efficient selection of transgenic fish by co- injection with a fluorescent reporter construct**. *Nature Protocols* 1(3): 1133-1139. ISSN: 1754-2189.
Descriptors: zebrafish, embryos, gene expression, models, plasmids, transgenic fish, *Danio rerio*, *Oryzias latipes*.

Renier, C., J.H. Faraco, P. Bourgin, T. Motley, P. Bonaventure, F. Rosa, and E. Mignot (2007). **Genomic and functional conservation of sedative-hypnotic targets in the zebrafish.** *PharmacoGenetics and Genomics* 17(4): 237-53. ISSN: 1744-6872.

Abstract: OBJECTIVES: The zebrafish is an ideally suited vertebrate animal model for large-scale genetic screens and is emerging as a model organism in pharmacological and behavioral research. We investigated the effects of sedative hypnotics commonly used in humans on zebrafish locomotor activity and identified the corresponding genomic and receptor binding targets. METHODS: We studied radioreceptor binding and behavioral responses to compounds with known sedative hypnotic properties representing multiple pharmacological classes. These included GABAergic hypnotics such as benzodiazepines, barbiturates, and baclofen; alpha-2 adrenergic agonists; and histaminergic H1 antagonists. An automated system was used to quantify behavioral effects. Zebrafish homologs of histamine receptor H1, gamma-amino-n-butyric acid type A (alpha-subunit), and gamma-amino-n-butyric acid type B (1 and 2) receptor genes were identified through translating queries of the zebrafish Zv4 database with human receptor protein sequences. A pilot screen of 154 N-ethyl-N-nitroso-urea-mutagenized F2 families was conducted with pentobarbital, flurazepam and mepyramine. RESULTS: Radioreceptor binding studies revealed high affinity binding sites for known gamma-amino-n-butyric acid type A, gamma-amino-n-butyric acid type B, and histaminergic ligands. Drug immersion of 5-7-day-old larvae reduced mobility and, in some cases, produced a complete state of unresponsive immobility similar to anesthesia. These effects were dose-dependent and rapidly reversible in water. As established in mammals, (R)-baclofen was more active behaviorally and had higher affinity in binding studies when compared with (S)-baclofen. In this model, (S)-baclofen only partially reduced activity at high dose and blocked (R)-baclofen behavioral hypnotic effects. Genomic sequences with high similarity to the corresponding pharmacological targets were identified, but no mutants were found in the pilot screen. CONCLUSIONS: These results demonstrate conservation of gene, protein and function for many established sedative hypnotic pathways. The results indicate feasibility of conducting large-scale pharmacogenomic screens to isolate novel proteins modulating susceptibility to hypnotic compounds in a vertebrate system.

Descriptors: zebrafish, hypnotics and sedatives pharmacology screening, amino acid sequence, baclofen chemistry, baclofen pharmacology, behavior drug effects, binding sites genetics, conserved sequence, hypnotics and sedatives chemistry, larva drug effects, larva metabolism and physiology, molecular sequence data, motor activity drug effects, motor activity genetics, pharmacogenetics, phylogeny, radioligand assay, GABA A receptors drug effects, genetics and metabolism, GABA B receptors drug effects and metabolism, histamine receptors h1 drug effects, genetics and metabolism, amino acid sequence homology.

Rentzsch, F., C. Kramer, and M. Hammerschmidt (2003). **Specific and conserved roles of TAp73 during zebrafish development.** *Gene* 323: 19-30. ISSN: 0378-1119.

NAL Call Number: QH442.A1G4

Abstract: p53, p63 and p73 are related transcription factors involved in the regulation of cell proliferation, survival and differentiation. Here, we report the isolation and characterization of p73 from zebrafish. While for zebrafish p63 only N-terminally truncated isoforms (DeltaNp63) have been reported, p73 appears to be predominantly or exclusively present in transactivating isoforms (TAp73). p73 shows a very restricted expression pattern during zebrafish development. Transcripts are found in a subset of cells of the olfactory system, the telencephalon, the dorsal diencephalon, and the pronephric ducts. In addition, p73 is expressed in differentiating slow muscle cells of the somites, and in the pharyngeal endoderm. We carried out TAp73 gain- and loss-of-function experiments, injecting either

TAp73alpha mRNA, or antisense morpholino oligonucleotides to suppress translation of TAp73 transcripts. The overexpression studies indicate that in contrast to p53, TAp73alpha has no pro-apoptotic effect in zebrafish embryos. However, TAp73 appears to be required for specific processes during the development of the olfactory system, the telencephalon and the pharyngeal arches. Together, our data point to both conserved and class-specific roles of p73 during vertebrate development.

Descriptors: zebrafish, developmental gene expression regulation, amino acid sequence, apoptosis genetics, complementary DNA chemistry and genetics, embryonic development, endoderm metabolism, in situ hybridization, kidney embryology and metabolism, molecular sequence data, muscles cytology, embryology and metabolism, olfactory bulb embryology and metabolism, pharynx embryology and metabolism, phylogeny, protein isoforms genetics and physiology, DNA sequence analysis, amino acid sequence homology, skull embryology and metabolism, telencephalon embryology and metabolism, transcription factors physiology and genetics.

Rentzsch, F., C. Guder, D. Vocke, B. Hobmayer, and T.W. Holstein (2007). **An ancient chordin-like gene in organizer formation of *Hydra*.** *Proceedings of the National Academy of Sciences, USA* 104(9): 3249-3254. ISSN: 0027-8424.

NAL Call Number: 500N21P

Descriptors: zebrafish, antagonism, antagonists, bone morphogenetic proteins, budding, chordin, developmental stages, differentiation, embryogenesis, evolution, freshwater fish, gene expression, genes, genetic diversity, Noggin protein, polarity, population genetics, regeneration, signal transduction, strains, *Danio rerio*, *Hydra*, metazoa, genetics and evolution, development and cell cycle.

Riley, B.B. (2003). **Genes controlling the development of the zebrafish inner ear and hair cells.** *Current Topics in Developmental Biology* 57: 357-88. ISSN: 0070 2153.

NAL Call Number: QL951.C8

Descriptors: zebrafish, inner ear growth and development, developmental gene expression regulation genetics , hair cells growth and development, cell differentiation, inner ear embryology, sensory ganglia growth and development, hair cells embryology, otolithic membrane and semicircular canals growth and development.

Rissone, A., M. Monopoli, M. Beltrame, F. Bussolino, F. Cotelli, and M. Arese (2007). **Comparative genome analysis of the neurexin gene family in *Danio rerio*: insights into their functions and evolution.** *Molecular Biology and Evolution* 24(1): 236-52. ISSN: 0707-4038.

NAL Call Number: QH506.M642

Abstract: Neurexins constitute a family of proteins originally identified as synaptic transmembrane receptors for a spider venom toxin. In mammals, the 3 known Neurexin genes present 2 alternative promoters that drive the synthesis of a long (alpha) and a short (beta) form and contain different sites of alternative splicing (AS) that can give rise to thousands of different transcripts. To date, very little is known about the significance of this variability, except for the modulation of binding to some of the Neurexin ligands. Although orthologs of Neurexins have been isolated in invertebrates, these genes have been studied mostly in mammals. With the aim of investigating their functions in lower vertebrates, we chose *Danio rerio* as a model because of its increasing importance in comparative biology. We have isolated 6 zebrafish homologous genes, which are highly conserved at the structural level and display a similar regulation of AS, despite about 450 Myr separating the human and zebrafish species. Our data indicate a strong selective pressure at the exonic level and on the intronic

borders, in particular on the regulative intronic sequences that flank the exons subject to AS. Such a selective pressure could help conserve the regulation and consequently the function of these genes along the vertebrates evolutive tree. AS analysis during development shows that all genes are expressed and finely regulated since the earliest stages of development, but mark an increase after the 24-h stage that corresponds to the beginning of synaptogenesis. Moreover, we found that specific isoforms of a zebrafish Neurexin gene (nrxn1a) are expressed in the adult testis and in the earliest stages of development, before the beginning of zygotic transcription, indicating a potential delivery of paternal RNA to the embryo. Our analysis suggests the existence of possible new functions for Neurexins, serving as the basis for novel approaches to the functional studies of this complex neuronal protein family and more in general to the understanding of the AS mechanism in low vertebrates.
Descriptors: zebrafish, molecular evolution, nerve tissue nerve tissue proteins metabolism, alternative splicing, amino acid sequence, molecular cloning, genome, molecular sequence data, nerve tissue proteins chemistry, phylogeny, protein isoforms genetics and metabolism, sequence alignment, metabolism.

Ro, H., Y. Jang, and M. Rhee (2004). **The RING domain of Siaz, the zebrafish homologue of *Drosophila* seven in absentia, is essential for cellular growth arrest.** *Molecules and Cells* 17(1): 160-65. ISSN: 1016-8478.
Abstract: Siah is a mammalian homologue of *Drosophila* seven in absentia (sina) that is required for R7 photoreceptor development. Both the SINA and Siah family interact with ubiquitin-conjugating enzymes via an N-terminal RING domain and the C-terminal domain of SINA/ Siahs interacts with proteins targeted for degradation. Siah induces cell growth arrest by promoting beta-catenin degradation in a phosphorylation-independent manner as a result of indirect binding to beta-catenin. We previously cloned a zebrafish homologue (Siaz) of Siah. Siaz shares high sequence homology with vertebrate Siah-2. We have now examined the role of Siaz in growth regulation using the trypan blue exclusion assay and flow cytometry and found that Siaz induces cellular growth arrest by inhibiting the G2/M transition. The C-terminal domain of Siaz that interacts with target proteins is not required for growth inhibition. We conclude that the N-terminal RING and central domain of Siaz are sufficient to block the G2/M phase transition.
Descriptors: zebrafish, nuclear proteins biosynthesis and chemistry and biosynthesis, Western blotting, cytoskeletal proteins chemistry, DNA metabolism, flow cytometry, g2 phase, glutathione transferase metabolism, HeLa cells, fluorescence microscopy, mitosis, nuclear proteins metabolism, phosphorylation , protein binding, tertiary protein structure, recombinant fusion proteins metabolism, trans activators chemistry, transfection, trypan blue pharmacology, ubiquitin protein ligases, proteins chemistry, beta catenin.

Ro, H., K.E. Kim, T.L. Huh, S.K. Lee, and M. Rhee (2003). **Expression pattern of Siaz gene during the zebrafish embryonic development.** *Gene Expression Patterns* 3(4): 483-8. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Siah is a mammalian homolog of *Drosophila* seven in absentia (SINA). Here we report the identification of a new member of the SINA/Siah gene family. This new gene, designated Siaz, was found in zebrafish, and its product is predicted to share extensive amino acid sequence homology with *Drosophila* SINA. Siaz is maternally inherited, with zygotic expression in all blastomeres starting at the mid-blastula transition. After the 20-somite stage, Siaz expression occurs in a stage-specific manner in particular regions, including the brain, eye, cranial cavity, otic vesicle, optic chiasm and gut.

Descriptors: zebrafish, developmental gene expression regulation, nuclear nuclear proteins metabolism, amino acid sequence, brain embryology, brain metabolism, molecular cloning, eye embryology and metabolism, gene library, in situ hybridization, intestines embryology, intestines metabolism, molecular sequence data, RNA probes, messenger RNA genetics and metabolism, rats, reverse transcriptase PCR, *Saccharomyces cerevisiae*, amino acid sequence homology, two hybrid system techniques, ubiquitin protein ligases.

Ro, H., K. Soun, E.J. Kim, and M. Rhee (2004). **Novel vector systems optimized for injecting in vitro-synthesized mRNA into zebrafish embryos.** *Molecules and Cells* 17(2): 373-6. ISSN: 1016-8478.

Abstract: Microinjection of nucleic acids or proteins is a useful way of studying embryonic development. In particular, injection of in vitro-transcribed capped RNA is commonly employed to achieve ectopic or increased expression of genes. Two vector systems, pCS2+ and pT7Ts, have been used for this purpose in zebrafish. However, they were initially optimized for Xenopus embryos not for zebrafish. Here we describe a vector, pcGlobin2, optimized for zebrafish, and its derivative, pcGlobin2-GST. This new vector system offers several advantages. First, pcGlobin 2 contains three critical elements 15' and 3' zebrafish beta-globin UTRs, and a poly(A) tail] for generating stable mRNAs and greatly improving translation efficiency. Second, subcloning and preparation of template DNA is easier because of the larger number of restriction sites. Third, protein-binding assays can be performed directly on the injected embryos with pcGlobin2-GST. Lastly, this vector system can be transfected into animal cells without additional subcloning.

Descriptors: zebrafish, genetic vectors, messenger RNA metabolism, transfection methods, reporter genes, in situ hybridization, microinjections.

Ro, H., M. Won, S.U. Lee, K.E. Kim, T.L. Huh, C.H. Kim, and M. Rhee (2005). **Sinup, a novel Siaz-interacting nuclear protein, modulates neural plate formation in the zebrafish embryos.** *Biochemical and Biophysical Research Communications* 332(4): 993-1003. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: Siah, the vertebrate homologue of the *Drosophila* seven in absentia (sina) gene, is well conserved from *Drosophila* to mammal and involved in ubiquitination and proteasome-dependent degradation of various target proteins. To identify cellular proteins interacting with Siah, we screened a zebrafish cDNA library with zebrafish Siah (Siaz) as bait in a yeast two-hybrid assay. We identified a cDNA encoding a novel protein composed of 145 amino acids and termed it as Sinup (Siaz-interacting-nuclear-protein). Sinup is a novel nuclear protein that binds to the highly conserved C-terminal protein-interacting domain of Siaz both in vivo and in vitro. During development, sinup transcripts are abundant from the one-cell stage to the early blastula and then markedly diminished, suggesting sinup largely exists as maternal transcripts. sinup overexpression induced lateral expansion of the neural plate and in consequence caused ectopic expression of otx-2 and hoxb1b during the late gastrula stage. In addition, the lateral/paraxial expression of wnt8 at the onset of gastrulation is suppressed by the forced expression of sinup while the expression levels of various dorso-ventral markers are unaffected. In contrast, interfering with sinup functions using sinup morpholino oligonucleotides gradually diminished the anterior neuroectoderm from the posterior region, and resulted in compete loss of hindbrain at the 3-somites stage. Our report suggests that sinup expression should be tightly regulated during early embryonic development for the proper neural plate formation.

Descriptors: zebrafish, carrier, developmental gene expression regulation, neural crest

embryology, nuclear proteins metabolism, amino acid base sequence, Northern blotting, carrier proteins metabolism, cell nucleus metabolism, cytoskeletal proteins biosynthesis, complementary DNA metabolism, ectoderm metabolism, gene library, glutathione transferase metabolism, green fluorescent proteins metabolism, homeodomain proteins biosynthesis, in situ hybridization, molecular sequence data, nuclear proteins chemistry, oligonucleotides chemistry, otx transcription factors, plasmids metabolism, protein binding, tertiary protein structure, RNA chemistry, reverse transcriptase PCR, signal transduction, time factors, transfection, two hybrid system techniques, ubiquitin protein ligases, Wnt proteins, proteins biosynthesis, proteins chemistry.

Robalino, J., B. Joshi, S.C. Fahrenkrug, and R. Jagus (2004). **Two zebrafish eIF4E family members are differentially expressed and functionally divergent.** *Journal of Biological Chemistry* 279(11): 10532-10541. ISSN: 0021 9258.
NAL Call Number: 381J824
Abstract: Eukaryotic translation initiation factor 4E (eIF4E) is an essential component of the translational machinery that binds m7GTP and mediates the recruitment of capped mRNAs by the small ribosomal subunit. Recently, a number of proteins with homology to eIF4E have been reported in plants, invertebrates, and mammals. Together with the prototypical translation factor, these constitute a new family of structurally related proteins. To distinguish the prototypical translation factor eIF4E from other family members, it has been termed eIF4E-1 (Keiper, B. D., Lamphear, B. J., Deshpande, A. M., Jankowska-Anyszka, M., Aamodt, E. J., Blumenthal, T., and Rhoads, R. E. (2000) J. Biol. Chem. 275, 10590-10596). We describe the characterization of two eIF4E family members in the zebrafish Danio rerio. Based on their relative identities with human eIF4E-1, these zebrafish proteins are termed eIF4E-1A (82%) and eIF4E-1B (66%). eIF4E-1B, originally termed eIF4E(L), has been reported previously as the zebrafish eIF4E-1 counterpart (Fahrenkrug, S. C., Dahlquist, M. O., Clark, K., and Hackett, P. B. (1999) Differentiation 65, 191-201; Fahrenkrug, S. C., Joshi, B., Hackett, P. B., and Jagus, R. (2000) Differentiation 66, 15-22). Sequence comparisons suggest that the two genes probably evolved from a duplication event that occurred during vertebrate evolution. eIF4E-1A is expressed ubiquitously in zebrafish, whereas expression of eIF4E-1B is restricted to early embryonic development and to gonads and muscle of the tissues investigated. The ability of these two zebrafish proteins to bind m7GTP, eIF4G, and 4E-BP, as well as to complement yeast conditionally deficient in functional eIF4E, show that eIF4E-1A is a functional equivalent of human eIF4E-1. Surprisingly, although eIF4E-1B possesses all known residues thought to be required for interaction with the cap structure, eIF4G, and 4E-BPs, it fails to interact with any of these components, suggesting that this protein serves a role other than that assigned to eIF4E.
Descriptors: zebrafish, *Danio rerio*, proteins, new eukaryotic translation initiation factor 4e proteins characterization, gene expression.

Robinson-Rechavi, M. (2005). **Fishes: a sucessful genome duplication.** *Biofutur* 254: 29-33. ISSN: 0294-3506.
Descriptors: zebrafish, genomes, karyotypes , phylogenetics, *Danio rerio*, *Oryzias latipes*, *Takifugu rubripes*, *Tetraodon nigroviridis*, Medaka.
Language of Text: French.

Robison, B.D. (2007). **Variation in behavior and global patterns of gene expression among wild and domesticated zebrafish: Implications for teleost aquaculture**. *Aquaculture* 272: 305. ISSN: 0044-8486.

NAL Call Number: SH1.A6
Descriptors: zebrafish, aquaculture, gene expression, *Danio rerio*, Teleostei.

Robles, V. and M.L. Cancela (2007). **Lipid-based transfection as a method for gene delivery in zebrafish (*Danio rerio*) embryos**. *Aquaculture Research* 38(12): 1317-1322. ISSN: 1355-557X.
NAL Call Number: SH1.F8
Descriptors: zebrafish, aquaculture techniques, cytoplasm, developmental stages, embryos, enhancers, fish culture, freshwater fish, gene transfer, genes, macromolecules , methodology, microinjection, models, plasmids, transfection, blastula, *Danio rerio.*

Rodriguez Mari, A., Y.L. Yan, R.A. Bremiller, C. Wilson, C. Canestro, and J.H. Postlethwait (2005). **Characterization and expression pattern of zebrafish Anti-Mullerian hormone (Amh) relative to sox9a, sox9b, and cyp19a1a, during gonad development.** *Gene Expression Patterns* 5(5): 655-67. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: The role of Anti-Mullerian hormone (Amh) during gonad development has been studied extensively in mammals, but is less well understood in other vertebrates. In male mammalian embryos, Sox9 activates expression of Amh, which initiates the regression of the Mullerian ducts and inhibits the expression of aromatase (Cyp19a1), the enzyme that converts androgens to estrogens. To better understand shared features of vertebrate gonadogenesis, we cloned amh cDNA from zebrafish, characterized its genomic structure, mapped it, analyzed conserved syntenies, studied its expression pattern in embryos, larvae, juveniles, and adults, and compared it to the expression patterns of sox9a, sox9b and cyp19a1a. We found that the onset of amh expression occurred while gonads were still undifferentiated and sox9a and cyp19a1a were already expressed. In differentiated gonads of juveniles, amh showed a sexually dimorphic expression pattern. In 31 days post-fertilization juveniles, testes expressed amh and sox9a, but not cyp19a1a, while ovaries expressed cyp19a1a and sox9b, but not amh. In adult testes, amh and sox9a were expressed in presumptive Sertoli cells. In adult ovaries, amh and cyp19a1a were expressed in granulosa cells surrounding the oocytes, and sox9b was expressed in a complementary fashion in the ooplasm of oocytes. The observed expression patterns of amh, sox9a, sox9b, and cyp19a1a in zebrafish correspond to the patterns expected if their regulatory interactions have been conserved with mammals. The finding that zebrafish sox9b and sox8 were not co-expressed with amh in oocytes excludes the possibility that amh expression in zebrafish granulosa cells is directly regulated by either of these two genes.
Descriptors: zebrafish, aromatase biosynthesis, developmental gene expression regulation, glycoproteins biosynthesis, glycogonads embryology, hmgb proteins biosynthesis, high mobility group proteins biosynthesis, testicular hormones biosynthesis and genetics , transcription factors biosynthesis, proteins biosynthesis, amino acid sequence, cell differentiation, chromosome mapping, molecular cloning, complementary DNA metabolism, granulosa cells metabolism, in situ hybridization, genetic models, molecular sequence data, oocytes metabolism, ovary embryology and metabolism, phylogeny, amino acid sequence homology, testis embryology and metabolism.

Rodriguez Martin, I., M.J. Herrero Turrion, E. Marron Fdez de Velasco, R. Gonzalez Sarmiento, and R.E. Rodriguez (2007). **Characterization of two duplicate zebrafish Cb2-like cannabinoid receptors.** *Gene* 389(1): 36-44. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4

Abstract: Several cannabinoid receptors have been detected in many organisms. The best known are CB1, mainly expressed in the central nervous system and CB2 which is almost exclusively expressed in the periphery. Here we report the molecular characterization of two duplicate CB2-like cannabinoid receptors from zebrafish (*Danio rerio*) (zebrafish Cb2a and zebrafish Cb2b). The amino acid sequences of these receptors present 56% identity with *Takifugu rubripes* CB2 sequence and 39% with human CB2 sequence and conserve some specific key residues for cannabinoid receptor function. Both duplicate receptors are expressed in peripheral tissues (gills, heart, intestine and muscle), immune tissue (spleen) and also in the central nervous system. Using in situ hybridization techniques zebrafish Cb2 mRNA expression was observed for the first time in the adenohypophysial cells of the rostral pars distalis and proximal pars distalis of the pituitary gland. Given the importance of the existence of duplication of genes in teleosts, the combined analysis of these two new cannabinoid receptors opens a new exciting door to investigate and understand cannabinoid function throughout evolution.

Descriptors: zebrafish, duplicate cannabinoid receptor, cb2 genetics, cannabinoid receptors genetics, amino acid base sequence, brain cytology, chromosomes, gene expression profiling and regulation, genome, in situ hybridization, molecular sequence data, phylogeny, messenger RNA genetics and metabolism, cannabinoid receptor, cb2 chemistry, cannabinoid receptor, cb2 metabolism, cannabinoid receptors chemistry and metabolism.

Roe, B.A., C. Lau, S. Oommen, J. Li, A. Hua, H.S. Lai, S. Kenton, J. White, and H. Wang (2003). **Comparative analysis of human chromosome 22q11.1-q12.3 with syntenic regions in the chimpanzee, baboon, bovine, mouse, pufferfish, and zebrafish genomes.** *Cold Spring Harbor Symposia on Quantitative Biology* 68: 265-74. ISSN: 0091 7451.

NAL Call Number: 442.9C672C

Descriptors: zebrafish, human chromosomes pair 22 genetics, cattle, DNA genetics, genomics, in situ hybridization, mice, *Pan troglodytes* genetics, *Papio* genetics, sequence alignment, species specificity, Tetraodontiformes genetics, puffer fish.

Roesner, A., T. Hankeln, and T. Burmester (2006). **Hypoxia induces a complex response of globin expression in zebrafish (*Danio rerio*).** *Journal of Experimental Biology* 209(Pt 11): 2129-37. ISSN: 0022-0949.

NAL Call Number: 442.8B77

Abstract: Unlike most mammals, many fish species live and survive in environments with low or changing levels of oxygen. Respiratory proteins like hemoglobin or myoglobin bind or store oxygen, thus enhancing its availability to the respiratory chain in the mitochondria. Here we investigate by means of quantitative real-time PCR the changes of hemoglobin, myoglobin, neuroglobin, cytoglobin and globin X mRNA in zebrafish (*Danio rerio*) exposed to mild (PO2=approximately 8.6 kPa) or severe (PO2=approximately 4.1 kPa) hypoxia. Neuroglobin and myoglobin protein levels were investigated by western blotting. Whereas mild hypoxia caused only minor changes of mRNA levels, strong hypoxia enhanced mRNA levels of the control genes (lactate dehydrogenase A and phosphoglycerate kinase 1). Surprisingly, levels of hemoglobin alpha and beta mRNA were significantly reduced under severe hypoxia. Myoglobin mRNA and protein in heart mildly increased, in line with its proposed oxygen supply function. Likewise, neuroglobin mRNA and protein significantly increased in brain (up to 5.7-fold at the protein level), but not in eye. This observation, firstly, suggests physiological differences of zebrafish eye and brain under hypoxia, and secondly, indicates an important role of neuroglobin in oxidative metabolism, probably oxygen supply within neurons. There was little change in the expression of the two cytoglobin genes. Globin X

mRNA significantly decreased under hypoxia, pointing to a functional linkage to oxygen-dependent metabolism.
Descriptors: zebrafish, anoxia genetics, gene expression regulation, globins genetics and metabolism, brain metabolism, eye metabolism, messenger RNA metabolism.

Roesner, A., C. Fuchs, T. Hankeln, and T. Burmester (2005). **A globin gene of ancient evolutionary origin in lower vertebrates: evidence for two distinct globin families in animals**. *Molecular Biology and Evolution* 22(1): 12-20. ISSN: 0737-4038.
NAL Call Number: QH506.M642
Descriptors: zebrafish, amino acid sequence, amphibiotic species, evolution, freshwater fish, genes, hemoglobinss, heme, hemoglobin, myoglobins, phylogenetics, structure, *Carassius auratus*, *Danio rerio*, *Silurana tropicalis*, *Tetraodon nigroviridis*, goldfish, cytoglobin, globin X, myoglobin, neuroglobin, taxonomy, systematics and evolutionary genetics.

Rohrschneider, M.R., G.E. Elsen, and V.E. Prince (2007). **Zebrafish Hoxb1a regulates multiple downstream genes including prickle1b.** *Developmental Biology* 309(2): 358-72. ISSN: 0012-1606.
NAL Call Number: 442.8D49
Abstract: Despite 30 years of Hox gene study, we have a remarkably limited knowledge of the downstream target genes that Hox transcription factors regulate to confer regional identity. Here, we have used a microarray approach to identify genes that function downstream of a single vertebrate Hox gene, zebrafish hoxb1a. This gene plays a critical and conserved role in vertebrate hindbrain development, conferring identity to hindbrain rhombomere (r) 4. For example, zebrafish Hoxb1a, similar to mouse Hoxb1, is required for the migration of r4-derived facial branchiomotor neurons into the posterior hindbrain. We have screened microarrays carrying more than 16,000 expressed sequence tags (ESTs) for genes that are differentially regulated in normal versus Hoxb1a-deficient r4 tissue. Using this approach, we have identified both positively and negatively regulated candidate Hoxb1a target genes. We have used in situ hybridization to validate twelve positively regulated Hoxb1a targets. These downstream targets are expressed in a variety of subdomains within r4, with one gene, a novel prickle homolog (pk1b), expressed specifically within the facial branchiomotor neurons. Using morpholino knock-down and cell transplantation, we demonstrate that the Hoxb1a target Prickle1b functions cell-autonomously to control facial neuron migration, a single aspect of r4 identity.
Descriptors: zebrafish, Hoxb1a, downstream, genes regulation, prickle1b, expressed sequence tags, ESTs.

Rojas, D.A., D.A. Perez Munizaga, L. Centanin, M. Antonelli, P. Wappner, M.L. Allende, and A.E. Reyes (2007). **Cloning of hif-1alpha and hif-2alpha and mRNA expression pattern during development in zebrafish.** *Gene Expression Patterns* 7(3): 339-45. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Hypoxia-inducible factors (HIFs) regulate gene expression in response to hypoxia and in vertebrates they are known to participate in several developmental processes, including angiogenesis, vasculogenesis, heart and central nervous system development. Over the last decade, major progress in unraveling the molecular mechanisms that mediate regulation of HIF proteins by oxygen tension has been reported, but our knowledge on their developmental regulation during embryogenesis in model organisms is limited. Expression of hif-1alpha and hif-2alpha genes has been characterized during normal mouse development and they were found to be expressed from stages E7.5, later in E9.5 and E15.5 in several

different tissues such as the brain, heart and blood vessels. However, there is no detailed temporal information on their expression at other embryonic stages, even though orthologous genes have been described in several different vertebrate species. In this study, we describe the cloning and detailed expression pattern of zebrafish hif-1alpha and hif-2alpha genes. Sequence analysis revealed that zebrafish Hif proteins are highly homologous to other vertebrate orthologues. Zebrafish hif-1alpha and hif-2alpha are both expressed throughout development in discrete territories in a dynamic pattern. Interestingly, in the notochord the expression of hif-1alpha is switched off, while hif-2alpha transcription is turned on, signifying that the two genes might have partially overlapping, although non-redundant functions in development. This is the first time that a detailed comparison of the expression of hif-1alpha and hif-2alpha is directly assessed in a vertebrate model system throughout development.

Descriptors: zebrafish, basic helix loop helix transcription factors genetics, molecular cloning, developmental gene expression regulation, hypoxia inducible factor 1, alpha subunit genetics, metabolism, gene expression profiling, in situ hybridization, organ specificity, phylogeny.

Rojo, I., Y. Castelruiz, A. Estonba, and M.A. Pardo (2005). **Identification of genes associated with resistance to *Listonella anguillarum* in the zebrafish *Danio rerio* Hamilton, 1822 animal model**. *Boletin Del Instituto Espanol De Oceanografia* 21(1-4): 131-135. ISSN: 0074-0195.

Descriptors: zebrafish, genes, resistance to *Listonella anguillarum*, *Danio rerio* Hamilton, animal model.

Rosemberg, D.B., E.P. Rico, M.R. Guidoti, R.D. Dias, D.O. Souza, C.D. Bonan, and M.R. Bogo (2007). **Adenosine deaminase-related genes: Molecular identification, tissue expression pattern and truncated alternative splice isoform in adult zebrafish (*Danio rerio*).** *Life Sciences* 81(21-22): 1526-34. ISSN: 0024-3205.

NAL Call Number: 442.8 L62

Abstract: Adenosine deaminase (ADA) is responsible for cleaving the neuromodulator adenosine to inosine. Two members of ADA subfamilies, known as ADA1 and ADA2, were described and evidence demonstrated another similar protein group named ADAL (adenosine deaminase "like"). Although the identification of ADA members seems to be consistent, the expression profile of ADA1, ADA2 and ADAL genes in zebrafish has not yet been reported. The aim of the present study was to map the expression pattern of ADA-related genes in various tissues of adult zebrafish (Danio rerio). An extensive search on zebrafish genome followed by a phylogenetic analysis confirmed the presence of distinct ADA-related genes (ADA1, ADAL and two orthologous genes of ADA2). Specific primers for each ADA member were designed, optimized semi-quantitative RT-PCR experiments were conducted and the relative amount of transcripts was determined. The tissue samples (brain, gills, heart, liver, skeletal muscle and kidney) were collected and the expression of ADA1, ADAL and ADA2 genes was characterized. ADA1 had a similar expression pattern, whereas ADAL was less expressed in the heart. The highest relative amount of ADA2-1 transcripts was observed in the brain, liver and gills and it was less expressed in the heart. RT-PCR assays revealed that the other ADA2 form (ADA2-2) was expressed ubiquitously and at comparable levels in zebrafish tissues. The strategy adopted also allowed the identification of an ADA2-1 truncated alternative splice isoform (ADA2-1/T), which was expressed at different intensities. These findings demonstrated the existence of different ADA-related genes, their distinct expression pattern and a truncated ADA2-1 isoform, which suggest a high degree of complexity in zebrafish adenosinergic system.

Descriptors: zebrafish, adenosine deaminase, genes, molecular identification, tissue expression, pattern, truncated alternative splice isoform.

Rosenthal, G.G. and M.J. Ryan (2005). **Assortative preferences for stripes in danios**. *Animal Behaviour* 70(5): 1063-1066. ISSN: 0003-3472.
NAL Call Number: 410 B77
Descriptors: zebrafish, color, color, freshwater fish, stripes pattern, gene expression, phenotypes, schooling behavior, shoals, *Danio*, *Danio albolineatus*, *Danio nigrofasciatus* , *Danio rerio.*

Rothenburg, S., N. Deigendesch, K. Dittmar, F. Koch Nolte, F. Haag, K. Lowenhaupt, and A. Rich (2005). **A PKR-like eukaryotic initiation factor 2alpha kinase from zebrafish contains Z-DNA binding domains instead of dsRNA binding domains.** *Proceedings of the National Academy of Sciences of the United States of America* 102(5): 1602-7. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: The double-stranded RNA (dsRNA)-dependent protein kinase (PKR) is induced as part of the IFN response in mammals and acts to shut down protein synthesis by the phosphorylation of eukaryotic initiation factor 2alpha (eIF2alpha). In fish, a PKR-like kinase activity has been detected, but the enzyme responsible has eluded characterization. Here, we describe a PKR-like kinase from zebrafish. Phylogenetic analysis shows that the C-terminal kinase domain is more closely related to the kinase domain of PKR than to any of the other three known eIF2alpha kinases. Surprisingly, instead of the two dsRNA binding domains found at the N terminus of PKR, there are two Zalpha domains. Zalpha domains specifically bind dsDNA and RNA in the left-handed Z conformation, often with high affinity. They have been found previously in two other IFN-inducible proteins, the dsRNA editing enzyme, ADAR1, and Z-DNA binding protein 1 (ZBP1), as well as in the poxvirus virulence factor, E3L. This previously undescribed kinase, designated PKZ (protein kinase containing Z-DNA binding domains), is transcribed constitutively at low levels and is highly induced after injection of poly(inosinic)-poly(cytidylic) acid, which simulates viral infection. Binding of Z-DNA by the Zalpha domain of PKZ was demonstrated by circular dichroism. PKZ inhibits translation in transfected cells; site-directed mutagenesis indicates that this inhibition depends on its catalytic activity. Identification of a gene combining Zalpha domains with a PKR-like kinase domain strengthens the hypothesis that the ability to bind left-handed nucleic acid plays a role in the host response to viruses.
Descriptors: zebrafish, Z form DNA metabolism, eIF 2 kinase metabolism and genetics, alternative splicing, amino acid and base sequence, binding sites, conserved sequence, molecular sequence data, phylogeny, poly I C pharmacology, sequence alignment, amino acid sequence homology.

Rottbauer, W., S. Just, G. Wessels, N. Trano, P. Most, H.A. Katus, and M.C. Fishman (2005). **VEGF-PLC gamma 1 pathway controls cardiac contractility in the embryonic heart**. *Genes and Development* 19(13): 1624-1634. ISSN: 0890 9369.
NAL Call Number: QH426.G46
Abstract: The strength of the heart beat can accommodate in seconds to changes in blood pressure or flow. The mechanism for such homeostatic adaptation is unknown. We sought the cause of poor contractility in the heart of the embryonic zebrafish with the mutation dead beat. We find through cloning that this is due to a mutation in the phospholipase C gamma 1 (plc gamma 1) gene. In mutant embryos, contractile function can be restored by PLC gamma 1 expression directed selectively to cardiac myocytes. In other situations, PLC

gamma 1 is known to transduce the signal from vascular endothelial growth factor (VEGF), and we show here that abrogation of VEGF also interferes with cardiac contractility. Somewhat unexpectedly, FLT-1 is the responsible VEGF receptor. We show that the same system functions in the rat. Blockage of VEGF-PLC gamma 1 signaling decreases calcium transients in rat ventricular cardiomyocytes, whereas VEGF imposes a positive inotropic effect on cardiomyocytes by increasing calcium transients. Thus, the muscle of the heart uses the VEGF-PLC gamma 1 cascade to control the strength of the heart beat. We speculate that this paracrine system may contribute to normal and pathological regulation of cardiac contractility. Copyright©Thomson Reuters 2009

Descriptors: zebrafish mutant embryos, cardiovascular system, transport and circulation, PLC gamma 1, VEGF receptor , development, cardiac contractility.

Roussigne, M. and P. Blader (2006). **Divergence in regulation of the PEA3 family of ets transcription factors**. *Gene Expression Patterns* 6(8): 777-782. ISSN: 1567 133X.

NAL Call Number: QH450.B73

Abstract: Here, we report the cloning of a cDNA encoding zebrafish ER81, a member of the PEA3 family of Ets transcription factors. Strikingly, the spatial and temporal expression of er81 is significantly different from its *Xenopus* orthologue, XER81, whose expression is more reminiscent of the FGF dependant zebrafish PEA3 family members. In keeping with this observation, while pea3, erm and XER81 require FGF activity for their expression, er81 does not require FGF signalling. Our results suggest that, since the vertebrate specific expansion of the PEA3 subfamily of Ets transcription factors, the regulation of PEA3 genes has been independently modified during the evolution of different vertebrate lineages. (c) 2006 Elsevier B.V. All rights reserved. Copyright©Thomson Reuters 2009

Descriptors: zebrafish , molecular genetics, cDNA, Ets transcription factors, PEA3 family.

Roy, N.M. and C.G. Sagerstrom (2004). **An early Fgf signal required for gene expression in the zebrafish hindbrain primordium.** *Brain Research* 148(1): 27-42. ISSN: 0006 8993.

NAL Call Number: QP376

Abstract: We have explored the role of fibroblast growth factor (Fgf) signaling in regulating gene expression in the early zebrafish hindbrain primordium. We demonstrate that a dominant negative Fgf receptor (FgfR) construct disrupts gene expression along the entire rostrocaudal axis of the hindbrain primordium and, using an FgfR antagonist, we find that this Fgf signal is required at early gastrula stages. This effect cannot be mimicked by morpholino antisense oligos to Fgf3, Fgf8 or Fgf24--three Fgf family members known to be secreted from signaling centers at the midbrain-hindbrain boundary (MHB), in rhombomere 4 and in caudal mesoderm at gastrula stages. We propose that an Fgf signal is required in the early gastrula to initiate hindbrain gene expression and that this is distinct from the later roles of Fgfs in patterning the hindbrain during late gastrula/early segmentation stages. We also find that blocking either retinoic acid (RA) or Fgf signaling disrupts hindbrain gene expression at gastrula stages, suggesting that both pathways are essential at this stage. However, both pathways must be blocked simultaneously to disrupt hindbrain gene expression at segmentation stages, indicating that these signaling pathways become redundant at later stages. Furthermore, exogenous application of RA or Fgf alone is sufficient to induce hindbrain genes in gastrula stage tissues, suggesting that the two-signal requirement can be overcome under some conditions. Our results demonstrate an early role for Fgf signaling and reveal a dynamic relationship between the RA and Fgf signaling pathways during hindbrain development.

Descriptors: zebrafish embryo, Fgf physiology, developmental gene expression regulation

physiology, rhombencephalon metabolism, signal transduction physiology, body patterning drug effects and physiology, cultured cells, cycloheximide pharmacology, DNA binding proteins genetics, Fgf chemistry, gastrula drug effects and metabolism, developmental gene expression regulation drug effects, immunohistochemistry methods, in situ hybridization methods, mesoderm drug effects and metabolism, microinjections methods, mitogen activated protein kinases metabolism, neurons drug effects, and physiology, antisense oligonucleotides pharmacology, drug effects and physiology, protein synthesis inhibitors pharmacology, pyrroles pharmacology, messenger RNA biosynthesis, fibroblast receptors growth factor antagonists, inhibitors, genetics and metabolism, retinoic acid receptors metabolism, reverse transcriptase PCR methods, rhombencephalon drug effects and embryology, signal transduction drug effects, spinal cord cytology and metabolism, time factors, tretinoin pharmacology.

Roy, S. (2007). **Genetic analysis of the vertebrate Hedgehog-signaling pathway using muscle cell fate specification in the zebrafish embryo.** *Methods in Molecular Biology* 397: 55-66. ISSN: 1064-3745.

Abstract: Over the recent years, a large number of embryological studies with the zebrafish have provided substantial evidence of its usefulness for the investigation of the genetic and cellular basis of vertebrate development. With regard to the Hedgehog (Hh) pathway, forward as well as reverse genetic approaches in this organism have not only validated the roles of evolutionarily conserved players of the signaling cascade, but have also contributed to the isolation of several novel components that had remained unidentified through screens in other animal models. Here, the author describes a whole mount antibody labeling method that allows the detection of three unique muscle cell fates in the zebrafish embryo, which are induced by distinct levels and timing of Hh-signaling activity. This technique provides a rapid and convenient assay that can be utilized for the evaluation of effects of loss- or gain-of-function of any gene on the levels of Hh pathway activation during embryogenesis.

Descriptors: zebrafish , Hedgehog, genetics, embryology.

Roy, S. and T. Ng (2004). **Blimp-1 specifies neural crest and sensory neuron progenitors in the zebrafish embryo.** *Current Biology* 14(19): 1772-7. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: Developmental origins of the neural crest (NC), a quintessential and pluripotent vertebrate cell type, has historically been a topic of extensive investigation but continues to remain poorly understood. In the zebrafish embryo, NC and primary sensory neurons are thought to segregate from a common population of progenitor cells in response to lateral inhibition. Here, we show that the zebrafish homolog of the B-lymphocyte-induced maturation protein (Blimp-1) gene, u-boot (ubo), is induced by BMP signaling in cells at the boundary of the neural plate and nonneural ectoderm. Loss of Ubo activity not only inhibits specification of the NC but also impairs development of the primary sensory neurons. Conversely, misexpression of ubo results in the generation of supernumerary primary sensory neurons consistent with this cell type representing the default fate within the progenitor equivalence group. These results establish a link between the activity of the transcriptional regulator Blimp-1 and the inductive effects of BMP signaling in the inception of NC progenitor fate.

Descriptors: zebrafish embryos, cell differentiation physiology, neural crest physiology, afferent neurons physiology, repressor, transcription factors physiology, bone morphogenetic proteins metabolism, DNA binding proteins genetics, proteins, developmental gene expression regulation physiology, immunohistochemistry, in situ hybridization, nuclear proteins,

repressor proteins metabolism, signal transduction physiology, transcription factors metabolism.

Ruuskanen, J.O., H. Xhaard, A. Marjamaki, E. Salaneck, T. Salminen, Y.L. Yan, J.H. Postlethwait, M.S. Johnson, D. Larhammar, and M. Scheinin (2004). **Identification of duplicated fourth alpha 2-adrenergic receptor subtype by cloning and mapping of five receptor genes in zebrafish**. *Molecular Biology and Evolution* 21(1): 14-28. ISSN: 0737 4038.
NAL Call Number: QH506.M642
Abstract: The alpha 2-adrenergic receptors (alpha 2-ARs) belong to the large family of rhodopsinlike G-protein-coupled receptors that share a common structure of seven transmembrane (TM) alpha -helices. The aims of this study were (1) to determine the number of alpha 2-AR genes in a teleost fish, the zebrafish (*Danio rerio*), (2) to study the gene duplication events that generated the alpha 2-AR subtypes, and (3) to study changes in receptor structure that have occurred since the divergence of the mammalian and fish lineages. Here, we report the cloning and chromosomal mapping of fish orthologs for all three mammalian alpha 2-ARs. In addition, we identified a fourth alpha 2-AR subtype with two duplicates in zebrafish. Chromosomal mapping showed that the zebrafish alpha 2-AR genes are located within conserved chromosomal segments, consistent with the origin of the four alpha 2-AR subtypes by two rounds of chromosome or block duplication before the divergence of the ray fin fish and tetrapod lineages. Thus, the fourth subtype has apparently been present in the common ancestor of vertebrates but has been deleted or is yet to be identified in mammals. The overall percentage identity between the fish and mammalian orthologs is 53% to 67%, and in the TM regions 80% to 87%. These values are clearly lower than what is observed between mammalian orthologs. Still, all of the residues thought to be important for alpha 2-adrenergic ligand binding are conserved across species and subtypes, and even the most divergent regions of the fish receptors show clear "molecular fingerprints" typical for orthologs of a given subtype. Copyright © 2008 CABI
Descriptors: zebrafish, alpha adrenergic receptors conserved accross species, ancestors, biochemical receptors, chromosome maps, gene mapping, genes, identification, *Danio rerio*.

Ryu, S., J. Holzschuh, S. Erhardt, A.K. Ettl, and W. Driever (2005). **Depletion of minichromosome maintenance protein 5 in the zebrafish retina causes cell-cycle defect and apoptosis.** *Proceedings of the National Academy of Sciences of the United States of America* 102(51): 18467-72. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: In multicellular organisms, the control of genome duplication and cell division must be tightly coordinated. Essential roles of the minichromosome maintenance (MCM) proteins for genome duplication have been well established. However, no genetic model has been available to address the function of MCM proteins in the context of vertebrate organogenesis. Here, we present positional cloning of a zebrafish mcm5 mutation and characterization of its retina phenotype. In the retina, mcm5 expression correlates closely with the pattern of cell proliferation. By the third day of development, mcm5 is down-regulated in differentiated cells but is maintained in regions containing retinal stem cells. We demonstrate that a gradual depletion of maternally derived MCM5 protein leads to a prolonged S phase, cell-cycle-exit failure, apoptosis, and reduction in cell number in mcm5(m850) mutant embryos. Interestingly, by the third day of development, increased apoptosis is detectable only in the retina, tectum, and hindbrain but not in other late-proliferating tissues, suggesting that different tissues may employ distinct cellular programs in responding to the depletion of MCM5.

Descriptors: zebrafish, apoptosis, cell cycle proteins metabolism, retina cytology and metabolism, cell cycle cell proliferation, molecular cloning, cytology, embryology, metabolism, developmental gene expression regulation, mutation genetics, retina embryology.

Ryu, S., J. Holzschuh, J. Mahler, and W. Driever (2006). **Genetic analysis of dopaminergic system development in zebrafish.** *Journal of Neural Transmission*(70): 61-6. ISSN: 0300-9564.
NAL Call Number: QP364.5
Abstract: Zebrafish have become an important model organism to study the genetic control of vertebrate nervous system development. Here, we present an overview on the formation of dopaminergic neuronal groups in zebrafish and compare the positions of DA neurons in fish and mammals using the neuromere model of the vertebrate brain. Based on mutant analysis, we evaluate the role of several signaling pathways in catecholaminergic neuron specification. We further discuss the prospect of identifying novel genes involved in dopaminergic development through forward genetics mutagenesis screens.
Descriptors: zebrafish, dopamine genetics, dopamine physiology, nervous system growth and development, signal transduction genetics, signal transduction physiology.

Sabaawy, H.E., M. Azuma, L.J. Embree, H.J. Tsai, M.F. Starost, and D.D. Hickstein (2006). **TEL-AML1 transgenic zebrafish model of precursor B cell acute lymphoblastic leukemia.** *Proceedings of the National Academy of Sciences of the United States of America* 103(41): 15166-71. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: Acute lymphoblastic leukemia (ALL) is a clonal disease that evolves through the accrual of genetic rearrangements and/or mutations within the dominant clone. The TEL-AML1 (ETV6-RUNX1) fusion in precursor-B (pre-B) ALL is the most common genetic rearrangement in childhood cancer; however, the cellular origin and the molecular pathogenesis of TEL-AML1-induced leukemia have not been identified. To study the origin of TEL-AML1-induced ALL, we generated transgenic zebrafish expressing TEL-AML1 either ubiquitously or in lymphoid progenitors. TEL-AML1 expression in all lineages, but not lymphoid-restricted expression, led to progenitor cell expansion that evolved into oligoclonal B-lineage ALL in 3% of the transgenic zebrafish. This leukemia was transplantable to conditioned wild-type recipients. We demonstrate that TEL-AML1 induces a B cell differentiation arrest, and that leukemia development is associated with loss of TEL expression and elevated Bcl2/Bax ratio. The TEL-AML1 transgenic zebrafish models human pre-B ALL, identifies the molecular pathways associated with leukemia development, and serves as the foundation for subsequent genetic screens to identify modifiers and leukemia therapeutic targets.
Descriptors: zebrafish, core bindingfactor alpha 2 subunit genetics, disease models, acute B cell leukemia genetics, leukemia, pre B cell genetics, oncogene proteins, fusion genetics, genetically modified, cell differentiation genetics, child, core bindingfactor alpha 2 subunit antagonists and inhibitors, core bindingfactor alpha 2 subunit biosynthesis, gene silencing, hematopoietic stem cells pathology, acute B cell leukemia metabolism, acute B cell leukemia pathology, leukemia, pre B cell metabolism, leukemia, pre B cell pathology, oncogene proteins, fusion antagonists and inhibitors, oncogene proteins, fusion biosynthesis, proto oncogene proteins C Bcl 2 biosynthesis, up regulation genetics, Bcl 2 associated X protein biosynthesis.

Sadler, K.C., K.N. Krahn, N.A. Gaur, and C. Ukomadu (2007). **Liver growth in the embryo and during liver regeneration in zebrafish requires the cell cycle regulator, uhrf1.** *Proceedings of the National Academy of Sciences of the United States of America* 104(5): 1570-5. ISSN:

0027-8424.
NAL Call Number: 500N21P
Abstract: In contrast to the deregulated hepatocellular division that is a feature of many hepatic diseases and malignancies, physiologic liver growth during embryonic development and after partial hepatectomy (PH) in adults is characterized by tightly controlled cell proliferation. We used forward genetic screening in zebrafish to test the hypothesis that a similar genetic program governs physiologic liver growth during hepatogenesis and regeneration after PH. We identified the uhrf1 gene, a cell cycle regulator and transcriptional activator of top2a expression, as required for hepatic outgrowth and embryonic survival. By developing a methodology to perform PH on adult zebrafish, we found that liver regeneration inuhrf1+/- adult animals is impaired.uhrf1 transcript levels dramatically increase after PH in both mice, and zebrafish and top2a is not up-regulated in uhrf1+/- livers after PH. This indicates that uhrf1 is required for physiologic liver growth in both embryos and adults and illustrates that zebrafish livers regenerate.
Descriptors: zebrafish, CCAAT enhancer binding, liver embryology, physiology and regeneration, trans activators biosynthesis and activators physiology, proteins biosynthesis, genetically modified, CCAAT enhancer binding proteins metabolism, cell cycle and proliferation, cyclin A physiology, developmental gene expression regulation, in situ hybridization, liver anatomy, histology and metabolism, mice, mutation, time factors.

Sadler, K.C., A. Amsterdam, and N. Hopkins (2004). **The genetic basis for hepatic outgrowth and hepatomegaly during zebrafish embryogenesis.** *Hepatology* 40(4, Suppl. 1): 221A. ISSN: 0270 9139.
Descriptors: zebrafish, development, digestive system, ingestion and assimilation, hepatomegaly, digestive system disease, cellular function, cell size, cycle, division and apoptosis, hepatic outgrowth.

Saera Vila, A., J.A. Calduch Giner, and J. Perez Sanchez (2005). **Duplication of growth hormone receptor (GHR) in fish genome: gene organization and transcriptional regulation of GHR type I and II in gilthead sea bream (*Sparus aurata*)**. *General and Comparative Endocrinology* 142(1/2): 193-203. ISSN: 0016 6480.
NAL Call Number: 444.8G28
Abstract: Nucleotide sequences encoding for functional growth hormone receptors (GHR) are now available in salmonids (coho and masu salmon) and other fish orders. Several authors have hypothesized a divergent evolution of salmonid GHRs, but the recent finding that a trout cDNA is related to non-salmonid (GHR type I) rather than to salmonid GHRs (GHR type II) points out a possible duplication of actively transcribed GHR genes. To address this issue, we search by RT-PCR for GHR type II in trout, gilthead sea bream, European sea bass, and turbot. Both in trout and gilthead sea bream, a cDNA sequence with all the characteristic features of masu and coho salmon GHRs (GHR type II) was found. Similarly, in European sea bass, a cDNA encoding for the intracellular domain of GHR type II was reported. No positive results were found in turbot, but searches in genome databases of fugu and zebrafish identified DNA sequences with a significant similarity to fish GHR type I and II, which are more related each other than to GHRs of tetrapods. Gene organization is, however, highly conserved through the evolution of vertebrates, and eight exons homologous to exons 2 and 4-10 of mammals were found in fish GHRs. Transcriptional regulation of GHR type I and II was also addressed by means of real-time PCR assays in gilthead sea bream. In liver and adipose tissue, GHR type I was the most abundant transcript, but the expression of GHR type I and II was practically equal in skeletal muscle. Both in liver and

adipose tissue, the two GHRs were down-regulated by fasting. In skeletal muscle, the expression of GHR type I remained unaltered in fasted fish, whereas a 2- to 3-fold increase was found for GHR type II. All this provides suitable evidence for a tissue-specific regulation of fish GHRs, but further research is needed to determine whether these duplicated genes are evolved in a new or redundant fashion. Copyright © 2008 CABI

Descriptors: zebrafish, adipose tissue, complementary DNA, gene expression, genes, genomes, hormone receptors, liver, nucleotide sequences, phylogeny, skeletal muscle, somatotropin, transcription, *Dicentrarchus labrax*, *Oncorhynchus kisutch*, *Pagrus aurata*, salmon, turbot.

Saito, R., Y. Tabata, A. Muto, K. Arai, and S. Watanabe (2005). **Melk-like kinase plays a role in hematopoiesis in the zebra fish.** *Molecular and Cellular Biology* 25(15): 6682-93. ISSN: 0270-7306.

NAL Call Number: QH506.M664

Abstract: A serine/threonine kinase, Melk, was initially cloned in mouse oocytes as a maternal gene, but whose function was unknown. In adult mice, Melk was strongly expressed in the thymus and bone marrow, suggesting a role for Melk in hematopoiesis. We cloned a Melk-like gene from zebra fish (zMelk). zMelk-like gene was expressed in the brain and lateral mesoderm at 12 hours postfertilization (hpf) and in several tissues of adult fish, including the kidney and spleen, both of which are known to be hematopoietic tissues in zebra fish. Abrogation of zMelk-like gene function by zMelk-like gene-specific Morpholino (MO) resulted in abnormal swelling around the tectum region. In addition, the start of blood circulation was severely delayed but, in contrast, the vessel formation seemed normal. Expression of scl, gata-1, and lmo-2 was down regulated at 12 to 14 hpf in the zMelk-like gene MO-injected embryos, and the coexpression of gata-1 rescued the anemic phenotype induced by zMelk-like gene MO. Expression of the zMelk-like gene in embryos enhanced gata-1 promoter-dependent enhanced green fluorescent protein expression, suggesting that the zMelk-like gene affects gata-1 expression at the transcriptional level. Taken together, our data suggest that the zMelk-like gene may play a role in primitive hematopoiesis by affecting the expression of genes critical for hematopoiesis.

Descriptors: zebrafish, hematopoiesis physiology, leucine zippers physiology, protein serine threonine kinases physiology, anemia genetics, base sequence , DNA binding proteins genetics and physiology, erythroid specific DNA binding proteins genetics, factors, eye embryology, gene expression profiling, hematopoiesis genetics, molecular sequence data, physiologic neovascularization genetics, antisense oligonucleotides, promoter regions genetics, protein serine threonine kinases biosynthesis and genetics, transcription factors genetics and physiology.

Sakai, N. (2006). **In vitro male germ cell cultures of zebrafish.** *Methods* 39(3): 239-45. ISSN: 0882-4010.

NAL Call Number: QP519.7.M47

Abstract: Transgenic modification of sperm before fertilization has the advantages of a much shorter timeline for the production of transgenic animals. A culture system using primary cultures of zebrafish male germ cells, in which the differentiation of spermatogonia to functional sperm can occur in vitro, allows us to introduce foreign DNA into the cultured sperm and to produce transgenics from the sperm. This chapter describes methods for the co-culture of male germ cells and a Sertoli cell feeder layer and the introduction of foreign DNA with retroviruses. This male germ cell culture system should prove useful not only in producing genetically modified sperm, but also in analyzing the regulatory function of Sertoli cells

for spermatogenesis in vertebrates.
Descriptors: zebrafish, coculture techniques, genetic engineering methods, spermatozoa cytology, zebrafish, genetically modified genetics, cell differentiation, fertilization in vitro, Retroviridae genetics, Sertoli cells cytology, spermatozoa enzymology, beta galactosidase metabolism.

Sakamoto, T. (2004). **Construction of genetic linkage maps and QTL analysis using microsatellite markers in fish**. *Nippon Suisan Gakkaishi* 70(4): 496-499. ISSN: 0021 5392.
Descriptors: zebrafish, gene mapping, genetic analysis, genetic markers, linkage, microsatellites, quantitative trait loci, ayu, brown trout, *Danio rerio*, fishes, *Ictalurus punctatus*, *Oncorhynchus kisutch*, *Oncorhynchus masou*, *Pagrus major*, *Paralichthys olivaceus*, rainbow trout, *Salmo trutta*, salmon, *Salvelinus*, *Salvelinus alpinus*, *Salvelinus fontinalis*, *Seriola quinqueradiata*, trout.
Language of Text: Japanese.

Salas Vidal, E., A.H. Meijer, Cheng Xi, and H.P. Spaink (2005). **Genomic annotation and expression analysis of the zebrafish Rho small GTPase family during development and bacterial infection**. *Genomics* 86(1): 25-37. ISSN: 0888 7543.
NAL Call Number: QH445.2.G45
Abstract: The zebrafish genomic sequence database was analyzed for the presence of genes encoding members of the Rho small GTPases. The analysis shows the presence of 32 zebrafish Rho genes representing one or more homologs of the human RHOA, RND3, RHOF, RHOG, RHOH, RHOJ, RHOU, RHOV, CDC42, RAC1, RAC2, RAC3, RND1, RHOBTB1, RHOBTB2, RHOBTB3, and RHOT1 genes. By expression analysis using reverse transcriptase-PCR we show that at least 20 of the predicted zebrafish small GTPase genes are expressed in the adult stage. Interestingly, only 5 of these were found to be expressed at early embryonic stages, including rhoab, rhoad, cdc42a, cdc42c, and rac1a. We observed a strong upregulation of zebrafish rhogb expression after *Mycobacterium marinum* infection of adult fish. This complete annotation study provides a firm basis for the use of zebrafish as a model for analysis of Rho GTPase function in vertebrate development and the innate immune system. Copyright © 2008 CABI
Descriptors: zebrafish, databases, DNA cloning, embryonic development, gene expression, genes, genomes, immune system, nucleotide sequences, *Danio rerio*, fishes, *Mycobacterium marinum*, bacterial pathogen.

Samani, N.J., H. Mahadeva, N. Chong, and S.Y. Shaw (2006). **Knockdown in zebrafish supports important role myocyte stress (MS1) in cardiac development and function**. *European Heart Journal* 27(Suppl. 1): 473. ISSN: 0195 668X.
Descriptors: zebrafish, development, cardiovascular system, transport and circulation, ischemia reperfusion injury, vascular disease, injury, genetics, gene sequencing, in situ hybridization, histology and cytology techniques, genetic techniques, gene cloning, gene amplification, cardiac function, cardiac development.

Sambrook, J.G., F. Figueroa, and S. Beck (2005). **A genome-wide survey of Major Histocompatibility Complex (MHC) genes and their paralogues in zebrafish.** *BioMed Central Medical Genomics* 6: 152. ISSN: 1755 8794.
Abstract: BACKGROUND: The genomic organisation of the Major Histocompatibility Complex (MHC) varies greatly between different vertebrates. In mammals, the classical MHC consists of a large number of linked genes (e.g. greater than 200 in humans) with

predominantly immune function. In some birds, it consists of only a small number of linked MHC core genes (e.g. smaller than 20 in chickens) forming a minimal essential MHC and, in fish, the MHC consists of a so far unknown number of genes including non-linked MHC core genes. Here we report a survey of MHC genes and their paralogues in the zebrafish genome. RESULTS: Using sequence similarity searches against the zebrafish draft genome assembly (Zv4, September 2004), 149 putative MHC gene loci and their paralogues have been identified. Of these, 41 map to chromosome 19 while the remaining loci are spread across essentially all chromosomes. Despite the fragmentation, a set of MHC core genes involved in peptide transport, loading and presentation are still found in a single linkage group. CONCLUSION: The results extend the linkage information of MHC core genes on zebrafish chromosome 19 and show the distribution of the remaining MHC genes and their paralogues to be genome-wide. Although based on a draft genome assembly, this survey demonstrates an essentially fragmented MHC in zebrafish.
Descriptors: zebrafish, genome, major histocompatibility complex genetics, chromosome mapping, cluster analysis, DNA metabolism, complementary DNA metabolism, gene duplication, human genone, genomics, haplotypes, linkage genetics, statistical models, molecular sequence data , genetic polymorphism, software.

Sansam, C.L., J.L. Shepard, K. Lai, A. Ianari, P.S. Danielian, A. Amsterdam, N. Hopkins, and J.A. Lees (2006). **DTL/CDT2 is essential for both CDT1 regulation and the early G2/M checkpoint**. *Genes & Development* 20(22): 3117-3129. ISSN: 0890-9369.
NAL Call Number: QH426.G46
Descriptors: zebrafish, cell cycle, DNA, DNA damage, freshwater fish, genetic diversity, population genetics, replication, S phase, ubiquitin-protein ligase, genomics, Danio rerio, development and cell cycle, genetics and evolution.

Santini, S. and G. Bernardi (2005). **Organization and base composition of tilapia Hox genes: implications for the evolution of Hox clusters in fish**. *Gene* 346: 51-61. ISSN: 0378 1119.
NAL Call Number: QH442.A1G4
Abstract: Hox genes encode DNA binding proteins that specify cell fate in the anterior-posterior axis of metazoan animal embryos. While each Hox cluster contains the same genes among the different mammalian species, this does not happen in ray-finned fish, in which both the number and organization of Hox genes and even Hox clusters are variables. Ray-finned fish are believed to have undergone an additional genome duplication that led to the presence of 8 Hox clusters (four twin pairs) in their ancestor. Here we describe the Tilapia (*Oreochromis niloticus*) Hox genes set in terms of gene content, clusters organization and base composition and compare it with those of pufferfish and zebrafish. We observed that in all these fish, when paralogous genes are conserved in both the twin clusters, the gene which has a lower GC level generally: (i) belongs to the less gene-rich (less conserved) cluster; (ii) has a reduced field of embryonic expression; or (iii) is a pseudogene. The relationship between the decrease of GC level and the loss of conservation and function of one of the paralogous genes from twin clusters is discussed. Copyright © 2008 CABI
Descriptors: zebrafish, DNA binding proteins genetics, proteins, evolution, gene expression, genes, genomes, fishes, *Oreochromis niloticus*.

Santos, F., G. MacDonald, E.W. Rubel, and D.W. Raible (2006). **Lateral line hair cell maturation is a determinant of aminoglycoside susceptibility in zebrafish (*Danio rerio*).** *Hearing Research* 213(1-2): 25-33. ISSN: 0378-5955.
Abstract: Developmental differences in hair cell susceptibility to aminoglycoside-induced

cell death has been observed in multiple species. Increased sensitivity to aminoglycosides has been temporally correlated with the onset of mechanotransduction-dependent activity. We have used in vivo fluorescent vital dye markers to further investigate the determinants of aminoglycoside induced hair cell death in the lateral line of zebrafish *(Danio rerio*). Labeling hair cells of the lateral line in vivo with the dyes FM 1-43, To-Pro-3, and Yo-Pro-1 served as reliable indicators of hair cell viability. Results indicate that hair cell maturation is a determinant of developmental differences in susceptibility. The age dependent differences in susceptibility to aminoglycosides are independent of the onset of mechanotransduction-dependent activity as measured by FM 1-43 uptake and independent of hair cell ability to take up fluorescently conjugated aminoglycosides.
Descriptors: zebrafish, mechanotransduction, activity, fM 1-43, uptake, hair cell, conjugated, aminoglycosides.

Sato, T., T. Hamaoka, H. Aizawa, T. Hosoya, and H. Okamoto (2007). **Genetic single-cell mosaic analysis implicates ephrinB2 reverse signaling in projections from the posterior tectum to the hindbrain in zebrafish.** *Journal of Neuroscience* 27(20): 5271-9. ISSN: 0270-6474.
Abstract: The optic tectum is a visual center in vertebrates. It receives topographically ordered visual inputs from the retina in the superficial layers and then sends motor outputs from the deeper layers to the premotor reticulospinal system in the hindbrain. Although the topographic patterns of the retinotectal projection are well known, it is not yet well understood how tectal efferents in the tectobulbar tract project to the hindbrain. The retinotectal and the tectobulbar projections were visualized in a zebrafish stable transgenic line Tg(brn3a-hsp70:GFP). Using a single-neuron labeling system in combination with the cre/loxP and Gal4/UAS systems, we showed that the tectal neurons that projected to rhombomeres 2 and 6 were distributed with distinctive patterns along the anterior-posterior axis. Furthermore, we found that ephrinB2a was critically involved in increasing the probability of neurons projecting to rhombomere 2 through a reverse signaling mechanism. These results may provide a neuroanatomical and molecular basis for the motor command map in the tectum.
Descriptors: zebrafish, ephrin B2 physiology, mosaicism embryology, neurons physiology, superior colliculus physiology, genetically modified, ephrin B2 genetics, neurons cytology, rhombencephalon cytology, embryology and physiology, signal transduction genetics, superior colliculus cytology and embryology, visual pathways cytology, embryology and physiopathology.

Sato, T. and M. Mishina (2003). **Representational difference analysis, high-resolution physical mapping, and transcript identification of the zebrafish genomic region for a motor behavior.** *Genomics* 82(2): 218-29. ISSN: 0888-7543.
NAL Call Number: QH445.2.G45
Abstract: Zebrafish is one of the best model organisms for investigating gene functions in vertebrates. By 4,5',8-trimethylpsoralen mutagenesis, we isolated a zebrafish mutant, vibrato, with defects in the spontaneous contraction and touch response. Whole genome subtraction between the wild-type and the mutant genomes by representational difference analysis yielded polymorphic markers tightly linked to the vibrato locus. Using these markers, we constructed a high-resolution physical map and localized the vibrato locus within a genomic region of 720 kb. Direct cDNA selection with the contig led to the identification of a novel gene, solo, encoding a protein with SEC14 and spectrin repeat domains. These domains of Solo shared significant amino acid sequence identities with those of mammalian Trio and Karilin. In addition, we found the zebrafish orthologs for mammalian TTN, COL5A2, and CED-6 in the vibrato region. Mapping of these genes localized human chromosomal regions

possibly involved in motor disorders. Our results suggest that representational difference analysis provides an efficient way to isolate mutated genomic regions in zebrafish.
Descriptors: zebrafish, chromosome mapping, genomics methods, movement physiology, touch genetics, amino acid sequence, Southern blotting, DNA primers, gene components genetics, molecular sequence data, mutagenesis genetics, radiation hybrid mapping, sequence alignment, DNA sequence analysis.

Sato, T., M. Takahoko, and H. Okamoto (2006). **HuC: Kaede, a useful tool to label neural morphologies in networks in vivo**. *Genesis* 44(3): 136-142 . ISSN: 1526 954X.
NAL Call Number: QH426.D32
Abstract: A simple and efficient procedure for labeling neurons is a prerequisite for investigating the development of neural networks in zebrafish. To label neurons we used Kaede, a fluorescent protein with a photoconversion property allowing conversion from green to red fluorescence following irradiation with UV or violet light. We established a zebrafish stable transgenic line, Tg(HuC:Kaede), expressing Kaede in neurons under the control of the HuC promoter. This transgenic line was used to label a small number of neurons in the trigeminal ganglion. Also, using embryos injected with the transgene, we labeled peripheral axon arbors of a Rohon-Beard neuron at 4 days postfertilization and observed the dendrite development of a tectal neuron for 3 days. These data indicate that Kaede is a useful tool to selectively label neural networks in zebrafish. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, Kaede, fluorescent protein, nervous system, neural coordination, irradiation.

Satoh, S., K. Arai, and S. Watanabe (2004). **Identification of a novel splicing form of zebrafish p73 having a strong transcriptional activity.** *Biochemical and Biophysical Research Communications* 325(3): 835-42. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: p73 is a transcriptional activator related to tumor suppressor p53 and regulates differentiation, cell-cycle arrest, and apoptosis. Recently, zebrafish p73 (zp73alpha) was isolated and shown to be required for zebrafish embryogenesis. In this study, we isolated a novel splicing-variant of zp73 mRNA, which was generated by the use of an alternative splicing acceptor site, and designated it as zp73theta;. The zp73theta; mRNA encoded a carboxy-terminal structure distinct from that of zp73alpha. Whereas the expression level of zp73theta; mRNA was much lower than that of zp73alpha in zebrafish adult tissues, it was relatively high and fluctuated during embryogenesis. Using Saos-2 cells for a transient reporter assay, we found that zp73theta;, but not zp73alpha, had strong transcriptional activity when the experiments were performed at 34 degrees C. In addition, zp73theta; had the ability to suppress the growth of Saos-2 cells and to cause the developmental defects in zebrafish. These data indicated that zp73theta; could work as a transcriptional activator in zebrafish.
Descriptors: zebrafish, gene expression regulation physiology, osteoblasts metabolism, trans activation genetics physiology, transcription factors chemistry and metabolism, proteins chemistry, amino acid sequence, cell line, molecular sequence data, organ specificity, protein isoforms genetics and metabolism, protein splicing genetics, recombinant recombinant proteins metabolism, amino acid sequence homology, tissue distribution, transcription factors genetics.

Saude (2005). **Terra is a left-right asymmetry gene required for left-right synchronization of the segmentation clock** . *Nature Cell Biology* 7(11): 918-1147. ISSN: 1465 7392.
NAL Call Number: QH573

Descriptors: zebrafish, molecular genetics, biochemistry, segmentation clock, Terra, asymmetry gene.

Savan, R. and M. Sakai (2006). **Genomics of fish cytokines**. *Comparative Biochemistry and Physiology D: Genomics and Proteomics* 1(1): 89-101. ISSN: 1744 117X.

Abstract: Cytokines are important regulators of immune system and finding these genes in fish have potential applications for the development of vaccines and or immunostimulants for aquaculture. Major hurdle in discovering cytokines from fish is the low identities of these molecules when compared to its mammalian counterparts. In fish, prior to pufferfish (*Fugu rubripes and Tetraodon nigroviridis*) and zebrafish (*Danio rerio*) genome sequencing, only a handful of cytokines like IL-1 beta , TNF- alpha , TGF beta , some CXC (including IL-8) and CC chemokine genes were identified. Post genome release of pufferfish and zebrafish, the rate of discovery of fish cytokines has accelerated tremendously. Fish and human share a certain degree of synteny in genomes. Exploiting this characteristic, cytokines have been searched for in the genome databases of pufferfish (http://fugu.hgmp.mrc.ac.uk) and zebrafish. Pro-inflammatory cytokines like TNF's, IL-6 and IL-17 family have been cloned. A new TNF gene has been found adjacent to TNF alpha gene from fugu and zebrafish. Among the TH1 type interleukins, IL-2, IL-15, IL-12 alpha , IL-12 beta , IL-18 have been cloned. Among IL-10 and its family members, IL-10, IL-19/20, IL-22 and IL-26 have been discovered. Interferon genes have also been identified by from fish. However, TH2 cytokines (IL-4, IL-5 and IL-13), IL-3, IL-7 and IL-9 are yet to be discovered from fish. This study shows that the type and diversification of fish cytokines are very similar to that found in mammals.
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Descriptors: zebrafish, chemokines, cytokines, DNA sequencing, genes, genomes, genomics, interferon, interleukins, reviews, tumor necrosis factor, *Danio rerio*, fishes, Tetraodontidae.
Notes: Review article.

Sawant, M.S., S.C. Zhang, Q.Y. Wang, and Y.J. Wang (2004). **Production of interspecies chimeras by transplanting rosy barb (*Puntius conchonius*) embryonic cells to zebrafish (*Danio rerio*) embryos.** *Indian Journal of Experimental Biology* 42(8): 830-6. ISSN: 0019-5189.
NAL Call Number: 442.8IN2

Abstract: Establishment of a cell-mediated gene transfer system has potential as a new breeding technique for commercially valuable fishes. As an important step toward developing an inter-species chimera, cells from blastula-stage embryos of rosy barb (*Puntius conchonius*) were transplanted into zebrafish (*Danio rerio*) blastula-stage embryos to observe the development of the recipient. From the total of 473 transplants obtained only a fraction of 13 chimeras appeared perfectly normal after one month. Over two in normal 13 chimeras showed some characters from the donor cells with scarce pigmentation. This is the first successful inter-species study on zebrafish by using blastula cell transplants from rosy barb both belonging to the same family cyprinidae.
Descriptors: zebrafish embryos, chimera genetics, Cyprinidae genetics and embryology, base sequence, breeding, chimera embryology, DNA primers genetics, gene transfer techniques, phenotype, pigmentation, stem cell transplantation.

Sawyer, S.J., K.A. Gerstner, and G.V. Callard (2006). **Real-time PCR analysis of cytochrome P450 aromatase expression in zebrafish: gene specific tissue distribution, sexdifferences, developmental programming, and estrogen regulation.** *General and Comparative Endocrinology* 147(2): 108-17. ISSN: 0016-6480.

NAL Call Number: 444.8G28
Abstract: In teleost fish, the predominant brain form of cytochrome P450 aromatase (P450aromB) is a neural marker of estrogen effect, and an entry point for studying the role of hormonal and environmental estrogens on neurodevelopment and neuroplasticity. As part of a project using zebrafish to investigate these issues, we developed and validated a rapid, sensitive, and reproducible real-time polymerase chain reaction (PCR) assay for quantifying and comparing P450aromB and P450aromA expression in unfertilized eggs, embryos/larvae, and dissected tissues of adult fish. Results confirm that P450aromB and -A predominate in brain and ovary, respectively, and further show that the degree of overlapping expression (ratio, B:A) is 100:1 in brain, 1:50 in ovary, 1:1 in eye, and 2:1 in testis. Sex differences were observed in eye only (female>male). When compared to whole ovaries, unfertilized eggs had similar levels of P450aromA but enrichment of P450aromB, which suggests preferential synthesis or accumulation in mature oocytes. Both of the maternally derived aromatase isoforms were rapidly degraded post-fertilization, but the onset of embryonic P450aromB expression (5 hpf) was much earlier than P450aromA (48 hpf), and reached higher maximum levels (e.g., 10-fold at 72 hpf). Consistent with earlier reports, P450aromB but not -A was estrogen-inducible, but the estrogen response system in embryos was far more robust than in adults (>100- vs. <4-fold maximal induction, respectively). Application of this real-time PCR assay to measurement of P450aromB and -A in zebrafish embryos has utility for routine screening of chemicals and environmental samples for estrogen-like bioactivity and neural effects.
Descriptors: zebrafish, aromatase genetics, PCR methods, aromatase drug effects, body patterning drug effects, body patterning genetics , brain drug effects and enzymology, enzymology, estradiol pharmacology, eye drug effects and enzymology, gonads drug effects and enzymology, ovum enzymology, protein isoforms analysis, sex characteristics, tissue distribution genetics, proteins drug effects.

Schafer, M., M. Rembold, J. Wittbrodt, M. Schartl, and C. Winkler (2005). **Medial floor plate formation in zebrafish consists of two phases and requires trunk-derived Midkine-a.** *Genes and Development* 19(8): 897-902. ISSN: 0890-9369.
NAL Call Number: QH426.G46
Abstract: The medial floor plate (MFP) organizes the specification of neurons and outgrowth of axons in the ventral spinal cord of vertebrates. We show that the growth factor Midkine-a, expressed in the paraxial mesoderm, is required for formation of the MFP in zebrafish. Our epistatic analyses demonstrate that development of MFP comprises two independent sequential phases. Following initial MFP induction in the gastrula organizer, Midkine-a regulates allocation of MFP cells during subsequent development. Thus in zebrafish, trunk-derived signals are required for complete MFP formation from a common pool of organizer-derived midline precursor cells.
Descriptors: zebrafish, cell differentiation physiology, cytokines metabolism, epistasis, genetic, mesoderm metabolism, nerve growth factors metabolism, spinal cord embryology, DNA primers, in situ hybridization, reverse transcriptase PCR.

Schaffeld, M., M. Knappe, C. Hunzinger, and J. Markl (2003). **cDNA sequences of the authentic keratins 8 and 18 in zebrafish.** *Differentiation* 71(1): 73-82. ISSN: 0301 4681.
NAL Call Number: QH607.D5
Abstract: From the zebrafish *Danio rerio*, we have cDNA cloned and sequenced a novel type II and a novel type I keratin, termed DreK8 and DreK18, respectively. We identified DreK8/18 as the true orthologs of the human keratin pair K8/18 as follows: (i) MALDI-MS

assignment to the biochemically identified K8 and K18 candidates that are co-expressed in simple epithelia and absent in epidermal keratinocytes; (ii) multiple sequence alignments and phylogenetic tree analysis, showing that DreK8, within the phylogenetic tree of type II keratins, forms a highly bootstrap-supported branch together with K8 from goldfish and rainbow trout, whereas DreK18, within the phylogenetic tree of type I keratins, groups with the K18 sequences from all other vertebrates studied; (iii) presence of a conserved motif in the tail domain of DreK8 (VxKxxETxDGxxVSESSxV) that is typical for all hitherto sequenced K8 orthologs. Moreover, several zebrafish type II keratin sequences published by other authors have now been assigned to epidermal keratins, previously identified biochemically.
Descriptors: zebrafish, keratins chemistry, proteins chemistry, amino acid sequence, molecular cloning, keratin 18 and 8, keratins genetics and metabolism, molecular sequence data, phylogeny, sequence alignment, tissue distribution.

Schebesta, M., C.L. Lien, F.B. Engel, and M.T. Keating (2006). **transcriptional profiling of caudal fin regeneration in zebrafish.** *TheScientificWorldJournal* 6(Suppl 1): 38-54. ISSN: 1537-744X.
Abstract: Regeneration of severed limbs in adult animals is restricted to urodele amphibians. Mammals, including humans, have very limited regenerative capabilities and even with proper treatment, only the tips of our digits can grow back. Teleost fish can regenerate amputated fins, the evolutionary ancestors of limbs. To elucidate the principles of limb-fin regeneration, we performed an Affymetrix microarray screen on regenerating caudal fins 12, 24, 48, and 72 h post amputation. Approximately 15,000 zebrafish transcripts were analyzed, identifying 829 transcripts as differentially expressed during regeneration. Of those, 563 were up-regulated and 266 were down-regulated. We constructed a comprehensive database containing expression data, functional assignment, and background information from the literature for each differentially expressed transcript. In order to validate our findings, we employed three approaches: (1) microarray expression analysis of genes previously implicated in fin regeneration, (2) RT-PCR analysis of genes newly identified as differentially expressed during regeneration, and (3) in situ hybridization of the up-regulated genes bambi, dlx5A, and her6. Moreover, we show that Smad 1/5/8 proteins, effector molecules of Bmp signaling, are phosphorylated during fin regeneration. Taken together, we provide a comprehensive database of fin regeneration that will serve as an important tool for understanding the molecular mechanisms of regeneration.
Descriptors: zebrafish, gene expression regulation, tail regeneration genetics and physiology, basic helix loop helix transcription factors genetics and metabolism, genetic databases, epidermis metabolism, gene expression profiling , homeodomain proteins metabolism, mesoderm metabolism, oligonucleotide array sequence analysis, reproducibility of results, reverse transcriptase PCR, signal transduction genetics, Smad proteins metabolism, tail metabolism, transcription factors genetics and metabolism, up regulation, wound healing genetics.

Schier, A.F. and A.J. Giraldez (2006). **MicroRNA function and mechanism: insights from zebra fish.** *Cold Spring Harbor Symposia on Quantitative Biology* 71: 195-203. ISSN: 0091 7451.
NAL Call Number: 442.9C672C
Abstract: MicroRNAs (miRNAs) are small RNAs that bind to the 3 UTR of mRNAs. We are using zebra fish as a model system to study the developmental roles of miRNAs and to determine the mechanisms by which miRNAs regulate target mRNAs. We generated zebra fish embryos that lack the miRNA-processing enzyme Dicer. Mutant embryos are devoid of mature miRNAs and have morphogenesis defects, but differentiate multiple cell types. Injection of miR-430 miRNAs, a miRNA family expressed at the onset of zygotic transcription,

rescues the early morphogenesis defects in dicer mutants. miR-430 accelerates the decay of hundreds of maternal mRNAs and induces the deadenylation of target mRNAs. These studies suggest that miRNAs are not obligatory components of all fate specification or signaling pathways but facilitate developmental transitions and induce the deadenylation and decay of hundreds of target mRNAs.
Descriptors: zebrafish mutant embryos, microRNAs genetics and metabolism, base sequence, developmental gene expression regulation, morphogenesis genetics, mutation, RNA interference, ribonuclease III deficiency and genetics.

Schier, A.F. and W.S. Talbot (2005). **Molecular genetics of axis formation in zebrafish.** *Annual Review of Genetics* 39: 561-613. ISSN: 0066 4197.
NAL Call Number: QH431.A1A5
Abstract: The basic vertebrate body plan of the zebrafish embryo is established in the first 10 hours of development. This period is characterized by the formation of the anterior-posterior and dorsal-ventral axes, the development of the three germ layers, the specification of organ progenitors, and the complex morphogenetic movements of cells. During the past 10 years a combination of genetic, embryological, and molecular analyses has provided detailed insights into the mechanisms underlying this process. Maternal determinants control the expression of transcription factors and the location of signaling centers that pattern the blastula and gastrula. Bmp, Nodal, FGF, canonical Wnt, and retinoic acid signals generate positional information that leads to the restricted expression of transcription factors that control cell type specification. Noncanonical Wnt signaling is required for the morphogenetic movements during gastrulation. We review how the coordinated interplay of these molecules determines the fate and movement of embryonic cells.
Descriptors: zebrafish, body patterning, endoderm cytology and metabolism, gastrula cytology and metabolism, developmental gene expression regulation, mesoderm cytology and metabolism.

Schier, A. (2006). **Molecular genetics of zebrafish embryogenesis: from nodal signals to microRNAs**. *Federation of American Societies for Experimental Biology Journal* 20(5, Part 2): A1300. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: During early development, the embryo is faced with multiple challenges - the switch from maternally provided gene products to activating its own genome, the specification and patterning of tissue progenitors, and the rearrangement of cells to generate the embryonic axes. We have used genetics, molecular biology, embryology and in vivo imaging to address this problem in zebrafish. Our studies have revealed how the extracellular interplay of Nodal signals, EGF-CFC proteins and Lefty antagonists controls mesoderm and endoderm formation and left-right patterning in zebrafish. Nodal signals can act as morphogens and instruct cells to form mesoderm and endoderm and internalize; EGF-CFC proteins act as essential coreceptors in this process; and Lefty attenuates mesoderm and endoderm induction by blocking Nodal signaling. More recently, we have identified a microRNA that regulates the degradation of a large number of maternal mRNAs and facilitates the maternal to zygotic transition. I will discuss how the interplay of microRNAs and signaling molecules regulates early development. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, molecular genetics, embryogenesis.

Schild Prufert, K., M. Giegerich, M. Schafer, C. Winkler, and G. Krohne (2006). **Structural and functional characterization of the zebrafish lamin B receptor.** *European Journal of Cell Biology* 85(8): 813-24. ISSN: 0171 9335.
NAL Call Number: QH573.C83
Abstract: The lamin B receptor (LBR) is an integral membrane protein of the inner nuclear membrane that is interacting with B-type lamins, chromatin and DNA. The complete loss of the protein in mouse mutants causes a reduced viability of embryos, and viable animals develop abnormalities of the skeleton. Here, we present the molecular characterization of the zebrafish LBR (zLBR) gene and the functional analysis of LBR during zebrafish embryogenesis. We found that the coding region of the LBR mRNA of zebrafish as well as of mammals is contained in 13 exons. At the protein level, human and zebrafish LBR exhibit a high sequence identity (57% and higher) in 8 of the 13 exons. Knockdown of zLBR by microinjection of 0.5-1.0 mM morpholino antisense oligonucleotides (MO) into 1- to 2-cell stage embryos reduced the amount of endogenous zLBR protein to approximately 10-20%. The viability of MO-injected embryos within 24 h was reduced to 70-77%. Surviving 1-day-old embryos exhibited morphological alterations including reduced growth of head structures, retardation of tail growth and a bent backbone and tail. Expression analysis of the transcription factors no tail (ntl) and goosecoid (gsc) by in situ hybridization suggests that these malformations are caused by altered cell migration during gastrulation. Our data indicate that the LBR of zebrafish and mammals are both required for correct development.
Descriptors: zebrafish, cytoplasmic receptors and nuclear genetics, metabolism, amino acid and base sequence, complementary DNA chemistry and genetics, polyacrylamide gel electrophoresis, cytology, embryology, metabolism, exons genetics, developmental gene expression regulation, gene silencing, goosecoid protein genetics and metabolism, immunoblotting, introns genetics, molecular sequence data, mutation, cytoplasmic receptors and nuclear metabolism, DNA sequence analysis, amino acid sequence homology, T-box domain proteins genetics and metabolism.

Schirmer, K. (2005). **Can gene expression in embryos of zebrafish (*Danio rerio*) predict chronic toxicity?** *Canadian Technical Report of Fisheries and Aquatic Sciences* 2617: 72-73. ISSN: 0706-652X.
NAL Call Number: 442.9C16J
Descriptors: zebrafish, toxicology, pollution assessment control and management, molecular genetics, quantitative real time PCR, genetic techniques, ecotoxicology.

Schloss, P.D., B.R. Larget, and J.o. Handelsman (2004). **Integration of microbial ecology and statistics: a test to compare gene libraries**. *Applied and Environmental Microbiology* 70(9): 5485-5492. ISSN: 0099-2240.
NAL Call Number: 448.3 Ap5
Descriptors: zebrafish, community composition, computer programs, freshwater fish, gene expression, gene libraries, genes, identification, illustrations, intestinal microflora, libraries, microbiology, population ecology, soil microorganisms, statistics, rRNA 16S, *Danio rerio*.
Notes: A review.

Schlueter, P.J., G. Peng, M. Westerfield, and C. Duan (2007). **Insulin-like growth factor signaling regulates zebrafish embryonic growth and development by promoting cell survival and cell cycle progression.** *Cell Death and Differentiation* 14(6): 1095-105. ISSN: 1350 9047.
NAL Call Number: QH671
Abstract: Although much is known about the global effects of insulin-like growth factor 1

receptor (IGF1R)-mediated signaling on fetal growth and the clinical manifestations resulting from IGF/IGF1R deficiencies, we have an incomplete understanding of the cellular actions of this essential pathway during vertebrate embryogenesis. In this study, we inhibited IGF1R signaling during zebrafish embryogenesis using antisense morpholino oligonucleotides or a dominant-negative IGF1R fusion protein. IGF1R inhibition resulted in reduced embryonic growth, arrested development and increased lethality. IGF1R-deficient embryos had significant defects in the retina, inner ear, motoneurons and heart. No patterning abnormalities, however, were found in the brain or other embryonic tissues. At the cellular level, IGF1R inhibition increased caspase 3 activity and induced neuronal apoptosis. Coinjection of antiapoptotic bcl2-like mRNA attenuated the elevated apoptosis and rescued the retinal and motoneuron defects, but not the developmental arrest. Subsequent cell cycle analysis indicated an increased percentage of cells in G1 and a decreased percentage in S phase in IGF1R-deficient embryos independent of apoptosis. These results provide novel insight into the cellular basis of IGF1R function and show that IGF1R signaling does not function as an anteriorizing signal but regulates embryonic growth and development by promoting cell survival and cell cycle progression.

Descriptors: zebrafish, cell cycle physiology, IGF receptor type 1 physiology, apoptosis genetics and physiology, Western blotting, body patterning genetics and physiology, caspase 3 metabolism, cell cycle genetics, cell survival genetics and physiology, flow cytometry, developmental gene expression regulation drug effects, immunohistochemistry, immunoprecipitation, in situ hybridization, insulin like growth factor I genetics and physiology, neurons cytology and metabolism, antisense oligonucleotides administration and dosage and pharmacology, IGF receptor type 1 genetics, signal transduction drug effects, genetics and physiology.

Schlueter, P.J., T. Royer, M.H. Farah, B. Laser, S.J. Chan, D.F. Steiner, and C. Duan (2006). **Gene duplication and functional divergence of the zebrafish insulin-like growth factor 1 receptors.** *Federation of American Societies for Experimental Biology Journal* 20(8): 1230-2. ISSN: 0892 6638.

NAL Call Number: QH301.F3

Abstract: Insulin-like growth factor (IGF) 1 receptor (IGF1R)-mediated signaling plays key roles in growth, development, and physiology. Recent studies have shown that there are two distinct ig f1r genes in zebrafish, termed ig f1ra and ig f1rb. In this study, we tested the hypothesis that zebrafish ig f1ra and ig f1rb resulted from a gene duplication event at the ig f1r locus and that this has led to their functional divergence. The genomic structures of zebrafish ig f1ra and ig f1rb were determined and their loci mapped. While zebrafish ig f1ra has 21 exons and is located on linkage group (LG) 18, zebrafish ig f1rb has 22 exons and mapped to LG 7. There is a strong syntenic relationship between the two zebrafish genes and the human IG F1R gene. Using a MO-based loss-of-function approach, we show that both Igf1ra and Igf1rb are required for zebrafish embryo viability and proper growth and development. Although Igf1ra and Igf1rb demonstrated a large degree of functional overlap with regard to cell differentiation in the developing eye, inner ear, heart, and muscle, they also exhibited functional distinction involving a greater requirement for Igf1rb in spontaneous muscle contractility. These findings suggest that the duplicated zebrafish ig f1r genes play largely overlapping but not identical functional roles in early development and provide novel insight into the functional evolution of the IGF1R/insulin receptor gene family.

Descriptors: zebrafish, gene duplication, somatomedins genetics and physiology, cell survival, chromosome mapping, inner ear embryology, exons, eye embryology, gene targeting, heart embryology, introns, motor neurons cytology, muscle contraction, IGF receptor type 1

antagonists, inhibitors, genetics and physiology, signal transduction, proteins antagonists and inhibitors.

Schmidt, K.U. and J.M. Starck (2003). **Patterns of variability during early embryonic development of zebrafish, *Danio rerio.*** *Integrative and Comparative Biology* 43(6): 1003. ISSN: 1540 7063.
NAL Call Number: QL1.I67
Descriptors: zebrafish, population genetics, population studies, development, reproduction, embryogenesis, genetic variance.

Schneider, V.A. and M. Granato (2007). **Genomic structure and embryonic expression of zebrafish lysyl hydroxylase 1 and lysyl hydroxylase 2.** *Matrix Biology* 26(1): 12-9. ISSN: 0945-053X.
NAL Call Number: QP552.C6
Abstract: Collagen biosynthesis in both invertebrates and vertebrates is critically dependent upon the activity of lysyl hydroxylase (LH) enzymes. In humans, mutations in the genes encoding LH1 and LH2 have been shown to cause two distinct connective tissue disorders, Ehlers-Danlos (Type VIA) and Bruck syndromes. While the biochemical properties of these enzymes have been intensively studied, their embryonic patterns of expression and developmental roles remain unknown. We now present the cloning and analyses of the genes encoding LH1 and LH2 in the zebrafish, Danio rerio. We find these genes to be similarly organized to other vertebrate lh (plod) genes, including the presence of an alternatively spliced exon in lh2. We also examine the mRNA expression patterns of lh1 and lh2 during embryogenesis and find them to exhibit unique and dynamic patterns of expression. These results strongly suggest that LH enzymes are not merely housekeeping enzymes, but play distinct developmental roles. The identification of these genes in the zebrafish, a genetic model organism whose development is well characterized, now provides the basis for the establishment of the first animal models for both Ehlers-Danlos (Type VIA) and Bruck syndromes.
Descriptors: zebrafish, metabolism, procollagen lysine, 2 oxoglutarate 5 dioxygenase genetics, amino acid sequence, molecular cloning, embryology, enzymology, exons genetics, gastrula metabolism, developmental and enzymologic gene expression regulation, isoenzymes genetics, mesoderm metabolism, molecular sequence data, notochord metabolism, amino acid sequence homology, somites metabolism.

Schneider, V. and M. Granato (2006). **The myotomal diwanka (lh3) glycosyltransferase and type xviii collagen are critical for motor growth cone migration**. *Neuron* 50(5): 683-695. ISSN: 0896 6273.
Abstract: The initial migration of motor growth cones from the spinal cord into the periphery requires extrinsic cues, yet their identities are largely unknown. In zebrafish diwanka mutants, motor growth cones are motile but fail to pioneer into the periphery. Here, we report on the positional cloning of diwanka and show that it encodes LH3, a myotomally expressed multifunctional enzyme with lysyl hydroxylase and glycosyltransferase domains. Cloning, expression analysis, and ubiquitous overexpression of other LH family members reveals that only diwanka (lh3) possesses a critical role in growth cone migration. We show that this unique role depends critically on the LH3 glycosyltransferase domain, and provide compelling evidence that diwanka (W) acts through myotomal type XVIII collagen, a ligand for neural-receptor protein tyrosine phosphatases that guide motor axons. Together, our results provide the first genetic evidence that glycosyltransferase modifications of the ECM playa critical role during vertebrate motor axon migration. Copyright©Thomson Reuters 2009

Descriptors: zebrafish diwanka mutants, development, molecular genetics: biochemistry and molecular biophysics, nervous system: neural coordination, axonogenesis, motor growth cone migration.

Schoft, V.K., A.J. Beauvais, C. Lang, A. Gajewski, K. Prufert, C. Winkler, M.A. Akimenko, M. Paulin Levasseur, and G. Krohne (2003). **The lamina-associated polypeptide 2 (LAP2) isoforms beta, gamma and omega of zebrafish: developmental expression and behavior during the cell cycle.** *Journal of Cell Science* 116(Pt 12): 2505-17. ISSN: 0021-9533.
NAL Call Number: QH301.J6
Abstract: Zebrafish lamina-associated polypeptides 2 (ZLAP2) beta, gamma and omega have in common an N-terminal region with a LEM domain, and in the C-terminal half of the molecule a lamina binding domain and a membrane spanning sequence. The maternally synthesized omega is the largest isoform and the only LAP2 present in the rapidly dividing embryonic cells up to the gastrula stage. ZLAP2omega levels decrease during development, concomitant with the increase of the somatic isoforms ZLAP2beta and gamma. In somatic zebrafish cells ZLAP2gamma is the predominant isoform, whereas only small amounts of ZLAP2beta are present. During early embryonic development, ZLAP2omega becomes associated with mitotic chromosomes before anaphase. The surface of these chromosomes is decorated with vesicles, and each chromosome assembles its own nuclear envelope at the end of mitosis (karyomere formation). Ectopically expressed ZLAP2omega-green fluorescent protein (GFP) fusion protein targets vesicles to mitotic chromosomes in *Xenopus* A6 cells, suggesting that ZLAP2omega is involved in karyomere formation during early zebrafish development. When ZLAP2beta and gamma were expressed as GFP fusion proteins in *Xenopus* A6 cells, the beta- but not the gamma-isoform was found in association with mitotic chromosomes, and ZLAP2beta-containing chromosomes were decorated with vesicles. Further analysis of ZLAP2-GFP fusion proteins containing only distinct domains of the ZLAP2 isoforms revealed that the common N-terminal region in conjunction with beta- or omega-specific sequences mediate binding to mitotic chromosomes in vivo.
Descriptors: zebrafish, DNA binding proteins genetics, proteins metabolism, membrane proteins metabolism, thymopoietins metabolism, amino acid sequence genetics, base sequence genetics, binding sites genetics, cell cycle genetics, cell line, chromosomes genetics and ultrastructure, complementary DNA analysis and genetics, DNA binding proteins genetics, proteins genetics, isolation and purification, ultrastructure, developmental gene expression regulation genetics, membrane membrane proteins isolation and purification, electron microscopy, mitosis genetics, molecular sequence data, mutation genetics, nuclear envelope genetics and ultrastructure, protein isoforms genetics, isolation, purification and metabolism, tertiary protein structure genetics, recombinant fusion proteins metabolism, thymopoietins genetics, isolation and purification, *Xenopus laevis*, proteins isolation and purification.

Scholey, J.M. and K.V. Anderson (2006). **Intraflagellar transport and cilium-based signaling**. *Cell* 125(3): 439-442. ISSN: 0092 8674.
NAL Call Number: QH573.C42
Descriptors: zebrafish, molecular genetics, cell biology, polycystic kidney disease, urologic disease, genetic disease, congenital disease, genetics, obesity, metabolic disease, nutritional disease, retinal degeneration, eye disease, Bardet Biedl syndrome, endocrine disease, gonads, behavioral and mental disorders, polydactyly, bone disease, intraflagellar transport, planar cell polarity signaling.

Scholpp, S., C. Groth, C. Lohs, M. Lardelli, and M. Brand (2004). **Zebrafish fgfr1 is a member of the fgf8 synexpression group and is required for fgf8 signalling at the midbrain-hindbrain boundary.** *Development Genes and Evolution* 214(6): 285-95. ISSN: 0949 944X.
NAL Call Number: QL951.R68
Abstract: FGFR1 is an important signalling molecule during embryogenesis and in adulthood. FGFR1 mutations in human may lead to developmental defects and pathological conditions, including cancer and Alzheimer's disease. Here, we describe cloning and expression analysis of the zebrafish fibroblast growth factor receptor 1 (fgfr1). Initially, fgfr1 is expressed in the adaxial mesoderm with transcripts distinctly localised to the anterior portion of each half-somite. Hereupon, fgfr1 is also strongly expressed in the otic vesicles, branchial arches and the brain, especially at the midbrain-hindbrain boundary (MHB). The expression patterns of fgfr1 and fgf8 are strikingly similar and knock-down of fgfr1 phenocopies many aspects observed in the fgf8 mutant acerebellar, suggesting that Fgf8 exerts its function mainly by binding to FgfR1.
Descriptors: zebrafish, Fgf metabolism, receptor protein tyrosine kinases genetics, fibroblast receptors growth factor genetics, amino acid sequence, molecular cloning, molecular evolution, fibroblast growth factor 8 and genetics, mesencephalon embryology and metabolism, molecular sequence data, phenotype, phylogeny, receptor protein tyrosine kinases metabolism, type 1 fibroblast growth factor receptors, fibroblast receptors growth factor metabolism, rhombencephalon embryology and metabolism, amino acid sequence homology, signal transduction.

Scholpp, S. and M. Brand (2004). **Endocytosis controls spreading and effective signaling range of fgf8 protein**. *Current Biology* 14(20): 1834-1841. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Abstract: Secreted signaling molecules released from a restricted source are of great importance during embryonic development because they elicit induction, proliferation, differentiation, and patterning events in target cells (1, 2). Fgf8 is a member of the fibroblast growth factor family with key inductive functions during vertebrate development of, for example, the forebrain (3), midbrain (4), cerebellum (5), heart (6), inner ear (7), and mesoderm (8). However, the mechanism by which the signaling range of Fgf8 is controlled in a field of target cells is unknown. We studied Fgf8 as a potential morphogen in the nascent neuroectoderm of living zebrafish embryos. We find that spreading of epitope-tagged Fgf8 through target tissue is carefully controlled by endocytosis and subsequent degradation in lysosomes, or "restrictive clearance," from extracellular spaces. If internalization is inhibited, Fgf8 protein accumulates extracellularly, spreads further, and activates target gene expression over a greater distance. Conversely, enhanced internalization increases Fgf8 uptake and shortens its effective signaling range. Our results suggest that Fgf8 spreads extracellularly by a diffusion-based mechanism and demonstrate that target cells can actively influence, through endocytosis and subsequent degradation, the availability of Fgf8 ligand to other target cells.
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Descriptors: zebrafish, development, embryonic development, endocytosis, gene expression.

Schonberger, J., L. Wang, J.T. Shin, S.D. Kim, F.F.S. Depreux, H. Zhu, L. Zon, A. Pizard, J.B. Kim, C. Macrae, A.J. Mungall, J.G. Seidman, and C.E. Seidman (2005). **Mutation in the transcriptional coactivator Eya4 causes dilated cardiomyopathy and sensorineural hearing loss**. *Nature Genetics* 37(4): 418-422. ISSN: 1061 4036.
NAL Call Number: QH426.N37
Abstract: We identified a human mutation that causes dilated cardiomyopathy and heart

failure preceded by sensorineural hearing loss (SNHL). Unlike previously described mutations causing dilated cardiomyopathy that affect structural proteins, this mutation deletes 4,846 bp of the human transcriptional coactivator gene EYA4. To elucidate the roles of eya4 in heart function, we studied zebrafish embryos injected with antisense morpholino oligonucleotides. Attenuated eya4 transcript levels produced morphologic and hemodynamic features of heart failure. To determine why previously described mutated EYA4 alleles(1,2) cause SNHL without heart disease, we examined biochemical interactions of mutant Eya4 peptides. Eya4 peptides associated with SNHL, but not the shortened 193 amino acid peptide associated with dilated cardiomyopathy and SNHL, bound wild-type Eya4 and associated with Six proteins. These data define unrecognized and crucial roles for Eya4-Six-mediated transcriptional regulation in normal heart function. Copyright©Thomson Reuters 2009

Descriptors: zebrafish , cardiovascular system, transport and circulation, molecular genetics, sense organs, sensory reception, heart failure, heart disease, sensorineural hearing loss, ear disease, dilated cardiomyopathy, heart disease, genetics.

Schorpp, M., M. Bialecki, D. Diekhoff, B. Walderich, J. Odenthal, H.M. Maischein, A.G. Zapata, and T. Boehm (2006). **Conserved functions of Ikaros in vertebrate lymphocyte development: genetic evidence for distinct larval and adult phases of T cell development and two lineages of B cells in zebrafish.** *Journal of Immunology* 177(4): 2463-76. ISSN: 0022-1767.

NAL Call Number: 448.8J8232

Abstract: Zebrafish has been advocated as an alternative animal model to study lymphocyte development, although the similarities in the genetic requirements of lymphopoiesis between fish and mammals have not yet been investigated. In this study, we examine the role of the transcription factor Ikaros in zebrafish lymphopoiesis. In fish larvae homozygous for an ikaros allele predicted to lack the C-terminal zinc fingers, T lymphopoiesis is absent; the presence of V(H)DmuJmu rearrangements in adolescent fish is delayed in mutants. In adolescent mutant fish, T cells expressing tcrb and tcrd and B cells expressing igm are formed with low efficiency and display an oligoclonal Ag receptor repertoire. By contrast, B cells expressing the igz isotype do not develop, providing genetic evidence for two separate B cell lineages in zebrafish. Thus, Ikaros appears to play similar roles in fish and mammalian lymphopoiesis.

Descriptors: zebrafish larva, B lymphocytes immunology, cell differentiation genetics, cell lineage genetics, conserved sequence, Ikaros transcription factor physiology, T lymphocytes immunology, zebrafish immunology, alleles, amino acid sequence, B lymphocytes cytology, base sequence, cell differentiation immunology, cell lineage immunology, nonsense codon, Ikaros transcription factor genetics, molecular sequence data, phenotype, chemokine receptors genetics, T lymphocytes cytology.

Schredelseker, J., V. Di Biase, G.J. Obermair, E. Felder, B.E. Flucher, C. Franzini-Armstrong, and M. Grabner (2005). **The beta sub(1a) subunit is essential for the assembly of dihydropyridine-receptor arrays in skeletal muscle**. *Proceedings of the National Academy of Sciences, USA* 102(47): 17219-17224. ISSN: 0027-8424.

NAL Call Number: 500N21P

Descriptors: zebrafish, calcium, excitation-contraction coupling, freeze-fracture, freshwater fish, hatching, immunocytochemistry, muscles, mutations, myotubes, point mutation, PCR, receptors, ryanodine receptors, skeletal muscle, tetrads, Western blotting, dihydropyridine receptors, *Danio rerio.*

Schredelseker, J., B.E. Flucher, G. Kugler, and M. Grabner (2004). **Paralytic zebrafish mutant relaxed (redts25) with dysgenic phenotype has an intact DHP-receptor alpha1s subunit.** *Biophysical Journal* 86(1): 63a. ISSN: 0006 3495.

NAL Call Number: 442.8B5238

Abstract: A defect in the skeletal muscle DHP-receptor alpha1S subunit has been suggested to be the cause of paralysis in the zebrafish mutant relaxed (redts25) (Ono et al., J. Neurosci., 2001, 21(15), 5439). Here we tested this hypothesis by analyzing the sequence of the wild type and mutant zebrafish alpha1S subunit and by examining its expression and targeting properties in zebrafish muscle. Wild type zebrafish alpha1S cDNA was analyzed by RT-PCR sequencing. The ORF codes for 1847 amino acids with 66% sequence identity to the rabbit alpha1S. Surprisingly, RT-PCR sequencing of the entire coding region of red alpha1S revealed no mutation. This was confirmed by sequencing genomic DNA coding for the putative voltage-sensing S4 transmembrane segments. Furthermore, immunofluorescence analysis of alpha1S red mutant embryonic fish muscles showed that the alpha1S protein was expressed at levels indistinguishable from wild type muscle. Based on the characteristic staining pattern of alpha1S and the correspondence with the labeling pattern of the ryanodine receptor it appears that alpha1S is correctly targeted into the triads of the mutant muscle. Together these data indicate that the alpha1S subunit of the skeletal muscle DHP-receptor is not the primary defect of the red mutant. The molecular analysis of the auxiliary zebrafish skeletal muscle DHP-receptor subunits is currently underway. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, molecular biophysics, muscular system, reverse transcriptase PCR, dysgenic phenotype, excitation contraction coupling, gene mutations, paralysis, causes, paralytic mutant relaxed, effects, phenotypes.

Schrim, L.M. and J. Sprague (2004). **Data mining the zebrafish genome.** *Methods in Cell Biology* 77: 415-37. ISSN: 0091-679X.

NAL Call Number: 442P92

Descriptors: zebrafish, computational biology methods, genomics methods, genetic databases, genetic markers genetics, genome, information storage and retrieval.

Schulz, R.W., J. Bogerd, R. Male, J. Ball, M. Fenske, L.C. Olsen, and C.R. Tyler (2007). **Estrogen-induced alterations in amh and dmrt1 expression signal for disruption in male sexual development in the zebrafish.** *Environmental Science and Technology* 41(17): 6305-10. ISSN: 0013 936X.

NAL Call Number: TD420.A1E5

Abstract: Dmrt1 and amh are genes involved in vertebrate sex differentiation. In this study, we cloned dmrt1 and amh cDNAs in zebrafish (Danio rerio) and investigated the effects of exposure to 17a-ethinylestradiol (EE2), during early life on their patterns of expression and impact on the subsequent gonadal phenotype. Expression of both amh and dmrt1 in embryos was detected as early as at 1 day post fertilization (dpf) and enhanced expression of amh from 25 dpf was associated with the period of early gonadal differentiation. Sex-dependent differences in enhanced green fluorescent protein transgene expression driven by the promoter of the germ cell-specific vas gene were exploited to show that at 28dpf and 56dpf both amh and dmrt1 mRNA were overexpressed in males compared with females. Exposure during early life to environmentally relevant concentrations of EE2 had a suppressive effect on the expression of both amh and dmrt1 mRNAs and this was associated with a cessation/retardation in male gonadal sex development. Our findings indicate that estrogen-induced suppression in expression of dmrt1 and amh during early life correlate with subsequent dis-

ruptive effects on the sexual phenotype in males.
Descriptors: zebrafish, estrogen, alterations, amh, dmrt1, expression, signal, disruption, male sexual development.

Schutte, A., D. Lottaz, E.E. Sterchi, W. Stocker, and C. Becker Pauly (2007). **Two alpha subunits and one beta subunit of meprin zinc-endopeptidases are differentially expressed in the zebrafish *Danio rerio*.** *Journal of Biological Chemistry* 388(5): 523-31. ISSN: 0021 9258.
NAL Call Number: 381J824
Abstract: Meprins are members of the astacin family of metalloproteases expressed in epithelial tissues, intestinal leukocytes and certain cancer cells. In mammals, there are two homologous subunits, which form complex glycosylated disulfide-bonded homo- and heterooligomers. Both human meprin alpha and meprin beta cleave several basement membrane components, suggesting a role in epithelial differentiation and cell migration. There is also evidence that meprin beta is involved in immune defence owing to its capability of activating interleukin-1beta and the diminished mobility of intestinal leukocytes in meprin beta-knockout mice. Here we show for the first time by reverse transcription PCR, immunoblotting and immunofluorescence analyses that meprins are expressed not only in mammals, but also in the zebrafish *Danio rerio*. In contrast to the human, mouse and rat enzymes, zebrafish meprins are encoded by three genes, corresponding to two homologous alpha subunits and one beta subunit. Observations at both the mRNA and protein level indicate a broad distribution of meprins in zebrafish. However, there are strikingly different expression patterns of the three subunits, which is consistent with meprin expression in mammals. Hence, *D. rerio* appears to be a suitable model to gain insight into the basic physiological functions of meprin metalloproteases.
Descriptors: zebrafish , metalloendopeptidases metabolism, zinc metabolism, amino acid sequence, catalysis, chromosomes genetics, conserved sequence, gene expression regulation, metalloendopeptidases chemistry and genetics, fluorescence microscopy, molecular sequence data, phylogeny, tertiary protein structure, protein subunits chemistry, protein subunits genetics and metabolism, messenger RNA genetics, sequence alignment, structural protein homology.

Schweitzer, J., C.G. Becker, M. Schachner, and T. Becker (2005). **Expression of collapsin response mediator proteins in the nervous system of embryonic zebrafish.** *Gene Expression Patterns* 5(6): 809-16. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Collapsin response mediator proteins (CRMPs also known as TUC, Drp, Ulip, TOAD-64) are cytosolic phosphoproteins that are involved in signal transduction during axon growth and in cytoskeletal dynamics. Here we report cloning and mRNA expression patterns of CRMP-1, -2, -3, -4 and, owing to a genome duplication in teleosts, two homologs of CRMP-5 (CRMP-5a and -5b) in embryonic zebrafish at 16 and 24h post-fertilization (hpf). CRMPs are evolutionarily conserved and zebrafish CRMPs show amino acid identities of 76-90% with their homologs in humans, with the exception of CRMP-3, which shows only 67% homology. Between 16 and 24hpf, expression of CRMPs generally increased in many regions of the CNS undergoing neuronal differentiation and axonogenesis, but not in the proliferative ventricular zone. Structures that were typically labeled by most, but not all the CRMP probes were the telencephalon, the nucleus of the tract of the post-optic commissure, the epiphysis, the nucleus of the medial longitudinal fascicle, clusters of hindbrain neurons, cranial ganglia, as well as Rohon-Beard neurons. No expression of CRMP mRNAs was observed outside the nervous system. Thus, expression patterns of different CRMP

family members correlate with neuronal differentiation and axonogenesis in embryonic zebrafish.
Descriptors: zebrafish, physiology, developmental gene expression regulation, nervous system embryology, semaphorin 3a physiology, amino acid sequence, conserved sequence, mice, molecular sequence data, morphogenesis, nerve tissue phylogeny, messenger RNA genetics, sequence alignment, amino acid sequence homology.

Schweitzer, J., T. Becker, C.G. Becker, and M. Schachner (2003). **Expression of protein zero is increased in lesioned axon pathways in the central nervous system of adult zebrafish.** *Glia* 41(3): 301-17. ISSN: 0894-1491.
Descriptors: zebrafish, myelin P0 protein genetics, nerve regeneration physiology, oligodendroglia physiology, optic nerve physiology, spinal cord physiology, age factors, molecular cloning, developmental gene expression regulation, molecular sequence data, myelin P0 protein metabolism, myelin sheath physiology, nerve crush, nerve fibers, myelinated physiology, optic nerve cytology, messenger RNA analysis, rhombencephalon cytology and physiology, amino acid sequence homology, spinal cord cytology.

Schweitzer, J., D. Gimnopoulos, B.C. Lieberoth, H.M. Pogoda, J. Feldner, A. Ebert, M. Schachner, T. Becker, and C.G. Becker (2007). **Contactin 1a expression is associated with oligodendrocyte differentiation and axonal regeneration in the central nervous system of zebrafish.** *Molecular and Cellular Neuroscience* 35(2): 194-207. ISSN: 1044-7431.
Abstract: Contactin1a (Cntn1a) is a zebrafish homolog of contactin1 (F3/F11/contactin) in mammals, an immunoglobulin superfamily recognition molecule of neurons and oligodendrocytes. We describe conspicuous Cntn1a mRNA expression in oligodendrocytes in the developing optic pathway of zebrafish. In adults, this expression is only retained in glial cells in the intraretinal optic fiber layer, which contains 'loose' myelin. After optic nerve lesion, oligodendrocytes re-express Cntn1a mRNA independently of the presence of regenerating axons and retinal ganglion cells upregulate Cntn1a expression to levels that are significantly higher than those during development. After spinal cord lesion, expression of Cntn1a mRNA is similarly increased in axotomized brainstem neurons and white matter glial cells in the spinal cord. In addition, reduced mRNA expression in the trigeminal/anterior lateral line ganglion in erbb3-deficient mutant larvae implies Cntn1a in Schwann cell differentiation. These complex regulation patterns suggest roles for Cntn1a in myelinating cells and neurons particularly in successful CNS regeneration.
Descriptors: zebrafish, cell adhesion molecules, neuronal metabolism, cell differentiation physiology, central nervous system pathology, developmental gene expression regulation physiology, nerve regeneration physiology, oligodendroglia physiology, genetically modified, newborn, cell adhesion molecules, neuronal genetics, central nervous system physiopathology eye enucleation methods, in situ hybridization methods, electron transmission microscopymethods, myelin p0 protein metabolism, myelin associated glycoprotein metabolism, neurons physiology, neurons ultrastructure, oligodendroglia ultrastructure, optic nerve injuries pathology, optic nerve injuries physiopathology, messenger RNA genetics and metabolism, erbb 3 receptor genetics, spinal cord injuries pathology and physiopathology.

Scott, A. and D.L. Stemple (2005). **Zebrafish notochordal basement membrane: signaling and structure.** *Current Topics in Developmental Biology* 65: 229-53. ISSN: 0070 2153.
NAL Call Number: QL951.C8
Descriptors: zebrafish, basement membrane anatomy and histology, basement membrane metabolism, notochord embryology and ultrastructure, signal transduction physiology, cell

differentiation, coatomer protein genetics and metabolism, laminin genetics and metabolism, morphogenesis, mutation, cell surface receptors metabolism.

Scott, E.K., L. Mason, A.B. Arrenberg, L. Ziv, N.J. Gosse, T. Xiao, N.C. Chi, K. Asakawa, K. Kawakami, and H. Baier (2007). **Targeting neural circuitry in zebrafish using GAL4 enhancer trapping.** *Nature Methods* 4(4): 323-6. ISSN: 1548-7091.
NAL Call Number: QH315
Abstract: We present a pilot enhancer trap screen using GAL4 to drive expression of upstream activator sequence (UAS)-linked transgenes in expression patterns dictated by endogenous enhancers in zebrafish. The patterns presented include expression in small subsets of neurons throughout the larval brain, which in some cases persist into adult. Through targeted photoconversion of UAS-driven Kaede and variegated expression of UAS-driven GFP in single cells, we begin to characterize the cellular components of labeled circuits.
Descriptors: zebrafish, brain cytology, embryology and metabolism, enhancer elements genetics, developmental gene expression regulation, neurons metabolism, *Saccharomyces cerevisiae* proteins genetics and biosynthesis, transcription factors genetics, genetically modified, pilot projects transcription factors biosynthesis, transgenes.

Seeley, M., W. Huang, Z. Chen, W.O. Wolff, X. Lin, and X. Xu (2007). **Depletion of zebrafish titin reduces cardiac contractility by disrupting the assembly of Z-discs and A-bands.** *Circulation Research* 100(2): 238-45. ISSN: 0009 7330.
Abstract: The genetic study of titin has been notoriously difficult because of its size and complicated alternative splicing routes. Here, we have used zebrafish as an animal model to investigate the functions of individual titin isoforms. We identified 2 titin orthologs in zebrafish, ttna and ttnb, and annotated the full-length genomic sequences for both genes. We found that ttna, but not ttnb, is required for sarcomere assembly in the heart as well as the subsequent establishment of cardiac contractility. In fact, ttna is the earliest sarcomeric mRNA that is expressed in the heart, which makes it an early molecular marker for cardiomyocyte differentiation. Surprisingly, ttna is required for later steps of sarcomere assembly, including the assembly of Z-discs and A-bands, but not for early steps such as the assembly of Z-bodies and nonstriated myosin filaments. Reduction of individual titin isoforms in vivo using morpholino-modified antisense oligonucleotides indicated that (1) both N2B exon-containing and N2A exon-containing isoforms of ttna are required for sarcomere assembly in the heart; (2) N2A exon-containing isoforms of both ttna and ttnb are required for sarcomere assembly in the somites; and (3) the N2B exon-containing isoforms of ttnb are expressed later than other titin isoforms and are probably involved in modulating their expression; however, these isoforms of ttnb are not required for sarcomere assembly. Collectively, our results reveal distinct functions of different titin isoforms and suggest that various phenotypes in "titinopathies" may be attributable to the disruption of different titin isoforms.
Descriptors: zebrafish, muscle proteins deficiency and genetics, myocardial contraction genetics, protein kinases deficiency and genetics, protein processing, post translational genetics, proteins deficiency, alternative splicing genetics, muscle proteins biosynthesis and metabolism, myocardial contraction physiology, protein isoforms deficiency, genetics, metabolism and physiology, protein kinases biosynthesis and metabolism, sarcomeres metabolism.

Semon, M. and K.H. Wolfe (2007). **Reciprocal gene loss between *Tetraodon* and zebrafish after whole genome duplication in their ancestor.** *Trends in Genetics* 23(3): 108-12. ISSN: 0168-9525.

NAL Call Number: QH426.T744
Abstract: The whole genome duplication that occurred in ray-finned fish coincided with the radiation of teleost species; consequently, these two phenomena have often been linked. Using the Tetraodon and zebrafish complete genome sequences, we tested a molecular hypothesis that can relate whole genome duplication to speciation in teleosts. We estimate that thousands of genes that remained duplicated when *Tetraodon* and zebrafish diverged underwent reciprocal loss subsequently in these two species, probably contributing to reproductive isolation between them.
Descriptors: zebrafish, gene deletion and duplication, genome, Tetraodontiformes genetics.

Semon, M. and K.H. Wolfe (2007). **Rearrangement rate following the whole-genome duplication in teleosts**. *Molecular Biology and Evolution* 24(3): 860-867. ISSN: 0737-4038.
NAL Call Number: QH506.M642
Descriptors: zebrafish, chromosomes, diploids, enumeration, evolution, freshwater fish, gene deletion, genomes, genomic instability, meiosis, molecular biology, nucleotide sequence, phylogeny, polyploids, polyploidy, population genetics, gene rearrangement, genomics, *Danio rerio*, *Takifugu*, Teleostei, *Tetraodon*, swellfishes.

Seok, S.H., J.H. Park, M.W. Baek, H.Y. Lee, D.J. Kim, H.M. Uhm, J.J. Hong, Y.R. Na, B.H. Jin, D.Y. Ryu, and J.H. Park (2006). **Specific activation of the human HSP70 promoter by copper sulfate in mosaic transgenic zebrafish.** *Journal of Biotechnology* 126(3): 406-13. ISSN: 0168-1656.
NAL Call Number: QH442.J69
Abstract: Heat shock proteins (HSPs) play a central role in cell protection and repair upon stresses, such as that caused by heat and heavy metals. Copper sulfate inducibility of a pHhsp70 construct expressing the enhanced green fluorescent protein (EGFP) gene under the control of the exogenous human hsp70 promoter was tested in transfected CHSE 214 cells and transgenic zebrafish (*Danio rerio*). We developed a transient expression system, using mosaically transgenic zebrafish, which allows rapid analysis of transgenic expression. Transfected CHSE 214 cells which had been exposed to 250 nM and 2.5 microM copper sulfate for up to 24h showed increased EGFP expression in a dose-dependent manner. The 1.5 microM copper sulfate caused stronger EGFP fluorescence than the 1.0 microM copper sulfate in transgenic zebrafish. Most of the expression was spotty and was detected in the gills, dorsal and ventral retina, myotubes of the trunk, and skin epithelium. Transgenic zebrafish exposed to copper sulfate exhibited gross dysmorphogenesis, edema and trunk abnormalities, such as spinal lordosis, in vertebral development 5 days after fertilization. This transgenic zebrafish system was sensitive enough to detect copper sulfate at doses below the median lethal concentration (the LC50 was calculated to be 1.2 microM (95% confidence interval of 0.6-1.9 microM)). These results indicate that zebrafish could be useful transgenic biosensor systems for the detection of xenobiotic toxicants in the environment.
Descriptors: zebrafish, copper sulfate pharmacology, HSP70 heat shock proeins genetics and metabolism, promoter regions genetics physiology, genetically modified, cultured cells, gene expression regulation drug effects and physiology, mosaicism, promoter regions genetics drug effects.

Seok, S.-H., D. Park, J.-H. Park, S.-A. Cho, M.-W. Baek, H.-Y. Lee, D.-J. Kim, B. Jin, D.-Y. Ryu, and J.-H. Park (2004). **Beta -naphthoflavonecaused up-regulation of AhR regulated GFP in transgenic zebrafish.** *Experimental Animals* 53(5): 479-483. ISSN: 1341-1357.
NAL Call Number: QL55.J55

Descriptors: transgenic zebrafish, aryl hydrocarbon receptors, fertilization, green fluorescent protein, reporter gene, spine, vertebrae, beta -Naphthoflavone, *Danio rerio.*

Seppola, M., J. Steinvik, K. Steiro, T. Solstad, B. Robertsen, and I. Jensen (2007). **Sequence and expression analysis of an interferon stimulated gene (ISG15) from Atlantic cod (*Gadus morhua L.*)**. *Developmental and Comparative Immunology* 31(2): 156-171. ISSN: 0145 305X.

NAL Call Number: QR180.D4

Abstract: Suppression subtractive hybridization was used to examine gene expression in Atlantic cod head kidney cells treated with polyinosinic polycytidylic acid (poly I:C). One of the most abundant genes was ISG15, showing 24-53% amino acid similarity to ISG15 from both mammals and teleosts. The promoter was cloned by genome walking and three potential interferon-stimulated response elements (ISREs) were identified. Analysis of the gene structure revealed a single intron in the 5' untranslated region (UTR) of cod ISG15, which also seems to be present in zebrafish and pufferfish ISG15. A quantitative real time PCR assay was established to monitor the gene expression of cod ISG15. Injection of cod with poly I:C strongly induced the expression of ISG15 in all organs investigated. Stimulation was most pronounced the first day with a gradual decline the following days. The expression of ISG15 in head kidney cells was also induced in vitro by treatment with poly I:C, but not significantly with LPS. However, injection of formalin killed Vibrio anguillarum-induced ISG15 expression in head kidney. Copyright © 2008 CABI

Descriptors: zebrafish, DNA cloning, gene expression, genes, genomes, interferon, kidneys, nucleotide sequences, promoters, fishes, *Gadus morhua*, *Listonella anguillarum.*

Shah, R., O. Medina Martinez, L.F. Chu, R.C. Samaco, and M. Jamrich (2006). **Expression of FoxP2 during zebrafish development and in the adult brain.** *International Journal of Developmental Biology, The* 50(4): 435-8. ISSN: 0214-6282.

Abstract: Fox (forkhead) genes encode transcription factors that play important roles in the regulation of embryonic patterning as well as in tissue specific gene expression. Mutations in the human FOXP2 gene cause abnormal speech development. Here we report the structure and expression pattern of zebrafish FoxP2. In zebrafish, this gene is first expressed at the 20-somite stage in the presumptive telencephalon. At this stage there is a significant overlap of FoxP2 expression with the expression of the emx homeobox genes. However, in contrast to emx1, FoxP2 is not expressed in the pineal gland or in the pronephric duct. After 72 hours of development, the expression of zebrafish FoxP2 becomes more complex in the brain. The developing optic tectum becomes the major area of FoxP2 expression. In the adult brain, the highest concentrations of the FoxP2 transcript can be observed in the optic tectum. In the cerebellum, only the caudal lobes show high levels of Foxp2 expression. These regions correspond to the vestibulocerebellum of mammals. Several other regions of the brain also show high levels of Foxp2 expression.

Descriptors: zebrafish, brain metabolism, forkhead transcription factors genetics, amino acid sequence, forkhead transcription factors biosynthesis, molecular sequence data, messenger RNA metabolism, superior colliculus embryology and metabolism , telencephalon embryology and metabolism, proteins biosynthesis, optic tectum.

Shankaran, S.S., A. Capell, A.T. Hruscha, K. Fellerer, M. Neumann, B. Schmid, and C. Haass (2008). **Missense mutations in the progranulin gene linked to frontotemporal lobar degeneration with ubiquitin-immunoreactive Inclusions reduce progranulin production and secretion**. *Journal of Biological Chemistry* 283(3): 1744-1753. ISSN: 0021-9258.

NAL Call Number: 381J824
Descriptors: zebrafish, cytoplasm, frontotemporal dementia, missense mutation, nonsense mutation, nuclei, proteolysis, secretion, nonsense-mediated mRNA decay, *Danio rerio.*

Sharma, M.K., E.M. Denovan Wright, M.E. Boudreau , and J.M. Wright (2003). **A cellular retinoic acid-binding protein from zebrafish (*Danio rerio*): cDNA sequence, phylogenetic analysis, mRNA expression, and gene linkage mapping.** *Gene* 311: 119-28. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: We report the sequence of a cDNA clone coding for a cellular retinoic acid-binding protein (CRABP) in zebrafish. The encoded polypeptide is 142 amino acids in length with an estimated molecular mass of 15.8 kDa and a calculated isoelectric point of 5.2. The zebrafish CRABP exhibits highest sequence identity to the pufferfish CRABPIIa (83%) and CRABPIIb (79%), and human CRABPII (74%) than to any other member of the intracellular lipid-binding protein (ILBP) family. A phylogenetic tree for different members of the ILBP multigene family including fatty acid-binding proteins (FABPs), cellular retinol-binding proteins (CRBPs) and CRABPs shows that the cloned zebrafish cDNA encodes a protein that clusters with CRABPs from other species and not with CRBPs and FABPs. Reverse-transcription polymerase chain reactions (RT-PCR), using oligonucleotide primers specific to the zebrafish CRABP cDNA made from total RNA of embryos collected at various developmental stages, did not detect the CRABP mRNA until 12 h post-fertilization. In adult zebrafish, CRABP mRNA was detected by RT-PCR in total RNA extracted from muscle, testes and skin, barely detectable in heart, ovary and brain and undetectable in liver, kidney and intestine. Quantitative RT-PCR (qRT-PCR) revealed a similar tissue-specific distribution for zebrafish CRABP mRNA with highest levels of CRABP mRNA in muscle followed by testes, skin, ovary and much lower levels in heart. Radiation hybrid mapping assigned the CRABP gene to linkage group 16 in the zebrafish genome. Comparison of the mapped zebrafish CRABP and human CRABPII genes revealed that zebrafish linkage group 16 has a syntenic relationship with human chromosome 1. Based on phylogenetic analysis and the syntenic relationship to the CRABPII gene in human, the zebrafish cDNA clone appears to code for a type II CRABP.
Descriptors: zebrafish, retinoic acid receptors genetics, amino acid and base sequence, Northern blotting, chromosome mapping, human chromosomes pair 1 genetics, complementary DNA chemistry and genetics, embryonic development, gene expression, developmental gene expression regulation, molecular sequence data, phylogeny, messenger RNA genetics and metabolism, radiation hybrid mapping, DNA sequence analysis, amino acid sequence homology, synteny.

Sharma, M.K., R.Z. Liu, C. Thisse, B. Thisse, E.M. Denovan Wright, and J.M. Wright (2006). **Hierarchical subfunctionalization of fabp1a, fabp1b and fabp10 tissue-specific expression may account for retention of these duplicated genes in the zebrafish (*Danio rerio*) genome.** *Federation of European Biochemical Societies Journal* 273(14): 3216-29. ISSN: 1742 464X.
NAL Call Number: QP501.E8
Abstract: Fatty acid-binding protein type 1 (FABP1), commonly termed liver-type fatty acid-binding protein (L-FABP), is encoded by a single gene in mammals. We cloned and sequenced cDNAs for two distinct FABP1s in zebrafish coded by genes designated fabp1a and fabp1b. The zebrafish proteins, FABP1a and FABP1b, show highest sequence identity and similarity to the human protein FABP1. Zebrafish fabp1a and fabp1b genes were assigned to linkage groups 5 and 8, respectively. Both linkage groups show conserved synte-

nies to a segment of mouse chromosome 6, rat chromosome 4 and human chromosome 2 harboring the FABP1 locus. Phylogenetic analysis further suggests that zebrafish fabp1a and fabp1b genes are orthologs of mammalian FABP1 and most likely arose by a whole-genome duplication event in the ray-finned fish lineage, estimated to have occurred 200-450 million years ago. The paralogous fabp10 gene encoding basic L-FABP, found to date in only non-mammalian vertebrates, was assigned to zebrafish linkage group 16. RT-PCR amplification of mRNA in adults, and in situ hybridization to whole-mount embryos to fabp1a, fabp1b and fapb10 mRNAs, revealed a distinct and differential pattern of expression for the fabp1a, fabp1b and fabp10 genes in zebrafish, suggesting a division of function for these orthogolous and paralogous gene products following their duplication in the vertebrate genome. The differential and complementary expression patterns of the zebrafish fabp1a, fapb1b and fabp10 genes imply a hierarchical subfunctionalization that may account for the retention of both the duplicated fabp1a and fabp1b genes, and the fabp10 gene in the zebrafish genome.
Descriptors: zebrafish, fatty acid binding proteins metabolism, developmental gene expression regulation, duplicate genes, genome, proteins metabolism, amino acid sequence, amino acid substitution, base sequence, initiator codon, terminator codon, conserved sequence, complementary DNA exons, fatty acid binding proteins chemistry, fatty acid binding ligands, linkage genetics, molecular sequence data, open reading frames, phylogeny, secondary protein structure, messenger RNA metabolism, radiation hybrid mapping, amino acid sequence homology, variation genetics.

Sharma, M.K., V. Saxena, R.Z. Liu, C. Thisse, B. Thisse, E.M. Denovan Wright, and J.M. Wright (2005). **Differential expression of the duplicated cellular retinoic acid-binding protein 2 genes (crabp2a and crabp2b) during zebrafish embryonic development.** *Gene Expression Patterns* 5(3): 371-9. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: The cellular retinoic acid-binding protein 2 (CRABP2) is believed to be involved in regulating access of retinoic acid to nuclear retinoic acid receptors. We have determined the cDNA sequence and the genomic organization of the duplicated crabp2 gene (crabp2b) in zebrafish. The crabp2b cDNA was 522bp in length and encodes a polypeptide consisting of 146 amino acids. Radiation hybrid mapping assigned the crabp2b gene to zebrafish linkage group 19. The comparison of the mapped human CRABP2 gene, zebrafish crabp2a and zebrafish crabp2b genes revealed that human chromosome 1 has a syntenic relationship to zebrafish linkage groups 16 and 19. Reverse transcription-polymerase chain reaction (RT-PCR) detected crabp2b mRNA in total RNA extracted from whole adult zebrafish, but not in any of the adult zebrafish tissues examined. The crabp2a mRNA was detected in total RNA extracted from whole adult zebrafish, adult zebrafish muscle, testes, and skin and to a lesser extent in heart, ovary and brain. No crabp2a mRNA-specific product was detected in kidney, liver or intestine of the adult zebrafish. Whole mount in situ hybridization detected crabp2b and crabp2a mRNA in a number of structures known to require retinoic acid signaling during embryonic development. The crabp2b mRNA was detected in the central nervous system, branchial arches, pectoral fins, retina (dorsal to the lens), epidermis and otic vesicle of the developing zebrafish. The crabp2a transcripts were detected by whole mount in situ hybridization in the central nervous system, epidermis, proliferative zone of the retina, intestinal bulb, esophagus, pectoral fins and branchial arches during zebrafish embryonic development.
Descriptors: zebrafish embryos, retinoic acid receptors biosynthesis, amino acid and base sequence, complementary DNA chemistry, gene duplication and expression, in situ hybrid-

ization, linkage genetics, molecular sequence data, retinoic acid receptors genetics, synteny, tissue distribution.

Shaw, S.Y., T.A. Peterson, D.J. Milan, T.P. Zong, C. Macrae, S.L. Schreiber, M.C. Fishman, and R.T. Peterson (2003). **Discovery of a novel pro-vasculogenic small molecule via a chemical genetic screen for suppressors of zebrafish gridlock mutants.** *Circulation* 108(17 Supplement): IV-33. ISSN: 0009 7322.
NAL Call Number: RC681.A1C8
Descriptors: zebrafish, cardiovascular system, transport and circulation, development, molecular genetics, vasculogenesis.

Shawber, C.J. and J. Kitajewski (2004). **Notch function in the vasculature: insights from zebrafish, mouse and man.** *BioEssays* 26(3): 225-34. ISSN: 0265 9247.
NAL Call Number: QH506.B365
Descriptors: zebrafish, blood vessels physiology, membrane proteins metabolism, signal transduction physiology, blood vessels pathology, blood vessels ultrastructure, calcium binding proteins, congenital heart defects genetics, congenital heart defects metabolism, homeostasis, intercellular signaling peptides and proteins, kidney abnormalities, ligands, membrane mice, physiologic neovascularization physiology, Notch receptors.

Shchedrina, V.A., S.V. Novoselov, M.Y. Malinouski, and V.N. Gladyshev (2007). **Identification and characterization of a selenoprotein family containing a diselenide bond in a redox motif**. *Proceedings of the National Academy of Sciences, USA* 104(35): 13919-13924 . ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, aquatic organisms , codons, cytosol, DNA, freshwater fish, insertion sequences, mammalian cells, oxidoreductases, phylogenetics, proteins, redox reactions, selenocysteine, thioredoxin, translation, oxidoreductase, selenoproteins, *Danio rerio*, genetics and evolution, biochemistry.

Shen, C.H. and L.A. Steiner (2004). **Genome structure and thymic expression of an endogenous retrovirus in zebrafish.** *Journal of Virology* 78(2): 899-911. ISSN: 0022-538X.
NAL Call Number: QR360.J6
Abstract: In a search for previously unknown genes that are required for lymphocyte development in zebrafish, a retroviral sequence was identified in a subtracted thymus cDNA library and in genomic DNA libraries. The provirus is 11.2 kb and contains intact open reading frames for the gag, pol, and env genes, as well as nearly identical flanking long terminal repeat sequences. As determined by in situ hybridization, the thymus appears to be a major tissue for retroviral expression in both larval and adult fish. Several viral transcripts were found by Northern blotting in the adult thymus. The provirus was found at the same genomic locus in sperm from four fish, suggesting that it is an endogenous retrovirus. Phylogenetic analysis indicates that it is closest to, yet distinct from, the cluster of murine leukemia virus-related retroviruses, suggesting that this virus represents a new group of retroviruses.
Descriptors: zebrafish, endogenous retroviruses genetics, viral genome, thymus gland virology, zebrafish virology, molecular cloning, endogenous retroviruses isolation and purification, gene library, genomic library, molecular sequence data, phylogeny, proviruses genetics, isolation and purification, viral RNAgenetics and metabolism, DNA sequence analysis, viral proteins metabolism.

Shentu, H., H.J. Wen, G.M. Her, C.J. Huang, J.L. Wu , and S.P. Hwang (2003). **Proximal upstream region of zebrafish bone morphogenetic protein 4 promoter directs heart expression of green fluorescent protein.** *Genesis* 37(3): 103-12. ISSN: 1526-954X.
NAL Call Number: QH426.D32
Descriptors: zebrafish embryos, bone morphogenetic developmental gene expression regulation, luminescent proteins biosynthesis, promoter regions genetics, embryonic development, green fluorescent proteins, heart physiology, indicators and reagents analysis, luminescent proteins analysis.

Shepard, J.L., J.F. Amatruda, H.M. Stern, A. Subramanian, D. Finkelstein, J. Ziai, K.R. Finley, K.L. Pfaff, C. Hersey, Y. Zhou, B. Barut, M. Freedman, C. Lee, J. Spitsbergen, D. Neuberg, G. Weber, T.R. Golub, J.N. Glickman, J.L. Kutok, J.C. Aster, and L.I. Zon (2005). **A zebrafish bmyb mutation causes genome instability and increased cancer susceptibility.** *Proceedings of the National Academy of Sciences of the United States of America* 102(37): 13194-9. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: A major goal of cancer research has been to identify genes that contribute to cancer formation. The similar pathology between zebrafish and human tumors, as well as the past success of large-scale genetic screens in uncovering human disease genes, makes zebrafish an ideal system in which to find such new genes. Here, we show that a zebrafish forward genetic screen uncovered multiple cell proliferation mutants including one mutant, crash&burn (crb), that represents a loss-of-function mutation in bmyb, a transcriptional regulator and member of a putative proto-oncogene family. crb mutant embryos have defects in mitotic progression and spindle formation, and exhibit genome instability. Regulation of cyclin B levels by bmyb appears to be the mechanism of mitotic accumulation in crb. Carcinogenesis studies reveal increased cancer susceptibility in adult crb heterozygotes. Gene-expression signatures associated with loss of bmyb in zebrafish are also correlated with conserved signatures in human tumor samples, and down-regulation of the B-myb signature genes is associated with retention of p53 function. Our findings show that zebrafish screens can uncover cancer pathways, and demonstrate that loss of function of bmyb is associated with cancer.
Descriptors: zebrafish, genomic instability, mutation, neoplasms genetics, proto oncogene proteins C myb genetics, cyclin B metabolism, embryos, genetic predisposition to disease, mitosis, mitotic spindle apparatus, neoplasms etiology, tumor suppressor protein p53.

Sherwood, N.M., B.A. Adams, E.R. Isaac, and S. Wu (2005). **Knocked down and out: pacap in development, reproduction and feeding**. *Regulatory Peptides* 130(3): 142. ISSN: 0167 0115.
NAL Call Number: QP552.P4
Descriptors: zebrafish, nutrition, development, molecular genetics, reproduction, feeding , reproduction.

Shestopalov, I.A., S. Sinha, and J.K. Chen (2007). **Light-controlled gene silencing in zebrafish embryos.** *Nature Chemical Biology* 3(10): 650-1. ISSN: 1552-4450.
Abstract: Functional genomic studies in zebrafish frequently use synthetic oligonucleotides called morpholinos that block RNA splicing or translation. However, the constitutive activity of these reagents limits their experimental utility. We report here the synthesis of a photoactivatable morpholino targeting the no tail (ntl) gene. This caged reagent permits spatiotemporal gene regulation in vivo and the photochemical generation of functionally mosaic

organisms.
Descriptors: zebrafish embryos, radiation effects, developmental gene expression regulation radiation effects, gene silencing radiation effects, light, RNA splicing drug effects, body patterning, physiology, developmental gene expression regulation physiology, gene silencing physiology, glycoproteins biosynthesis and chemistry, translational glycopeptide chain initiation, RNA splicing physiology, ultraviolet rays.

Shi, K., L. Zhang, and A. Meng (2004). **Cloning and expression analysis of zebrafish tob1 gene.** *Development Genes and Evolution* 214(6): 309-11 . ISSN: 0949 944X.
NAL Call Number: QL951.R68
Abstract: Many members of the Btg/Tob family, which possess anti-proliferation activity, have been identified in mammals but their physiological functions are poorly understood. We have identified a zebrafish tob1 gene, which encodes a peptide of 322 amino acids. The expression pattern of zebrafish tob1 during early embryogenesis is examined by RT-PCR and whole-mount in situ hybridization. The maternal tob1 transcript is abundant in fertilized eggs and blastulas. The expression of tob1 ceases during midgastrulation and early segmentation, when it is expressed and restricted to the notochord and somites. On day 2, expression occurs in the pectoral fin bud but not in other tissues. It will be interesting to investigate whether Tob1 is required for controlling cell proliferation during vertebrate embryogenesis.
Descriptors: zebrafish, molecular cloning, amino acid sequence, conserved sequence, gene expression profiling, in situ hybridization, molecular sequence data, sequence alignment, amino acid sequence homology.

Shields, C.R., J. Klooster, Y. Claassen, M. Kamermans, G. Zoidl, and R. Dermietzel (2005). **Horizontal cell-specific expression of connexin 52.6 & Connexin 55.5 In the zebrafish retina**. *Investigative Ophthalmology & Visual Science* 46(Suppl. S): 599. ISSN: 0146 0404.
Abstract: Purpose: Connexin (Cx) hemichannels and gap junctions in retinal horizontal cells are believed to play a vital role in visual information processing. Because it is uncertain which of the many subtypes of zebrafish Cx's are involved inthis processing, we investigated the distribution of two Cx's believed to be retina-specific, Cx52.6 and Cx55.5. Methods: We performed promoter expression and immunohistochemical studies to investigate the distribution Cx52.6 and Cx55.5. Constructs containing either the Cx55.5 or Cx52.6 promoters and an EGFP reporter were injected into one to two cell stage zebrafish embryos, which weresubsequently examined for EGFP expression by fluorescence, confocal, and immuno-electron microscopy. Additionally, we generated antibodies against Cx52.6 and studied its labeling of the retina alone and in combination with other cell-specific markers. Results: Confocal fluorescence and immuno-electron microscopy studies revealed that both the Cx52.6 and Cx55.5 promoters drove EGFP expression in retinal horizontal cells, but not in any other cell types in the zebrafish. Immunohistochemical studies using two distinct antibodies directed against Cx52.6 confirmed that horizontal cells, but not other retinal neurons, express this Cx subtype. Light microscopy showed Cx52.6 staining in the outer plexiform layer (OPL), but not in other synaptic or cellular layers of the retina. Double labeling for Cx52.6 and tyrosine hydroxylase (TH), the G protein Go-alpha, the ionotropic glutamate receptor GluR2, the GABA C receptor rho subunit, or protein kinase C (PKC) suggested that Cx52.6 protein was confined to horizontal cells. Pre-embedding immuno-electron microscopy confirmed the specific expression of Cx52.6 in horizontal cells. Furthermore, electron microscopy demonstrated the presence of Cx52.6-containing hemichannels in horizontal cell spinules and lateral dendritic elements and of Cx52.6-containing gap junctions between horizontal cells. Conclusions: Our results provide clear evidence that Cx52.6 and Cx55.5 are expressed spe-

cifically in zebrafish retinal horizontal cells. Furthermore, our data indicate that Cx52.6 is the Cx that forms gap junctions between horizontal cells. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, biochemistry and molecular biophysics, sense organs, light microscopy, connexin, imaging and microscopy techniques.

Shih, L.J., Y.F. Lu, Y.H. Chen, C.C. Lin, J.A. Chen, and S.P. Hwang (2007). **Characterization of the agr2 gene, a homologue of X. laevis anterior gradient 2, from the zebrafish, *Danio rerio*.** *Gene Expression Patterns* 7(4): 452-60. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: We characterized a zebrafish (*Danio rerio*) anterior gradient 2 homologue (agr2) gene. agr2 contains an open reading frame of 513bp encoding 171 amino acids. Deduced amino acid sequence comparison showed that the zebrafish agr2 protein shares high (80-89%) amino acid sequence similarity with those homologues of anterior gradient 2 (HAGR2, MAgr2, Tagr2, and Sagr2) from the human, mouse, pufferfish, and Atlantic salmon, while sharing less (67-71%) sequence similarity with those anterior gradient 2 genes (XAG-2, XAG-1, XAgr2, MAgr3, and HAGR3) from *Xenopus laevis*, mouse, and human. Both phylogenetic and syntenic analyses indicate that zebrafish agr2 is the orthologue of human AGR2 and mouse Agr2 genes. Whole-mount in situ hybridization indicated that zebrafish agr2 is expressed in most organs, such as epidermis, olfactory bulbs, otic vesicles, pharynx, esophagus, pneumatic duct, swim bladder, and intestine, which contain mucus-secreting cells. Moreover, semi-quantitative RT-PCR demonstrated agr2 is expressed in the gill, pharynx/esophagus, swim bladder/pneumatic duct, and intestine in the adult fish. In contrast, *Xenopus* anterior gradient 2 homologues are mainly expressed in ectoderm-derived organs including the cement gland and otic vesicles, while human and mouse anterior gradient 2 orthologues are mainly distributed in endoderm-derived organs including the trachea, lungs, stomach, intestines, and colon.
Descriptors: zebrafish, amino acid and base sequence, metabolism, gene expression, molecular sequence data, phylogeny, sequence alignment, amino acid sequence homology, synteny.

Shike, H., C. Shimizu, X. Lauth, and J.C. Burns (2004). **Organization and expression analysis of the zebrafish hepcidin gene, an antimicrobial peptide gene conserved among vertebrates.** *Developmental and Comparative Immunology* 28(7-8): 747-54. ISSN: 0145 305X.
NAL Call Number: QR180.D4
Abstract: Hepcidin is an antimicrobial peptide and iron-regulatory molecule that is conserved among vertebrates. Mutations or over-expression of the human hepcidin gene have been found in patients with hemochromatosis and refractory anemia. To further understand the function and regulation of hepcidin, animal models are needed. We sequenced cDNA, genes and upstream regions of zebrafish hepcidin and analyzed gene expression by kinetic PCR. Zebrafish hepcidin genes consist of two introns and three exons that encode a prepropeptide (91 amino acids). The amino acid sequences and gene organization were remarkably conserved between zebrafish and other species. Elevated gene expression was observed in abdominal organs, skin, and heart in fish that developed signs of infection following bacterial injection. Zebrafish may be a suitable model organism for further study of hepcidin gene regulation.
Descriptors: zebrafish , antimicrobial cationic peptides genetics , complementary DNA genetics, exons and introns genetics, amino acid sequence, iron metabolism, molecular sequence data.

Shimada, N. and S.J. Moorman (2005). **Effects of microgravity on hsp 70 gene expression in developing lens**. *Federation of American Societies for Experimental Biology Journal* 19(4, Suppl. S, Part 1): A802. ISSN: 0892 6638.

NAL Call Number: QH301.F3

Abstract: Gravity has been an important physical factor for the evolution and development of living organisms on Earth. Using a bioreactor NASA designed for cells in culture, we have been studying effects of simulated-microgravity on gene expression in transgenic zebrafish embryos expressing GFP under the influence of gene-specific promoters. This allows us to visualize the target gene's expression and development in live embryos. We selected Heat shock protein 70 (hsp70) encoding a molecular chaperon because hsp70 functions as an indicator of various physical stress responses. We placed eggs from transgenic zebrafish having the gfp gene under the control of hsp70 promoter in the bioreactor at differentdevelopmental stages for defined developmental periods. There was a significant increase in GFP expression in lens (45%) compared with controls when hsp70:gfp embryos were incubated in the bioreactor during a time period that included the lens differentiation stage (32 to 80 hours post-fertilization at 20 degrees C). This increase was lens specific since the whole embryo showed only a 4% increase in GFP expression. We also compared the expression level of hsp70 mRNA by both northern blot and whole mount in situ hybridization. The results were similar to those for hsp70:gfp expression, indicating that the combinationof bioreactor and transgenic fish is a powerful system for analyzing the effect of microgravity on gene expression. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryo, development, sense organs, sensory reception, microgravity , heat shock protein 70, lens.

Shimizu, T., Y.K. Bae, O. Muraoka, and M. Hibi (2005). **Interaction of Wnt and caudal-related genes in zebrafish posterior body formation.** *Developmental Biology* 279(1): 125-41. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: Although Wnt signaling plays an important role in body patterning during early vertebrate embryogenesis, the mechanisms by which Wnts control the individual processes of body patterning are largely unknown. In zebrafish, wnt3a and wnt8 are expressed in overlapping domains in the blastoderm margin and later in the tailbud. The combined inhibition of Wnt3a and Wnt8 by antisense morpholino oligonucleotides led to anteriorization of the neuroectoderm, expansion of the dorsal organizer, and loss of the posterior body structure-a more severe phenotype than with inhibition of each Wnt alone-indicating a redundant role for Wnt3a and Wnt8. The ventrally expressed homeobox genes vox, vent, and ved mediated Wnt3a/Wnt8 signaling to restrict the organizer domain. Of posterior body-formation genes, expression of the caudal-related cdx1a and cdx4/kugelig, but not bmps or cyclops, was strongly reduced in the wnt3a/wnt8 morphant embryos. Like the wnt3a/wnt8 morphant embryos, cdx1a/cdx4 morphant embryos displayed complete loss of the tail structure, suggesting that Cdx1a and Cdx4 mediate Wnt-dependent posterior body formation. We also found that cdx1a and cdx4 expression is dependent on Fgf signaling. hoxa9a and hoxb7a expression was down-regulated in the wnt3a/wnt8 and cdx1a/cdx4 morphant embryos, and in embryos with defects in Fgf signaling. Fgf signaling was required for Cdx-mediated hoxa9a expression. Both the wnt3a/wnt8 and cdx1a/cdx4 morphant embryos failed to promote somitogenesis during mid-segmentation. These data indicate that the cdx genes mediate Wnt signaling and play essential roles in the morphogenesis of the posterior body in zebrafish.

Descriptors: zebrafish embryos, developmental gene expression regulation, homeodomain

intercellular signaling peptides and transcription factors genetics, body patterning, physiology, morphogenesis, Wnt proteins.

Shimomura, S., T. Nagamine, T. Nimura, N. Sueyoshi, Y. Shigeri, and I. Kameshita (2007). **Expression, characterization, and gene knockdown of zebrafish doublecortin-like protein kinase.** *Archives of Biochemistry and Biophysics* 463(2): 218-30. ISSN: 0003 9861.
NAL Call Number: 381 Ar2
Abstract: Doublecortin-like protein kinase (DCLK) is a protein Ser/Thr kinase expressed in brain and believed to play crucial roles in neuronal development. To investigate the biological significance of DCLK, we isolated cDNA clones for zebrafish DCLK (zDCLK) and found that there were five splice variants of the kinase. In this study, the catalytic properties of a major isoform of zDCLK, which we designated as zDCLK1, and of an N-terminal truncated mutant retaining the kinase domain were examined by expressing them in *Escherichia coli*. Mutational analysis of recombinant zDCLK suggested that the kinase was activated not only by phosphorylation at Thr-576 in the activation loop but also by autophosphorylation at the other site(s) in the catalytic domain. zDCLK significantly phosphorylated protein substrates such as myelin basic protein, histones, and synapsin I. Subcellular localization of zDCLK and its N-terminal deletion mutant implicated that microtubule-association of zDCLK is mediated through N-terminal doublecortin like domain of this enzyme. Western blotting analysis and whole mount in situ hybridization revealed that zDCLK was highly expressed in brain and eyes after 24-h post fertilization. Gene knockdown of zDCLK using morpholino-based antisense oligonucleotides induced significant increase of apoptotic cells in the central nervous systems and resulted in the increase of the morphologically abnormal embryos in a dose-dependent manner. These results suggest that zDCLK may play crucial roles in the central nervous systems during the early stage of embryogenesis.
Descriptors: mutant zebrafish, amino acid sequence, catalysis, central nervous system embryology, molecular cloning, gene expression, molecular sequence data, protein serine threonine kinases physiology, analysis and genetics , sequence alignment, tissue distribution, metabolism.

Shin, J., H.C. Park, J.M. Topczewska, D.J. Mawdsley, and B. Appel (2003). **Neural cell fate analysis in zebrafish using olig2 BAC transgenics.** *Methods in Cell Science* 25(1-2): 7-14. ISSN: 1381-5741.
NAL Call Number: QH573.T52
Abstract: We describe here simple methods for producing transgenic zebrafish reporter lines using BAC clones. The use of BAC clones facilitates creation of useful transgenics as the large amounts of genomic DNA they contain increase the likelihood that reporter gene expression will be properly regulated. Combined with recent advances in live embryo image analysis, this strategy has the potential to greatly advance the investigation of neural cell behavior during development.
Descriptors: zebrafish, developmental gene expression regulation physiology, motor neurons metabolism, peripheral nervous system metabolism, Purkinje cells metabolism, molecular cloning, cytology, metabolism, reporter genetic vectors, green fluorescent proteins, luminescent proteins metabolism, microinjections, motor neurons cytology, peripheral nervous system cytology, Purkinje cells cytology.

Shin, J.T., J.R. Priest, I. Ovcharenko, A. Ronco, R.K. Moore, C.G. Burns, and C.A. MacRae (2005). **Human-zebrafish non-coding conserved elements act in vivo to regulate transcription.** *Nucleic Acids Research* 33(17): 5437-45. ISSN: 0305-1048.

NAL Call Number: QD341.A2N8

Abstract: Whole genome comparisons of distantly related species effectively predict biologically important sequences--core genes and cis-acting regulatory elements (REs)--but require experimentation to verify biological activity. To examine the efficacy of comparative genomics in identification of active REs from anonymous, non-coding (NC) sequences, we generated a novel alignment of the human and draft zebrafish genomes, and contrasted this set to existing human and fugu datasets. We tested the transcriptional regulatory potential of candidate sequences using two in vivo assays. Strict selection of non-genic elements which are deeply conserved in vertebrate evolution identifies 1744 core vertebrate REs in human and two fish genomes. We tested 16 elements in vivo for cis-acting gene regulatory properties using zebrafish transient transgenesis and found that 10 (63%) strongly modulate tissue-specific expression of a green fluorescent protein reporter vector. We also report a novel quantitative enhancer assay with potential for increased throughput based on normalized luciferase activity in vivo. This complementary system identified 11 (69%; including 9 of 10 GFP-confirmed elements) with cis-acting function. Together, these data support the utility of comparative genomics of distantly related vertebrate species to identify REs and provide a scaleable, in vivo quantitative assay to define functional activity of candidate REs.

Descriptors: zebrafish, gene expression regulation, human genone, response elements, genetically modified, base sequence, conserved sequence, metabolism, genomics, luciferases, firefly analysis, luciferases, firefly genetics, luciferases, renilla analysis and genetics, luminescent agents, genetic transcription.

Shiomi, K., H. Uchida, K. Keino Masu, and M. Masu (2003). **Ccd1, a novel protein with a DIX domain, is a positive regulator in the Wnt signaling during zebrafish neural patterning.** *Current Biology* 13(1): 73-7. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: Wnt signaling plays a crucial role in directing cell differentiation, polarity, and growth. In the canonical pathway, Wnt receptors activate Dishevelled (Dvl), which then blocks the degradation of a key signal transducer, beta-catenin, leading to the nuclear accumulation of beta-catenin and induction of Wnt target genes through TCF/LEF family transcription factors. Here we identified a novel zebrafish gene encoding Ccd1, which possesses a DIX (Dishevelled-Axin) domain. DIX domains are essential for the signal transduction of two major Wnt downstream mediators, Dvl and Axin. Ccd1 formed homomeric and heteromeric complexes with Dvl and Axin and activated TCF-dependent transcription in vitro. In addition, overexpression of ccd1 in zebrafish embryos led to a reduction in the size of the eyes and forebrain (posteriorization), as seen with wnt8 overexpression, whereas a dominant-negative ccd1 (DN-ccd1) caused the opposite phenotype. Furthermore, the Wnt activation phenotype induced by ccd1 was inhibited by the expression of axin1 or DN-ccd1, and the wnt8 overexpression phenotype was rescued by DN-ccd1, suggesting that Ccd1 functions downstream of the Wnt receptor and upstream of Axin. These results indicate that Ccd1 is a novel positive regulator in this Wnt signaling pathway during zebrafish neural patterning.

Descriptors: zebrafish, body patterning physiology, nervous system embryology, repressor proteins, signal transduction, proteins metabolism, amino acid sequence, binding sites, cytoskeletal proteins, DNA binding proteins genetics, proteins metabolism eye abnormalities genetics, eye proteins, developmental gene expression regulation, homeodomain lymphoid enhancer bindingfactor 1, molecular sequence data, nervous system metabolism, paired box transcription factors, tertiary protein structure, protein serine threonine kinases genetics,

proto oncogene proteins C myc genetics and metabolism, amino acid sequence homology, transcription factors metabolism, transfection, Wnt proteins.

Shirak, A., E. Seroussi, N. Zilberman, G. Hulata, M. Ron, A. Cnaani, and T.D. Kocher (2006). **Genetic basis of sex determination in fishes: Searching for master key regulator genes in the sex determination pathway of tilapias**. *Israeli Journal of Aquaculture/Bamidgeh [Isr. J. Aquacult./Bamidgeh]. Vol. 58, No. 4, P. 350. Dec 2006.* 58(4): 350. ISSN: 0792-156X.
NAL Call Number: SH117.I75B36
Descriptors: zebrafish, aromatase, DNA, databases, differentiation, exons, fish culture, freshwater fish, gene mapping, genes, genotypes, gonads, quantitative trait loci, sex determination, expressed sequence tags, cichlidae, *Danio rerio* , pisces, tilapia, African mouthbrooders, cichlids, fish culture, development and cell cycle.

Shkumatava, A. and C.J. Neumann (2005). **Shh directs cell-cycle exit by activating p57Kip2 in the zebrafish retina.** *European Molecular Biology Organization Journal Reports* 6(6): 563-9. ISSN: 1469 221X.
NAL Call Number: QH506 .E463
Abstract: The Hedgehog (Hh) family of signalling proteins control both differentiation and proliferation during animal development. Previous studies have shown that Hh signalling has a stimulatory effect on the cell cycle in several organs by controlling core cell-cycle components. Here, we show that Sonic hedgehog (Shh) signalling has the opposite effect in the zebrafish retina, where it leads to cell-cycle exit, and that this is mediated by transcriptional activation of the cyclin kinase inhibitor p57Kip2. The loss of p57Kip2 activity strongly resembles the Shh mutant eye phenotype, and overexpression of p57Kip2 rescues cell-cycle exit in Shh mutants, indicating that p57Kip2 is both necessary and sufficient to mediate Shh-induced cell-cycle exit in the retina. These findings raise the possibility that stimulation of cell-cycle exit through regulation of core cell-cycle components may be part of a general mechanism required for Hh-directed differentiation.
Descriptors: mutant zebrafish, developmental gene expression regulation, nuclear proteins metabolism, retina metabolism, signal transduction physiology, trans activators metabolism, bromodeoxyuridine, cell cycle, cell differentiation physiology, cyclin dependent kinase inhibitor p57, genetic vectors, green fluorescent proteins, Hedgehog proteins, in situ hybridization and nick end labeling, microinjections, antisense oligonucleotides, retina embryology.

Shmukler, B.E., C.E. Kurschat, G.E. Ackermann, L.W. Jiang, Y. Zhou, B. Barut, A.K. Stuart Tilley, J.H. Zhao, L.I. Zon, I.A. Drummond, D.H. Vandorpe, B.H. Paw, and S.L. Alper (2005). **Zebrafish slc4a2/ae2 anion exchanger: cDNA cloning, mapping, functional characterization, and localization**. *American Journal of Physiology* 289(4(2)): F835-F849. ISSN: 0002 9513.
NAL Call Number: 447.8Am3
Abstract: Although the zebrafish has been used increasingly for the study of pronephric kidney development, studies of renal ion transporters and channels of the zebrafish remain few. We report the cDNA cloning and characterization of the AE2 anion exchanger ortholog from zebrafish kidney, slc4a2/ae2. The ae2 gene in linkage group 2 encodes a polypeptide of 1,228 aa exhibiting 64% aa identity with mouse AE2a. The exon-intron boundaries of the zebrafish ae2 gene are nearly identical to those of the rodent and human genes. Whole-mount in situ hybridization detects ae2 mRNA in prospective midbrain as early as the five-somite stage, then later in the pronephric primordia and the forming pronephric duct, where it persists through 72 h postfertilization (hpf). Zebrafish Ae2 expressed in *Xenopus*

laevis oocytes mediates Na+-independent, electroneutral 36Cl-/Cl- exchange moderately sensitive to inhibition by DIDS, is inhibited by acidic intracellular pH and by acidic extracellular pH, but activated by (acidifying) ammonium and by hypertonicity. Zebrafish Ae2 also mediates Cl-/HCO3- exchange in *X. laevis* oocytes and accumulates in or near the plasma membrane in transfected HEK-293 cells. In 24-48 hpf zebrafish embryos, the predominant but not exclusive localization of Ae2 polypeptide is the apical membrane of pronephric duct epithelial cells. Thus Ae2 resembles its mammalian orthologs in function, mechanism, and acute regulation but differs in its preferentially apical expression in kidney. These results will inform tests of the role of Ae2 in zebrafish kidney development and function. Copyright © 2008 CABI

Descriptors: zebrafish, biological development, DNA cloning, genes, kidneys, localization, polypeptides, *Danio rerio.*

Sidi, S., R.W. Friedrich, and T. Nicolson (2003). **NompC TRP channel required for vertebrate sensory hair cell mechanotransduction**. *Science* 301(5629): 96-99. ISSN: 0036-8075.

NAL Call Number: 470Sci2

Descriptors: zebrafish, audition, auditory organs, balance organs, deafness, endocytosis, fish larvae, hair cells, mechanoreceptors, mechanotransduction, sense organs, transduction, *Danio rerio*, Nompc protein, TRP protein, hair cells, mechanotransduction channel, NompC TMP channel, physiology, biochemistry, biophysics, developmental genetics, auditory and vestibular systems (including echolocation).

Sie, R.J., J.Y. Chen, J.L. Wu, W.D. Huang, and C.M. Kuo (2005). **Application RNAi Technology for Inhibition zebrafish FSH and LH Gene Expression and their Promoter**. *Journal of the Fisheries Society of Taiwan* 32(1): 10. ISSN: 0379-4180.

NAL Call Number: SH135.T3

Descriptors: zebrafish, cages, double-stranded RNA, fish culture, follicle-stimulating hormone, freshwater fish, gene expression, hormones, inhibitors, population genetics, post-transcription, promoters, RNA-mediated interference, specificity, sterility, transgenic fish, Danio rerio, genetics and evolution, Taiwan.

Sieger, D., D. Tautz, and M. Gajewski (2004). **Her11 is involved in the somitogenesis clock in zebrafish.** *Development Genes and Evolution* 214(8): 393-406. ISSN: 0949 944X.

NAL Call Number: QL951.R68

Abstract: Somitogenesis requires an intricate process of pre-patterning, which is driven by an oscillator mechanism consisting of the Delta-Notch pathway and hairy- (h) and Enhancer of split- [E(spl)] related genes. With the aim of unravelling the complex mechanism of somite pre-patterning, we have conducted an extensive search for h/E(spl)-related genes in the third release of the *Danio rerio* genomic sequence. We identified 14 new h/E(spl) genes and analysed them by in situ hybridisation for their potential role in the somitogenesis process. We describe here the functional analysis of one of these genes, which we have named her11. her11 is a paralogue of her1 and, similar to her1, is arranged in a head to head fashion with another her gene, namely the previously described her5. It shares an expression in the midbrain-hindbrain boundary with her5, but is in addition cyclically expressed in patterns overlapping those of her1 and her7 and complementary to those of hey1. Furthermore it is expressed in the anterior half of the most caudally formed somites. We show that Delta-Notch pathway genes and fused somites (fss) are necessary for the control of her11 expression. However, some aspects of the her11 regulation suggest that at least one additional as yet unknown gene of the Delta-Notch cascade is required to explain its expression.

Morpholino-oligonucleotide-mediated knockdown of her11 shows that it is involved in the zebrafish somitogenesis clock via an interaction with her1 and her7. We have also studied the role of hey1 by morpholino injection, but could not find a direct function for this gene, suggesting that it reflects the output of the clock rather than being a core component of the mechanism.

Descriptors: zebrafish, biological clocks physiology, developmental gene expression regulation, morphogenesis genetics, phylogeny, somites physiology, transcription factors genetics, amino acid and base sequence, basic helix loop helix transcription factors, biological clocks genetics, brain metabolism, cluster analysis, computational biology, DNA primers, gene duplication, helix loop helix motifs, in situ hybridization, molecular sequence data, sequence alignment, DNA sequence analysis, transcription factors metabolism.

Siekmann, A.F. and N.D. Lawson (2007). **Notch signalling limits angiogenic cell behaviour in developing zebrafish arteries.** *Nature* 445(7129): 781-4. ISSN: 0028-0836.

NAL Call Number: 472 N21

Abstract: Recent evidence indicates that growing blood-vessel sprouts consist of endothelial cells with distinct cell fates and behaviours; however, it is not clear what signals determine these sprout cell characteristics. Here we show that Notch signalling is necessary to restrict angiogenic cell behaviour to tip cells in developing segmental arteries in the zebrafish embryo. In the absence of the Notch signalling component Rbpsuh (recombining binding protein suppressor of hairless) we observed excessive sprouting of segmental arteries, whereas Notch activation suppresses angiogenesis. Through mosaic analysis we find that cells lacking Rbpsuh preferentially localize to the terminal position in developing sprouts. In contrast, cells in which Notch signalling has been activated are excluded from the tip-cell position. In vivo time-lapse analysis reveals that endothelial tip cells undergo a stereotypical pattern of proliferation and migration during sprouting. In the absence of Notch, nearly all sprouting endothelial cells exhibit tip-cell behaviour, leading to excessive numbers of cells within segmental arteries. Furthermore, we find that flt4 (fms-related tyrosine kinase 4, also called vegfr3) is expressed in segmental artery tip cells and becomes ectopically expressed throughout the sprout in the absence of Notch. Loss of flt4 can partially restore normal endothelial cell number in Rbpsuh-deficient segmental arteries. Finally, loss of the Notch ligand dll4 (delta-like 4) also leads to an increased number of endothelial cells within segmental arteries. Together, these studies indicate that proper specification of cell identity, position and behaviour in a developing blood-vessel sprout is required for normal angiogenesis, and implicate the Notch signalling pathway in this process.

Descriptors: zebrafish, arteries cytology and embryology, physiologic neovascularization physiology, Notch receptors metabolism, signal transduction, cell lineage, vascular endothelium cytology and metabolism, developmental gene expression regulation, immunoglobulin j recombination signal sequence binding protein deficiency, genetics and metabolism, vascular endothelial growth factor receptor 3 metabolism.

Simoes, B., N. Conceicao, C.S. Viegas, J.P. Pinto, P.J. Gavaia, L.D. Hurst, R.N. Kelsh, and M.L. Cancela (2006). **Identification of a promoter element within the zebrafish colXalpha1 gene responsive to runx2 isoforms Osf2/Cbfa1 and til-1 but not to pebp2alphaA2.** *Calcified Tissue International* 79(4): 230-44. ISSN: 0171 967X.

NAL Call Number: QP88.C3

Abstract: Type X collagen is a short chain collagen specifically expressed by hypertrophic chondrocytes during endochondral ossification. We report here the functional analysis of the zebrafish (*Danio rerio*) collagen Xalpha1 gene (colXalpha1) promoter with the identifica-

tion of a region responsive to two isoforms of the runt domain transcription factor runx2. Furthermore, we provide evidence for the presence of dual promoter usage in zebrafish, a finding that should be important to further understanding of the regulation of its restricted tissue distribution and spatial-temporal expression during early development. The zebrafish colXalpha1 gene structure is comparable to that recently identified by comparative genomics in takifugu and shows homology with corresponding mammalian genes, indicating that its general architecture has been maintained throughout vertebrate evolution. Our data suggest that, as in mammals, runx2 plays a role in the development of the osteogenic lineage, supporting zebrafish as a model for studies of bone and cartilage development.
Descriptors: zebrafish, bone development genetics, collagen type X genetics, core bindingfactor alpha 1 subunit metabolism , developmental gene expression regulation , protein isoforms metabolism, amino acid and base sequence, molecular cloning, gene expression, gene expression profiling , in situ hybridization, molecular sequence data, phylogeny, promoter regions genetics , messenger RNA analysis, reverse transcriptase PCR, sequence homology, trans activation genetics.

Sison, M., J. Cawker, C. Buske, and R. Gerlai (2006). **Fishing for genes influencing vertebrate behavior: zebrafish making headway.** *Lab Animal* 35(5): 33-9. ISSN: 0093-7375.
Abstract: The zebrafish (*Danio rerio*) has been a favorite model of developmental biologists and geneticists, but only recently have investigators begun to appreciate its usefulness in behavior genetics. Papers focusing on the behavior or brain function of this species were once extremely rare, but during the past decade rapid growth has taken place. Despite the increased interest, however, the number of studies devoted to the analysis of the behavior of this species is still orders of magnitude less than those conducted on more traditional laboratory subjects including the rat and the mouse. The authors review selected literature and demonstrate that zebrafish is an excellent subject for behavior genetics research, especially in the area of forward genetics (mutagenesis).
Descriptors: zebrafish, behavior physiology, behavioral genetics, models, mutagenesis.

Sivasubbu, S., D. Balciunas, A. Amsterdam, and S.C. Ekker (2007). **Insertional mutagenesis strategies in zebrafish.** *Genome Biology* 8(Suppl 1): S9. ISSN: 1465-6906.
NAL Call Number: QH431.G452
Abstract: We review here some recent developments in the field of insertional mutagenesis in zebrafish. We highlight the advantages and limitations of the rich body of retroviral methodologies, and we focus on the mechanisms and concepts of new transposon-based mutagenesis approaches under development, including prospects for conditional 'gene trapping' and 'gene breaking' approaches.
Descriptors: zebrafish, insertional mutagenesis methods, DNA transposable elements, embryonic stem cells metabolism, Retroviridae, zebrafish virology.
Notes: A review.

Slatter, C.A., H. Kanji, C.A. Coutts, and D.W. Ali (2005). **Expression of PKC in the developing zebrafish, *Danio rerio*.** *Journal of Neurobiology* 62(4): 425-38. ISSN: 0022-3034.
NAL Call Number: QP351.J55
Descriptors: zebrafish, developmental gene expression regulation physiology, protein kinase C biosynthesis, metabolism, immunohistochemistry, larva enzymology and growth and development, protein kinase C alpha.

Slusarski, D.C. and T. Westfall (2004). **Wnt/calcium functions as a negative regulator of beta-catenin activity**. *Federation of American Societies for Experimental Biology Journal* 18(4-5): Abst. 67.3. ISSN: 0892 6638.

NAL Call Number: QH301.F3

Abstract: Progression from egg to embryo requires a dynamic interaction of signal transduction networks. We combine cellular, molecular and genetic analysis with high-resolution calcium imaging to investigate how signaling networks influence embryonic patterning. Inhibition of endogenous Ca2+ modulation induces hyper-dorsalized phenotypes in the zebrafish (*Danio rerio*). We provide genetic evidence defining a role for Non-canonical Wnt function in vertebrate axis formation. Wnt-4, -5 and -11 stimulate Ca2+ release, defining the Wnt/Ca2+ class. We describe genetic interaction between two Wnt/Ca2+ members, Wnt-5 (pipetail) and Wnt-11, and a reduced Ca2+ release in pipetail. Embryos depleted of Wnt-5 exhibit cell movement and dorsalized phenotypes. Ectopic dorsal-signaling centers are generated as demonstrated by altered Beta-catenin localization and concomitant expression of Beta-catenin target genes. Wnt-5 activity is antagonistic to Beta-catenin signaling. We also investigate overlap between noncanonical Wnt signaling pathways. Control of epithelial planar cell polarity (PCP) involves a group of genes including frizzled, dishevelled, prickle and strabismus and we determine if these components also signal via Ca2+ modulation. Our long-range goal is to unravel the signaling networks initiated by the Wnt/ Ca2+ class. We also investigate the regulation of downstream signaling components such as G-proteins and Ca2+-sensing proteins. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, biochemistry, cell biology, Wnt, calcium, beta-catenin activity.

Smear, M.C., H.W. Tao, W. Staub, M.B. Orger, N.J. Gosse, Y. Liu, K. Takahashi, M.M. Poo, and H. Baier (2007). **Vesicular glutamate transport at a central synapse limits the acuity of visual perception in zebrafish.** *Neuron* 53(1): 65-77 . ISSN: 0896-6273.

Abstract: The neural circuitry that constrains visual acuity in the CNS has not been experimentally identified. We show here that zebrafish blumenkohl (blu) mutants are impaired in resolving rapid movements and fine spatial detail. The blu gene encodes a vesicular glutamate transporter expressed by retinal ganglion cells. Mutant retinotectal synapses release less glutamate, per vesicle and per terminal, and fatigue more quickly than wild-type in response to high-frequency stimulation. In addition, mutant axons arborize more extensively, thus increasing the number of synaptic terminals and effectively normalizing the combined input to postsynaptic cells in the tectum. This presumably homeostatic response results in larger receptive fields of tectal cells and a degradation of the retinotopic map. As predicted, mutants have a selective deficit in the capture of small prey objects, a behavior dependent on the tectum. Our studies successfully link the disruption of a synaptic protein to complex changes in neural circuitry and behavior.

Descriptors: zebrafish, presynaptic terminals metabolism, retinal ganglion cells metabolism, synaptic transmission genetics, vesicular glutamate transport protein 2 metabolism, vision disorders genetics, developmental gene expression regulation genetics, glutamic acid metabolism, mutation genetics, predatory behavior physiology, presynaptic terminals ultrastructure, retinal ganglion cells ultrastructure, superior colliculus abnormalities, metabolism and physiopathology, vesicular glutamate transport protein 2 genetics, vision genetics, vision disorders metabolism and physiopathology, visual pathways abnormalities, visual pathways metabolism and physiopathology.

Smith, A.A., K. Wyatt, J. Vacha, T.S. Vihtelic, J.S.J. Zigler, G.J. Wistow, and M. Posner (2006). **Gene duplication and separation of functions in alphaB-crystallin from zebrafish (*Danio rerio*).** *Federation of European Biochemical Societies Journal* 273(3): 481-90. ISSN: 1742 464X.

NAL Call Number: QP501.E8

Abstract: We previously reported that zebrafish alphaB-crystallin is not constitutively expressed in nervous or muscular tissue and has reduced chaperone-like activity compared with its human ortholog. Here we characterize the tissue expression pattern and chaperone-like activity of a second zebrafish alphaB-crystallin. Expressed sequence tag analysis of adult zebrafish lens revealed the presence of a novel alpha-crystallin transcript designated cryab2 and the resulting protein alphaB2-crystallin. The deduced protein sequence was 58.2% and 50.3% identical with human alphaB-crystallin and zebrafish alphaB1-crystallin, respectively. RT-PCR showed that alphaB2-crystallin is expressed predominantly in lens but, reminiscent of mammalian alphaB-crystallin, also has lower constitutive expression in heart, brain, skeletal muscle and liver. The chaperone-like activity of purified recombinant alphaB2 protein was assayed by measuring its ability to prevent the chemically induced aggregation of alpha-lactalbumin and lysozyme. At 25 degrees C and 30 degrees C, zebrafish alphaB2 showed greater chaperone-like activity than human alphaB-crystallin, and at 35 degrees C and 40 degrees C, the human protein provided greater protection against aggregation. 2D gel electrophoresis indicated that alphaB2-crystallin makes up approximately 0.16% of total zebrafish lens protein. Zebrafish is the first species known to express two different alphaB-crystallins. Differences in primary structure, expression and chaperone-like activity suggest that the two zebrafish alphaB-crystallins perform divergent physiological roles. After gene duplication, zebrafish alphaB2 maintained the widespread protective role also found in mammalian alphaB-crystallin, while zebrafish alphaB1 adopted a more restricted, nonchaperone role in the lens. Gene duplication may have allowed these functions to separate, providing a unique model for studying structure-function relationships and the regulation of tissue-specific expression patterns.

Descriptors: zebrafish, alternative splicing, gene duplication, alpha crystallin B chain genetics and physiology, amino acid sequence, molecular cloning, two dimensional gel electrophoresis, polyacrylamide gel electrophoresis, gene expression regulation, molecular sequence data, phylogeny, reverse transcriptase PCR, sequence alignment, temperature, time factors.

Smith, T.H., K. Stedronsky, B. Morgan, and R.A. McGowan (2006). **Identification and isolation of a BTB-POZ-containing gene expressed in oocytes and early embryos of the zebrafish *Danio rerio*.** *Genome* 49(7): 808-14. ISSN: 0831-2796.

NAL Call Number: QH431.G452

Abstract: In this report, we describe the cloning of a cDNA from the zebrafish *Danio rerio* encoding a protein containing a BTB-POZ domain closely resembling the BTBD1 and BTBD2 proteins previously identified in mammals. However, unlike other BTB-POZ-containing genes, expression of this gene in adults is most abundant in oocytes, where the RNA can be detected at all stages of oogenesis examined. The presence of the RNA persists through early cleavage, but is decreased significantly by gastrulation. Although the function of this gene has yet to be determined, its resemblance to the BTB-POZ family of genes coupled with its expression pattern suggests that it may have an important function in oogenesis and (or) early zebrafish development.

Descriptors: zebrafish, oocytes metabolism, proteins isolation and purification, amino acid sequence, carrier molecular cloning, complementary DNA isolation and purification, DNA

binding proteins genetics, metabolism, molecular sequence data, oogenesis, organ specificity, tertiary protein structure, RNA biosynthesis, transcription factors genetics.

Smolen, G.A., B.J. Schott, R.A. Stewart, S. Diederichs, B. Muir, H.L. Provencher, A. Look, D.C. Sgroi, R.T. Peterson, and D.A. Haber (2007). **A Rap GTPase interactor, RADIL, mediates migration of neural crest precursors**. *Genes & Development* 21(17): 2131-2136. ISSN: 0890-9369.
NAL Call Number: QH426.G46
Descriptors: zebrafish, cartilage, cell adhesion, cell migration, developmental stages, embryogenesis, embryonic development, enteric nervous system, freshwater fish, guanosinetriphosphatase, neural crest, neural stem cells, neurons, pigments, *Danio rerio*, development and cell cycle, reproduction and development.

Smyth, V. and B.N. Kennedy (2005). **Identification of an enhancer within the 5 ' flanking region of the conetransducin a-subunit**. *Investigative Ophthalmology & Visual Science* 46(Suppl. S): 3070. ISSN: 0146 0404.
Abstract: Purpose: The alpha-subunit of cone transducin (T alpha C) is expressed exclusively in cone photoreceptors both in the eye and pinealocytes in the pineal. Talpha C specificity makes it an ideal tool to identify cis-elements driving cone specific expression. This study examines the cis-elements responsible for the cell specific expression pattern of T alpha C in vivo. Methods: To identify cis-elements responsible for driving T alpha C expression, a series of deletions of the 5 ' flanking region of the zebrafish T alpha C (zT alpha CP) genewere constructed upstream of the enhanced green fluorescent protein (EGFP) and tested in vivo. A transient assay was carried out by injecting these zT alpha CP-EGFP deletion reporter constructs, into one to two cell stage zebrafish embryos. Injected embryos were anaesthetised and analyzed by fluorescence microscopy at 5 days post fertilization (dpf). The zT alpha CP deletion constructs activity was measured by counting the number of GFP cells within embryos eyes at 5 dpf. Zebrafish embryos with greater than 50 GFP cells in the eye were scored as (+++), fish with 5-50 cells expressing GFP were scored as (++), 1-5 fluorescent cells as (+) and fish with no GFP were scored as (-). Results: A 1.2 Kb 5 ' flanking region of zT alpha CP was initially identified as driving strong EGFP expression in cone photoreceptors. Deletion analysis of the 5 ' flanking region refined this to a 200 base pair (bp) region and eventually to a 50 bp region. Significant loss of GFP expression is observed when this 50 bp enhancer region is deleted. Conclusions: A 0.05 Kb region of the zebrafish T alpha C 5 ' flanking region acts as an enhancer. Progress in characterising this enhancer by electrophoretic mobility shift assay (EMSA) and the enhancer's ability to drive heterologous promoters will also be presented. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics, sense organs, sensory reception.

Snekser, J.L., S.P. McRobert, C.E. Murphy, and E.D. Clotfelter (2006). **Aggregation behavior in wildtype and transgenic zebrafish**. *Ethology* 112(2): 181-187. ISSN: 0179 1613.
NAL Call Number: QL750.E74
Abstract: Recent advances in the development and availability of genetically modified animals enable researchers to examine the effects of phenotypic characters on social behavior. In fish, shoaling behavior is known to be influenced by characteristics such as body coloration, striping pattern, body shape, and size. GloFishTM are genetically engineered zebrafish (Danio rerio) that express red fluorescent protein (RFP), resulting in on overall red coloration under the dark longitudinal stripes. The GloFish pattern is distinct from the light body coloration underlying the dark longitudinal stripes seen in wildtype zebrafish. We presented

wildtype and transgenic RFP zebrafish with same-sex shoals of both strains of fish in dichotomous choice tests. No preference for either of the shoals was shown, however, both strains showed significant preferences for swimming near shoals vs. swimming near an empty tank compartment. When presented with opposite-sex individuals of both strains, no preference was shown by either sex of either strain. Thus, the red body coloration of transgenic zebrafish does not appear to affect choice of social partner, in either a shoaling or a potentially reproductive context. Copyright © 2008 CABI

Descriptors: zebrafish, aggregation, behavior, mating behavior, pigmentation, reproductive behavior, sex differences, transgenics, *Danio rerio.*

So, W.K., H.F. Kwok, and W. Ge (2005). **Zebrafish gonadotropins and their receptors: II. Cloning and characterization of Zebrafish follicle-stimulating hormone and luteinizing hormone subunits--their spatial-temporal expression patterns and receptor specificity.** *Biology of Reproduction* 72(6): 1382-96. ISSN: 0006 3363.

NAL Call Number: QD415.F4

Abstract: Gonadotropins, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) play critical roles in vertebrate reproduction. In the present study, we cloned and characterized zebrafish FSHbeta (fshb), LHbeta (lhb), and GTHalpha (cga) subunits. Compared with the molecules of other teleosts, the cysteine residues and potential glycosylation sites are fully conserved in zebrafish Lhb and Cga but not in Fshb, whose cysteines exhibit unique distribution. Interestingly, in addition to the pituitary, fshbeta, lhbeta, and cga were also expressed in some extrapituitary tissues, particularly the gonads and brain. In situ hybridization showed that zebrafish fshbeta and lhbeta were expressed in two distinct populations of gonadotrophs in the pituitary. Real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis revealed that all the three subunits increased expression before ovulation (0100-0400) when the germinal vesicles in the full-grown follicles were migrating toward the periphery, but the levels dropped at 0700, when ovulation occurred. Recombinant zebrafish FSH (zfFSH) and LH (zfLH) were produced in the Chinese hamster ovary (CHO) cells and their effects on the cognate receptors (zebrafish Fshr and Lhr) tested. Interestingly, zfFSH specifically activated zebrafish Fshr expressed together with a cAMP-responsive reporter gene in the CHO cells, whereas zfLH could stimulate both Fshr and Lhr. In conclusion, the present study systematically investigated gonadotropins in the zebrafish in terms of their structure, spatial-temporal expression patterns, and receptor specificity. These results, together with the availability of recombinant zfFSH and zfLH, provide a solid foundation for further studies on the physiological relevance of FSH and LH in the zebrafish, one of the top biological models in vertebrates.

Descriptors: zebrafish, follicle stimulating hormone and luteinizing hormone, beta subunit genetics and metabolism, FSH receptors metabolism and genetics, LH receptors metabolism and genetics, amino acid and base sequence, CHO cells, molecular cloning, cricetinae, cricetulus, follicle stimulating hormone, beta subunit metabolism, gene expression regulation, gonadotropins, pituitary genetics and metabolism, molecular sequence data, organ specificity, ovulation physiology, pituitary gland physiology, recombinant proteins metabolism.

Sohn, K.C. and S.I. Do (2005). **Transcriptional regulation and O-GlcNAcylation activity of zebrafish OGT during embryogenesis.** *Biochemical and Biophysical Research Communications* 337(1): 256-63. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: Zebrafish OGT (zOGT) sequence was identified in zebrafish (*Danio rerio*) genome and six different transcriptional variants of zOGT, designated var1 to var6, were

isolated. Here we describe the developmental regulation of zOGT variants at transcriptional level and characterization of their OGT activities of protein O-GlcNAcylation. OGT transcriptional variants in zebrafish were differentially generated by alternative splicing and in particular, var1 and var2 were contained by 48 bp intron as a novel exon sequence, demonstrating that this form of OGT was not found in mammals. Transcript analysis revealed that var1 and var2 were highly expressed at early phase of development including unfertilized egg until dome stage whereas var3 and var4 were begins to be expressed at sphere stage until late phase of development. Our data indicate that var1 and var2 are likely to be maternal transcripts. The protein expression assay in Escherichia coli-p62 system showed that OGT activities of var3 and var4 were found to be only active whereas those of other variants were inactive.

Descriptors: zebrafish, n acetylglucosaminyltransferases genetics, n acetylglucosaminyltransferases metabolism, alternative splicing, amino acid and base sequence, embryonic development, *Escherichia coli* genetics, developmental gene expression regulation, molecular sequence data, n acetylglucosaminyltransferases classification, phylogeny, sequence alignment, genetic transcription, variation genetics, metabolism, proteins classification.

Sollner, C., G.J. Rauch, J. Siemens, R. Geisler, S.C. Schuster, U. Muller, and T. Nicolson (2004). **Mutations in cadherin 23 affect tip links in zebrafish sensory hair cells.** *Nature* 428(6986): 955-9. ISSN: 0028-0836.

NAL Call Number: 472 N21

Abstract: Hair cells have highly organized bundles of apical projections, or stereocilia, that are deflected by sound and movement. Displacement of stereocilia stretches linkages at the tips of stereocilia that are thought to gate mechanosensory channels. To identify the molecular machinery that mediates mechanotransduction in hair cells, zebrafish mutants were identified with defects in balance and hearing. In sputnik mutants, stereociliary bundles are splayed to various degrees, with individuals displaying reduced or absent mechanotransduction. Here we show that the defects in sputnik mutants are caused by mutations in cadherin 23 (cdh23). Mutations in Cdh23 also cause deafness and vestibular defects in mice and humans, and the protein is present in hair bundles. We show that zebrafish Cdh23 protein is concentrated near the tips of hair bundles, and that tip links are absent in homozygous sputnik(tc317e) larvae. Moreover, tip links are absent in larvae carrying weak alleles of cdh23 that affect mechanotransduction but not hair bundle integrity. We conclude that Cdh23 is an essential tip link component required for hair-cell mechanotransduction.

Descriptors: sputnik mutants zebrafish, cadherins metabolism and genetics, hair cells metabolism, mutation genetics, alleles, cilia metabolism and ultrastructure, gene expression profiling, hair cells ultrastructure, hearing genetics, hearing physiology, larva genetics, larva physiology, mechanotransduction, cellular genetics and physiology, messenger RNA genetics and metabolism.

Sollner, C., H. Schwarz, R. Geisler, and T. Nicolson (2004). **Mutated otopetrin 1 affects the genesis of otoliths and the localization of Starmaker in zebrafish.** *Development Genes and Evolution* 214(12): 582-90. ISSN: 0949 944X.

NAL Call Number: QL951.R68

Abstract: Otoliths in bony fishes and otoconia in mammals are composite crystals consisting of calcium carbonate and proteins. These biominerals are part of the gravity and linear acceleration detection system of the inner ear. Mutations in otopetrin 1 have been shown to result in lack of otoconia in tilted and mergulhador mutant mice. The molecular function of Otopetrin 1, a novel protein that contains ten predicted transmembrane domains, however,

has remained elusive. Here we show that a mutation in the orthologous gene in zebrafish is responsible for the complete absence of otoliths in backstroke mutants. We examined the localization of Starmaker, a secreted protein that is highly abundant in otoliths in backstroke mutants. Starmaker protein accumulated within cells of the otic epithelium, indicating a possible defect in secretion. Our data suggest that Otopetrin 1 in zebrafish may be involved in the protein trafficking of components required for formation of biominerals in the ear.
Descriptors: zebrafish, membrane, otolithic membrane embryology, proteins analysis and phusiology, amino acid sequence, molecular cloning, inner ear cytology, epithelium chemistry and metabolism, gene expression drug effects, membrane molecular sequence data, mutation, oligodeoxyribonucleotides, antisense genetics and pharmacology, otolithic membrane metabolism, protein transport genetics and physiology, messenger RNA analysis and metabolism.

Solnica Krezel, L. (2006). **Gastrulation in zebrafish -- all just about adhesion?** *Current Opinion in Genetics and Development* 16(4): 433-41. ISSN: 0959 437X.
NAL Call Number: QH426.C88
Abstract: During vertebrate gastrulation, the evolutionarily conserved morphogenetic movements of epiboly, internalization, convergence and extension cooperate to generate germ layers and to sculpt the body plan. In zebrafish, these movements are driven by a variety of cell behaviors, including slow and fast directed migration, radial and mediolateral intercalation, and cell shape changes. Whereas some signaling pathways are required for a subset of these behaviors, other molecules, such as E-cadherin or Galpha12 and Galpha13 proteins, appear to have a widespread role in different gastrulation cell behaviors.
Descriptors: zebrafish, gastrula cytology and physiology, cadherins physiology, cell adhesion, cell movement genetics, cytoskeleton physiology, developmental gene expression regulation, mesoderm cytology.

Son, O.L., H.T. Kim, M.H. Ji, K.W. Yoo, M. Rhee, and C.H. Kim (2003). **Cloning and expression analysis of a Parkinson's disease gene, uch-L1, and its promoter in zebrafish.** *Biochemical and Biophysical Research Communications* 312(3): 601-7. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: Three genes, alpha-synuclein, parkin, and ubiquitin C-terminal hydrolase L1 (UCH-L1), have been associated with inherited forms of Parkinson's disease (PD), although their in vivo functions have remained largely unknown. To develop an animal model for the molecular study of PD, we cloned zebrafish uch-L1 cDNA and its gene promoter. Sequence analysis revealed that the zebrafish Uch-L1 is highly homologous (79%) to the human UCH-L1, which is a member of the deubiquitinating enzymes. By whole-mount in situ hybridization, we examined the spatiotemporal expression of uch-L1 mRNA in developing zebrafish embryos. The uch-L1 mRNAs are detected in neuronal cells at the first day of embryo development. The expression domain of uch-L1 overlaps with that of tyrosine hydroxylase, a molecular marker for dopaminergic neurons, in the ventral diencephalon, an equivalent structure to the substantia nigra where PD progresses in human. To further analyze the tissue-specific regulation of uch-L1 gene expression, we also tested its gene promoter activity and showed a preferential neuronal expression in transient transgenic zebrafish embryos. These results suggest that uch-L1 may have an important role in the development of neuronal cells in early embryos as well as in the degeneration and disease of neuronal cells in late adult brain.
Descriptors: zebrafish, gene expression profiling methods, developmental gene expression regulation, enzymologic gene expression regulation, Parkinson disease genetics and metabo-

lism, ubiquitin thiolesterase chemistry, genetics and metabolism, amino acid sequence, molecular cloning, mice, models, molecular sequence data, promoter regions genetics, sequence homology, species specificity, tissue distribution.

Song, H.D., X.J. Sun, M. Deng, G.W. Zhang, Y. Zhou , X.Y. Wu, Y. Sheng, Y. Chen, Z. Ruan, C.L. Jiang , H.Y. Fan, L.I. Zon, J.P. Kanki, T.X. Liu, A.T. Look, and Z. Chen (2004). **Hematopoietic gene expression profile in zebrafish kidney marrow.** *Proceedings of the National Academy of Sciences of the United States of America* 101(46): 16240-5. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: The zebrafish kidney marrow is considered to be the organ of definitive hematopoiesis, analogous to the mammalian bone marrow. We have sequenced 26,143 ESTs and isolated 304 cDNAs with putative full-length ORF from a zebrafish kidney marrow cDNA library. The ESTs formed 7,742 assemblies, representing both previously identified zebrafish ESTs (56%) and recently discovered zebrafish ESTs (44%). About 30% of these EST assemblies have orthologues in humans, including 1,282 disease-associated genes in the Online Mendelian Inheritance in Man (OMIM) database. Comparison of the effective and regulatory molecules related to erythroid functions across species suggests a good conservation from zebrafish to human. Interestingly, both embryonic and adult zebrafish globin genes showed higher homology to the human embryonic globin genes than to the human fetal/adult ones, consistent with evo-devo correlation hypothesis. In addition, conservation of a whole set of transcription factors involved in globin gene switch suggests the regulatory network for such remodeling mechanism existed before the divergence of the teleost and the ancestor of mammals. We also carried out whole-mount mRNA in situ hybridization assays for 493 cDNAs and identified 80 genes (16%) with tissue-specific expression during the first five days of zebrafish development. Twenty-six of these genes were specifically expressed in hematopoietic or vascular tissues, including three previously unidentified zebrafish genes: coro1a, nephrosin, and dab2. Our results indicate that conserved genetic programs regulate vertebrate hematopoiesis and vasculogenesis, and support the role of the zebrafish as an important animal model for studying both normal development and the molecular pathogenesis of human blood diseases.

Descriptors: zebrafish, hematopoiesis genetics, complementary DNA genetics, isolation and purification, erythropoiesis genetics, evolution, expressed sequence tags, gene expression profiling, gene library, in situ hybridization, metalloendopeptidases genetics, molecular sequence data, open reading frames, phylogeny, messenger RNA genetics and metabolism, species specificity.

Song, L. and R.S. Tuan (2006). **MicroRNAs and cell differentiation in mammalian development**. *Birth Defects Research* 78(2): 140-149. ISSN: 1542 0752.

NAL Call Number: QL991.T4

Abstract: MicroRNAs (miRNAs) are a group of recently discovered small RNAs produced by the cell using a unique process, involving RNA polymerase II, Microprocessor protein complex, and the RNAase III/Dicer endonuclease complex, and subsequently sequestered in an miRNA ribonucleoprotein complex. The biological functions of miRNAs depend on their ability to silence gene expression, primarily via degradation of the target mRNA and/or translational suppression, mediated by the RNA-induced silencing complex (RISC). First discovered in *Caenorhabditis elegans* (lin-4), miRNAs have now been identified in a wide array of organisms, including plants, zebrafish, *Drosophila*, and mammals. The expression of miRNAs in multicellular organisms exhibits spatiotemporal, and tissue- and cell-specificity,

suggesting their involvement in tissue morphogenesis and cell differentiation. More than 200 miRNAs have been identified or predicted in mammalian cells. Recent studies have demonstrated the importance of miRNAs in embryonic stem cell differentiation, limb development, adipogenesis, myogenesis, angiogenesis and hematopoiesis, neurogenesis, and epithelial morphogenesis. Overexpression (gain-of-function) and inactivation (loss-of-function) are currently the primary approaches to -studying miRNA functions. Another family of small RNAs related to miRNAs is the small interfering RNAs (siRNAs), generated by Dicer from long double-stranded RNAs (dsRNAs), and produced from an induced transgene, a viral intruder, or a rogue genetic element. siRNAs silence genes via either mRNA degradation, using the RISC, or DNA methylation. siRNAs are actively being applied in basic, functional genetic studies, particularly in the generation of gene knockdown animals, as well as in gene knockdown studies of cultured cells. These studies have provided invaluable information on the specific function(s) of individual genes. siRNA technology also presents exciting potential as a therapeutic approach in disease prevention and treatment, as suggested by a recent study targeting apolipoprotein B (ApoB) in primates. Further elucidation of how miRNAs and other small RNAs interact with known and yet-to-be identified gene regulatory pathways in the cell should provide us with a more in-depth understanding of the mechanisms regulating cellular function and differentiation, and facilitate the application of small RNA technology in disease control and treatment. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, nervous system, neural coordination, development, molecular genetics and system, movement and support, cell differentiation, angiogenesis, tissue morphogenesis, adipogenesis , neurogenesis, myogenesis.

Song, W., Z. Zou, F. Xu, X. Gu, X. Xu, and Q. Zhao (2006). **Molecular cloning and expression of a second zebrafish aldehyde dehydrogenase 2 gene (aldh2b).** *DNA Sequence* 17(4): 262-9. ISSN: 1042 5179.

NAL Call Number: QH435.D57

Abstract: Aldehyde dehydrogenase 2 (ALDH2) is primarily responsible for detoxification of short-chain aldehydes in vivo. Previously it was reported that zebrafish has an aldh2 gene. Here we report the presence of a second aldh2 gene (aldh2b) in zebrafish. Zebrafish aldh2b locates adjacently to aldh2 on Chromosome 5 and the two genes share the same genomic organizations. aldh2b was predicted to encode a protein comprising 516 amino acids. The protein exhibits 95% amino acid identity with zebrafish ALDH2 and more than 76% identity with other vertebrate ALDH2s, respectively. Employing RT-PCR analysis, we demonstrated that both aldh2 and aldh2b mRNAs were present in embryos at cleavage stage (2 hpf: hour post fertilization) throughout protruding-mouth stage (72 hpf) and in different adult tissues of zebrafish. Taken together, our results reveal that zebrafish has two orthologues of aldh2 gene and the two genes share similar expression patterns during early development and in adult tissues.

Descriptors: zebrafish, aldehyde dehydrogenase genetics, phylogeny, amino acid and base sequence, chromosome mapping, molecular cloning, enzymology, gene components, molecular sequence data, reverse transcriptase PCR, DNA sequence analysis and primers.

Song, Y., G. Golling, T.L. Thacker, and R.D. Cone (2003). **Agouti-related protein (AGRP) is conserved and regulated by metabolic state in the zebrafish, *Danio rerio*.** *Endocrine* 22(3): 257-65. ISSN: 0969 711X.

NAL Call Number: QP187.A1

Abstract: Agouti-related protein (AGRP) and proopiomelanocortin (POMC) genes encode secreted hypothalamic factors regulated by metabolic state in mammals and are involved in

energy homeostasis. The zebrafish, Danio rerio, is a model system for forward genetics in vertebrates: POMC and AGRP in this organism have not been well characterized. Toward this end, AGRP and POMC were cloned from zebrafish. Zebrafish AGRP cDNA encodes a 127-amino-acid protein 36% and 40% identical to human and mouse AGRP, respectively. Zebrafish POMC cDNA encodes a 222-amino-acid preprohormone. Sequence identity to the mammalian ortholog is about 50%. Zebrafish AGRP and POMC transcripts were detected at 24 h post-fertilization (hpf) by RTPCR, and in situ hybridization demonstrated zebrafish AGRP mRNA exclusively in hypothalamus and POMC mRNA in hypothalamus and pituitary. Fasting did not alter POMC transcript levels, while AGRP transcripts were significantly upregulated. The ratio of AGRP/POMC transcripts in adult brain was increased up to threefold by fasting. These results demonstrate that the melanocortin system is regulated by metabolic state in zebrafish, and this finding thus provides a vertebrate system for the genetic analysis of the role of the melanocortin system in energy homeostasis.
Descriptors: zebrafish, developmental gene expression regulation physiology, amino acid and base sequence, molecular cloning, food deprivation, hypothalamus metabolism and physiology, intercellular signaling peptides and proteins, molecular sequence data, pro opiomelanocortin genetics, RNA chemistry and genetics, reverse transcriptase PCR, sequence alignment, DNA sequence analysis.

Sood, R., M.A. English, M. Jones, J. Mullikin, D.M. Wang, M. Anderson, D. Wu, S.C. Chandrasekharappa, J. Yu, J. Zhang, and P. Paul Liu (2006). **Methods for reverse genetic screening in zebrafish by resequencing and TILLING.** *Methods* 39(3): 220-7. ISSN: 0882-4010.
NAL Call Number: QP519.7.M47
Abstract: Animal models provide an in vivo system to study gene function by transgenic and knockout approaches. Targeted knockout approaches have been very successful in mice, but are currently not feasible in zebrafish due to the inability to grow embryonic stem cells. As an alternative, a reverse genetic approach that utilizes screening by resequencing and/or TILLING (Targeting Induced Local Lesions INGenomes) of mutagenized genomes has recently gained popularity in the zebrafish field. Spermatogonia of healthy males are mutagenized using ENU (N-ethyl-N-nitrosourea) and F1 progeny is collected by breeding treated males with healthy wild type females. Sperm and DNA banks are generated from F1 males. DNA is screened for ENU-induced mutations by sequencing or TILLING. These mutations can then be studied by in vitro fertilization (IVF) from the cryopreserved sperm of the corresponding F1 male followed by breeding to homozygosity. A high-throughput method of screening for rare heterozygotes and efficient recovery of mutant lines are important in identification of a large number of mutations using this approach. This article provides optimized protocols for resequencing and TILLING based on our experiences. We performed a pilot screen on 1235 F1 males by resequencing 54 exons from 17 genes and analyzed the sequencing data using multiple programs to maximize the mutation detection with minimal false positive detection. As an alternative to sequencing, we developed the protocols for TILLING by capillary electrophoresis using an ABI Genetic analyzer 3100 platform followed by fragment analysis using GeneScan and Genotyper softwares. PCR products generated by fluorescently labeled universal primers and tailed exon-specific primers were pooled 4-fold prior to heteroduplex formation. Overall, our pilot screen shows that a combination of TILLING and sequencing is optimal for achieving cost-effective, high-throughput screening of a large number of samples. Amplicons with fewer common SNPs are ideal for TILLING whereas amplicons with multiple SNPs and in/del polymorphisms are best suited for sequencing followed by analysis with SNPdetector.

Descriptors: zebrafish, DNA mutational analysis methods, gene targeting methods, mutagenesis, computational biology, cryopreservation methods, ethylnitrosourea pharmacology , fertilization in vitro, genome, single nucleotide polymorphism, semen preservation methods, spermatogonia drug effects, testis, zebrafish growth and development software.

Spence, R., W.C. Jordan, and C. Smith (2006). **Genetic analysis of male reproductive success in relation to density in the zebrafish, *Danio rerio.*** *Frontiers in Zoology* 3: 5. ISSN: 1742-9994.

NAL Call Number: QL1 F76

Abstract: BACKGROUND: We used behavioural and genetic data to investigate the effects of density on male reproductive success in the zebrafish, *Danio rerio*. Based on previous measurements of aggression and courtship behaviour by territorial males, we predicted that they would sire more offspring than non-territorial males. RESULTS: Microsatellite analysis of paternity showed that at low densities territorial males had higher reproductive success than non-territorial males. However, at high density territorial males were no more successful than non-territorials and the sex difference in the opportunity for sexual selection, based on the parameter Imates, was low. CONCLUSION: Male zebrafish exhibit two distinct mating tactics; territoriality and active pursuit of females. Male reproductive success is density dependent and the opportunity for sexual selection appears to be weak in this species.

Descriptors: zebrafish, reproduction, male, genetics, mating behavior, sexual selection, population density effects.

Spencer, M., J. Kanungo, S. Swamynathan, and J. Piatigorsky (2005). **Two gelsolin genes in zebrafish: differences in structure and expression pattern**. *Investigative Ophthalmology & Visual Science* 46(Suppl. S): 2178. ISSN: 0146 0404.

Abstract: Purpose: In zebrafish, gelsolin-1 (previously called C/L-gelsolin) is expressed highly in cornea (50% of water-soluble protein) and slightly in lens and early cleavage-stage embryos. Since this is an atypical expression pattern for vertebrate gelsolin, we searched for a second gelsolin gene. Methods: Gelsolin-2was cloned by PCR based on EST data available from the GenBank database. Sequences for in situ hybridization probes were constructed in pBS-sk(-) for gelsolin-2 and pCS2 for gelsolin-1. Probes were labeled with the DIG kit from Roche. Multiple alignments were generated with clustalW from sequences obtained from GeneBank. Dendograms were created from the clustalW results and formatted with Tree View. Results: We cloned a second zebrafish gelsolin (gelsolin-2) which exhibits 56% identity to corneal-preferred zebrafish gelsolin-1 at the protein level. The gelsolin-2 protein has 71% identity to human gelsolin compared to 58% for gelsolin-1. As expected, gelsolin-2 exhibits six conserved gelsolin motifs. Phylogenetic analysis demonstrates that gelsolin-1 and gelsolin-2 diverged early in vertebrate evolution and that gelsolin-2 gave rise to the other vertebrate gelsolins. Gelsolin-2 mRNA is widely expressed at a low level in the embryo and adult; it does not accumulate in the cornea as gelsolin-1. Comparison of up to 10 kb of the upstream regions of these two genes indicated that only a few short stretches of sequence are comparable. In addition, the gene structures of the gelsolin-1 and gelsolin-2 differ, with 18 introns and 20 exons, and 16 introns and 17 exons for gelsolin-1 and gelsolin-2, respectively. Conclusions: The differences in gene structures and upstream putative regulatorysequences driving their diverse patterns of expression suggest that the two zebrafish gelsolin genes have undergone adaptive changes in regulation, consistent with functional differences for the proteins, following gene duplication. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, molecular genetics, PCR, genetic techniques, in situ hybridization, genetic techniques, exons, introns, body pattern diversity.

Srinivas, S. (2006). **The anterior visceral endoderm-turning heads**. *Genesis* 44(11): 565-572. ISSN: 1526 954X.
NAL Call Number: QH426.D32
Abstract: The Anterior Visceral Endoderm is an extra-embryonic tissue that plays a pivotal role during embryogenesis, being responsible for the proper orientation of the anterior-posterior axis of the embryo and for appropriate pattering of adjacent embryonic tissue. In this review I discuss the formation and migration of the AVE, and attempt to place some recent findings in the context of a working model. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics, development, embryogenesis, tissue formation and migration, working model.
Notes: A review.

Starr, C.J., J.A. Kappler, D.K. Chan, R. Kollmar, and A.J. Hudspeth (2004). **Mutation of the zebrafish choroideremia gene encoding Rab escort protein 1 devastates hair cells.** *Proceedings of the National Academy of Sciences of the United States of America* 101(8): 2572-7. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: To identify genes important for hair-cell function, we conducted a mutagenic screen in zebrafish. Larvae from one mutant line, ru848, were unresponsive to acoustic stimuli and unable to balance. The mutation results in a 90% reduction in hair-cell number and partial retinal degeneration by 5 days postfertilization. We localized the recessive ru848 mutation by positional cloning to the zebrafish homolog of the human Choroideremia gene, which encodes Rab escort protein 1. This protein is essential for the normal prenylation of Rabs. Mutations in the human gene induce choroideremia, a disease marked by slow-onset degeneration of rod photoreceptors and retinal pigment epithelial cells. The degenerative phenotype resulting from a null mutation in the zebrafish gene indicates that hair cells and retinal cells require Rab escort protein 1 for survival.
Descriptors: zebrafish larva, alkyl and aryl transferases genetics, hair cells cytology, mutation, amino acid sequence, cell survival, molecular cloning, conserved sequence, genetic crosses, inner ear physiology, recessive genes, heterozygote, larva, molecular sequence data, phenotype, retina cytology, sequence alignment, growth and development.

Stehr, C.M., K.A. Lefebvre, T. Kao, J. Incardona, and N.L. Scholz (2004). **Rapid phenotypic screening in zebrafish: A model for identifying developmental toxicity in native fish species**. *2003 Georgia basin/Puget sound research conference,March 3, 1931-April 3, 2003, Vancouver, BC (Canada),* Puget Sound Action Team: Olympia WA 98504 USA,
Descriptors: zebrafish, embryonic development, endemic species, fish larvae, freshwater fish, herring, larvae, larval development, model studies, phenotypes, pollutants, pollution indicators, salmon, screening, screens, sounds, stages, toxicants, toxicity, toxicity tests, toxins, water pollution effects, water Quality, *Danio rerio*, Canada, British columbia, Georgia Basin, Pacific, USA.

Steinke, D., W. Salzburger, I. Braasch, and A. Meyer (2006). **Many genes in fish have species-specific asymmetric rates of molecular evolution**. *Bio Med Central Genomics* 7(20) ISSN: 1471 2164.
Online: http://www.biomedcentral.com/content/pdf/1471-2164-7-20.pdf
NAL Call Number: QH447.B63
Abstract: Background: Gene and genome duplication events increase the amount of genetic material that might then contribute to an increase in the genomic and phenotypic

complexity of organisms during evolution. Thus, it has been argued that there is a relationship between gene copy number and morphological complexity and/or species diversity. This hypothesis implies that duplicated genes have subdivided or evolved novel functions compared to their pre-duplication proto-orthologs. Such a functional divergence might be caused by an increase in evolutionary rates in one ortholog, by changes in expression, regulatory evolution, insertion of repetitive elements, or due to positive Darwinian selection in one copy. We studied a set of 2466 genes that were present in *Danio rerio, Takifugu rubripes, Tetraodon nigroviridis* and *Oryzias latipes* to test (i) for forces of positive Darwinian selection; (ii) how frequently duplicated genes are retained, and (iii) whether novel gene functions might have evolved. Results: 25% (610) of all investigated genes show significantly smaller or higher genetic distances in the genomes of particular fish species compared to their human ortholog than their orthologs in other fish according to relative rate tests. We identified 49 new paralogous pairs of duplicated genes in fish, in which one of the paralogs is under positive Darwinian selection and shows a significantly higher rate of molecular evolution in one of the four fish species, whereas the other copy apparently did not undergo adaptive changes since it retained the original rate of evolution. Among the genes under positive Darwinian selection, we found a surprisingly high number of ATP binding proteins and transcription factors. Conclusion: The significant rate difference suggests that the function of these rate-changed genes might be essential for the respective fish species. We demonstrate that the measurement of positive selection is a powerful tool to identify divergence rates of duplicated genes and that this method has the capacity to identify potentially interesting candidates for adaptive gene evolution. Copyright © 2008 CABI

Descriptors: zebrafish, evolution, genes, genetic distance, genome analysis, genomes, molecular genetics, *Danio rerio*, fishes, *Oryzias*, *Tetraodontiformes.*

Stenkamp, D.L. and S.L. Wardwell (2004). **Developmental genetics of the rod photoreceptor lineage in zebrafish**. *Investigative Ophthalmology & Visual Science* 45(Suppl. 2): U630. ISSN: 0146 0404.

Abstract: Purpose: Expression of hedgehog genes in the retinal pigmented epithelium, andactivity of the Hedgehog (Hh) signal transduction pathway, are necessary for the differentiation of rod and cone photoreceptors in the developing zebrafish retina. Experimental knockdown of Hh signaling results in reduced opsin expression, as well as failed expression of the transcription factor rx1 in the photoreceptor layer (Stenkamp et al., 2003). Because the rx gene product can regulate photoreceptor gene expression (Kimura et al., 2000), we hypothesize that the effects of Hh signaling on photoreceptor differentiation may be mediated by rx1. The rx1 gene is expressed in cone photoreceptors of the zebrafish (Chuang et al., 1999), but expression in the rod photoreceptor lineage has not beenevaluated. The goals of this study were to unambiguously identify the rod I lineage of the embryonic zebra fish, and to determine whether rx I is expressedin this lineage.Methods: In zebrafish, rod neurogenesis continues after other retinal neurons have become post-mitotic, providing an experimental window for selective labeling of the rod lineage with bromodeoxy uridine (BrdU). BrdU was injected into zebrafish embryos, and after various survival times, embryos were fixed, sectioned and doubly-labeled for BrdU and for cell-specific markers.Results: Injected BrdU accumulates in multipotent progenitor cells and their progeny at the retinal margin, and in isolated cells in the inner nuclear layer(INL) and outer nuclear layer (ONL), as well as in radial clusters of cells spanning the INL and ONL. Some of the BrdU positive cells in the ONL were also rod opsin positive, confirming that the BrdU-labeled lineage gave rise to rod photoreceptors. Some of the BrdU positive cells in the INL were also pax:6 positive, confirming the earlier findings of Otteson et al. (2001), that a pax6-expressing cell constitutes the

apex of the rod lineage.Conclusions: This study verifies that the rod photoreceptor lineage can be identified in the zebrafish embryo and provides a means by which to further characterize the expression pattern of rx1. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics, sense organs, sensory reception, neurogenesis.

Stern, H.M., R.D. Murphey, J.L. Shepard, J.F. Amatruda, C.T. Straub, K.L. Pfaff, G. Weber, J.A. Tallarico, R.W. King, and L.I. Zon (2005). **Small molecules that delay S phase suppress a zebrafish bmyb mutant.** *Nature Chemical Biology* 1(7): 366-70. ISSN: 1552-4450.
Abstract: Bmyb is a ubiquitously expressed transcription factor involved in cellular proliferation and cancer. Loss of bmyb function in the zebrafish mutant crash&burn (crb) results in decreased cyclin B1 expression, mitotic arrest and genome instability. These phenotypic observations in crb mutants could be attributed to the decreased expression of cyclin B1, a cell-cycle regulatory protein that is responsible for driving cell progression from G2 through mitosis. To identify small molecules that interact with the bmyb pathway, we developed an embryo-based suppressor screening strategy. In 16 weeks we screened a diverse approximately 16,000 compound library, and discovered one previously unknown compound, persynthamide (psy, 1), that suppressed bmyb-dependent mitotic defects. Psy-treated embryos showed an S-phase delay, and knockdown of the cell-cycle checkpoint regulator ataxia telangiectasia--and Rad-related kinase (ATR) abrogated the suppression of crb. The DNA synthesis inhibitors aphidicolin (2) and hydroxyurea (3) also suppressed crb. S-phase inhibition upregulated cyclin B1 mRNA, promoting the progression of cells through mitosis. Our study demonstrates that chemical suppressor screening in zebrafish can identify compounds with cell-cycle activity and can be used to identify pathways that interact with specific cell-cycle phenotypes.
Descriptors: mutant zebrafish, adamantane analogs and derivatives, proto oncogene proteins C myb genetics, pyridines pharmacology, adamantane chemistry and pharmacology, cell cycle drug effects and physiology, cyclin B drug effects and genetics, developmental gene expression regulation drug effects, mitosis drug effects, phenotype, proto oncogene proteins C myb metabolism, pyridines chemistry, messenger RNA drug effects and genetics, S phase drug effects and genetics.

Stet, R.J.M., T. Hermsen, A.H. Westphal, J. Jukes, M. Engelsma, B.M.L. Verburg van Kemenade, J. Dortmans, J. Aveiro, and H.F.J. Savelkoul (2005). **Novel immunoglobulin-like transcripts in teleost fish encode polymorphic receptors with cytoplasmic ITAM or ITIM and a new structural Ig domain similar to the natural cytotoxicity receptor NKp44**. *Immunogenetics* 57(1/2): 77-89. ISSN: 0093 7711.
NAL Call Number: QR184.I4
Abstract: Members of the immunoglobulin superfamily (IgSF) include a group of innate immune receptors located in the leukocyte receptor complex (LRC) and other small clusters such as the TREM/NKp44 cluster. These receptors are characterised by the presence of immunoglobulin domains, a stalk, a transmembrane domain, and a cytoplasmic region containing either an immunoreceptor tyrosine-based inhibitory motif (ITIM) or are linked to an adapter molecule with an activation motif (ITAM) for downstream signalling. We have isolated two carp cDNA sequences encoding receptors in which the extracellular Ig domain structurally resembles the novel V-type Ig domain of NKp44. This is supported by a homology model. The cytoplasmic regions contain either an ITAM (Cyca-NILT1) or ITIMs (Cyca-NILT2). The tissue expression of these receptors is nearly identical, with the highest expression in the immunological organs. Peripheral blood leucocytes showed no detectable expression, but upon in vitro culture expressed NILT1, the activating receptor, and not the

inhibitory NILT2 receptor. Southern blot analysis indicated that the NILT1 and NILT2 sequences belong to a multigene family. Analysis of the NILT Ig domain-encoding sequences amplified from both genomic DNA and cDNA revealed extensive haplotypic and allelic polymorphism. Database mining of the zebrafish genome identified several homologs on Chromosome 1, which also contains a cluster of class I major histocompatibility genes. This constellation is reminiscent of the TREM/NKp44 gene cluster and the HLA complex located on human Chromosome 6. The carp NILT genes form a unique cluster of innate immune receptors, which are highly polymorphic, and characterised by a new Ig structural subfamily and are distinct from the novel immune-type receptors (Nitrs) found in other fish species. Copyright © 2008 CABI

Descriptors: zebrafish, alleles, biochemical receptors, complementary DNA, cytotoxicity, gene expression, genes, genetic polymorphism, haplotypes, immunity, immunogenetics, immunoglobulins, leukocytes, tissue distribution, carp.

Steven, C., N. Lehnen, K. Kight, S. Ijiri, U. Klenke , W.A. Harris, and Y. Zohar (2003). **Molecular characterization of the GnRH system in zebrafish (*Danio rerio*): cloning of chicken GnRH-II, adult brain expression patterns and pituitary content of salmon GnRH and chicken GnRH-II.** *General and Comparative Endocrinology* 133(1): 27-37. ISSN: 0016-6480.

NAL Call Number: 444.8G28

Abstract: The zebrafish has proven to be a model system with unparalleled utility in vertebrate genetic and developmental studies. Substantially less attention has been paid to the potential role that zebrafish can play in answering important questions of vertebrate reproductive endocrinology. As an initial step towards exploiting the advantages that the zebrafish model offers, we have characterized their gonadotropin-releasing hormone (GnRH) system at the molecular level. GnRHs comprise a family of highly conserved decapeptide neurohormones widely recognized to orchestrate the hormonal control of reproduction in all vertebrates. We have isolated the gene and cDNA encoding chicken GnRH-II (cGnRH-II) from zebrafish, as well as several kilobases of upstream promoter sequence for this gene. As the gene encoding salmon GnRH (sGnRH) has been previously isolated (Torgersen et al, 2002), this is the second GnRH gene isolated from zebrafish to date. We have localized expression of these two genes in the brains of reproductively mature zebrafish using in situ hybridization. sGnRH is localized to the olfactory bulb-terminal nerve region (OB-TN), the ventral telencephalon-preoptic area (VT-POA) and, as we report here for the first time in any teleost species, the hindbrain. cGnRH-II is expressed exclusively in the midbrain, as has been found in all other jawed vertebrate species examined. Finally, the levels of both GnRH peptides in pituitaries of reproductively mature zebrafish were quantified using specific ELISAs. sGnRH pituitary peptide levels were shown to be 3- to 4-fold higher than cGnRH-II pituitary peptide. The cumulative results of these experiments allow us to conclude that zebrafish express just two forms of GnRH in a site-specific manner within the brain, and that sGnRH is the hypophysiotropic GnRH form. This work lays the foundation for further research into the control of reproduction in zebrafish, such as the functional significance of multiple GnRHs in vertebrates, and the molecular mechanisms controlling tissue-specific GnRH expression.

Descriptors: zebrafish, brain metabolism, chickens metabolism, molecular cloning, gonadotropin releasing hormone genetics and metabolism, pituitary gland metabolism, salmon metabolism, brain cytology, chickens genetics, complementary DNA isolation and purification, ELISA, in situ hybridization, neurons metabolism, protein isoforms genetics and metabolism, tissue distribution.

Stevens, C.B. and M.C. Halloran (2005). **Developmental expression of sema3G, a novel zebrafish semaphorin.** *Gene Expression Patterns* 5(5): 647-53. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: The semaphorins are a large, evolutionarily conserved family of signaling molecules with broad functions during development. The class 3 semaphorins are a subclass of secreted semaphorins found in vertebrates. There have been six class 3 semaphorins identified to date (sema3A to sema3F) and some have been shown to function in axon guidance and cardiovascular development. However, the functions of many class 3 semaphorins and their potential interactions in vivo are still not well understood. As a step toward understanding the actions of all class 3 semaphorins in vivo, we have cloned and analyzed the developmental expression pattern of a novel zebrafish class 3 semaphorin, sema3H [corrected] sema3H [corrected] is expressed in a dynamic pattern throughout the first 3 days of development. It is expressed in the adaxial cells of the somite during somitogenesis. In the brain, sema3H [corrected] is expressed in cell clusters in the midbrain and diencephalon, and is expressed in the telencephalon in close proximity to the olfactory epithelium. sema3H [corrected] also is expressed in the pharyngeal arches, the pectoral fin bud, and the developing pronephros. These results provide a basis for studying how expression of multiple semaphorins could be essential for aspects of early development.
Descriptors: zebrafish , developmental gene expression regulation , nerve growth factors biosynthesis and genetics, semaphorins biosynthesis and genetics, axons metabolism, brain embryology, cardiovascular system, molecular cloning, immunoglobulins metabolism, in situ hybridization, kidney embryology, optic nerve metabolism, phylogeny, messenger RNA metabolism, somites, telencephalon metabolism, time factors.

Straehle, U., C.S. Lam, R. Ertzer, and S. Rastegar (2004). **Vertebrate floor-plate specification: variations on common themes.** *Trends in Genetics* 20(3): 155-162. ISSN: 0168 9525.
NAL Call Number: QH426.T744
Abstract: Situated at the ventral-most part of the vertebrate neural tube, the floor plate (FP) is an important signalling centre that controls the regional differentiation of neurons in the nervous system. It secretes guidance molecules that direct ventrally navigating axons crucial for the correct wiring of neuronal circuits. Although the function of the FP is well-conserved from fish to humans, discrepancies exists with respect to both the signalling system involved in FP induction, and the origin of the FP in various vertebrate species. Recent findings from the embryos of zebrafish, chicken and mouse provide insights that reconcile previous results and suggest common themes in vertebrate FP specification. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, molecular genetics, nervous system, neural coordination, floor plate specification, gene expression.

Strahle, U. and V. Korzh (2004). **Development of the primary nervous system of the zebrafish embryo**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd. Singapore, Singapore , p. 185-215. ISBN: 9812388214.
NAL Call Number: QL638.C94 F57
Abstract: The early zebrafish embryo develops a simple primary nervous system that controls motility. Similar to that of the nematode *C. elegans*, this primary nervous system is composed of a relatively low number of neurons and is amenable to observation and experimental manipulation at the single cell level. The primary nervous system is derived from neurogenic regions in the neural plate. Several conserved gene loci are required for the development of

the primary neurons, suggesting that the molecular mechanisms underlying neurogenesis are strongly related to that of the fruitfly *Drosophila melanogaster* and higher vertebrates. Since inhibition of gene activity or mutations in the zebrafish emulate many human hereditary disorders, it renders the zebrafish an attractive model for the study of vertebrate nervous system development and human hereditary diseases. Copyright © 2008 CABI

Descriptors: zebrafish, animal anatomy and models, embryonic neuron development, embryos, genetic disorders, morphology, nervous system, neurophysiology, regulatory genes, *Danio rerio*.

Strussmann, C.A., M. Karube, L.A. Miranda, R. Patino, G.M. Somoza, D. Uchida and M. Yamashita (2005). **Methods of sexcontrol in fishes and an overview of novel hypotheses concerning the mechanisms of sexdifferentiation**. T.J. Pandian, C.A. Strussmann and M.P. Marian *Fish Genetics and Aquaculture Biotechnology,* Enfield, USA: Science Publishers, Inc., p. 65-79. ISBN: 1578083729.

NAL Call Number: SH155.5.F57

Abstract: This chapter discusses the methods of sex control and current knowledge of the mechanisms of gonadal sex differentiation in fishes. Although our understanding of sex differentiation in fishes has improved greatly in recent years, it is still inadequate either to allow generalizations about its mechanisms or to develop practical, reliable and safe methods of sex control. The scarcity of information arises from the fact that studies on sex differentiation are generally conducted on a few traditional species and focus on a limited set of hypotheses. Therefore, the current knowledge does not account for the variation in the forms of sexual expression in fishes and for the various degrees of plasticity of phenotypic sex that are found among fishes. In order to broaden the discussion about the mechanisms of sex differentiation, we present here an overview of our studies on the sex differentiation of three species, the pejerrey (*Odontesthes bonariensis*), the channel catfish (*Ictalurus punctatus*), and the zebrafish (*Danio rerio*), and introduce some novel views about this process. The hypotheses being explored are that: (a) temperature-dependent sex determination/differentiation is under the control of the central nervous system; (b) differential sex-linked distribution of cells expressing the oestrogen receptor plays a role in early gonadal sex differentiation; and (c) apoptosis is involved in the death of somatic or germ cells during sex differentiation. Copyright © 2008 CABI

Descriptors: zebrafish, apoptosis, gonads, methodology, estrogen receptors, reproduction, sex control, sex determination, sex differentiation, water temperature, *Danio rerio*, fishes, *Ictalurus punctatus*, channel catfish.

Notes: A review.

Stuckenholz, C., P.E. Ulanch, and N. Bahary (2005). **From guts to brains: using zebrafish genetics to understand the innards of organogenesis.** *Current Topics in Developmental Biology* 65: 47-82. ISSN: 0070 2153.

NAL Call Number: QL951.C8

Descriptors: zebrafish embryology, molecular biology methods, morphogenesis drug effects, DNA mutational analysis, digestive system anatomy, histology, embryology, growth and development, gene expression regulation, mutagenesis, mutagens pharmacology, nervous system anatomy, histology, embryology, growth and development.

Stuermer, C.A.D., E. Malaga-Trillo, and H. Plattner (2004). **The 'lipid raft' microdomain proteins reggie-1 and reggie-2 are scaffolds for protein interaction and signalling**. *Biochemical Society Transactions* 32(4): 14A. ISSN: 0300 5127.

NAL Call Number: QD415.A1B58
Descriptors: zebrafish, axon regeneration, protein interaction, transmembrame signal transduction.

Su, K.C. and Y.C. Lin (2005). **Microtas**. *Design and Experimental Verification of the Electroporation Microchip for Transgenic Zebrafishes Utilizing Genes and Quantum Dots,* Vol. 2, ROYAL SOCIETY OF CHEMISTRY: THOMAS GRAHAM HOUSE, SCIENCE PARK, CAMBRIDGE CB4 4WF, CAMBS, UK, p. 324-326. ISBN: 0854048960.
Abstract: This study focused on design and experimental verification of an electroporation (EP) microchip for transgenic zebrafishes. (CdSe)ZnS quantum dots, QDs, genes coding for green fluorescence protein, GFP, and trypan-blue dye were used to verify the successful transfection to the zebrafish embryos. The experimental results show that the delivery rate was upto 62% when using an EP microchip to deliver trypa-blue dye, QDs or GFP genes into zebrafish embryos. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, methods and techniques, cell biology, microchip electrophoresis, techniques.

Sugahara, T., C.C. Liu, G. Carter, T. Govind Pai, and M.C. Liu (2003). **cDNA cloning, expression, and functional characterization of a zebrafish SULT1 cytosolic sulfotransferase.** *Archives of Biochemistry and Biophysics* 414(1): 67-73. ISSN: 0003 9861.
NAL Call Number: 381 Ar2
Abstract: Using the reverse transcriptase-polymerase chain reaction technique, a full-length cDNA encoding a novel zebrafish sulfotransferase was cloned and sequenced. Sequence analysis indicated that this zebrafish sulfotransferase belongs to the SULT1 cytosolic sulfotransferase gene family. The recombinant form of the zebrafish sulfotransferase, purified from *Escherichia coli* cells, displayed sulfating activities toward a number of endogenous compounds, in particular dopamine and thyroid hormones, in addition to xenobiotics including some flavonoids, isoflavonoids, and other phenolic compounds. The zebrafish sulfotransferase exhibited substrate dependence in pH optimum. In comparison with those determined with dopamine as substrate, the zebrafish sulfotransferase displayed much lower K(m) and higher V(max) with n-propyl gallate as substrate. A thermostability experiment revealed the enzyme to be relatively stable over a temperature range between 20 and 43 degrees C. Among 10 divalent metal cations tested, Hg(2+), Co(2+), Zn(2+), Cd(2+), Cu(2+), and Pb(2+) exhibited dramatic inhibitory effects on the activity of the zebrafish sulfotransferase.
Descriptors: zebrafish, complementary DNA genetics, sulfotransferases genetics and metabolism, base sequence, divalent cations chemistry, molecular cloning methods, cytosol enzymology and metabolism, enzyme activation and stability, *Escherichia coli* enzymology, genetics, isolation and purification, enzymologic gene expression regulation, hydrogen ion concentration, metals chemistry, molecular sequence data, recombinant fusion proteins biosynthesis, chemistry, isolation and purification, reverse transcriptase PCR methods, sulfotransferases chemistry, isolation and purification, thermostability.

Sugahara, T., C.C. Liu, T. Govind Pai, and M.C. Liu (2003). **Molecular cloning, expression, and functional characterization of a novel zebrafish cytosolic sulfotransferase.** *Biochemical and Biophysical Research Communications* 300(3): 725-30. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: By searching the zebrafish expressed sequence tag (EST) database, we have identified a cDNA clone encoding a putative zebrafish cytosolic sulfotransferase (ST). This cDNA was isolated and subjected to nucleotide sequencing. Analysis of the sequence data revealed

that this novel zebrafish ST displays 32-35% amino acid sequence identity to members of all major cytosolic ST gene families. Therefore, this zebrafish ST, while belonging to the cytosolic ST gene superfamily, appears to be independent from all known constituent ST gene families. Recombinant zebrafish ST, expressed using the pET23c prokaryotic expression vector and purified from transformed *Escherichia coli* cells, migrated as a 34-kDa protein upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Purified zebrafish ST displayed sulfating activities toward dopamine and thyroid hormones (T(3) and T(4)), with a pH optimum spanning 7-9. The enzyme also exhibited activities toward a number of xenobiotics including some flavonoids, isoflavonoids, and other phenolic compounds. A thermostability experiment revealed the enzyme to be relatively stable over a temperature range between 20 and 48 degrees C. Among 10 divalent metal cations tested, Fe(++), Hg(++), Co(++), Zn(++), Cu(++), and Cd(++) exhibited dramatic inhibitory effects on the activity of the enzyme. These results constitute a first study on the cloning, expression, and characterization of a zebrafish cytosolic ST.

Descriptors: zebrafish, cytosol enzymology, sulfotransferases chemistry and genetics, proteins chemistry and genetics, amino acid and base sequence, divalent cations pharmacology, molecular cloning, polyacrylamide gel electrophoresis, enzyme activation drug effects, enzyme stability physiology, hydrogen ion concentration, molecular sequence data, molecular weight, DNA sequence analysis, amino acid sequence homology, substrate specificity, sulfotransferases biosynthesis, thermostability, proteins biosynthesis.

Sugahara, T., C.C. Liu, T.G. Pai, P. Collodi, M. Suiko, Y. Sakakibara, K. Nishiyama, and M.C. Liu (2003). **Sulfation of hydroxychlorobiphenyls. Molecular cloning, expression, and functional characterization of zebrafish SULT1 sulfotransferases.** *Federation of European Biochemical Societies Journal* 270(11): 2404-11. ISSN: 1742 464X.

NAL Call Number: QP501.E8

Abstract: As a first step toward developing a zebrafish model for investigating the role of sulfation in counteracting environmental estrogenic chemicals, we have embarked on the identification and characterization of cytosolic sulfotransferases (STs) in zebrafish. By searching the zebrafish expressed sequence tag database, we have identified two cDNA clones encoding putative cytosolic STs. These two zebrafish ST cDNAs were isolated and subjected to nucleotide sequencing. Sequence data revealed that the two zebrafish STs are highly homologous, being approximately 82% identical in their amino acid sequences. Both of them display approximately 50% amino acid sequence identity to human SULT1A1, rat SULT1A1, and mouse SULT1C1 ST. These two zebrafish STs therefore appear to belong to the SULT1 cytosolic ST gene family. Recombinant zebrafish STs (designated SULT1 STs 1 and 2), expressed using the pGEX-2TK prokaryotic expression system and purified from transformed Escherichia coli cells, migrated as approximately 35 kDa proteins on SDS/PAGE. Purified zebrafish SULT1 STs 1 and 2 displayed differential sulfating activities toward a number of endogenous compounds and xenobiotics including hydroxychlorobiphenyls. Kinetic constants of the two enzymes toward two representative hydroxychlorobiphenyls, 3-chloro-4-biphenylol and 3,3',5,5'-tetrachloro-4,4'-biphenyldiol, and 3,3',5-triiodo-l-thyronine were determined. A thermostability experiment revealed the two enzymes to be relatively stable over the range 20-43 degrees C. Among 10 different divalent metal cations tested, Co2+, Zn2+, Cd2+, and Pb2+ exhibited considerable inhibitory effects, while Hg2+ and Cu2+ rendered both enzymes virtually inactive.

Descriptors: zebrafish, arylsulfotransferase, biphenyl compounds metabolism, sulfotransferases metabolism, amino acid sequence, Western blotting, cations, molecular cloning, cytosol metabolism, DNA primers chemistry, complementary DNA metabolism, polyacryl-

amide gel electrophoresis, *Escherichia coli* metabolism , hydrogen ion concentration, kinetics, molecular sequence data, phylogeny, recombinant proteins chemistry and metabolism, amino acid sequence homology, sulfotransferases chemistry, temperature.

Sugahara, T., Y.S. Yang, C.C. Liu, T.G. Pai, and M.C. Liu (2003). **Sulphonation of dehydroepiandrosterone and neurosteroids: molecular cloning, expression, and functional characterization of a novel zebrafish SULT2 cytosolic sulphotransferase.** *Biochemical Journal, The* 375(Pt 3): 785-91. ISSN: 0264 6021.
NAL Call Number: 382B52
Abstract: By searching the zebrafish EST (expressed-sequence tag) database, we have identified two partial cDNA clones encoding the 5' and 3' regions of a putative zebrafish sulphotransferase (ST). Using the reverse transcription-PCR technique, a full-length cDNA encoding this zebrafish ST was successfully cloned. Sequence analysis revealed that this novel zebrafish ST displays 44%, 43% and 40% amino acid identity with mouse SULT2B1, human SULT2B1b and human SULT2A1 ST respectively. This zebrafish ST therefore appears to belong to the SULT2 cytosolic ST gene family. Recombinant zebrafish ST, expressed using the pGEX-2TK prokaryotic expression system and purified from transformed *Escherichia coli* cells, migrated as a 34 kDa protein upon SDS/PAGE. Purified zebrafish ST displayed a strong sulphonating activity toward DHEA (dehydroepiandrosterone), with a optimum pH of 9.5. The enzyme also exhibited activities toward several neurosteroids with differential K(m) and V(max) values. A thermostability experiment revealed the enzyme to be relatively stable over a temperature range between 20 degrees C and 43 degrees C. Among ten different divalent metal cations tested, Fe2+ and Cd(2+ exhibited small, but significant, stimulatory effects, whereas Hg2+ and Cu2+ displayed considerably stronger inhibitory effects on the DHEA-sulphonating activity of the enzyme. These results constitute the first study on the molecular cloning, expression, and characterization of a zebrafish cytosolic SULT2 ST.
Descriptors: zebrafish, dehydroepiandrosterone metabolism, steroids metabolism, sulfotransferases genetics, amino acid and base sequence, divalent cations pharmacology, molecular cloning, cytosol drug effects, enzymology and metabolism, complementary DNA chemistry and genetics, enzyme stability, *Escherichia coli* genetics, enzymologic gene expression regulation, hydrogen ion concentration, kinetics, molecular sequence data, phylogeny, recombinant proteins isolation and purification, recombinant proteins metabolism, DNA sequence analysis, substrate specificity, sulfonic acids metabolism, sulfotransferases isolation and purification, sulfotransferases metabolism, thermostability, proteins isolation and purification.

Sugano, T., M. Kajikawa, and N. Okada (2006). **Isolation and characterization of retrotransposition-competent LINEs from zebrafish.** *Gene* 365: 74-82. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: Long interspersed elements (LINEs) are a type of retroposon and are widely distributed in most eukaryotic genomes. LINEs are classified into two groups, the stringent type and relaxed type, based on the recognition of the 3' tail of their own RNA by reverse transcriptase (RT) during retrotransposition. Although most LINEs are thought to belong to the stringent type, retrotransposition studies of the stringent type LINEs are relatively limited compared with those of the relaxed type. We have now isolated two retrotransposition-competent LINEs (ZfL2-1 and ZfL2-2) from the zebrafish genome. Both ZfL2-1 and ZfL2-2 are members of the L2 clade; ZfL2-1 encodes two open reading frames (ORFs) and ZfL2-2 encodes one ORF, and each of the ORFs is required for retrotransposition. Using a

retrotransposition assay in HeLa cells, we established that both ZfL2-1 and Zfl2-2 belong to the stringent type. We also demonstrated that an esterase (ES) domain encoded by ZfL2-1 ORF1 strongly enhances its own retrotransposition. The ES domain is encoded only in ORF1 of LINEs classified in the CR1 and L2 clades, although its function or significance in retrotransposition has not been elucidated. Thus, this is the first experimental evidence that the ES domain has an enhancing function during retrotransposition. These zebrafish LINEs will be useful for determining the function of ORF1 and the retrotransposition mechanism of stringent-type LINEs.

Descriptors: zebrafish, long interspersed nucleotide elements genetics, retroelements genetics, 5' untranslated regions, amino acid motifs, amino acid and base and consensus sequence, DNA genetics, gene library, genome, HeLa cells, genetic models, molecular sequence data, open reading frames, phylogeny, point mutation, tertiary protein structure, genetic templates.

Sultana, N., K. Nag, K. Hoshijima, D.W. Laird, A. Kawakami, and S. Hirose (2008). **Zebrafish early cardiac connexin, Cx36.7/Ecx, regulates myofibril orientation and heart morphogenesis by establishing Nkx2.5 expression**. *Proceedings of the National Academy of Sciences, USA* 105(12): 4763-4768. ISSN: 0027-8424.

NAL Call Number: 500N21P

Descriptors: zebrafish, cardiac connexin Ecx, signaling, Nkx2.5 expression, unidirectional, parallel, alignment, myofibrils, heart morphogenesis.

Sumanas, S. and S. Lin (2006). **Ets1-related protein is a key regulator of vasculogenesis in zebrafish.** *Public Library of Science Biology* 4(1): E10. ISSN: 1544-9173.

NAL Call Number: QH301.P56

Abstract: During embryonic development, multiple signaling pathways control specification, migration, and differentiation of the vascular endothelial cell precursors, angioblasts. No single gene responsible for the commitment of mesenchymal cells to the angioblast cell fate has been identified as yet. Here we report characterization and functional studies of Etsrp, a novel zebrafish ETS domain protein. etsrp embryonic expression is only restricted to vascular endothelial cells and their earliest precursors. Morpholino knockdown of Etsrp protein function resulted in the complete absence of circulation in zebrafish embryos. Angioblasts in etsrp-morpholino-injected embryos (morphants) failed to undergo migration and differentiation and did not coalesce into functional blood vessels. Expression of all vascular endothelial molecular markers tested was severely reduced in etsrp morphants, whereas hematopoietic markers were not affected. Overexpression of etsrp RNA caused multiple cell types to express vascular endothelial markers. etsrp RNA restored expression of vascular markers in cloche mutants, defective in hematopoietic and endothelial cell formation, arguing that etsrp functions downstream of cloche in angioblast formation. etsrp gene function was also required for endothelial marker induction by the vascular endothelial growth factor (vegf) and stem cell leukemia (scl/tal1). These results demonstrate that Etsrp is necessary and sufficient for the initiation of vasculogenesis.

Descriptors: zebrafish, basic helix loop helix transcription factors biosynthesis, blood vessels embryology, vascular endothelium embryology, GATA1 transcription factor biosynthesis, molecular sequence data, proto oncogene proteins biosynthesis, signal transduction physiology, vascular endothelial growth factor receptor 2 biosynthesis.

Notes: Comment in: PLoS Biol. 2006 Jan; 4 (1):e24

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Sun, L., Z. Zou, P. Collodi, F. Xu, X. Xu, and Q. Zhao (2005). **Identification and characterization of a second fibronectin gene in zebrafish.** *Matrix Biology* 24(1): 69-77. ISSN: 0945-053X.
NAL Call Number: QP552.C6

Abstract: Fibronectin (FN) is a highly conserved extracellular matrix protein that plays crucial roles in vertebrate embryogenesis. Previously, it was reported that zebrafish possess a single FN gene (fn1a). Here we report the presence of a second zebrafish FN gene (fn1b) that encodes a protein with a predicted primary structure that is similar to FNs identified in other vertebrates possessing 12 type I, 2 type II and 17 type III repeats including two alternative splice sites (EIIIA and EIIIB) and a variable region (V). Zebrafish FN1b exhibits 62.0% amino acid identity with zebrafish FN1a, 54% with human and 55% with Xenopus laevis FNs respectively. Employing RT-PCR analysis, we demonstrate that EIIIB- and V- isoforms are produced by alternative splicing of a single fn1b transcript. The FN1b EIIIA- isoform was not detected in zebrafish embryos or adult tissues nor were EIIIA, EIIIB or V region splice variants of fn1a found. FN1b mRNA was detected by RT-PCR in embryos at the gastrula-stage (8hpf) through 72hpf and in various adult tissues. EIIIB- and V+ are the predominant forms of FN1b present in the zebrafish embryo. Unlike FN1a, which is present at a relatively high amount at the embryonic stages before gastrulation, the FN1b isoforms are present at very low amounts at the early cleavage stage. The presence of multiple isoforms of FN1b along with a different pattern of expression compared to FN1a indicates that the two fn genes have separate roles in zebrafish development.

Descriptors: zebrafish, fibronectins chemistry and genetics, alternative splicing, amino acid sequence, molecular cloning, conserved sequence, complementary DNA metabolism, exons, genome, molecular sequence data, phylogeny, protein isoforms, tertiary protein structure, RNA metabolism, reverse transcriptase PCR, DNA sequence analysis, amino acid sequence homology, time factors, *Xenopus.*

Sun, S.N., Y.H. Gui, Y.X. Wang, L.X. Qian, Q. Jiang, D. Liu, and H.Y. Song (2007). **Effect of dihydrofolate reductase gene knock-down on the expression of heart and neural crest derivatives expressed transcript 2 in zebrafish cardiac development.** *Chinese Medical Journal* 120(13): 1166-71. ISSN: 0578 1337.

Abstract: BACKGROUND: Folic acid is very important for embryonic development and dihydrofolate reductase is one of the key enzymes in the process of folic acid performing its biological function. Therefore, the dysfunction of dihydrofolate reductase can inhibit the function of folic acid and finally cause the developmental malformations. In this study, we observed the abnormal cardiac phenotypes in dihydrofolate reductase (DHFR) gene knock-down zebrafish embryos, investigated the effect of DHFR on the expression of heart and neural crest derivatives expressed transcript 2 (HAND2) and explored the possible mechanism of DHFR knock-down inducing zebrafish cardiac malformations. METHODS: Morpholino oligonucleotides were microinjected into fertilized eggs to knock down the functions of DHFR or HAND2. Full length of HAND2 mRNA which was transcribed in vitro was microinjected into fertilized eggs to overexpress HAND2. The cardiac morphologies, the heart rates and the ventricular shortening fraction were observed and recorded under the microscope at 48 hours post fertilization. Whole-mount in situ hybridization and real-time PCR were performed to detect HAND2 expression. RESULTS: DHFR or HAND2 knock-down caused the cardiac malformation in zebrafish. The expression of HAND2 was obviously reduced in DHFR knock-down embryos ($P < 0.05$). Microinjecting HAND2 mRNA into fertilized eggs can induce HAND2 overexpression. HAND2 overexpression rescued the cardiac malformation phenotypes of DHFR knock-down embryos. CONCLUSIONS: DHFR plays a crucial role in cardiac development. The down-regulation of

HAND2 caused by DHFR knock-down is the possible mechanism of DHFR knock-down inducing the cardiac malformation.
Descriptors: zebrafish, basic helix loop helix transcription factors genetics, heart embryology, congenital heart defects etiology, tetrahydrofolate dehydrogenase physiology, basic helix loop helix transcription factors physiology, tetrahydrofolate dehydrogenase genetics.

Sun, Z., A. Amsterdam, G.J. Pazour, and N. Hopkins (2004). **A genetic screen in zebrafish identifies cilia genes as a principal cause of cystic kidney and links the cilium to size control of epithelial tubes**. *Molecular Biology of the Cell* 15(Suppl. S): 359A. ISSN: 1059 1524.
NAL Call Number: QH604.C452
Descriptors: zebrafish, methods and techniques, molecular genetics, urinary system, polycystic kidney disease, urologic, genetic disease, congenital disease, insertional mutagenesis, genetic techniques, cystogenesis.

Sun, Z., P. Jin, T. Tian, Y. Gu, Y.G. Chen, and A. Meng (2006). **Activation and roles of ALK4/ALK7-mediated maternal TGFbeta signals in zebrafish embryo.** *Biochemical and Biophysical Research Communications* 345(2): 694-703. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: Activin, Nodal, and Vg1, members of the transforming growth factor beta (TGFbeta) superfamily, transduce signal through type I receptors ALK4 or ALK7 and play important roles in mesoderm induction and patterning during vertebrate embryogenesis. However, the timing and magnitude of the ALK4/ALK7-mediated maternal TGFbeta signals are not clear. SB-431542 is identified as an inhibitor of the ALK4/ALK5/ALK7-mediated TGFbeta signals and its specificity in vertebrate embryos has not been reported. We demonstrate that SB-431542 is able to specifically and reproducibly block the Smad2/3-mediated TGFbeta signals in zebrafish embryo. Embryos exposed to SB-431542 exhibit various defects phenocopying Nodal-deficient mutants. SB-431542 treatments starting at different cell cycles before the midblastula transition lead to different degrees of developmental defects in mesoderm induction and patterning, suggesting that maternal TGFbeta signals are activated right after fertilization and required for mesoderm formation and patterning.
Descriptors: zebrafish, activin receptors metabolism, benzamides pharmacology, cell cycle drug effects, dioxoles pharmacology, developmental gene expression regulation drug effects, signal transduction drug effects, transforming growth factor beta metabolism, blastula physiology, cell cycle physiology, mesoderm drug effects and metabolism, mutation genetics, signal transduction physiology, Smad2 and Smad3 protein metabolism, transforming growth factor beta antagonists and inhibitors and genetics, vertebrates embryology and genetics.

Sundstrom, G., T.A. Larsson, S. Brenner, B. Venkatesh, and D. Larhammar (2005). **Ray-fin fish tetraploidization gave rise to pufferfish duplicates of NPY and PYY, but zebrafish NPY duplicate was lost.** *Annals of the New York Academy of Sciences* 1040: 476-8. ISSN: 0077 8923.
NAL Call Number: 500 N484
Abstract: We have used sequence information and gene location to identify NPY family genes in the pufferfish, *Takifugu rubripes* (fugu), and zebrafish. Fugu has two copies of NPY, presumably resulting from the ray-fin fish tetraploidization. Zebrafish has probably lost one of the copies. Both species have two copies of PYY, the second of which was previously named PY. The two fugu NPY genes are predominantly expressed in brain. The two PYY genes are expressed in a broad range of tissues including brain and gonads. Thus, the NPY system appears to be more complex in teleosts than in tetrapods.

Descriptors: zebrafish, duplicate neuropeptide Y genetics, peptide YY genetics, *Takifugu* genetics, polyploidy, species specificity.

Sussman, R. (2007). **DNA repair capacity of zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 104(33): 13379-83. ISSN: 0027 8424.
NAL Call Number: 500N21P
Abstract: Damage to the genome is unavoidable in living creatures, because of sunlight exposure as well as environmental chemicals present in food and drinking water. There is a need to monitor and purify the drinking water; therefore, several methods of detection have been developed. A very promising model system for this purpose is the zebrafish (Danio rerio), which is endowed with special qualities for detecting external as well as internal abnormalities. Grossman and Wei's assay [Grossman L, Wei Q (1995) Clin Chem 12:1854-1863], which measures the expression level of a nonreplicating recombinant plasmid DNA containing a UV-damaged luciferase reporter gene, shows that zebrafish can repair chromosomal lesions to a much greater extent than the human population. This vertebrate model is still very promising after possible down-regulation of the DNA repair enzymes.
Descriptors: zebrafish, DNA, repair, radiation, chemicals, luciferase reporter gene.

Sutherland, V., K. Brannen, M.A. Jacobs, T.L. Danberry, S.L. Rayhon, P. Garrison-Borowski, C.L. Goodwin, L. Olitan, K.J.M. Panzica, R. Seethala, S. Sloan, U. Hanumegowda, and K.A. Augustine (2006). **Assessment of rodent whole embryo and zebrafish embryo culture assays as a predictive model for midline facial defects**. *Birth Defects Research* 76(5): 398. ISSN: 1542 0752.
NAL Call Number: QL991.T4
Descriptors: zebrafish, toxicology, development, midline facial defect, predictive model, cell culture assays, congenital disease.

Sutter, S.B., M.O. Raeker, A.B. Borisov, and M.W. Russell (2004). **Orthologous relationship of obscurin and Unc-89: phylogeny of a novel family of tandem myosin light chain kinases**. *Development Genes and Evolution* 214(7): 352-359. ISSN: 0949-944X.
NAL Call Number: QL951.R68
Descriptors: zebrafish, amino acid sequence, cardiac muscle, conserved sequence, cytoskeleton, DNA, evolution, exons, freshwater fish, gene families, immunoglobulins, light chains, myocytes, myofibrils, myosin-light-chain kinase, phylogenetics, phylogeny, protein interaction, signal transduction, skeletal muscle, *Caenorhabditis elegans*, *Danio rerio*, *Drosophila* , *Drosophila melanogaster*, developmental genetics.

Suzuki, T., Y. Takagi, H. Osanai, L. Li, M. Takeuchi, Y. Katoh, M. Kobayashi, and M. Yamamoto (2005). **Pi class glutathione S-transferase genes are regulated by Nrf 2 through an evolutionarily conserved regulatory element in zebrafish.** *Biochemical Journal, The* 388(Pt 1): 65-73. ISSN: 0264 6021.
NAL Call Number: 382B52
Abstract: Pi class GSTs (glutathione S-transferases) are a member of the vertebrate GST family of proteins that catalyse the conjugation of GSH to electrophilic compounds. The expression of Pi class GST genes can be induced by exposure to electrophiles. We demonstrated previously that the transcription factor Nrf 2 (NF-E2 p45-related factor 2) mediates this induction, not only in mammals, but also in fish. In the present study, we have isolated the genomic region of zebrafish containing the genes gstp1 and gstp2. The regulatory regions of zebrafish gstp1 and gstp2 have been examined by GFP (green fluorescent protein)-reporter

gene analyses using microinjection into zebrafish embryos. Deletion and point-mutation analyses of the gstp1 promoter showed that an ARE (antioxidant-responsive element)-like sequence is located 50 bp upstream of the transcription initiation site which is essential for Nrf 2 transactivation. Using EMSA (electrophoretic mobility-shift assay) analysis we showed that zebrafish Nrf 2-MafK heterodimer specifically bound to this sequence. All the vertebrate Pi class GST genes harbour a similar ARE-like sequence in their promoter regions. We propose that this sequence is a conserved target site for Nrf 2 in the Pi class GST genes.
Descriptors: zebrafish, Pi class glutathione S-transferase genes, Nrf 2, EMSA, electrophoretic mobility-shift assay.

Suzuki, T., A.S. Srivastava, H. Hashimoto, and T. Kurokawa (2006). **Structural comparison of promoter and coding sequence of type I collagen alpha 1 chain gene duplicates between zebrafish and flounder/fugu lineages.** *Comparative Biochemistry and Physiology D Genomics and Proteomics* 1(1): 20-27. ISSN: 1744 117X.
Descriptors: zebrafish, *Danio rerio*, *Oncorhynchus mykiss*, *Paralichthys olivaceus, Takifugu rubripes, Tetraodon nigroviridis*, nucleic acids, type 1 collagen alpha 1 gene duplicates, proteins, molecular genetics, structural comparison of promoter and coding sequence.

Szabo, M., F. Muller, J. Kiss, C. Balduf, U. Strahle, and F. Olasz (2003). **Transposition and targeting of the prokaryotic mobile element IS30 in zebrafish.** *Federation of European Biochemical Societies Journal* 550(1-3): 46-50. ISSN: 1742 464X.
NAL Call Number: QP501.E8
Abstract: We provide evidence that a prokaryotic insertion sequence (IS) element is active in a vertebrate system. The transposase of Escherichia coli element IS30 catalyzes both excision and integration in extrachromosomal DNA in zebrafish embryos. The transposase has a pronounced target preference, which is shown to be modified by fusing the enzyme to unrelated DNA binding proteins. Joining the transposase to the cI repressor of phage lambda causes transposition primarily into the vicinity of the lambda operator in *E. coli*, and linking to the DNA binding domain of Gli1 also directs the recombination activity of transposase near to the Gli1 binding site in zebrafish. Our results demonstrate the possibility of fusion transposases to acquire novel target specificity in both prokaryotes and eukaryotes.
Descriptors: zebrafish, DNA transposable elements , DNA binding proteins genetics, proteins, binding sites, DNA metabolism *Escherichia coli* genetics, genetic techniques, HeLa cells, oncogene proteins genetics and metabolism, prokaryotic cells physiology, genetic recombination, repressor proteins genetics and metabolism, substrate specificity, trans activators, transcription factors genetics and metabolism, transposases genetics and metabolism, viral proteins.

Szeto, D.P. and D. Kimelman (2006). **The regulation of mesodermal progenitor cell commitment to somitogenesis subdivides the zebrafish body musculature into distinct domains.** *Genes and Development* 20(14): 1923-32. ISSN: 0890-9369.
NAL Call Number: QH426.G46
Abstract: The vertebrate musculature is produced from a visually uniform population of mesodermal progenitor cells (MPCs) that progressively bud off somites populating the trunk and tail. How the MPCs are regulated to continuously release cells into the presomitic mesoderm throughout somitogenesis is not understood. Using a genetic approach to study the MPCs, we show that a subset of MPCs are set aside very early in zebrafish development, and programmed to cell-autonomously enter the tail domain beginning with the 16th somite. Moreover, we show that the trunk is subdivided into two domains, and that entry into the

anterior trunk, posterior trunk, and tail is regulated by interactions between the Nodal and bone morphogenetic protein (Bmp) pathways. Finally, we show that the tail MPCs are held in a state we previously called the Maturation Zone as they wait for the signal to begin entering somitogenesis.

Descriptors: zebrafish, mesoderm cytology, muscles embryology, stem cells physiology, body patterning physiology, bone morphogenetic proteins genetics and metabolism, developmental gene expression regulation, mesoderm metabolism, muscles cytology, signal transduction, skeleton, somites, tail embryology, transforming growth factor beta genetics and metabolism.

Tada, M.N., K. Senzaki, Y. Tai, H. Morishita, Y.Z. Tanaka, Y. Murata, Y. Ishii, S. Asakawa, N. Shimizu, H. Sugino, and T. Yagi (2004). **Genomic organization and transcripts of the zebrafish protocadherin genes.** *Gene* 340(2): 197-211. ISSN: 0378-1119.

NAL Call Number: QH442.A1G4

Abstract: We have examined the protocadherin (Pcdh) gene clusters of the zebrafish (*Danio rerio*). At least three sets of the Pcdh gene cluster were found in the zebrafish genome. Here, we describe the complete organization of the DrPcdh2 gene clusters. Classification by phylogenetic and transcript analyses revealed 7 DrPcdh2omicron, 20 DrPcdh2alphaa, 12 DrPcdh2alphab, and 1 DrPcdh2alphac variable exons upstream of the DrPcdh2alpha constant region exons in the DrPcdh2 gene cluster. The constant regions of the DrPcdh1alpha and DrPcdh2alpha genes in zebrafish were orthologs of those of the mammalian Pcdhalpha. These exons all encoded plural PXXP motifs in their cytoplasmic tails. The sequences of the variable exons were highly conserved within each family: DrPcdh2omicron, DrPcdh2alphaa, and DrPcdh2alphab. Transcript analysis revealed that zebrafish Pcdhs had alternatively spliced variants in the constant region that were not found in mammals. More gene clusters, more variable exons, and more alternative splicing variants were found in zebrafish than in mammals. Thus, although the Pcdhalpha families were common to diverse vertebrates, their gene number, structure, and transcripts were different between teleosts and mammals.

Descriptors: zebrafish, cadherins genetics, protein precursors genetics, genetic transcription genetics, alternative splicing, amino acid and base sequence, molecular cloning, DNA chemistry, DNA genetics, DNA isolation and purification, complementary DNA chemistry, genetics, isolation and purification, exons genetics, mice, molecular sequence data, multigene family genetics, phylogeny , protein isoforms genetics, radiation hybrid mapping, sequence alignment, DNA sequence analysis, amino acid sequence homology, *Takifugu* genetics.

Takagi, Y., M. Kobayashi, L. Li, T. Suzuki, K. Nishikawa, and M. Yamamoto (2004). **MafT, a new member of the small Maf protein family in zebrafish.** *Biochemical and Biophysical Research Communications* 320(1): 62-9. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: Small Maf proteins play critical roles on morphogenesis and homeostasis through associating with CNC proteins. To date, three small Maf proteins, MafF, MafG, and MafK, have been reported in vertebrates, which share redundant functions. In this study, we tried to identify and characterize small Maf proteins in zebrafish to elucidate their conservation and diversity in the fish kingdom. We identified homolog genes of MafG and MafK but not MafF in zebrafish, indicating the former two are conserved among vertebrates. In addition, a novel type of small Maf protein MafT was identified. MafT protein bound MARE sequence as a homodimer or heterodimers with zebrafish Nrf2 or p45 Nfe2. Co-overexpression of MafT and Nrf2 synergistically activated MARE-mediated gene expression in zebrafish embryos. These results indicated that MafT is a new member of small Maf proteins and

involved in the Nrf2-dependent gene regulation in cellular defense system.
Descriptors: zebrafish, enzymologic gene expression regulation physiology, protein sequence analysis, transcription factors chemistry, transcription factors metabolism, proteins chemistry, amino acid sequence, cultured cells, conserved sequence, DNA binding proteins genetics, proteins metabolism, Maf transcription factors, small, molecular sequence data, nf e2 related factor 2, phylogeny, amino acid sequence homology, trans activators metabolism, transcription factors genetics.

Takahashi, N. and I.B. Dawid (2005). **Characterization of zebrafish Rad52 and replication protein A for oligonucleotide-mediated mutagenesis**. *Nucleic Acids Research* 33(13): e120. ISSN: 0305 1048.
NAL Call Number: QD341.A2N8
Abstract: Zebrafish has become a favorite model organism not only in genetics and developmental biology, but also for the study of cancer, neuroscience and metabolism. However, strategies for reverse genetics in zebrafish are mostly limited to the use of antisense oligonucleotides, and therefore the development of other targeting methods is highly desirable. Here, we report an approach to gene targeting in this system in which single-stranded oligonucleotides and zebrafish Rad52 protein are employed. It has been proposed that a single-stranded oligonucleotide containing a mutation can be incorporated into the genome by annealing to the single-stranded region of the lagging strand of the replication fork. Rad52 is expected to accelerate the annealing step. In vitro experiments using purified truncated Rad52 proteins and replication protein A (RPA) showed that annealing of oligonucleotides is accelerated by Rad52 in the presence of RPA. We developed a simple and sensitive PCR-based method to detect point mutations in the genome. In exploratory experiments, we found that microinjection of single-stranded oligonucleotide targeted to a specific gene together with truncated Rad52 into zebrafish embryos resulted in a low level of recombinant copies in 3 of the 80 embryos tested under these conditions. Copyright © 2008 CABI
Descriptors: zebrafish embryos, gene expression, genes, genomes, methodology, mutagenesis, mutations, oligonucleotides, PCR, proteins, *Danio rerio.*

Takayama, S., U. Hostick, M. Haendel, J. Eisen, and B. Darimont (2008). **An F-domain introduced by alternative splicing regulates activity of the zebrafish thyroid hormone receptor alpha.** *General and Comparative Endocrinology* 155(1): 176-89. ISSN: 0016-6480.
NAL Call Number: 444.8G28
Abstract: Thyroid hormones (THs) play an important role in vertebrate development; however, the underlying mechanisms of their actions are still poorly understood. Zebrafish (*Danio rerio*) is an emerging vertebrate model system to study the roles of THs during development. In general, the response to THs relies on closely related proteins and mechanisms across vertebrate species, however some species-specific differences exist. In contrast to mammals, zebrafish has two TRalpha genes (thraa, thrab). Moreover, the zebrafish thraa gene expresses a TRalpha isoform (TRalphaA1) that differs from other TRs by containing additional C-terminal amino acids. C-terminal extensions, called "F domains", are common in other members of the nuclear receptor superfamily and modulate the response of these receptors to hormones. Here we demonstrate that the F-domain constrains the transcriptional activity of zebrafish TRalpha by altering the selectivity of this receptor for certain coactivator binding motifs. We found that the F-domain of zebrafish TRalphaA1 is encoded on a separate exon whose inclusion is regulated by alternative splicing, indicating a regulatory role of the F-domain in vivo. Quantitative expression analyses revealed that TRalphaA1 is primarily expressed in reproductive organs whereas TRalphaB and the TRalphaA isoform that

lacks the F-domain (TRalphaA1-2) appear to be ubiquitous. The relative expression levels of these TRalpha transcripts differ in a tissue-specific manner suggesting that zebrafish uses both alternative splicing and differential expression of TRalpha genes to diversify the cellular response to THs.
Descriptors: zebrafish, thyroid hormone, receptor alpha, tralpha transcripts, f-domain.

Takechi, M. and S. Kawamura (2005). **Temporal and spatial changes in the expression pattern of multiple red and green subtype opsin genes during zebrafish development.** *Journal of Experimental Biology* 208(Pt 7): 1337-45. ISSN: 0022-0949.
NAL Call Number: 442.8B77
Abstract: Zebrafish have two red, LWS-1 and LWS-2, and four green, RH2-1, RH2-2, RH2-3 and RH2-4, opsin genes encoding photopigments with distinct absorption spectra. Occurrence of opsin subtypes by gene duplication is characteristic of fish but little is known whether the subtypes are expressed differently in the retina, either spatially or temporally. Here we show by in situ hybridization the dynamic expression patterns of the opsin subtypes in the zebrafish retina. Expression of red type opsins is initiated with the shorter-wavelength subtype LWS-2, followed by the longer-wavelength subtype LWS-1. In the adult retina, LWS-2 was expressed in the central to dorsal area and LWS-1 in the ventral and peripheral areas. Expression patterns of green type opsins were similar to those of the red type opsins. The expression started with the shortest wavelength subtype RH2-1 followed by the longer wavelength ones, and in the adult retina, the shorter wavelength subtypes (RH2-1 and RH2-2) were expressed in the central to dorsal area and longer wavelength subtypes (RH2-3 and RH2-4) in the ventral and peripheral areas. These results provide the framework for subsequent studies of opsin gene regulation and for probing functional rationale of the developmental changes by using the power of zebrafish genetics.
Descriptors: zebrafish, developmental gene expression regulation, opsin metabolism, retina metabolism, photopigments and genetics, visual opsin genes, duplicate in situ hybridization.

Takechi, M. and S. Kawamura (2004). **Differential expression of duplicated visual opsin genes in zebrafish retina**. *Zoological Science* 21(12): 1346-1347. ISSN: 0289 0003.
NAL Call Number: 442P92
Descriptors: zebrafish, development, molecular genetics, sense organs, sensory reception, visual opsin genes, retina.

Takeda, H. and Y. Saga (2004). **Somite segmentation: a view from fish**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd. Singapore, Singapore , p. 261-293. ISBN: 9812388214.
NAL Call Number: QL638.C94 F57
Abstract: Somite formation, a process in which reiterated epithelial structures are progressively demarcated from the mesenchymal presomitic mesoderm (PSM) in a anterior-posterior sequence, is the earliest manifestation of segmentation and is a feature shared by all vertebrate embryos. The temporal and spatial regulation of this process requires a molecular oscillator, the segmentation clock. The mechanisms driving and regulating the oscillation in PSM cells have been actively studied in zebrafish, chick and mouse. The oscillator is comprised of genetic circuit involving the Notch signaling pathway and its target genes her1 and her7 in zebrafish. Converting clock oscillation into the periodic arrangement of segment boundaries is achieved at the 'wavefront' located in the anterior PSM. The level of Fgf/MAPK activation (highest in the posterior PSM) serves as a positional cue within the PSM to restrict the wave-

front to the anterior PSM. Once the level of Fgf/MAPK signaling declines in the anterior PSM, the wavefront activity mediated by a transcription factor, Fss/Tbx24, arrests the oscillation and leads to activation of a number of key genes required for subsequent sequences of somite formation. In the anterior PSM or wavefront, a complicated gene network centered on Mesp, a bHLH transcription factor, finally establishes a rostrocaudal subdivisions within somite primordium, which is prerequisite for formation of morphological distinct somite boundaries. Copyright © 2008 CABI
Descriptors: mutant zebrafish, embryonic development, embryos, gene expression, genes, signal transduction, transcription factors, *Danio rerio*.

Takeda, H., T. Yoshida, N. Matsuda, and M. Mishina (2006). **Inducible expression of dominant-negative creb by tet-on system suppressed lens differentiation during zebrafish development**. *Journal of Pharmacological Sciences* 100(Suppl. 1): 292P. ISSN: 1347 8613.
Descriptors: zebrafish, development, molecular genetics, marine ecology, environmental sciences.

Tallafuss, A., L.A. Hale, Y.L. Yan, L. Dudley, J.S. Eisen, and J.H. Postlethwait (2006). **Characterization of retinoid-X receptor genes rxra, rxrba, rxrbb and rxrg during zebrafish development.** *Gene Expression Patterns* 6(5): 556-65. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: During development of vertebrate embryos, retinoic acid plays a variety of roles that are mediated by binding to retinoic acid receptors (Rars) and their heterodimerization partners, the retinoid receptors (Rxrs). Here, we characterize the expression patterns of four zebrafish rxr genes during development and provide an analysis of the phylogenetic relationships between zebrafish and tetrapod Rxr genes based on sequence similarities and conserved syntenies. This analysis prompted the renaming of several of the zebrafish rxr genes to match their tetrapod orthologs. Understanding phylogenetic relationships among Rxr genes and their expression patterns during development provides a foundation for future studies of Rxr functions.
Descriptors: zebrafish, retinoid X receptors genetics, base sequence, molecular cloning, DNA primers, gene expression profiling, in situ hybridization, phylogeny.

Tamimi, Y., J.M. Skarie, T. Footz, F.B. Berry, B. Link, and M.A. Walter (2006). **FGF19 is a target for FOXC1 regulation in ciliary body-derived cells**. *Human Molecular Genetics* 15(21): 3229-3240. ISSN: 0964 6906.
NAL Call Number: RB155.5.H87
Abstract: The forkhead C1 (FOXC1) transcription factor is involved in the development and regulation of several organs, including the eye, where FOXC1 alterations cause iris, trabecular meshwork and corneal anomalies. Using nickel agarose chromatin enrichment with human anterior segment cells, we previously identified the fibroblast growth factor 19 (FGF19) locus as a gene potentially regulated by FOXC1. Here, we demonstrate that FGF19 is a direct target of FOXC1 in the eye. FOXC1 positively regulates FGF19 expression in corneal and periocular mesenchymal cells in cell culture and in zebrafish embryos. Through the FGFR4 tyrosine kinase, FGF19 promotes MAPK phosphorylation in the developing and mature cornea. During development, loss of either FOXC1 or FGF19 results in complementary, but distinct, anterior segment dysgeneses. This study reveals an important role for FOXC1 in the direct regulation of the FGF19-FGFR4-MAPK pathway to promote both the development and maintenance of anterior segment structures within the eye. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, molecular biophysics, sense organs, sensory reception, eye development, iris, trabecular and corneal anomalies, FgF19, dysgeneses, FOXC1.

Tamura, M., M. Kajikawa, and N. Okada (2007). **Functional splice sites in a zebrafish LINE and their influence on zebrafish gene expression.** *Gene* 390(1-2): 221-31. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: Long interspersed elements (LINEs) are transposable elements that exist in many kinds of eukaryotic genomes, where they have a large effect on genome evolution. There are several thousands to hundreds of thousands of LINE copies in each eukaryotic genome. LINE elements are amplified by a mechanism called retrotransposition, in which a LINE-encoded protein reverse transcribes (copies) its own RNA. We previously isolated two retrotransposition-competent LINEs, ZfL2-1 and ZfL2-2, from zebrafish. Although it has generally been thought that LINEs do not have 'introns' (because the LINE RNA is used as the template during retrotransposition), we now show that these two LINEs contain multiple putative functional splice sites. We further show that at least one pair of these splice sites is actually functional in zebrafish cells. Moreover, some of these splice sites are coupled with the splicing signal of a host endogenous gene, thereby generating a new chimeric spliced mRNA variant for this gene. Our results suggest the possible role of these LINE splice sites in modulating retrotransposition and host gene expression.
Descriptors: zebrafish, long interspersed nucleotide elements, RNA splice sites, base sequence, cell line, chimera genetics, DNA genetics, genetic databases, expressed sequence tags, gene expression, HeLa cells, introns, molecular sequence data, RNA genetics, sequence deletion, nucleic acid sequence homology.

Tanaka, M., D. Murayama, M. Nagashima, T. Higashi, K. Mawatari, T. Matsukawa, and S. Kato (2007). **Purpurin expression in the zebrafish retina during early development and after optic nerve lesion in adults.** *Brain Research* 1153: 34-42. ISSN: 0006-8993.
NAL Call Number: QP376
Abstract: Purpurin, a retina-specific protein, is known to play a role in cell adhesion during development of the chicken retina. Although purpurin has been significantly detected in adult chicken retina, its function in the matured retina is not well understood. Therefore, to determine the expression pattern of purpurin in the retina, we simultaneously investigated expression patterns of purpurin in the zebrafish retina during development in larvae and optic nerve regeneration after nerve transection in adults. In early development, levels of purpurin suddenly increased in the zebrafish retina 3 to 5 days after fertilization, and purpurin-positive immunoreactivity was diffusely located in all retinal layers. In contrast, levels of purpurin mRNA rapidly increased in the adult retina 1-3 days after optic nerve transection, and rapidly declined by 10 days after injury. Signal for purpurin mRNA was seen only in photoreceptors. Immunohistochemistry showed that levels of purpurin protein were also increased in the retina 1-3 days after nerve injury, but positive staining was located in photoreceptors and ganglion cells, and the staining in ganglion cells was stronger than that in photoreceptors. Thus, the transient expression of purpurin protein was greatly different during development and optic nerve regeneration. In the former, purpurin may be required in all retinal layers, whereas in the latter, purpurin may be required for injured ganglion cells.
Descriptors: adult zebrafish, purpurin in retina, expression, early eye development, optic nerve, lesion.

Tang, R., A. Dodd, D. Lai, W.C. McNabb, and D.R. Love (2007). **Validation of zebrafish (*Danio rerio*) reference genes for quantitative real-time RT-PCR normalization.** *Acta Biochimica Et Biophysica Sinica* 39(5): 384-90. ISSN: 1672 9145.

Abstract: The normalization of quantitative real time RT-PCR (qRT-PCR) is important to obtain accurate gene expression data. The most common method for qRT-PCR normalization is to use reference, or housekeeping genes. However, there is emerging evidence that even reference genes can be regulated under different conditions. qRT-PCR has only recently been used in terms of zebrafish gene expression studies and there is no validated set of reference genes. This study characterizes the expression of nine possible reference genes during zebrafish embryonic development and in a zebrafish tissue panel. All nine reference genes exhibited variable expression. The beta-actin, EF1alpha and Rpl13alpha genes comprise a validated reference gene panel for zebrafish developmental time course studies, and the EF1alpha, Rpl13alpha and 18S rRNA genes are more suitable as a reference gene panel for zebrafish tissue analysis. Importantly, the zebrafish GAPDH gene appears unsuitable as reference gene for both types of studies.

Descriptors: zebrafish, developmental gene expression regulation, reverse transcriptase PCR methods, actins metabolism, DNA primers chemistry, complementary DNA metabolism, genetic techniques, nucleic acid hybridization, peptide elongation factor 1 metabolism, RNA metabolism, reference values, time factors.

Tang, X., S. Maegawa, E.S. Weinberg, and I.J. Dmochowski (2007). **Regulating gene expression in zebrafish embryos using light-activated, negatively charged peptide nucleic acids.** *Journal of the American Chemical Society* 129(36): 11000-010. ISSN: 0002-7863.

NAL Call Number: 381 AM33

Descriptors: zebrafish, developmental gene expression regulation drug effects, glycoproteins genetics , intercellular signaling peptides and proteins gentics, peptide nucleic acids chemistry and pharmacology, glycoproteins metabolism, intercellular signaling peptides and proteins metabolism, light, peptide nucleic acids radiation effects, RNA.

Tani, H., T. Yoshida, and M. Mishina (2006). **Molecular cloning and expression patterns of zebrafish Syncam1 genes**. *Journal of Pharmacological Sciences* 100(Suppl. 1): 246P. ISSN: 1347 8613.

Descriptors: zebrafish, nervous system, neural coordination, molecular genetics, cloning, genetic techniques, reverse transcriptase PCR, genetic techniques, in situ hybridization, histology and cytology techniques, genetic techniques, synapse formation.

Tasinato, A., D. Belet, F.L. Munier, S. Neuhauss, and D.F. Schorderet (2005). **Knockdown of TGFBI/BIGH3 corneal dystrophy-linked gene leads to developmental impairment in zebrafish**. *Investigative Ophthalmology & Visual Science* 46(Suppl. S): 4934. ISSN: 0146 0404.

Abstract: Purpose: Over 25 specific mutations in the Transforming Growth Factor beta-induced gene (TGFBI, BIGH3) are so far known to be associated with 13 different inherited clinically and histopathologically distinct phenotypes of corneal dystrophies. Despite the ubiquitous expression of the encoded keratoepithelin (KE), no other specific disease has been described in tissues other than the cornea. KE plays an important role in the extra cellular matrix, where it is mainly found and interfere with cell adhesion processes by interacting with specific integrins. Until now, no loss-of-function mutation has been observed and itwould be useful to know the exact function of BIGH3. In mouse embryo, KE is expressed mainly in tissues derived from the mesoderm, including developing bones and cartilages.

Still the exact function of KE remains elusive. Our aim is to investigate the role of KE during development, using the zebrafish as model. Methods: We injected the mRNA inhibitor KE-morpholino into 1 to 2-cell zebrafish embryos. Standard control-morpholino was separately injected as negative control and chordin-morpholino as positive control. At 6 days post-fertilization, larvae were fixed in 4% paraformaldehyde and embedded either in Technovit and sectioned into 5 um slices for the morphology determination, or in sucrosefor cryosection and immunohistochemistry. Results: Hundreds of zebrafish embryos were microinjected using different concentrations of the various morpholinos. Injection efficacy was monitored according to the chordin morphological change. The appropriate non lethal dose for the KE-morpholino showed broad development retardation in KE morphants. The eye morphology appeared to be normal but smaller in size. Dorsal and caudal fins were underdeveloped. The notochord was smaller in size and the pericardium was enlarged. Conclusions: The effectsobserved in zebrafish were in agreement with expression studies in the mouse embryos. Silencing of the TGFBI/BIGH3 gene in this model lead to widespread development retardation, suggesting a primary role for KE. Fine analysis of the treated zebrafish will help elucidate the physiological role of TGFBI/BIGH3. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, molecular genetics, sensory reception, corneal dystrophy, eye disease , development retardation.

Taylor, M.R., S. Kikkawa, A. Diez Juan, V. Ramamurthy, K. Kawakami, P. Carmeliet, and S.E. Brockerhoff (2005). **The zebrafish pob gene encodes a novel protein required for survival of red cone photoreceptor cells.** *Genetics* 170(1): 263-73. ISSN: 0016-6731.
NAL Call Number: 442.8G28
Abstract: The zebrafish mutant, partial optokinetic response b (pob), was isolated using an N-ethyl N-nitrosourea (ENU)-based screening strategy designed to identify larvae with defective optokinetic responses in red but not white light. Previous studies showed that red-light blindness in pob is due to the specific loss of long-wavelength photoreceptor cells via an apoptotic mechanism. Here, we used positional cloning to identify the mutated pob gene. We find that pob encodes a highly conserved 30-kDa protein of unknown function. To demonstrate that the correct gene was isolated, we used the Tol2 transposon system to generate transgenic animals and rescue the mutant phenotype. The Pob protein contains putative transmembrane regions and protein-sorting signals. It is localized to the inner segment and synapse in photoreceptor cells, and when expressed in COS-7 cells it localizes to intracellular compartments. We also show that the degeneration of red cone photoreceptors in the mutants occurs independently of light. On the basis of our findings, we propose that Pob is not involved in phototransduction but rather plays an essential role in protein sorting and/or trafficking.
Descriptors: zebrafish, retina cones metabolism, membrane proteins genetics, amino acid sequence, COS cells, cell survival genetics, *Cercopithecus aethiops*, immunohistochemistry, in situ hybridization, membrane, mice, molecular sequence data, metabolism.

Te Velthuis, A.J., E.B. Ott, I.J. Marques, and C.P. Bagowski (2007). **Gene expression patterns of the ALP family during zebrafish development.** *Gene Expression Patterns* 7(3): 297-305. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: The actinin-associated LIM protein (ALP) genes belong to the PDZ/LIM protein family which is characterized by the presence of both a PDZ and a LIM domain. The ALP subfamily in mammals has four members: ALP, Elfin, Mystique and RIL. In this study, we have annotated and cloned the zebrafish ALP gene family and identified a zebrafish-specific

fifth member of the family, the alp-like gene. We compared the zebrafish sequences to their human and mouse orthologues. A phylogenetic analysis based on the amino acid sequences showed the overall high degree of conservation within the family. We describe here the expression patterns for all five ALP family genes during zebrafish development. Whole mount in situ hybridization results revealed common and distinct expression patterns for the five genes. With the exception of elfin, all genes were expressed as maternal RNAs at early developmental stages. Gene expression for all of them appeared regulated and localized in specific regions at the eight different developmental stages studied. Expression for all five genes was observed in the central nervous system (CNS), which led us to further investigate brain-specific expression in sections of embryos at 2 days of development. In summary, we identified the zebrafish orthologues of the ALP family and determined their gene expression patterns during zebrafish embryogenesis. Finally, we compare our results to the limited expression data available for this gene family during mammalian development.
Descriptors: zebrafish, developmental gene expression regulation, microfilament amino acid sequence, molecular cloning, embryonic development, gene expression profiling, molecular sequence data, phylogeny.

Teh, C., S.W. Chong, and V. Korzh (2003). **DNA delivery into anterior neural tube of zebrafish embryos by electroporation.** *BioTechniques* 35(5): 950-4. ISSN: 0736 6205.
NAL Call Number: RB37.1B56
Abstract: The zebrafish is widely used for functional studies of vertebrate genes. It is accessible to manipulations during all stages of embryogenesis because the embryo develops externally and is optically transparent. However, functional studies conducted on the zebrafish have been generally limited to the earliest phase of activity of the gene of interest, which is a limitation in studies of genes that are expressed at various stages of embryonic development. It is therefore necessary to develop methods that allow for the modulation of gene activity during later stages of zebrafish development while leaving earlier functions intact. We have successfully electroporated the green fluorescent protein (GFP) reporter gene into the neural tube of the zebrafish embryo in a unidirectional or bilateral manner. This approach can be used for the functional analysis of the late role of developmental genes in the neural tube of zebrafish embryo and larvae.
Descriptors: zebrafish, brain embryology, brain metabolism, DNA administration and dosage, DNA pharmacokinetics, electroporation instrumentation and methods, gene transfer techniques, DNA genetics, electrodes, equipment design, equipment failure analysis, green fluorescent luminescent proteins, tissue distribution.

Tendeng, C. and C. Houart (2006). **Cloning and embryonic expression of five distinct sfrp genes in the zebrafish *Danio rerio*.** *Gene Expression Patterns* 6(8): 761-71. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Recently, a new member of the secreted frizzled-related protein (sFRP) family, named tlc, has been identified as expressed by the anterior neural border (ANB) cells in the zebrafish *Danio rerio*. Tlc plays an important role in telencephalic induction and patterning. In absence of Tlc, formation of the telencephalon is severely delayed, but not abolished. This prompted us to clone the other zebrafish sfrp family members and analyse their expression patterns, in search of a family member that may partly functionally overlap with Tlc. Except sizzled, expression profile of sfrp genes in zebrafish has not been reported so far. Here, we describe the cloning of full-length cDNA for sfrp1a, sfrp1b, sfrp2, sfrp3 and sfrp5 gene transcripts and we examine their expression at different embryonic stages. Only sfrp1a is expressed in the anterior neural plate including the ANB cells where and when tlc

is expressed. Interestingly, compared to both tlc and sfrp1a, wnt genes are complementary expressed more posteriorly in the neural plate. Later, both sfrp1a and sfrp5 expression profiles are overlapping, in particular at pharyngula stage these genes are expressed in the ventral part of the forebrain, midbrain and hindbrain. sfrp1b, sfrp2 and sfrp3 are mainly expressed in mesodermal and endodermal embryonic tissues. Expression profiles of these different genes in zebrafish gave interesting clues on the possible function and evolution of sFRPs in zebrafish and other organisms.
Descriptors: zebrafish, molecular cloning methods, glycoproteins genetics, central nervous system embryology and metabolism, conserved sequence molecular evolution, gastrula metabolism, gene expression profiling, developmental gene expression regulation, glycoproteins metabolism, intercellular signaling peptides and proteins metabolism, phylogeny, somites metabolism, spinal cord embryology and metabolism.

Teng, P., Y. Cao, W.X. Wang, S.X. Zhu, A.M. Meng , and J.P. Zhang (2004). **The Shh promoter of zebrafish directs the expression of GFP in notochord**. *Acta Genetica Sinica* 31(1): 39-42. ISSN: 0379-4172.
Abstract: In vitro experiment showed that HNF3beta was the direct regulator of sonic hedgehog (shh) promoter. To investigate the activity of zebrafish shh promoter in vivo, we constructed the expression vector pShh-EGFP with ligating a 538 bp zebrafish shh promoter,which contained two HNF3beta binding sites, to EGFP. The pShh-EGFP DNA was microinjected into one-cell stage embryos of the zebrafish and the embryos were observed for GFP expression with fluorescent microscopy. GFP expression started during gastrulation in the axial hypoblast layer. During segmentation, GFP was detected in the notochord but not in the foor plate. Our experiment demonstrated that the 538 bp shh promoter containing two HNF3beta binding sites is able to confer the notochord-expressing activity.
Descriptors: zebrafish, luminescent notochord metabolism, promoter regions genetics, trans activators genetics, green fluorescent proteins, Hedgehog proteins.
Language of Text: Chinese.

Teraoka, H., W. Dong, Y. Tsujimoto, H. Iwasa, D. Endoh, N. Ueno, J.J. Stegeman, R.E. Peterson, and T. Hiraga (2003). **Induction of cytochrome P450 1A is required for circulation failure and edema by 2,3,7,8-tetrachlorodibenzo-p-dioxin in zebrafish.** *Biochemical and Biophysical Research Communications* 304(2): 223-8. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: The mechanism of toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is thought to result from changes in gene expression via the aryl hydrocarbon receptor (AHR). The induction of cytochrome P450 1A (CYP1A) in various organs is a cardinal effect of TCDD. However, whether CYP1A is involved in endpoints of TCDD toxicity is controversial. We investigated the role of CYP1A in TCDD-induced developmental toxicities using gene knock-down with morpholino antisense oligos. Exposure of zebrafish embryos to TCDD, at concentrations eliciting the hallmark endpoints of developmental toxicity, induced CYP1A in the heart and vascular endothelium throughout the body. This induction by TCDD was markedly inhibited by morpholinos to zebrafish arylhydrocarbon receptor 2 (zfAHR2-MO) and to zebrafish CYP1A (zfCYP1A-MO). The zfAHR2-MO but not the zfCYP1A-MO inhibited zfCYP1A mRNA expression, indicating the specificities of these morpholinos. Injection of either zfAHR2-MO or zfCYP1A-MO blocked the representative signs of TCDD developmental toxicity in zebrafish, pericardial edema and trunk circulation failure. The morpholinos appeared do not affect normal development in TCDD-untreated

embryos. These results suggest a mediatory role of zfCYP1A induction through zfAHR2 activation in causing circulation failure by TCDD in zebrafish. This is the first molecular evidence demonstrating an essential requirement for CYP1A induction in TCDD-evoked developmental toxicities in any vertebrate species.

Descriptors: zebrafish, blood circulation drug effects, cytochrome P450 enzyme system biosynthesis and physiology, teratogens toxicity, tetrachlorodibenzodioxin toxicity, chemically induced cardiomyopathies and pathology, cultured cells, cytochrome P450 enzyme system genetics, chemically induced edema, edema pathology, embryo anatomy and histology, embryo drug effects, antisense oligonucleotides pharmacology, pericardium pathology, aryl hydrocarbon receptors genetics and physiology.

Teraoka, H., C. Russell, J. Regan, A. Chandrasekhar, M.L. Concha, R. Yokoyama, K. Higashi, M. Take Uchi, W. Dong, T. Hiraga, N. Holder, and S.W. Wilson (2004). **Hedgehog and Fgf signaling pathways regulate the development of tphR-expressing serotonergic raphe neurons in zebrafish embryos.** *Journal of Neurobiology* 60(3): 275-88. ISSN: 0022-3034.

NAL Call Number: QP351.J55

Abstract: Serotonin (5HT) plays major roles in the physiological regulation of many behavioral processes, including sleep, feeding, and mood, but the genetic mechanisms by which serotonergic neurons arise during development are poorly understood. In the present study, we have investigated the development of serotonergic neurons in the zebrafish. Neurons exhibiting 5HT-immunoreactivity (5HT-IR) are detected from 45 h postfertilization (hpf) in the ventral hindbrain raphe, the hypothalamus, pineal organ, and pretectal area. Tryptophan hydroxylases encode rate-limiting enzymes that function in the synthesis of 5HT. As part of this study, we cloned and analyzed a novel zebrafish tph gene named tphR. Unlike two other zebrafish tph genes (tphD1 and tphD2), tphR is expressed in serotonergic raphe neurons, similar to tph genes in mammalian species. tphR is also expressed in the pineal organ where it is likely to be involved in the pathway leading to synthesis of melatonin. To better understand the signaling pathways involved in the induction of the serotonergic phenotype, we analyzed tphR expression and 5HT-IR in embryos in which either Hh or Fgf signals are abrogated. Hindbrain 5HT neurons are severely reduced in mutants lacking activity of either Ace/Fgf8 or the transcription factor Noi/Pax2.1, which regulates expression of ace/fgf8, and probably other genes encoding signaling proteins. Similarly, serotonergic raphe neurons are absent in embryos lacking Hh activity confirming a conserved role for Hh signals in the induction of these cells. Conversely, over-activation of the Hh pathway increases the number of serotonergic neurons. As in mammals, our results are consistent with the transcription factors Nk2.2 and Gata3 acting downstream of Hh activity in the development of serotonergic raphe neurons. Our results show that the pathways involved in induction of hindbrain serotonergic neurons are likely to be conserved in all vertebrates and help establish the zebrafish as a model system to study this important neuronal class.

Descriptors: zebrafish, Fgf physiology, developmental gene expression regulation, neurons metabolism, raphe nuclei cytology, trans activators physiology, genetically modified, base sequence, molecular cloning methods enzyme inhibitors pharmacology, fertilization, green fluorescent proteins, Hedgehog proteins, homeodomain proteins metabolism, in situ hybridization methods, luminescent proteins metabolism, nerve tissue proteins metabolism, opsin metabolism, pyrroles pharmacology, raphe nuclei embryology, sequence alignment methods, serotonin metabolism, signal transduction physiology, time factors, trans activators genetics and metabolism, tryptophan hydroxylase genetics and metabolism, veratrum alkaloids pharmacology.

Tessmar-Raible, K., F. Raible, F. Christodoulou, K. Guy, M. Rembold, H. Hausen, and D. Arendt (2007). **Conserved sensory-neurosecretory cell types in Annelid and fish forebrain: Insights into hypothalamus evolution**. *Cell* 129(7): 1389-1400. ISSN: 0092-8674.
NAL Call Number: QH573.C42
Descriptors: zebrafish, animal physiology, aquatic insects, brain, evolution, forebrain, freshwater fish, hypothalamus, neurons, phylogeny, transcription factors, vasopressin, miRNA, vasotocin, *Annelida*, *bilateria*, *Danio rerio*, *Platynereis dumerilii*.

Tetreault, M.L., D. Henry, D.M. Horrigan, G. Matthews, and A.L. Zimmerman (2006). **Characterization of a novel cyclic nucleotide-gated channel from zebrafish brain.** *Biochemical and Biophysical Research Communications* 348(2): 441-9. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: Cyclic nucleotide-gated (CNG) channels have been well characterized in the sensory receptors of vision and olfaction, but their characteristics in other tissues remain largely unknown. Here, we report characterization of a novel brain-specific CNG channel from zebrafish. Unique among CNG channels, the transcript is expressed mainly in the brain. When expressed in *Xenopus* oocytes, the channel's electrophysiological properties are distinct compared to CNG channels from either rods (CNGA1), olfactory receptors (CNGA2), or cones (CNGA3). The channel is less sensitive to cAMP than cGMP (K(1/2) of 280 and 7 microM, respectively), with a maximum cAMP efficacy at least 80% of that with saturating levels of cGMP. The single-channel conductance of 58pS is larger than most other CNG channels. Like other CNG channels the channel is relatively nonselective among monovalent cations. However, unlike other CNG channels, there was rundown of the macroscopic current within 30-100 min after patch excision.
Descriptors: zebrafish, brain chemistry, ion channels physiology, amino acid sequence, cyclic AMP pharmacology, cyclic GMP pharmacology, ion channels chemistry and drug effects, molecular sequence data, sequence alignment, proteins chemistry and drug effects.

Thisse, B., V. Heyer, A. Lux, V. Alunni, A. Degrave, I. Seiliez, J. Kirchner, J.P. Parkhill, and C. Thisse (2004). **Spatial and temporal expression of the zebrafish genome by large-scale in situ hybridization screening.** *Methods in Cell Biology* 77: 505-19. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, gene expression profiling methods, genomics methods, in situ hybridization methods, digoxigenin chemistry, fluorescein chemistry, genome, RNA probes chemistry, antisense RNA chemistry, messenger RNA analysis and metabolism, uridine triphosphate chemistry.

Thisse, C., A. Degrave, G.V. Kryukov, V.N. Gladyshev, S. Obrecht Pflumio, A. Krol, B. Thisse, and A. Lescure (2003). **Spatial and temporal expression patterns of selenoprotein genes during embryogenesis in zebrafish.** *Gene Expression Patterns* 3(4): 525-32. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Selenium is important for embryogenesis in vertebrates but little is known about the expression patterns and biological functions of most selenoprotein genes. Taking advantage of the zebrafish model, systematic analysis of selenoprotein gene expression was performed by in situ hybridization on whole-mount embryos at different developmental stages. Twenty-one selenoprotein mRNAs were analyzed and all of them exhibited expression patterns restricted to specific tissues. Moreover, we demonstrated that highly similar selenoprotein paralogs were expressed within distinct territories. Therefore, tissue- and

development-specific expression patterns provided new information for selenoproteins of unknown function.
Descriptors: zebrafish, developmental gene expression regulation, in situ hybridization, molecular sequence data, RNA probes, messenger RNA metabolism, selenoproteins, tissue distribution, proteins.

Thisse, C. and B. Thisse (2008). **High-resolution in situ hybridization to whole-mount zebrafish embryos.** *Nature Protocols* 3(1): 59-69. ISSN: 1754-2189.
Descriptors: zebrafish, in situ hybridization, Ish, technique, genes, digoxigenin, antisense RNA, probes, whole-mount zebrafish embryos , PCR-amplified.

Thomas, K.A. and D.L. Stemple (2004). **Development of the zebrafish organizer and notochord**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd. Singapore, Singapore , p. 87-120. ISBN: 9812388214.
NAL Call Number: QL638.C94 F57
Abstract: The major axes of a zebrafish embryo are established early in its development. One key structure, centrally involved in the specification of these axes, is the dorsal Organizer. The Organizer becomes apparent at the beginning of gastrulation and generates signals that pattern the mesoderm to generate dorsal structures and specifies the neuroectoderm. In addition to providing inductive signals, the Organizer itself will eventually differentiate to form the axial mesendoderm tissues prechordal plate and notochord. These tissues provide signals to pattern surrounding tissues and, in the case of the notochord, mechanical support required for locomotion of zebrafish larvae. Thus, how the Organizer forms and functions has been a source of great interest in developmental biology. Modern molecular and genetic studies are now providing a detailed picture of the events controlling both formation of the Organizer and its activity. Focusing on these events during zebrafish development, but drawing on results from a variety of other experimental systems, we review dorsal Organizer establishment and function, differentiation of Organizer tissue to chordamesoderm and notochord and finally, the patterning and mechanical functions of the notochord. Copyright © 2008 CABI
Descriptors: zebrafish, blastomere, cell differentiation, cleavage, embryonic development, embryos, gene expression, genes, grafts, morphology, mutations, signal transduction, transcription factors, transforming growth factor, *Danio rerio*, dorsal Organizer.

Thummel, R., C.T. Burket, and D.R. Hyde (2006). **Two different transgenes to study gene silencing and re-expression during zebrafish caudal fin and retinal regeneration.** *The Scientific World Journal* 6(Suppl 1): 65-81. ISSN: 1537-744X.
Abstract: We used the 500-bp *Xenopus* ef1-alpha promoter and the 2-kb zebrafish histone 2A.F/Z promoter to generate several independent transgenic zebrafish lines expressing EGFP. While both promoters drive ubiquitous EGFP expression in early zebrafish development, they are systematically silenced in several adult tissues, including the retina and caudal fin. However, EGFP expression is temporarily renewed in the adult during either caudal fin or retinal regeneration. In the Tg(H2A.F/Z:EGFP)nt line, EGFP is moderately expressed in both the wound epithelium and blastema of the regenerating caudal fin. In the Tg(ef1-alpha:EGFP)nt line, EGFP expression is reinitiated and restricted to the blastema of the regenerating caudal fin and colabels with BrdU, PCNA, and msxc-positive cells. Thus, these two ubiquitous promoters drive EGFP transgene expression in different cell populations during caudal fin regeneration. We further analyzed the ability of the ef1-alpha:EGFP trans-

gene to label nonterminally differentiated cells during adult tissue regeneration. First, we demonstrated that the transgene is highly methylated in adult zebrafish caudal fin tissue, but not during fin regeneration, implicating methylation as a potential means of transgene silencing in this line. Next, we determined that the ef1-alpha:EGFP transgene is also re-expressed during adult retinal regeneration. Specifically, the ef1-alpha:EGFP transgene colabels with PCNA in the Muller glia, a specialized cell that is the source of neuronal progenitors during zebrafish retinal regeneration. Thus, we concluded that Tg(ef1-alpha:EGFP)nt line visually marks nonterminally differentiated cells in multiple adult regeneration environments and may prove to be a useful marker in tissue regeneration studies in zebrafish.
Descriptors: zebrafish, gene expression regulation, regeneration genetics, retina physiology, tail physiology, genetically modified, DNA methylation, gene silencing , green fluorescent proteins analysis, green fluorescent histones genetics, neuroglia metabolism, neurons cytology, promoter regions genetics, retina cytology and metabolism, stem cells metabolism, transgenes.

Thummel, R., M. Ju, M.P.J. Sarras, and A.R. Godwin (2007). **Both Hoxc13 orthologs are functionally important for zebrafish tail fin regeneration.** *Development Genes and Evolution* 217(6): 413-20. ISSN: 0949 944X.
NAL Call Number: QL951.D38
Abstract: Hox genes are re-expressed during regeneration in many species. Given their important role in body plan development, it has been assumed, but not directly shown, that they play a functional role in regeneration. In this paper we show that morpholino-mediated knockdown of either Hoxc13a or Hoxc13b during the process of zebrafish tail fin regeneration results in a significant reduction of regenerative outgrowth. Furthermore, cellular proliferation within the blastema is directly affected in both knockdowns. Hence, similar to the demonstration of unique functions of multiple Hox genes during limb formation, both Hoxc13 orthologs have distinct functions in regeneration.
Descriptors: zebrafish, regeneration , electroporation , *Danio rerio*, Hoxc13, morpholino.

Tian Jing and Karuna Sampath (2004). **Formation and functions of the floor plate**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* World Scientific Publishing Co. Pte. Ltd.: Singapore, Singapore: p. 121-163. ISBN: 9812388214.
NAL Call Number: QL638.C94 F57
Abstract: The most ventral cells of the vertebrate neural tube, the floor plate, comprise a specialized group of cells distinct in form and function from the rest of the neural tube. These cells have been ascribed many functions, ranging from the differentiation of motor neurons that innervate specific muscle cell types in the body, to providing cues for the correct pathfinding of various axons. Here, we summarize the process by which the floor plate develops in a few model vertebrate organisms, the various functions of the floor plate, and the molecular nature of signals emanating from the floor plate. Finally, we assess the prevailing models of how the cells of the floor plate are thought to arise. Copyright © 2008 CABI
Descriptors: zebrafish embryo, central nervous system, embryonic development, neurons, neurophysiology, signal transduction, *Danio rerio*, fishes, vertebrates, floor plate development.

Timmerman, L.A., J. Grego-Bessa, A. Raya, E. Bertran, J.M. Perez-Pomares, J. Diez, S. Aranda, S. Palomo, F. Mccormick, J.C. Izpisua-Belmonte, and J.L. DeLaPompa (2004). **Notch promotes epithelial-mesenchymal transition during cardiac development and oncogenic**

transformation. *Genes and Development* 18(1): 99-115. ISSN: 0890 9369.
NAL Call Number: QH426.G46
Abstract: Epithelial-to-mesenchymal transition (EMT) is fundamental to both embryogenesis and tumor metastasis. The Notch intercellular signaling pathway regulates cell fate determination throughout metazoan evolution, and overexpression of activating alleles is oncogenic in mammals. Here we demonstrate that Notch activity promotes EMT during both cardiac development and oncogenic transformation via transcriptional induction of the Snail repressor, a potent and evolutionarily conserved mediator of EMT in many tissues and tumor types. In the embryonic heart, Notch functions via lateral induction to promote a selective transforming growth factor-beta (TGFbeta)-mediated EMT that leads to cellularization of developing cardiac valvular primordia. Embryos that lack Notch signaling elements exhibit severely attenuated cardiac snail expression, abnormal maintenance of intercellular endocardial adhesion complexes, and abortive endocardial EMT in vivo and in vitro. Accordingly, transient ectopic expression of activated Notch1 (N1IC) in zebrafish embryos leads to hypercellular cardiac valves, whereas Notch inhibition prevents valve development. Overexpression of N1IC in immortalized endothelial cells in vitro induces EMT accompanied by oncogenic transformation, with corresponding induction of snail and repression of VE-cadherin expression. Notch is expressed in embryonic regions where EMT occurs, suggesting an intimate and fundamental role for Notch, which may be reactivated during tumor metastasis. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, cardiovascular system, transport and circulation, development, tumor biology, neoplasms, oncogenic transformation, neoplastic disease, pathology, Notch intercellular signaling pathway, cardiac development, cell fate determination, epithelial mesenchymal transition, promotion , lateral induction.

Tingaud Sequeira, A., M. Andre, J. Forgue, C. Barthe, and P.J. Babin (2004). **Expression patterns of three estrogen receptor genes during zebrafish *(Danio rerio*) development: evidence for high expression in neuromasts.** *Gene Expression Patterns* 4(5): 561-8. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: The estrogen receptor (ER) genes encode a group of nuclear enhancer proteins, which are important ligand-activated transcription factors, modulating estrogen-target gene transcription. In this study we analyzed expression patterns of three zebrafish ER genes, esr1, esr2a, and esr2b, during development using whole-mount in situ hybridization. High levels of esr2a and esr2b of maternal origin are inherited and segregated to the blastomers. After the mid-blastula transition, the three genes exhibit similar spatio-temporal patterns of expression. In 24 h postfertilization (hpf) embryos, high levels of esr2a and esr2b and low levels of esr1 mRNAs are detected in the epidermis, pectoral fin buds, hatching gland and, to a lesser extent, developing brain. From 24 hpf onward, the expression of the three genes is down-regulated in the epidermis. By 60 hpf, esr2a mRNA is abundant in mature primary neuromasts of the anterior line system and by 3 days postfertilization (dpf), all mature primary neuromasts in both the anterior and posterior lateral line systems express significant levels of esr2a and esr2b transcripts. Histological sections show a high level of esr2a transcripts in both mechanoreceptive hair cells and supporting cells. The transcripts are still detected after neomycin-induced hair cell death, consistent with the presence of esr2a transcripts in supporting cells. From 6 dpf onward, esr2a and esr2b transcripts are robustly co-expressed in primary neuromasts, branchial arches, pectoral fins, and anal papilla, while slight labeling is observed for esr1 transcripts.
Descriptors: zebrafish, gene expression, neuroepithelial cells metabolism, estrogen receptors, metabolism, DNA primers, complementary DNA genetics, metabolism, developmental

gene expression regulation, histological techniques, in situ hybridization, reverse transcriptase PCR.

Tingaud Sequeira, A. and J. Cerda (2007). **Phylogenetic relationships and gene expression pattern of three different cathepsin L (Ctsl) isoforms in zebrafish: Ctsla is the putative yolk processing enzyme.** *Gene* 386(1-2): 98-106. ISSN: 0378-1119.

NAL Call Number: QH442.A1G4

Abstract: Certain cysteine proteases, such as cathepsin L (Ctsl), have been involved in yolk processing mechanisms in oocytes and embryos of lower vertebrates. In zebrafish *(Danio rerio*), three different ctsl genes, ctsla, ctslb and ctslc, have been found in the genome, but their pattern of expression, as well as information on which the encoded enzymes are potentially involved in yolk absorption during embryogenesis, is unknown. Here, phylogenetic and gene structure analysis revealed that zebrafish ctsla and ctslb genes are similar, showing a highly conserved structure in comparison with human ctsl, while ctslc presents different exon organization together with an earlier evolution. Thus, ctslc appears to be evolved from a common ancestral ctsl-like gene, possibly through an early duplication event, whereas ctsla and ctslb may be originated from a second duplication mechanism. Zebrafish ctsla, ctslb and ctslc also showed different patterns of mRNA expression during embryogenesis and in adult tissues. While Ctsla transcripts were accumulated in embryos throughout development and in the adult ovary, those encoding Ctslb were detected only in embryos around the time of hatching as previously reported, and those for Ctslc appeared only in larvae and in some adult tissues, but not in the ovary. In zebrafish and killifish (*Fundulus heteroclitus*) embryos, Ctsla mRNA was first detected in blastomers, and later in development it was localized in cells of the yolk syncytial layer, an embryonic structure involved in yolk absorption. These data therefore suggested that Ctsla is most likely the putative protease involved in yolk processing in fish embryos, while Ctslc seems not to be required during early embryogenesis in zebrafish.

Descriptors: zebrafish, cathepsins genetics, cysteine endopeptidases genetics, egg yolk enzymology, gene expression regulation physiology, phylogeny, amino acid sequence, cathepsins biosynthesis and physiology, cysteine endopeptidases biosynthesis and physiology, egg yolk chemistry, molecular evolution, Fundulidae genetics and metabolism, isoenzymes biosynthesis and genetics, molecular sequence data, vitellogenesis, proteins biosynthesis.

Titus, T.A., D.R. Selvig, B. Qin, C. Wilson, A.M. Starks, B.A. Roe, and J.H. Postlethwait (2006). **The Fanconi anemia gene network is conserved from zebrafish to human.** *Gene* 371(2): 211-23. ISSN: 0378-1119.

NAL Call Number: QH442.A1G4

Abstract: Fanconi anemia (FA) is a complex disease involving nine identified and two unidentified loci that define a network essential for maintaining genomic stability. To test the hypothesis that the FA network is conserved in vertebrate genomes, we cloned and sequenced zebrafish *(Danio rerio*) cDNAs and/or genomic BAC clones orthologous to all nine cloned FA genes (FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, and FANCL), and identified orthologs in the genome database for the pufferfish *Tetraodon nigroviridis*. Genomic organization of exons and introns was nearly identical between zebrafish and human for all genes examined. Hydrophobicity plots revealed conservation of FA protein structure. Evolutionarily conserved regions identified functionally important domains, since many amino acid residues mutated in human disease alleles or shown to be critical in targeted mutagenesis studies are identical in zebrafish and human. Comparative genomic analysis demonstrated conserved syntenies for all FA genes. We conclude that the

FA gene network has remained intact since the last common ancestor of zebrafish and human lineages. The application of powerful genetic, cellular, and embryological methodologies make zebrafish a useful model for discovering FA gene functions, identifying new genes in the network, and identifying therapeutic compounds.

Descriptors: zebrafish, Fanconi anemia genetics and complementation group D2 protein genetics, amino acid sequence, artificial bacterial chromosomes, molecular sequence data, amino acid sequence homology.

To, T.T., S. Hahner, G. Nica, K.B. Rohr, M. Hammerschmidt, C. Winkler, and B. Allolio (2007). **Pituitary-interrenal interaction in zebrafish interrenal organ development.** *Molecular Endocrinology* 21(2): 472-85. ISSN: 0888-8809.

NAL Call Number: QP187.3M64M6

Abstract: To further elucidate pituitary adrenal interactions during development, we studied the organogenesis of the interrenal organ, the teleost homolog of the mammalian adrenal gland, in zebrafish. To this end we compared wild-type zebrafish interrenal development with that of mutants lacking pituitary cell types including corticotrophs. In addition, we studied the effects of ACTH receptor (Mc2r) knockdown and dexamethasone (dex) on interrenal development and pituitary feedback. Until 2 d post fertilization (2 dpf) interrenal development assessed by transcripts of key steroidogenic genes (cyp11a1, mc2r, star) is independent of proopiomelanocortin (Pomc) as demonstrated in aal/eya1and lia/fgf3 mutants. However, at 5 dpf lack of pituitary cells leads to reduced expression of steroidogenic genes at both the transcriptional and the protein level. Pituitary control of interrenal development resides in corticotrophs, because pit1 mutants lacking pituitary cells except corticotrophs have a phenotype similar to that of wild-type controls. Furthermore, development in mc2r knockdown morphants does not differ from aal/eya1 and lia/fgf3 mutants. Inhibition of steroidogenesis by mc2r knockdown induces up-regulation of pomc expression in the anterior domain of pituitary corticotrophs. Accordingly, dex suppresses pomc in the anterior domain only, leading to impaired expression of steroidogenic genes commencing at 3 dpf and interrenal hypoplasia via reduced interrenal proliferation. In contrast, negative feedback on pituitary corticotrophs by dex is evident at 2 dpf and precedes effects of Pomc on the interrenal primordium. These data demonstrate a gradual transition from early pituitary-independent interrenal organogenesis to developmental control by the anterior domain of pituitary corticotrophs acting via Mc2 receptors.

Descriptors: wild-type zebrafish mutant, interrenal gland embryology, pituitary gland embryology, genetically modified, cell proliferation, cholesterol side chain cleavage enzyme metabolism, corticotrophs cytology and metabolism, dexamethasone pharmacology, interrenal gland metabolism, mutation, phosphoproteins metabolism, pituitary gland cytology and gland metabolism, pro opiomelanocortin metabolism, corticotropin receptors metabolism.

Tohya, S., A. Mochizuki, and Y. Iwasa (2003). **Difference in the retinal cone mosaic pattern between zebrafish and medaka: cell-rearrangement model.** *Journal of Theoretical Biology* 221(2): 289-300. ISSN: 0022-5193.

NAL Call Number: 442.8J8223

Abstract: In fish retina, four kinds of photoreceptor cells (or cones) are two-dimensionally arranged in a very regular manner, forming cone mosaics. Mosaic pattern differs between species--two typical patterns are “row mosaic” and “square mosaic”, exemplified by the cone mosaics in zebrafish and in medaka, respectively. In this paper, we study a cell-rearrangement model. Cells with pre-fixed fate exchange their locations between nearest neighbors and form regular mosaic patterns spontaneously, if the adhesive force between nearest neighbors

and between next-nearest neighbors depend on their cell types in an appropriate manner. The same model can produce both row and square mosaic patterns. However, if the cell-cell interaction is restricted to nearest neighbors only, the square mosaic (medaka pattern) cannot be generated, showing the importance of interaction between next-nearest neighbors. In determining whether row mosaic (zebrafish pattern) or square mosaic (medaka pattern) is to be formed, two shape factors are very important, which control the way adhesions in different geometric relations are combined. We also developed theoretical analysis of the parameter ranges for the row mosaic and the square mosaic to have higher total adhesion than alternative spatial patterns.

Descriptors: zebrafish, retina cones cytology, *Oryzias* anatomy and histology, cell adhesion and communication, retina cones metabolism, gene expression, opsin genetics.

Tong, S.K. and B.C. Chung (2003). **Analysis of zebrafish cyp19 promoters**. *Journal of Steroid Biochemistry and Molecular Biology* 86(3/5): 381-386. ISSN: 0960 0760.

NAL Call Number: QD426.A1J6

Abstract: Cyp19 encodes P450 aromatase, the key enzyme catalysing the conversion of androgens into oestrogens. Oestrogens play a crucial role in the anatomical, functional and behavioural characteristics of sexually dimorphic development. In zebrafish, two cyp19 genes, cyp19a and cyp19b, expressed in ovary and brain, respectively, were found. We have isolated the promoter regions of the zebrafish cyp19 genes from a bacterial artificial chromosome library to search for regulatory sequences that bind to transcription factors. Sequences like arylhydrocarbon receptor (AhR) recognition site, oestrogen receptor recognition half sites (1/2ERE) and c-AMP responsive elements were found in the 5'-flanking regions of both cyp19 genes. For ovarian-specific expression, we found binding sites for steroidogenic factor-1 (SF-1), GATA transcription factor 4 (GATA-4) and Wilm tumour 1 (WT1-KTS) on the promoter region of cyp19a but not cyp19b. For brain-specific expression of the cyp19b gene, sequences for recognition of chicken ovalbumin upstream promoter-transcription factor (COUP) and Ptx-1 were detected in the promoter. The importance of these putative control elements in ovary and brain-specific promoter has been assessed by sequence comparison among various species. Copyright © 2008 CABI

Descriptors: zebrafish, androgens, brain, enzyme activity, enzymes, genes, genetic analysis, estrogens, ovalbumin, ovaries, promoters, regulatory sequences, transcription factors, *Danio rerio.*

Tong, Y., T. Shan, Y.K. Poh, T. Yan, H. Wang, S.H. Lam, and Z. Gong (2004). **Molecular cloning of zebrafish and medaka vitellogenin genes and comparison of their expression in response to 17beta-estradiol.** *Gene* 328: 25-36. ISSN: 0378-1119.

NAL Call Number: QH442.A1G4

Abstract: In the present study, both zebrafish and medaka vitellogenin genes have been isolated and used as a biomarker to compare the two small aquarium fish in response to estrogen treatment and thus to evaluate the two fish models in development of a biomonitoring system for environmental estrogens. The isolated zebrafish vitellogenin gene, zvtg1, is the most abundantly expressed vitellogenin gene in zebrafish and its complete protein sequence of 1360 amino acids was deduced from a genomic and a cDNA clone. The isolated medaka vitellogenin (mvtg1) genomic clone covers 1053 amino acids in the N-terminal. Both zebrafish zvtg1 and medaka mvtg1 are specifically expressed in female liver and their expression can be induced by 17beta-estradiol (E2) in male fish both by intramuscular injection and immersion treatment. A real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay was developed for quantification of vitellogenin mRNA level in both control

fish and E2-treated fish. The lowest-observed-effect concentrations of E2 for the induction of vitellogenin mRNAs were observed at 1 microg/l for zebrafish and 0.1 microg/l for medaka in a 2-day exposure experiment. Further kinetics studies of the two fish models indicated that medaka was able to respond much faster to E2 treatment than zebrafish, while the zebrafish can attain a much higher level of vitellogenin mRNAs than medaka after a long-term E2 treatment. The implication of these observations may be that the medaka system is better in monitoring acute treatment while the zebrafish system is better in monitoring chronic exposure.

Descriptors: zebrafish, estradiol pharmacology, gene expression drug effects, *Oryzias* genetics, vitellogenins genetics, amino acid and base sequence, molecular cloning, DNA chemistry, genetics and isolation and purification, complementary DNA chemistry, genetics, isolation and purification, exons, gene expression genetics, introns, molecular sequence data, messenger RNA drug effects, genetics and metabolism, reverse transcriptase PCR, sequence alignment, DNA sequence analysis, amino acid sequence homology, nucleic acid sequence homology, time factors.

Topczewska, J.M., J. Topczewski, A. Szostak, L. Solnica Krezel, and B.L. Hogan (2003). **Developmentally regulated expression of two members of the Nrarp family in zebrafish.** *Gene Expression Patterns* 3(2): 169-71. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Delta-Notch signaling is essential for somitogenesis in vertebrate embryos. In a search for genes that control somite formation in zebrafish we have identified two paralogues encoding proteins related to Nrarp (Notch regulated ankyrin repeat protein). Zebrafish nrarp-a and-b encode small proteins with two ankyrin repeat domains. Here, we report the expression patterns of both genes in normal and mutant embryos.

Descriptors: zebrafish, gene expression regulation, amino acid sequence, gene expression profiling, molecular sequence data, multigene family, mutation, protein biosynthesis.

Tostivint, H., L. Joly, I. Lihrmann, J.M. Conlon, M. Ekker, and H. Vaudry (2005). **Linkage mapping of the [Pro2]somatostatin-14 gene in zebrafish: evolutionary perspectives.** *Annals of the New York Academy of Sciences* 1040: 486-9. ISSN: 0077 8923.

NAL Call Number: 500 N484

Abstract: Radiation hybrid mapping assigned the zebrafish [Pro(2)]somatostatin-14 (also termed somatostatin 2; SS2) gene to linkage group 23 of the zebrafish genome, close to the marker nadl1.2. Comparative genomic analysis revealed conserved syntenies of the SS2 gene locus with part of the human 1p36 region, where the cortistatin gene is located. This observation strongly suggests that the SS2 gene in nonmammalian species and the cortistatin gene in mammals are orthologous.

Descriptors: zebrafish, chromosome mapping methods, molecular evolution, somatostatin genetics, analogs and derivatives.

Tostivint, H., L. Joly, I. Lihrmann, M. Ekker, and H. Vaudry (2004). **Chromosomal localization of three somatostatin genes in zebrafish. Evidence that the [Pro2]-somatostatin-14 isoform and cortistatin are encoded by orthologous genes.** *Journal of Molecular Endocrinology* 33(3): 819-826. ISSN: 0952 5041.

NAL Call Number: QP187.3.M64J68

Abstract: There is now evidence for the existence of two somatostatin genes in most vertebrate species, and even three somatostatin genes in teleosts. To help clarify the evolutionary relationships between the different somatostatin isoforms currently known, we character-

ized the somatostatin loci in a teleost species, the zebrafish *Danio rerio*, and compared them with the corresponding regions in the human and pufferfish genomes. The occurrence of three somatostatin genes, termed SS1, SS2 and SSII, has been previously demonstrated in the zebrafish. Radiation hybrid mapping assigned these three genes to linkage groups 15, 23 and 2, respectively. Conserved synteny of the zebrafish SS2 gene and the human cortistatin gene was revealed by comparative genomic analysis, indicating that mammalian cortistatin is orthologous to the SS2 variant of non-mammalian species. In contrast, using a similar approach, it was not possible to identify the evolutionary relationships between the atypical SSII gene of zebrafish and the other teleost SSII genes.
Descriptors: zebrafish, *Danio rerio*, chromosomes, molecular genetics, somatostatin gene family, chromosomal localization and evolutionary relationships within vertebrata, evolution, somatostatin gene family evolution, phylogeny, somatostatin gene phylogeny.

Toyama, R., D.M. Gomez, M.D. Mana, and I.B. Dawid (2004). **Sequence relationships and expression patterns of zebrafish zic2 and zic5 genes.** *Gene Expression Patterns* 4(3): 345-50. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: The zinc finger motif forms a DNA binding domain that is found in a wide variety of proteins. Among them, the members of the zic gene family are highly conserved throughout metazoans. We report here the isolation of two new members of this gene family in zebrafish, zic2.2 and zic5, isolated during random screening for tissue-specific genes. Zic2.2 is closely related to the previously reported zic2 gene, which we propose to rename zic2.1; these two genes form a subfamily with other vertebrate zic2 genes. We compare here the expression patterns of zic2.1, zic2.2, and zic5. All three genes showed dynamic expression patterns starting after the initiation of zygotic transcription, predominantly in the developing neural tube. Compared to zic2.1, zic2.2 was expressed in a similar but distinct manner during early development, particularly in the retina and the forming somites. A zic2.2 ortholog has not been identified in other vertebrate species, suggesting that the zic2.1/zic2.2 pair resulted from a genome duplication event during the evolution of the zebrafish lineage.
Descriptors: zebrafish, DNA binding proteins genetics, amino acid sequence, central nervous system embryology, DNA binding proteins genetics, proteins metabolism, gene duplication, developmental gene expression regulation, molecular sequence data, phylogeny, sequence alignment, somites metabolism, proteins metabolism, zinc fingers genetics.

Toyama, R., M. Rebbert, A. Dey, K. Ozato, and I.B. Dawid (2004). **The brd4 protein associates with transcriptionally inactive chromosomes during early embryogenesis**. *Molecular Biology of the Cell* 15(Suppl. S): 330A. ISSN: 1059 1524.
NAL Call Number: QH604.C452
Descriptors: zebrafish embryos, development, molecular genetics, cell biology, carcinoma, neoplastic disease, immunostaining, histology and cytology techniques, immunologic techniques, in situ hybridization, histology and cytology techniques, genetic techniques, cell cycle progression, mitosis, embryogenesis, midblastula transition, maternal gene expression.

Tran, T., B. Sneed, J. Haider, D. Blavo, A. White, T. Aiyejorun, T.C. Baranowski, A.M.Y.L. Rubinstein, T.N. Doan, R. Dingledine, and E.M. Sandberg (2007). **Automated, quantitative screening assay for antiangiogenic compounds using transgenic zebrafish**. *Cancer Research* 67(23): 11386-11392. ISSN: 0008-5472.
Descriptors: transgenic zebrafish, algorithms, angiogenesis, blood vessels, cancer, drug dis-

covery, endothelial cells, image processing, imaging, umbilical vein, *Danio rerio*, molecular techniques.

Trede, N.S., J. Medenbach, A. Damianov, L.H. Hung, G.J. Weber, B.H. Paw, Y. Zhou, C. Hersey, A. Zapata, M. Keefe, B.A. Barut, A.B. Stuart, T. Katz, C.T. Amemiya, L.I. Zon, and A. Bindereif (2007). **Network of coregulated spliceosome components revealed by zebrafish mutant in recycling factor p110.** *Proceedings of the National Academy of Sciences of the United States of America* 104(16): 6608-13. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: The spliceosome cycle consists of assembly, catalysis, and recycling phases. Recycling of postspliceosomal U4 and U6 small nuclear ribonucleoproteins (snRNPs) requires p110/SART3, a general splicing factor. In this article, we report that the zebrafish earl grey (egy) mutation maps in the p110 gene and results in a phenotype characterized by thymus hypoplasia, other organ-specific defects, and death by 7 to 8 days postfertilization. U4/U6 snRNPs were disrupted in egy mutant embryos, demonstrating the importance of p110 for U4/U6 snRNP recycling in vivo. Surprisingly, expression profiling of the egy mutant revealed an extensive network of coordinately up-regulated components of the spliceosome cycle, providing a mechanism compensating for the recycling defect. Together, our data demonstrate that a mutation in a general splicing factor can lead to distinct defects in organ development and cause disease.
Descriptors: zebrafish, dead box RNA helicases genetics and metabolism, RNA binding proteins metabolism and genetics, spliceosomes physiology, lethal genes, mutagenesis, organ specificity genetics, phenotype, U4 U6 ribonucleoprotein, small nuclear genetics and metabolism, thymus gland abnormalities.

Trevarrow, B. and B. Robison (2004). **Genetic backgrounds, standard lines, and husbandry of zebrafish.** *Methods in Cell Biology* 77: 599-616 . ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, animal husbandry methods, cryopreservation methods, genetic markers.

Triqueneaux, G., S. Thenot, T. Kakizawa, M.P. Antoch, R. Safi, J.S. Takahashi, F. Delaunay, and V. Laudet (2004). **The orphan receptor Rev-erbalpha gene is a target of the circadian clock pacemaker**. *Journal of Molecular Endocrinology* 33(3): 585-608. ISSN: 0952 5041.
NAL Call Number: QP187.3.M64J68
Abstract: Rev-erbalpha is a ubiquitously expressed orphan nuclear receptor which functions as a constitutive transcriptional repressor and is expressed in vertebrates according to a robust circadian rhythm. We report here that two Rev-erbalpha mRNA isoforms, namely Rev-erbalpha1 and Rev-erbalpha2, are generated through alternative promoter usage and that both show a circadian expression pattern in an in vitro system using serum-shocked fibroblasts. Both promoter regions P1 (Rev-erbalpha1) and P2 (Rev-erbalpha2) contain several E-box DNA sequences which function as response elements for the core circadian-clock components: CLOCK and BMAL1. The CLOCK-BMAL1 heterodimer stimulates the activity of both P1 and P2 promoters in transient transfection assay by 3-6-fold. This activation was inhibited by the overexpression of CRY1, a component of the negative limb of the circadian transcriptional loop. Critical E-box elements were mapped within both promoters. This regulation is conserved in vertebrates since we found that the CLOCK-BMAL1 heterodimer also regulates the zebrafish Rev-erbalpha, gene. In line with these data Rev-erbalpha circadian expression was strongly impaired in the livers of Clock mutant mice and in the pineal glands

of zebrafish embryos treated with Clock and Bmal1 antisense oligonucleotides. Together these data demonstrate that CLOCK is a critical regulator of Rev-erba circadian gene expression in evolutionarily distant vertebrates and suggest a role for Rev-erbalpha in the circadian clock output. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics, circadian clock, gene expression, heterodimer, orphan receptor.

Tsai HuaiJen (2003). **Transgenic fish: researches and applications**. *Journal of the Fisheries Society of Taiwan* 30(4): 263-277. ISSN: 0379 4180.
NAL Call Number: SH135.T3
Abstract: This article discusses researches and applications of transgenics in fishes. The gene transfer technology applied in commercially important aquatic animals is used to enhance the quality or to improve the genetic characters of aquaculture broodstocks. The anti-freezing transgenic salmon and the fast-growing transgenic fish and shellfish have been reported and are considered as the most successful cases until now. It highlights a new access to the modification of food fish and shellfish. However, the issues of genetically modified organisms (GMO) are still controversial. Food safety and ecological impacts are the major concerns before GMO are put in the market. For research purposes, zebrafish *Danio rerio* and medaka *Oryzias latipes* have become the most used model organisms since they are primitive vertebrates yet have many advantages over the other. Transgenesis, in vivo transient assay, mutagenesis and knock-down translation of zebrafish embryos are the most effective ways to map expression profiles, to dissect the regulatory cis-elements and to identify the gene functions of vertebral genes. Germ-line transmission is much more easily accessible. Transgenic lines carrying a specific tissue tagged by green fluorescent protein (GFP) live marker become a powerful tool to study developmental biology because they permit the recapitulation of the expression of endogenous genes. Furthermore, the transparent embryos are available for observation and tracing of dynamic expression of a specific gene during embryogenesis. Transgenic fish can also serve as a simple animal model to study human diseases because some zebrafish mutants can faithfully phenocopy human diseases. For application purposes, GFP lines are potentially applied as biosensors for detecting the environmental pollutants. Interestingly, new pet fish species that glow colourfully in the dark can be achieved by transferring fluorescent protein genes. These advantages make the transgenic model fish apt for modern biomedical researches and applications as new materials. Copyright © 2008 CABI
Descriptors: zebrafish, aquaculture, biological development, fish culture, gene expression, gene transfer, genes, germ line, ontogeny.
Language of Text: Chinese.

Tsai, S.B., V. Tucci, J. Uchiyama, N.J. Fabian, M.C. Lin, P.E. Bayliss, D.S. Neuberg, I.V. Zhdanova, and S. Kishi (2007). **Differential effects of genotoxic stress on both concurrent body growth and gradual senescence in the adult zebrafish.** *Aging Cell* 6(2): 209-24. ISSN: 1474-9718.
Abstract: Among vertebrates, fish and mammals show intriguing differences in their growth control properties with age. The potential for unlimited or indeterminate growth in a variety of fish species has prompted many questions regarding the senescent phenomena that appear during the aging process in these animals. Using zebrafish as our model system, we have attempted in our current study to examine the growth phenomena in fish in relation to the onset of senescence-associated symptoms, and to evaluate the effects of genotoxic stress on these processes. We observed in the course of these analyses that the zebrafish undergoes continuous growth, irrespective of age, past the point of sexual maturation with gradually

decreasing growth rates at later stages. Animal population density, current body size and chronological age also play predominant roles in regulating zebrafish growth and all inversely influence the growth rate. Interestingly, the induction of genotoxic stress by exposure to ionizing radiation (IR) did not adversely affect this body growth ability in zebrafish. However, IR was found to chronically debilitate the regeneration of amputated caudal fins and thereby induce high levels of abnormal fin regeneration in the adult zebrafish. In addition, by resembling and mimicking the natural course of aging, IR treatments likewise enhanced several other symptoms of senescence, such as a decline in reproductive abilities, increased senescence-associated beta-galactosidase activity and a reduction in melatonin secretion. Our current data thus suggest that during the lifespan of zebrafish, the onset of senescence-associated symptoms occurs in parallel with continuous growth throughout mid-adulthood. Moreover, our present findings indicate that genotoxic DNA damage may play a role as a rate-limiting factor during the induction of senescence, but not in the inhibition of continuous, density-dependent growth in adult zebrafish.

Descriptors: zebrafish, aging genetics, DNA damage and aging physiology, brain metabolism and radiation effects, gills physiology and radiation effects, melatonin metabolism, phenotype, ionizing radiation, regeneration, reproduction, beta galactosidase metabolism.

Tsinkalovsky, O., A.O. Vik Mo, S. Ferreira, O.D. Laerum, and A. Fjose (2007). **Zebrafish kidney marrow contains ABCG2-dependent side population cells exhibiting hematopoietic stem cell properties.** *Differentiation* 75(3): 175-83. ISSN: 0301 4681.

NAL Call Number: QH607.D5

Abstract: Zebrafish (*Danio rerio*) has emerged as a powerful genetic model for the study of vertebrate hematopoiesis. However, methods for detection and isolation of hematopoietic stem cells (HSCs) have not yet been reported. In mammals, the combination of Hoechst 33342 staining with flow cytometry can be used for separation of a bone marrow side population (SP), which is highly enriched for HSCs. We applied a similar procedure to hematopoietic kidney marrow cells from adult zebrafish, and identified a segregated cohort of SP cells, which demonstrate a set of features typical of stem cells. SP cells show extremely low scatter characteristics, and are small in size with a minimum of cytoplasm. Treatment of zebrafish kidney marrow cells with reserpine or fumitremorgin C, which inhibit the ABCG2 transporter responsible for Hoechst 33342 efflux, caused a clear reduction in the number of SP cells. Consistent with the quiescent state of HSCs, the SP cells are strongly resistant to the myelosuppressive agent 5-fluorouracil. In addition, SP cells specifically demonstrate higher expression of genes known to be markers of HSCs of mammals. Hence, our results show that the SP phenotype is conserved between mammals and teleosts, and the properties of the zebrafish SP cells indicate a significant enrichment for HSCs. These rapid flow cytometric methods for purification of HSCs from zebrafish may greatly facilitate genetic analysis of stem cells using the advantages of this vertebrate model.

Descriptors: zebrafish, ATP binding cassette transporters metabolism, hematopoietic stem cells cytology, kidney cytology, metabolism, ATP binding cassette transporters antagonists and inhibitors, benzimidazoles metabolism, cell differentiation, flow cytometry, hematopoietic stem cells drug effects and metabolism, indoles pharmacology, kidney drug effects, kidney metabolism, phenotype, reserpine pharmacology, proteins antagonists and inhibitors.

Tsujikawa, M. and J. Malicki (2004). **Genetics of photoreceptor development and function in zebrafish.** *International Journal of Developmental Biology, The* 48(8-9): 925-34. ISSN: 0214-6282.

Abstract: The vertebrate photoreceptor is a cell of unique morphology and function. It

is an exquisite light detector, both sensitive and adaptable. Several unusual morphological features facilitate photoreceptor function. Signal detection is accomplished by a specialized apical structure, the outer segment. There, the capture of light produces fluctuations in cell membrane potential, which are then transmitted to the downstream circuitry of the retina via a rare type of synaptic junction, the ribbon synapse. The development, maintenance and function of the vertebrate photoreceptor cell have been studied mainly in four model organisms, ranging from an amphibian to man. A teleost fish, the zebrafish, is an important recent addition to this group. Genetic screens in zebrafish have identified an impressive collection of photoreceptor cell mutants, including the absence or malformation of specific morphological features as well as functional abnormalities. These mutant strains are currently studied using both molecular and embryological tools and provide important insights into photoreceptor biology.

Descriptors: zebrafish embryo, eye embryology, vision developmental gene expression regulation, vertebrate photoreceptors physiology, body patterning, cell differentiation, cell lineage, light, mutation, time factors.

Tsujimura, T., A. Chinen, and S. Kawamura (2007). **Identification of a locus control region for quadruplicated green-sensitive opsin genes in zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 104(31): 12813-8. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: Duplication of opsin genes has a crucial role in the evolution of visual system. Zebrafish have four green-sensitive (RH2) opsin genes (RH2-1, RH2-2, RH2-3, and RH2-4) arrayed in tandem. They are expressed in the short member of the double cones (SDC) but differ in expression areas in the retina and absorption spectra of their encoding photopigments. The shortest and the second shortest wavelength subtypes, RH2-1 and RH2-2, are expressed in the central-to-dorsal retina. The longer wavelength subtype, RH2-3, is expressed circumscribing the RH2-1/RH2-2 area, and the longest subtype, RH2-4, is expressed further circumscribing the RH2-3 area and mainly occupying the ventral retina. The present report shows that a 0.5-kb region located 15 kb upstream of the RH2 gene array is an essential regulator for their expression. When the 0.5-kb region was deleted from a P1-artificial chromosome (PAC) clone encompassing the four RH2 genes and when one of these genes was replaced with a reporter GFP gene, the GFP expression in SDCs was abolished in the zebrafish to which a series of the modified PAC clones were introduced. Transgenic studies also showed that the 0.5-kb region conferred the SDC-specific expression for promoters of a non-SDC (UV opsin) and a nonretinal (keratin 8) gene. Changing the location of the 0.5-kb region in the PAC clone conferred the highest expression for its proximal gene. The 0.5-kb region was thus designated as RH2-LCR analogous to the locus control region of the L-M opsin genes of primates.

Descriptors: zebrafish, opsin genetics, opsin metabolism, vision genetics, metabolism, chromosomes genetics, color, DNA genetics, gene expression regulation, reporter locus control region, promoter regions genetics.

Tsutsumi, M. and M. Itoh (2007). **Novel transcript nort is a downstream target gene of the Notch signaling pathway in zebrafish.** *Gene Expression Patterns* 7(3): 227-32. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: The Notch signaling pathway plays important roles in the regulation of diverse developmental processes. Although many Notch-signal target genes with different specificities have been identified, their regulation and functions are not fully understood. Here, we conducted a microarray screen to search for novel downstream target genes of the Notch

pathway in zebrafish. From the screen, we isolated nort (Notch-regulated transcript) as a transcript whose expression was reduced by the inhibition of Notch signaling. The expression level of nort increased when Notch signaling was activated. nort was expressed in hypoblast cells and the developing nervous system. We found its expression pattern to be similar to that of her4, but it showed some differences, at least in the anterior and posterior neural plate at the 3-somite stage. The nort transcript did not contain any long open-reading frame (ORF) of more than 300 nt, and its ORF-encoded sequence showed no significant homology with the proteins in databases. However, nort has one SPS (suppressor of hairless paired binding site) in its 5'-flanking region. These data suggest that nort is a putative noncoding RNA regulated by Notch signaling.

Descriptors: zebrafish, developmental gene expression regulation, untranslated RNA genetics, Notch receptors metabolism, signal transduction, proteins metabolism , 5' flanking region genetics, metabolism, nervous system embryology and metabolism, oligonucleotide array sequence analysis, untranslated RNA metabolism.

Tucker, B., C. Hepperle, D. Kortschak, B. Rainbird, S. Wells, A.C. Oates, and M. Lardelli (2007). **Zebrafish Angiotensin II receptor-like 1a (agtrl1a) is expressed in migrating hypoblast, vasculature, and in multiple embryonic epithelia.** *Gene Expression Patterns* 7(3): 258-65. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: The human gene AGTRL1 is an angiotensin II receptor-like gene expressed in vasculature, which acts as the receptor for the small peptide APELIN, and a co-receptor for Human Immunodeficiency Virus. Mammalian AGTRL1 has been shown to modulate cardiac contractility, venous and arterial dilation, and endothelial cell migration in vitro, but no role in the development of the vasculature, or other tissues, has been described. We report the identification and expression of the zebrafish ortholog of the human gene AGTRL1. Zebrafish agtrl1a is first expressed before epiboly in dorsal precursors. During epiboly it is expressed in the enveloping layer, yolk syncytial layer and migrating mesendoderm. During segmentation stages, expression is observed in epithelial structures such as adaxial cells, border cells of the newly formed somites, developing lens, otic vesicles and venous vasculature.

Descriptors: zebrafish, epithelium embryology, developmental gene expression regulation, angiotensin type 1 receptor genetics, amino acid sequence, blastula metabolism, blood vessels embryology, blood vessels metabolism, cleavage stage, ovum metabolism, molecular cloning, metabolism, epithelium metabolism, gastrula metabolism, in situ hybridization, mesoderm metabolism, molecular sequence data, phylogeny, sequence alignment, somites cytology and metabolism.

Tucker, B., R. Richards, and M. Lardelli (2004). **Expression of three zebrafish orthologs of human FMR1-related genes and their phylogenetic relationships.** *Development Genes and Evolution* 214(11): 567-74. ISSN: 0949 944X.

NAL Call Number: QL951.R68

Abstract: The human fragile X mental retardation syndrome is caused by expansions of a CGG repeat in the FMR1 gene. FXR1 and FXR2 are autosomal paralogs of FMR1. The products of the three genes, FMRP, FXR1P, and FXR2P, respectively, belong to a family of RNA-binding proteins. While the FMR1-related gene family is well described in human, mouse and *Drosophila*, little is known about zebrafish (*Danio rerio*) orthologs of these genes. Here we collate the known FMR1-related gene sequences from zebrafish, examine their regions of structural conservation, and define their orthologies with the human genes. We

demonstrate that zebrafish possess only three FMR1-related genes, fmr1, fxr1 and fxr2, and these are orthologous to the human FMR1, FXR1 and FXR2 genes respectively. We examine the spatiotemporal pattern of transcription of the zebrafish genes from 0 hours post fertilisation (hpf) until 24 hpf. Expression of fmr1, fxr1 and fxr2 is widespread throughout this time. However, relative to surrounding tissues, expression of fxr2 is raised in adaxial and somitic cells by 12 hpf while fxr1 expression is high in the anterior of the embryo, and is raised in adaxial cells by 12 hpf. Distinct patterns (and levels) of expression are seen for the different genes later in development. At 24 hpf, fxr1 and fxr2 transcripts show complex distribution patterns in somites. The expression of the FMR1-related gene family in zebrafish tissues is broadly consistent with expression in mouse and human, supporting the idea that zebrafish should be an excellent model organism in which to study the functions of the vertebrate FMR1-related gene family.
Descriptors: zebrafish, multigene family, nerve tissue RNA binding proteins genetics, amino acid sequence, genetic databases, fragile X mental retardation protein, in situ hybridization, molecular sequence data, organ specificity, reverse transcriptase PCR.

Tzeng, C.M., C.D. Hsiao, and P.P. Hwang (2005). **Molecular evolution of Aquaporin Gene Family in zebrafish**. *Journal of the Fisheries Society of Taiwan* 32(1): 85-86. ISSN: 0379-4180.
NAL Call Number: SH135.T3
Descriptors: zebrafish, cell membranes, chromosomes, freshwater fish, genomes, gills, intestines, kidneys , molecular structure, respiration, *Danio rerio*, Taiwan.

Uckun, F.M. and A.O. Benyumov (2005). **Transgenic zebrafish embryo model for hematopoiesis and lymphoproliferative disorders**. *Official Gazette of the United States Patent and Trademark Office* 800-10 ISSN: 0098 1133.
NAL Call Number: T223.A21
Abstract: A transgenic zebrafish animal model is disclosed. The model can be used for study of hematopoetic cell differentiation, control, and screening of therapeutic agents and can include a transgenic zebrafish expressing a heterologous Ikaros protein. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, lymphoproliferative disease, neoplastic disease, immune system disease, blood and lymphatic disease.
Notes: Patent No.US06953875.

Uechi, T., Y. Nakajima, A. Nakao, H. Torihara, A. Chakraborty, K. Inoue, and N. Kenmochi (2006). **Ribosomal protein gene knockdown causes developmental defects in zebrafish.** *Public Library of Science One* 1: E37. ISSN: 1817-101X.
Abstract: The ribosomal proteins (RPs) form the majority of cellular proteins and are mandatory for cellular growth. RP genes have been linked, either directly or indirectly, to various diseases in humans. Mutations in RP genes are also associated with tissue-specific phenotypes, suggesting a possible role in organ development during early embryogenesis. However, it is not yet known how mutations in a particular RP gene result in specific cellular changes, or how RP genes might contribute to human diseases. The development of animal models with defects in RP genes will be essential for studying these questions. In this study, we knocked down 21 RP genes in zebrafish by using morpholino antisense oligos to inhibit their translation. Of these 21, knockdown of 19 RPs resulted in the development of morphants with obvious deformities. Although mutations in RP genes, like other housekeeping genes, would be expected to result in nonspecific developmental defects with widespread phenotypes, we found that knockdown of some RP genes resulted in phenotypes specific

to each gene, with varying degrees of abnormality in the brain, body trunk, eyes, and ears at about 25 hours post fertilization. We focused further on the organogenesis of the brain. Each knocked-down gene that affected the morphogenesis of the brain produced a different pattern of abnormality. Among the 7 RP genes whose knockdown produced severe brain phenotypes, 3 human orthologs are located within chromosomal regions that have been linked to brain-associated diseases, suggesting a possible involvement of RP genes in brain or neurological diseases. The RP gene knockdown system developed in this study could be a powerful tool for studying the roles of ribosomes in human diseases.
Descriptors: zebrafish, ribosomal proteins, RPs, brain phenotypes, 3 human orthologs.

Ueno, S., G. Weidinger, T. Osugi, A.D. Kohn, J.L. Golob, L. Pabon, H. Reinecke, R.T. Moon, and C.E. Murry (2007). **Biphasic role for Wnt/beta-catenin signaling in cardiac specification in zebrafish and embryonic stem cells.** *Proceedings of the National Academy of Sciences of the United States of America* 104(23): 9685-90. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: Understanding pathways controlling cardiac development may offer insights that are useful for stem cell-based cardiac repair. Developmental studies indicate that the Wnt/beta-catenin pathway negatively regulates cardiac differentiation, whereas studies with pluripotent embryonal carcinoma cells suggest that this pathway promotes cardiogenesis. This apparent contradiction led us to hypothesize that Wnt/beta-catenin signaling acts biphasically, either promoting or inhibiting cardiogenesis depending on timing. We used inducible promoters to activate or repress Wnt/beta-catenin signaling in zebrafish embryos at different times of development. We found that Wnt/beta-catenin signaling before gastrulation promotes cardiac differentiation, whereas signaling during gastrulation inhibits heart formation. Early treatment of differentiating mouse embryonic stem (ES) cells with Wnt-3A stimulates mesoderm induction, activates a feedback loop that subsequently represses the Wnt pathway, and increases cardiac differentiation. Conversely, late activation of beta-catenin signaling reduces cardiac differentiation in ES cells. Finally, constitutive overexpression of the beta-catenin-independent ligand Wnt-11 increases cardiogenesis in differentiating mouse ES cells. Thus, Wnt/beta-catenin signaling promotes cardiac differentiation at early developmental stages and inhibits it later. Control of this pathway may promote derivation of cardiomyocytes for basic research and cell therapy applications.
Descriptors: zebrafish, Wnt, beta-catenin, cardiac, embryonic stem cells, cardiomyocytes, cell therapy, applications.

Ulrich, F. and C.P. Heisenberg (2004). **Gastrulation in zebrafish**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd. Singapore, Singapore , p. 39-86. ISBN: <41 ISBN>.
NAL Call Number: QL638.C94 F57
Descriptors: mutant zebrafish, biochemical pathways, blastomere, cell cycle, cell division, cytoadherence, embryonic development, embryos, endocytosis, enzyme activity, fibronectins, gene expression, genes, glycoproteins, kinases, molecular biology, morphogenesis, morphology, nuclei, platelet derived growth factor, protein kinase, signal transduction, techniques, transcription factors, transforming growth factor, yolk sac, *Danio rerio.*

Unger, J.L. and E. Glasgow (2003). **Expression of isotocin-neurophysin mRNA in developing zebrafish.** *Gene Expression Patterns* 3(1): 105-8. ISSN: 1567-133X.
NAL Call Number: QH450.B73

Descriptors: zebrafish, neurophysins genetics, oxytocin analogs and derivatives, oxytocin genetics, messenger RNA metabolism, amino acid sequence, molecular sequence data, neurophysins biosynthesis, oxytocin biosynthesis, phylogeny.

Uraiqat, C.A., N.C. Bird, and L.P. Hernandez (2003). **Pharyngeal arch development in midline and Hedgehog signaling mutants: implications for morphogenesis of the visceral skeleton**. *Integrative and Comparative Biology* 43(6): 942. ISSN: 1540 7063.
NAL Call Number: QL1.I67
Descriptors: zebrafish, nervous system, neural coordination, molecular genetics, skeletal system, movement and support, development, dental and oral system, ingestion and assimilation, cyclopia, congenital disease, chondrogenesis.

Urasaki, A. and K. Kawakami (2005). **Remobilization of the Tol2 transposable element in zebrafish**. *Genes and Genetic Systems* 80(6): 486. ISSN: 1341 7568.
NAL Call Number: QH426.G462
Descriptors: zebrafish, molecular genetics, f1 progeny, chromosomal insertion.

Urasaki, A., G. Morvan, and K. Kawakami (2006). **Functional dissection of the Tol2 transposable element identified the minimal cis-sequence and a highly repetitive sequence in the subterminal region essential for transposition**. *Genetics* 174(2): 639-649. ISSN: 0016 6731.
NAL Call Number: 442.8G28
Abstract: The Tol2 element is a naturally occurring active transposable element found in vertebrate genomes. The Tol2 transposon system has been to be active from fish to mammals and considered to be a useful gene transfer vector in vertebrates. However, cis-sequences essential for transposition have not been characterized. Here we report the characterization of the minimal cis-sequence of the Tol2 element. We constructed Tol2 vectors containing various lengths of DNA from both the left (5') and the right (3') ends and tested their transpositional activities both by the transient excision assay using zebrafish embryos and by analyzing chromosomal transposition in the zebrafish germ lineage. We demonstrated that Tol2 vectors with 200 bp from the left end and 150 bp from the right end were capable of transposition without reducing the transpositional efficiency and found that these sequences, including the terminal inverted repeats (TIRs) and the subterminal regions, are sufficient and required for transposition. The left and right ends were not interchangeable. The Tol2 vector carrying an insert of > 11 kb could transpose, but a certain length of spacer, < 276 but > 18 bp, between the left and right ends was necessary for excision. Furthermore, we found that a 5-bp sequence, 5'-(A/G)AGTA-3', is repeated 33 times in the essential subterminal region. Mutations in the repeat sequence at 13 different sites in the subterminal region, as well as mutations in TIRs, severely reduced the excision activity, indicating that they play important roles in transposition. The identification of the minimal cis-sequence of the Tol2 element and the construction of mini-Tol2 vectors will facilitate development of useful transposon tools in vertebrates. Also, our study established a basis for further biochemical and molecular biological studies for understanding roles of the repetitive sequence in the subterminal region in transposition. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics, functional dissection, terminal inverted repeat, cis sequence , To12 transposable element.

Vallone, D., S.B. Gondi, D. Whitmore, and N.S. Foulkes (2004). **E-box function in a period gene repressed by light**. *Proceedings of the National Academy of Sciences, USA* 101(12): 4106-4111. ISSN: 0027-8424.

NAL Call Number: 500N21P
Descriptors: zebrafish, bioluminescence, chronometers, circadian rhythms, freshwater fish, genes, light effects, mutagenesis, nucleotide sequence, period gene, phase shift, promoters, repression, cDNA, *Danio rerio*, migrations and rhythms.

Vallone, D., C. Santoriello, S.B. Gondi, and N.S. Foulkes (2007). **Basic protocols for zebrafish cell lines: maintenance and transfection.** *Methods in Molecular Biology* 362: 429-41. ISSN: 1064-3745.
Abstract: Cell lines derived from zebrafish embryos show great potential as cell culture tools to study the regulation and function of the vertebrate circadian clock. They exhibit directly light-entrainable rhythms of clock gene expression that can be established by simply exposing cultures to light-dark cycles. Mammalian cell lines require treatments with serum or activators of signaling pathways to initiate transient, rapidly dampening clock rhythms. Furthermore, zebrafish cells grow at room temperature, are viable for long periods at confluence, and do not require a CO2-enriched atmosphere, greatly simplifying culture conditions. Here we describe detailed methods for establishing zebrafish cell cultures as well as optimizing transient and stable transfections. These protocols have been successfully used to introduce luciferase reporter constructs into the cells and thereby monitor clock gene expression in vivo. The bioluminescence assay described here lends itself particularly well to high-throughput analysis.
Descriptors: zebrafish embryos, cell culture techniques methods, cell line, circadian rhythm genetics, cryopreservation, electroporation, gene expression, luciferases genetics, transfection methods.

Van der Meer, D.L., G.E. van den Thillart, F. Witte, M.A. de Bakker, J. Besser, M.K. Richardson, H.P. Spaink, J.T. Leito, and C.P. Bagowski (2005). **Gene expression profiling of the long-term adaptive response to hypoxia in the gills of adult zebrafish.** *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 289(5): R1512-9. ISSN: 0363 6119.
Abstract: Low oxygen levels (hypoxia) play a role in clinical conditions such as stroke, chronic ischemia, and cancer. To better understand these diseases, it is crucial to study the responses of vertebrates to hypoxia. Among vertebrates, some teleosts have developed the ability to adapt to extremely low oxygen levels. We have studied long-term adaptive responses to hypoxia in adult zebrafish. We used zebrafish that survived severe hypoxic conditions for 3 wk and showed adaptive behavioral and phenotypic changes. We used cDNA microarrays to investigate hypoxia-induced changes in expression of 15,532 genes in the respiratory organs (the gills). We have identified 367 differentially expressed genes of which 117 showed hypoxia-induced and 250 hypoxia-reduced expressions. Metabolic depression was indicated by repression of genes in the TCA cycle in the electron transport chain and of genes involved in protein biosynthesis. We observed enhanced expression of the monocarboxylate transporter and of the oxygen transporter myoglobin. The hypoxia-induced group further included the genes for Niemann-Pick C disease and for Wolman disease [lysosomal acid lipase (LAL)]. Both diseases lead to a similar intra- and extracellular accumulation of cholesterol and glycolipids. The Niemann-Pick C protein binds to cholesterol from internal lysosomal membranes and is involved in cholesterol trafficking. LAL is responsible for lysosomal cholesterol degradation. Our data suggest a novel adaptive mechanism to hypoxia, the induction of genes for lysosomal lipid trafficking and degradation. Studying physiological responses to hypoxia in species tolerant for extremely low oxygen levels can help identify novel regulatory genes, which may have important clinical implications.

Descriptors: zebrafish, physiological adaptation genetics, anoxia genetics, gene expression profiling and regulation physiology, gills physiology, physiological adaptation , gills ultrastructure, microarray analysis, Niemann Pick diseases metabolism, reverse transcriptase PCR, time factors, Wolman disease metabolism.

Van der Meulen, T. (2005). ***Epigenetics of the locomotory system in zebrafish***. 191 p.

Abstract: The epigenetics of muscle and bone in the locomotory system of zebrafish has been studied, with special reference to mechanical load. Five experiments have been conducted to determine the effects of mechanical load. The objectives, materials and methods and results of each experiment are presented in the 5 chapters of this supplement. Runx2a and Runx2b genes are excellent markers for studying the effects of mechanical load on bone development in the zebrafish. The white muscle fibres reorient during development. It is hypothesized that this may occur under the influence of the self-imposed mechanical load. The information required to develop the basal muscle components is independent of external signalling from muscle activity. Endurance training accelerates the ossification of tail bones. Strain eventually decreases the proliferation and/or increases apoptosis because of the morphological adaptation of the cells. Copyright © 2008 CABI

Descriptors: zebrafish, apoptosis, bones, epigenetics, exercise, fibroblasts, gene expression, genes, genetic markers, heart, molecular genetics, morphology, muscle fibres, muscles, musculoskeletal system, orientation, strain, *Danio rerio.*

Language of Text: Dutch.

Notes: ISBN:9085043220.

Van der Meulen, T., S. Kranenbarg, H. Schipper, J. Samallo, J.L. van Leeuwen, and H. Franssen (2005). **Identification and characterisation of two Runx2 homologues in zebrafish with different expression patterns.** *Biochimica Et Biophysica Acta* 1729(2): 105-17. ISSN: 0006 3002.

NAL Call Number: 381B522

Abstract: Genome and gene duplications are considered to be the impetus to generate new genes, as the presence of multiple copies of a gene allows for paralogues to adopt novel function. After at least two rounds of genome/gene duplication, the Runt gene family consists of three members in vertebrates, instead of one in invertebrates. One of the family members, Runx2, plays a key role in the development of bone, a tissue that first occurs in vertebrates. The family has thus gained new gene function in the course of evolution. Two Runx2 genes were cloned in the vertebrate model system the zebrafish (*Danio rerio*). The expression patterns of the two genes differ and their kinetics differ up to four fold. In addition, splice forms exist that are novel when compared with mammals. Together, these findings comprise opportunities for selection and retention of the paralogues towards divergent and possibly new function.

Descriptors: zebrafish, transcription factors genetics, 5' untranslated regions genetics, amino acid sequence, bone regeneration, developmental gene expression regulation, molecular sequence data, protein isoforms biosynthesis and genetics, transcription factors biosynthesis, variation genetics, proteins biosynthesis.

Van der Meulen, T., H. Schipper, J.L. van Leeuwen, and S. Kranenbarg (2005). **Effects of decreased muscle activity on developing axial musculature in nicb107 mutant zebrafish (*Danio rerio*).** *Journal of Experimental Biology* 208(19): 3675-87. ISSN: 0022 0949.

NAL Call Number: 442.8B77

Abstract: The present paper discusses the effects of decreased muscle activity (DMA) on

embryonic development in the zebrafish. Wild-type zebrafish embryos become mobile around 18 h post-fertilisation, long before the axial musculature is fully differentiated. As a model for DMA, the nic(b107) mutant was used. In nic(b107) mutant embryos, muscle fibres are mechanically intact and able to contract, but neuronal signalling is defective and the fibres are not activated, rendering the embryos immobile. Despite the immobility, distinguished slow and fast muscle fibres developed at the correct location in the axial muscles, helical muscle fibre arrangements were detected and sarcomere architecture was generated. However, in nic(b107) mutant embryos the notochord is flatter and the cross-sectional body shape more rounded, also affecting muscle fibre orientation. The stacking of sarcomeres and myofibril arrangement show a less regular pattern. Finally, expression levels of several genes were changed. Together, these changes in expression indicate that muscle growth is not impeded and energy metabolism is not changed by the decrease in muscle activity but that the composition of muscle is altered. In addition, skin stiffness is affected. In conclusion, the lack of muscle fibre activity did not prevent the basal muscle components developing but influenced further organisation and differentiation of these components.
Descriptors: zebrafish embryo, body patterning physiology, developmental gene expression regulation, skeletal muscle embryology, peptide fragments genetics, nicotinic receptors genetics, body patterning genetics, electron microscopy, muscle fibers ultrastructure, skeletal muscle physiology, skeletal muscle ultrastructure, mutation genetics, PCR.

Van 't Padje, S., B. Engels, L. Blonden, L.A. Severijnen, F. Verheijen, B.A. Oostra, and R. Willemsen (2005). **Characterisation of Fmrp in zebrafish: evolutionary dynamics of the fmr1 gene.** *Development Genes and Evolution* 215(4): 198-206. ISSN: 0949 944X.
NAL Call Number: QL951.R68
Abstract: Fragile X syndrome is the most common inherited form of mental retardation. It is caused by the lack of the Fragile X Mental Retardation Protein (FMRP), which is encoded by the FMR1 gene. Although Fmr1 knockout mice display some characteristics also found in fragile X patients, it is a complex animal model to study brain abnormalities, especially during early embryonic development. Interestingly, the ortholog of the FMR1 gene has been identified not only in mouse, but also in zebrafish (*Danio rerio*). In this study, an amino acid sequence comparison of FMRP orthologs was performed to determine the similar regions of FMRP between several species, including human, mouse, frog, fruitfly and zebrafish. Further characterisation of Fmrp has been performed in both adults and embryos of zebrafish using immunohistochemistry and western blotting with specific antibodies raised against zebrafish Fmrp. We have demonstrated a strong Fmrp expression in neurons of the brain and only a very weak expression in the testis. In brain tissue, a different distribution of the isoforms of Fmrp, compared to human and mouse brain tissue, was shown using western blot analysis. Due to the high similarity between zebrafish Fmrp and human FMRP and their similar expression pattern, the zebrafish has great potential as a complementary animal model to study the pathogenesis of the fragile X syndrome, especially during embryonic development.
Descriptors: zebrafish, molecular evolution, developmental gene expression regulation, nerve tissue RNA binding proteins genetics, amino acid and base sequence, Western blotting, brain chemistry genetics, COS cells, *Cercopithecus aethiops*, conserved sequence fragile X mental retardation protein, fragile X syndrome, immunohistochemistry, models, molecular sequence data, nerve tissue proteins chemistry, nuclear localization signals genetics, tertiary protein structure, RNA binding proteins chemistry, amino acid sequence homology, nucleic acid sequence homology, transfection.

Vaulont, S. and L. Viatte (2006). **La peche au gene chez le poisson zebre: identification du transporteur du fer mitochondrial. [Gene fishing in zebrafish: identification of the iron mitochondrial transporter]**. *Medecine Sciences* 22(5): 466-8. ISSN: 0767-0974.
Descriptors: zebrafish, iron metabolism, mitochondrial membrane transport proteins genetics, membrane transport mitochondrial membrane transport proteins isolation and purification, proteins isolation and purification.
Language of Text: French.

Veeman, M.T., D.C. Slusarski, A. Kaykas, S.H. Louie, and R.T. Moon (2003). **Zebrafish prickle, a modulator of noncanonical Wnt/Fz signaling, regulates gastrulation movements.** *Current Biology* 13(8): 680-5. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Abstract: In addition to the canonical Wnt/beta-catenin signaling pathway, at least two noncanonical Wnt/Fz pathways have been described: the planar cell polarity (PCP) pathway in Drosophila [1] and the Wnt/calcium pathway in vertebrate embryos [2]. Recent work suggests that a vertebrate pathway homologous to the PCP pathway acts to regulate the convergent extension movements of gastrulation [3-7]. To further test this hypothesis, we have identified two zebrafish homologs of the *Drosophila* PCP gene prickle (pk) [8], both of which show discrete and dynamic expression patterns during gastrulation. Both gain and loss of pk1 function cause defects in convergent extension. Pk1 localizes to both the cytoplasm and the cell membrane, and its normal localization is partially dependent on its C-terminal prenylation motif. At the cell membrane, Pk1 is frequently localized asymmetrically around the cell and can colocalize with the signaling molecule Dishevelled (Dsh). In overexpression assays, Pk1 is able to activate AP-1-mediated transcription and inhibit activation of Wnt/beta-catenin signaling. Like noncanonical Wnts [9-10], overexpression of Pk1 increases the frequency of calcium transients in zebrafish blastulae. Our results support the idea that a vertebrate PCP pathway regulates gastrulation movements and suggest that there is overlap between the PCP and Wnt/calcium pathways.
Descriptors: zebrafish, cell movement physiology, DNA binding proteins genetics, *Drosophila* gastrula physiology, signal transduction physiology, signal transducing adaptor proteins, cell movement genetics, frizzled receptors, gastrula metabolism, gene expression, glycoproteins metabolism, immunohistochemistry, in situ hybridization, phosphoproteins metabolism, Wnt proteins.

Veeramachaneni, V. and W. Makalowski (2005). **Database of evolutionary Distances**. *Nucleic Acids Research* 33(Suppl.1): D442-D446. ISSN: 0305-1048.
NAL Call Number: QD341.A2N8
Descriptors: zebrafish, data collections, databases, evolution, genes, genetic distance, nucleic acids, nucleotide sequence, phylogenetics, phylogeny, proteins, *Danio rerio*, Fugu , physical and computer methods and assays , genetics and evolution, theoretical genetics, bioinformatics.
Notes: A review.

Verri, T., G. Kottra, A. Romano, N. Tiso, M. Peric, M. Maffia, M. Boll, F. Argenton, H. Daniel, and C. Storelli (2003). **Molecular and functional characterisation of the zebrafish (*Danio rerio*) PEPT1-type peptide transporter.** *Federation of European Biochemical Societies Journal* 549(1-3): 115-22. ISSN: 1742 464X.
NAL Call Number: QP501.E8
Abstract: We report the molecular and functional characterisation of a novel peptide trans-

porter from zebrafish, orthologue to mammalian and avian PEPT1. Zebrafish PEPT1 is a low-affinity/high-capacity system. However, in contrast to higher vertebrate counterparts in which maximal transport activity is independent of extracellular pH, zebrafish PEPT1 maximal transport rates unexpectedly increase at alkaline extracellular pH. Zebrafish pept1 is highly expressed in the proximal intestine since day 4 post-fertilisation, thus preceding functional maturation of the gut, first feeding and complete yolk resorption. Zebrafish PEPT1 might help to understand the evolutionary and functional relationships among vertebrate peptide transporters. Moreover, zebrafish pept1 can be a useful marker for screening mutations that affect gut regionalisation, differentiation and morphogenesis.

Descriptors: zebrafish, carrier, symporters, base sequence, carrier proteins genetics and metabolism, electrophysiology, embryonic and fetal development, hydrogen ion concentration, intestines metabolism, kinetics, molecular sequence data, oocytes, phylogeny, tissue distribution, *Xenopus*.

Vianello, S., L. Brazzoduro, L. Dalla Valle, P. Belvedere, and L. Colombo (2003). **Myostatin expression during development and chronic stress in zebrafish (*Danio rerio*).** *Journal of Endocrinology* 176(1): 47-59. ISSN: 0022-0795.

Abstract: Myostatin, a member of the transforming growth factor-beta superfamily, is a negative regulator of skeletal muscle mass in mammals. We have studied myostatin expression during embryonic and post-hatching development in zebrafish by semiquantitative RT-PCR. The transcript is present in just-fertilized eggs and declines at 8 h post-fertilization (hpf), suggesting a maternal origin. A secondary rise occurs at 16 hpf, indicating the onset of embryonic transcription at the time of muscle cell differentiation. The level of myostatin mRNA decreases slightly at 24 hpf, when somitogenesis is almost concluded, and rises again at and after hatching, during the period of limited muscle hyperplastic growth that is typical of slow-growing, small fish. In the adult muscle, we found the highest expression of myostatin mRNA and protein, which were detectable by Northern and Western blot analyses respectively. Although only the precursor protein form was revealed in the adult lateral muscle, we demonstrated that zebrafish myostatin is proteolytically processed and secreted in cultured cells, as is its mammalian counterpart. These results suggest that myostatin may play an important regulatory role during myogenesis and muscle growth in fish, as it does in mammals. In chronically stressed fish, grown from 16 days post-fertilization to adulthood in an overcrowded environment, we observed both depression of body growth and a diminished level of myostatin mRNA in the adult muscle, as compared with controls. We propose that chronic stunting in fish brings about a general depression of muscle protein synthesis which does not spare myostatin.

Descriptors: zebrafish, skeletal muscle metabolism, stress metabolism, transforming growth factor beta genetics, metabolism, Northern blotting methods, Southern blotting methods, body weight, gene expression, in situ hybridization methods , messenger RNA analysis, reverse transcriptase PCR, stress physiopathology, transforming growth factor beta analysis.

Vidal, G., B. Scheffler, A. Michel, and S.J. D'Surney (2004). **Genomic and phylogenic comparisons of the alpha-globin and beta-globin intergenic sequences between zebra fish (*Danio rerio*) and six closely related Cyprinindae species.** *DNA and Cell Biology* 23(5): 325-34. ISSN: 1044 5498.

NAL Call Number: QH443.D5

Abstract: The alpha-globin and beta-globin genes of the zebrafish are tightly linked on the same chromosome in a 3'-5' and 5'-3' configuration, respectively. Although the location of the controlling sequences has been mapped to the intergenic region, analysis determining

the uniqueness of this unusual arrangement to zebrafish has not been undertaken. To explore this, we isolated, sequenced, and phylogenetically analyzed the intergenic region between globin gene families of seven Cyprinindae species including zebrafish. These species were grouped into an in group (immediate relatives, not so distant relatives), and an out group (distant relative). Cellulose acetate electrophoresis of hemoglobin (Hb) detected multiple variants in each species, but a band with electrophoretic mobility (EM) of 6.7 x 10(-5) cm(2).volt(-1).sec(-1) was shared between species. Polymerase chain reaction (PCR) amplification of the intergenic globin gene region also detected a 1.0-kb fragment that was repeated in the in group and a 1.2-kb fragment in the out group. Sequence comparison confirmed that the genetic orientation and controlling sequences location were conserved throughout this region in all seven species. This phylogenic footprinting indicated that the configuration was not exclusive to zebrafish. To confirm sequence alignment, maximum parsimony phylogenic analysis, was performed. Only one member of that group the giant danio, was not closely clustered, being located almost equidistance between the immediate relative and the species of the other clusters. This may represent an ancestral configuration prior to transposition of the alpha globin and beta-globin genes families to nonsynteny.
Descriptors: zebrafish, Cyprinidae genetics, DNA, intergenic, globins genetics, phylogeny, algorithms, base sequence, conserved sequence, cellulose acetate electrophoresis methods, erythrocytes physiology, GC rich sequence, hemoglobins genetics, molecular sequence data, regulatory sequences, nucleic acid, sequence alignment, nucleic acid sequence homology.

Vihtelic, T.S., J.M. Fadool, J. Gao, K.A. Thornton, D.R. Hyde, and G. Wistow (2005). **Expressed sequence tag analysis of zebrafish eye tissues for NEIBank.** *Molecular Vision* 11: 1083-100. ISSN: 1090-0535.
NAL Call Number: QP475 .M65
Abstract: PURPOSE: To characterize gene expression patterns in various tissues of the zebrafish (*Danio rerio*) eye and identify zebrafish orthologs of human genes by expressed sequence tag (EST) analysis for NEIBank. METHODS: mRNA was extracted from adult zebrafish eye tissues, including lenses, anterior segments (minus lens), retinas, posterior segments lacking retinas, and whole eyes. Five different cDNA libraries were constructed in the pCMVSport6 vector. Approximately 4,000 clones from each library were sequenced and analyzed using various bioinformatics programs. RESULTS: The analysis yielded approximately 2,500 different gene clusters for each library. Combining data from the five libraries produced 10,392 unique gene clusters. GenBank accession numbers were identified for 37.6% (3,906) of the total gene clusters in the combined libraries and approximately 50% were linked to Unigene clusters in the current database. Several new crystallin genes, including two gammaN-crystallins, and a second major intrinsic protein (MIP) were identified in the lens library. In addition, a zebrafish homolog of cochlin (COCH), a gene that may play a role in the pathogenesis of human glaucoma, was identified in the anterior segment library. Surprisingly, no clear ortholog of the major retinal transcription factor Nrl was identified. CONCLUSIONS: The zebrafish eye tissue cDNA libraries are a useful resource for comparative gene expression analysis. These libraries will complement the cDNA libraries made for the Zebrafish Gene Collection (ZGC) and provide an additional source for gene identification and characterization in the vertebrate eye.
Descriptors: zebrafish, genetic databases, expressed sequence tags, eye metabolism, eye ophthalmology organization and administration, computational biology organization and administration, gene expression, gene library, in situ hybridization, molecular biology, ., RNA genetics, DNA sequence analysis, National Institutes of Health United States.

Von der Hardt, S., J. Bakkers, A. Inbal, L. Carvalho, L. Solnica Krezel, C.P. Heisenberg, and M. Hammerschmidt (2007). **The Bmp gradient of the zebrafish gastrula guides migrating lateral cells by regulating cell-cell adhesion.** *Current Biology* 17(6): 475-87. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: BACKGROUND: Bone morphogenetic proteins (Bmps) are required for the specification of ventrolateral cell fates during embryonic dorsoventral patterning and for proper convergence and extension gastrulation movements, but the mechanisms underlying the latter role remained elusive. RESULTS: Via bead implantations, we show that the Bmp gradient determines the direction of lateral mesodermal cell migration during dorsal convergence in the zebrafish gastrula. This effect is independent of its role during dorsoventral patterning and of noncanonical Wnt signaling. However, it requires Bmp signal transduction through Alk8 and Smad5 to negatively regulate Ca(2+)/Cadherin-dependent cell-cell adhesiveness. In vivo, converging mesodermal cells form lamellipodia that attach to adjacent cells. Bmp signaling diminishes the Cadherin-dependent stability of such contact points, thereby abrogating subsequent cell displacement during lamellipodial retraction. CONCLUSIONS: We propose that the ventral-to-dorsal Bmp gradient has an instructive role to establish a reverse gradient of cell-cell adhesiveness, thereby defining different migratory zones and directing lamellipodia-driven cell migrations during dorsal convergence in lateral regions of the zebrafish gastrula.

Descriptors: zebrafish, bone morphogenetic, cell adhesion and movement drug effects, gastrula metabolism, body patterning, bone morphogenetic proteins genetics and metabolism, cadherins metabolism, calcium metabolism, cell differentiation, chelating agents pharmacology, gastrula cytology and drug effects, signal transduction.

Von Hofsten, J., C. Modig, A. Larsson, J. Karlsson, and P.E. Olsson (2005). **Determination of the expression pattern of the dual promoter of zebrafish fushi tarazu factor-1a following microinjections into zebrafish one cell stage embryos.** *General and Comparative Endocrinology* 142(1-2): 222-6. ISSN: 0016-6480.

NAL Call Number: 444.8G28

Abstract: The zebrafish fushi tarazu factor-1a (ff1a) is a transcription factor belonging to the NR5A subgroup of nuclear receptors. The NR5A receptors bind DNA as monomers and are considered to be orphans due to their ability to promote transcription of downstream genes without ligands. In zebrafish, four ff1 homologues (Ff1a, Ff1b, Ff1c, and Ff1d) have been identified so far. The gene coding for Ff1a is driven by two separate promoters, and give rise to four splice variants. Ff1a is expressed in the somites and pronephric ducts during somitogenesis and in the brain, liver, and mandibular arch during later embryonic stages. In adults the gene is highly expressed in gonads, liver, and intestine, but can be detected in most tissues. The broad variety of embryonic expression domains indicates several important developmental features. One of the mammalian fushi tarazu factor-1 genes, steroidogenic factor-1 (SF-1), is essential for the development of gonads and adrenals. SF-1 is together with Sox9, WT1, and GATA4 a positive transcriptional regulator of human anti-mullerian hormone (AMH) and thereby linked to the male sex-determining pathway. The zebrafish ff1a dual promoter contains several GATA binding sites and E-boxes, a site for DR4, XFD2, MyoD, Snail, HNF3, S8, and an HMG-box recognition site for Sox9. In a first attempt to dissect the ff1a promoter in vivo we have produced first generation transgenes in order to determine the correlation between the expression of the endogenous ff1a gene and the microinjected ff1a dual promoter coupled to the pEGFP reporter vector. Our results show that the microinjected constructs are expressed in the correct tissues.

Descriptors: zebrafish, DNA binding proteins genetics, DNA binding proteins genetics, proteins pharmacology, physiology, gene expression regulation physiology, transcription factors genetics and pharmacology, genetically modified, base sequence, DNA binding proteins genetics, proteins administration and dosage, genetic vectors, homeodomain proteins, in situ hybridization, microinjections, molecular sequence data, promoter regions genetics, epidermal growth factor receptos metabolism, cytoplasmic receptors and nuclear, reverse transcriptase PCR, transcription factors administration and dosage.

Von Hofsten, J. and P.E. Olsson (2005). **Zebrafish sex determination and differentiation: involvement of FTZ-F1 genes.** *Reproductive Biology and Endocrinology* 3: 63. ISSN: 1477-7827.
NAL Call Number: QP1
Abstract: Sex determination is the process deciding the sex of a developing embryo. This is usually determined genetically; however it is a delicate process, which in many cases can be influenced by environmental factors. The mechanisms controlling zebrafish sex determination and differentiation are not known. To date no sex linked genes have been identified in zebrafish and no sex chromosomes have been identified. However, a number of genes, as presented here, have been linked to the process of sex determination or differentiation in zebrafish. The zebrafish FTZ-F1 genes are of central interest as they are involved in regulating interrenal development and thereby steroid biosynthesis, as well as that they show expression patterns congruent with reproductive tissue differentiation and function. Zebrafish can be sex reversed by exposure to estrogens, suggesting that the estrogen levels are crucial during sex differentiation. The Cyp19 gene product aromatase converts testosterone into 17 beta-estradiol, and when inhibited leads to male to female sex reversal. FTZ-F1 genes are strongly linked to steroid biosynthesis and the regulatory region of Cyp19 contains binding sites for FTZ-F1 genes, further linking FTZ-F1 to this process. The role of FTZ-F1 and other candidates for zebrafish sex determination and differentiation is in focus of this review.
Descriptors: zebrafish, fushi tarazu transcription factors genetics, sex determination genetics, sex differentiation physiology, aromatase genetics, DNA binding proteins genetics, erythroid specific DNA binding proteins genetics, factors genetics, glycohmgb homeodomain genetic models, cytoplasmic receptors and nuclear genetics, testicular hormones genetics, transcription factors genetics, Wt1 proteins genetics.

Wada, Y., J. Sugiyama, T. Okano, and Y. Fukada (2006). **GRK1 and GRK7: unique cellular distribution and widely different activities of opsin phosphorylation in the zebrafish rods and cones.** *Journal of Neurochemistry* 98(3): 824-37. ISSN: 0022-3042.
NAL Call Number: QP351.J6
Abstract: Retinal cone cells exhibit distinctive photoresponse with a more restrained sensitivity to light and a more rapid shutoff kinetics than those of rods. To understand the molecular basis for these characteristics of cone responses, we focused on the opsin deactivation process initiated by G protein-coupled receptor kinase (GRK) 1 and GRK7 in the zebrafish, an animal model suitable for studies on retinal physiology and biochemistry. Screening of the ocular cDNAs identified two homologs for each of GRK1 (1A and 1B) and GRK7 (7-1 and 7-2), and they were classified into three GRK subfamilies, 1 A, 1B and 7 by phylogenetic analysis. In situ hybridization and immunohistochemical studies localized both GRK1B and GRK7-1 in the cone outer segments and GRK1A in the rod outer segments. The opsin/GRKs molar ratio was estimated to be 569 in the rod and 153 in the cone. The recombinant GRKs phosphorylated light-activated rhodopsin, and the Vmax value of the major cone subtype, GRK7-1, was 32-fold higher than that of the rod kinase, GRK1A. The reinforced activity of the cone kinase should provide a strengthened shutoff mechanism of

the light-signaling in the cone and contribute to the characteristics of the cone responses by reducing signal amplification efficiency.
Descriptors: zebrafish, retina cones metabolism, opsin metabolism, protein serine threonine kinases and rhodopsin kinase biosynthesis, retinal rods metabolism, proteins biosynthesis, cell line, retina cones chemistry, eye proteins analysis and biosynthesis, eye molecular sequence data, opsin chemistry, phosphorylation, protein serine threonine kinases genetics, rhodopsin kinase genetics, retinal rods chemistry.

Wagle, M. and S. Jesuthasan (2003). **Baculovirus-mediated gene expression in zebrafish.** *Marine Biotechnology* 5(1): 58-63. ISSN: 1436-2228.
NAL Call Number: TP248.27.M37M37
Abstract: In an effort to misexpress genes in zebrafish, we tested the ability of baculovirus to infect and drive gene expression in embryos. By injecting virus into specific tissues and using appropriate promoters, both the location and time of gene expression could be controlled. Using a virus with 2 different promoters, LacZ and GFP could be expressed independently. The efficiency of expression appears to depend on the promoter used. As a test of this system, baculovirus was used to ectopically express ephrinB2a in the presomitic mesoderm. EphrinB2a is normally expressed in the posterior region of developing somites, and baculovirus-mediated misexpression caused abnormal somite boundary formation. Baculovirus can thus be used as a tool for gene misexpression experiments in the zebrafish, especially when localized misexpression is required late in development.
Descriptors: zebrafish, Baculoviridae genetics, developmental gene expression regulation, genetic vectors genetics, transfection methods.

Wagner, G.P., K. Takahashi, V. Lynch, S.J. Prohaska, C. Fried, P.F. Stadler, and C. Amemiya (2005). **Molecular evolution of duplicated ray finned fish HoxA clusters: increased synonymous substitution rate and asymmetrical co-divergence of coding and non-coding sequences**. *Journal of Molecular Evolution* 60(5): 665-676. ISSN: 0022-2844.
NAL Call Number: QH359.J6
Descriptors: mutant zebrafish, asymmetry, base composition, evolution, evolutionary genetics, freshwater fish , genes, mutation rates, nucleotide sequence, phylogeny, rAg1 protein, codon bias and usage, gene duplication, molecular evolution, *Danio rerio*, fugu genetics and evolution.

Wakasugi, K. and I. Morishima (2005). **Preparation and characterization of a chimeric zebrafish-human neuroglobin engineered by module substitution.** *Biochemical and Biophysical Research Communications* 330(2): 591-7. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: Neuroglobin (Ngb) is a recently discovered vertebrate heme protein that can reversibly bind oxygen that is expressed in the brain. Zebrafish and human Ngb share about 50% amino acid sequence identity. These Ngb proteins consist of four compact protein structural unit "modules" referred to as M1-M4. In the present study, we investigated the effects of module substitution on the properties of Ngb. Specifically, we prepared and characterized a chimeric ZHZZ Ngb in which the heme-binding module M2 of zebrafish Ngb was replaced by the comparable human Ngb module. Our results showed that the chimeric ZHZZ was stable and formed almost the identical heme-environmental and alpha-helical structure as the human and zebrafish Ngb proteins, suggesting that the structure of Ngb has been evolutionarily conserved.
Descriptors: zebrafish, globins chemistry, nerve tissue proteins chemistry, recombinant

fusion proteins chemistry, amino acid and base sequence, circular dichroism, DNA primers, polyacrylamide gel electrophoresis, molecular sequence data, biomolecular nuclear magnetic resonance, protein denaturation, amino acid sequence homology.

Wallace, B.M. and H. Wallace (2003). **Synaptonemal complex karyotype of zebrafish.** *Heredity* 90(2): 136-40. ISSN: 0018-067X.
NAL Call Number: 443.8H42
Abstract: Meiotic cells of zebrafish have been prepared to show synaptonemal complexes (SCs) by light and electron microscopy. Completely paired SCs from both spermatocytes and oocytes were measured to produce an SC karyotype. The SC karyotype resembles the somatic karyotype of zebrafish and has no recognisable sex bivalent. Measurements of total SC length indicate that SCs grow longer and develop centromeres during pachytene. Oocytes consistently have longer SCs than spermatocytes, presumably correlated with the reported higher recombination frequency in females than in males.
Descriptors: zebrafish, chromosomes, synaptonemal complex, karyotyping, meiosis, genetic recombination, sex characteristics, sex chromosomes, sex determination genetics.

Wan, H., S. Korzh, Z. Li, S.P. Mudumana, V. Korzh, Y.J. Jiang, S. Lin, and Z. Gong (2006). **Analyses of pancreas development by generation of gfp transgenic zebrafish using an exocrine pancreas-specific elastaseA gene promoter.** *Experimental Cell Research* 312(9): 1526-39. ISSN: 0014 4827.
NAL Call Number: 442.8EX7
Abstract: In contrast to what we know on development of endocrine pancreas, the formation of exocrine pancreas remains poorly understood. To create an animal model that allows observation of exocrine cell differentiation, proliferation, and morphogenesis in living animals, we used the zebrafish elastaseA (elaA) regulatory sequence to develop transgenic zebrafish that display highly specific exocrine pancreas expression of GFP in both larvae and adult. By following GFP expression, we found that the pancreas in early development was a relatively compact organ and later extended posterior along the intestine. By transferring the elaA:gfp transgene into slow muscle omitted mutant that is deficient in receiving Hedgehog signals, we further showed that Hedgehog signaling is required for exocrine morphogenesis but not for cell differentiation. We also applied the morpholino knockdown and toxin-mediated cell ablation approaches to this transgenic line. We showed that the development of exocrine pancreas is Islet-1 dependent. Injection of the diphtheria toxin A (DTA) construct under the elastaseA promoter resulted in selective ablation of exocrine cells while the endocrine cells and other endodermal derivatives (liver and intestine) were not affected. Thus, our works demonstrated the new transgenic line provided a useful experimental tool in analyzing exocrine pancreas development.
Descriptors: transgenic zebrafish, green fluorescent proteins genetics, exocrine pancreas metabolism, pancreatic elastase genetics, promoter regions genetics, amino acid sequence, genetically modified, base sequence, diphtheria toxin genetics, fatty acid binding gene deletion, developmental gene expression regulation, homeodomain molecular sequence data, nerve tissue antisense oligonucleotides genetics, exocrine pancreas embryology, exocrine pancreas growth and development, G protein Coupled receptors genetics, recombinant fusion amino acid sequence homology, somatostatin genetics, transferrin genetics.

Wang, D.S., B. Jiao, C. Hu, X. Huang, Z. Liu, and C.H.K. Cheng (2008). **Discovery of a gonad-specific IGF subtype in teleost**. *Biochemical and Biophysical Research Communications* 367(2): 336-341. ISSN: 0006-291X.

NAL Call Number: 442.8B5236
Descriptors: zebrafish, Igf-3, cDNA, clone, medaka genome, EST databases, Igf-1, Igf-2.

Wang, D., L.E. Jao, N. Zheng, K. Dolan, J. Ivey, S. Zonies, X. Wu, K. Wu, H. Yang, Q. Meng, Z. Zhu, B. Zhang, S. Lin, and S.M. Burgess (2007). **Efficient genome-wide mutagenesis of zebrafish genes by retroviral insertions.** *Proceedings of the National Academy of Sciences of the United States of America* 104(30): 12428-33. ISSN: 0027 8424.
NAL Call Number: 500N21P
Abstract: Using a combination of techniques we developed, we infected zebrafish embryos using pseudotyped retroviruses and mapped the genomic locations of the proviral integrations in the F offspring of the infected fish. From F fish, we obtained 2,045 sequences representing 933 unique retroviral integrations. A total of 599 were mappable to the current genomic assembly (Zv6), and 233 of the integrations landed within genes. By inbreeding fish carrying proviral integrations in 25 different genes, we were able to demonstrate that in [almost equal to]50% of the gene "hits," the mRNA transcript levels were reduced by >=70%, with the highest probability for mutation occurring if the integration was in an exon or first intron. Based on these data, the mutagenic frequency for the retrovirus is nearly one in five integrations. In addition, a strong mutagenic effect is seen when murine leukemia virus integrates specifically in the first intron of genes but not in other introns. Three of 19 gene inactivation events had embryonic defects. Using the strategy we outlined, it is possible to identify 1 mutagenic event for every 30 sequencing reactions done on the F fish. This is a 20- to 30-fold increase in efficiency when compared with the current resequencing approach [targeting induced local lesions in genomes (TILLING)] used in zebrafish for identifying mutations in genes. Combining this increase in efficiency with cryopreservation of sperm samples from the F fish, it is now possible to create a stable resource that contains mutations in every known zebrafish gene.
Descriptors: zebrafish, mutagenesis, genes, pseudotyped retroviruses, F offspring.

Wang, H., E.M. Lee, S.M. Sperber, S. Lin, M. Ekker, and Q. Long (2007). **Isolation and expression of zebrafish zinc-finger transcription factor gene tsh1.** *Gene Expression Patterns* 7(3): 318-22. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: We report the expression patterns of tsh1, a zebrafish homologue of the Drosophila homeotic gene teashirt. Expression of tsh1 is first detected at the 2-somite stage (10h post-fertilization, hpf) at the anterior end of the spinal cord. Expression expands toward the posterior spinal cord, and by the prim-5 stage (24 hpf) tsh1 transcripts are detected throughout spinal cord. Between the 14- and 25-somite stage (16-24 hpf), spinal cord expression shows a clear anterior boundary at the rostral margin of rhombomere 7. Around the prim-25 stage (36 hpf), while the spinal expression of tsh1 decreases, new expression is detected in the pectoral fin buds and dorsal forebrain. By the long-pec stage (48 hpf), spinal cord expression is undetectable, but strong expression is observed in the rhombencephalon, telencephalon, tectum opticum, midbrain-hindbrain boundary, in the first pharyngeal arch and in the eyes. This expression persists at least until the larval stages. Retinoic acid signaling influences tsh1 expression. Zebrafish tsh1 expression was induced in the anterior neural tube in embryos treated briefly with exogenous retinoic acid. Furthermore, tsh1 expression was down-regulated in the spinal cord in the zebrafish neckless mutant in which RA signaling is disrupted due to a missense mutation in the gene encoding retinaldehyde dehydrogenase type 2.
Descriptors: zebrafish, brain metabolism, developmental gene expression regulation, spinal cord metabolism, transcription factors genetics, amino acid sequence, brain embryology,

brain growth and development, metabolism, molecular sequence data, missense mutation, phenotype, retinal dehydrogenase genetics, spinal cord embryology, spinal cord growth and development, transcription factors chemistry, tretinoin metabolism.

Wang, H., J.T. Tan, A. Emelyanov, V. Korzh, and Z. Gong (2005). **Hepatic and extrahepatic expression of vitellogenin genes in the zebrafish, *Danio rerio*.** *Gene* 356: 91-100. ISSN: 0378-1119.

NAL Call Number: QH442.A1G4

Abstract: Vitellogenins (Vtgs) are yolk precursor proteins in oviparous species and are cleaved into three portions-lipovitellin I (LVI), phosvitin (PV), and lipovitellin II (LVII)-in oocytes in vertebrates. In the present study, we found that the zebrafish genome contains at least seven vtg genes (vtg1-7) encoding heterogeneous vitellogenins with three distinct types of Vtgs: type I (Vtg1, 4-7) contains all the three major portions but lacks the C-terminal half of LVII; type II (Vtg2) is the only one including intact three portions; type III (Vtg3) lacks both PV and the LVII C-terminal half. The seven vtgs were located in two different chromosomes: one (vtg3) in LG11 and the rest closely linked in LG22, probably arisen from local gene duplication events. All of the seven vtgs are predominantly expressed in female liver and can be induced in male liver by 17beta-estradiol (E2). The level of vtg1 mRNA was about 100x and 1000x higher than those of vtg2 and vtg3 mRNAs. We also found vtg mRNAs in several non-liver tissues, but the expression level is generally <10% of that in the liver. In situ hybridization experiments confirmed that the extrahepatic expression was actually in adipocytes associated with several organs such as the intestine, ovary, and E2-induced testis.

Descriptors: zebrafish, gene expression profiling, liver metabolism, vitellogenins genetics, adipocytes metabolism, amino acid sequence, chromosome mapping methods, complementary DNA chemistry, complementary DNA genetics, estradiol pharmacology, expressed sequence tags, gene expression regulation drug effects, hepatocytes metabolism, in situ hybridization, liver cytology and drug effects, molecular sequence data, phylogeny, messenger RNA genetics and metabolism, sequence alignment, DNA sequence analysis, amino acid sequence homology.

Wang, H., Q. Zhou, J.W. Kesinger, C. Norris, and C. Valdez (2007). **Heme regulates exocrine peptidase precursor genes in zebrafish.** *Experimental Biology and Medicine* 232(9): 1170-80. ISSN: 1535 3702.

NAL Call Number: QP1 .S8

Abstract: We previously determined that yquem harbors a mutation in the gene encoding uroporphyrinogen decarboxylase (UROD), the fifth enzyme in heme biosynthesis, and established zebrafish yquem (yqe(tp61)) as a vertebrate model for human hepatoery-thropoietic porphyria (HEP). Here we report that six exocrine peptidase precursor genes, carboxypeptidase A, trypsin precursor, trypsin like, chymotrypsinogen B1, chymotrypsinogen 1-like, and elastase 2 like, are downregulated in yquem/urod (-/-), identified initially by microarray analysis of yquem/urod zebrafish and, subsequently, confirmed by in situ hybridization. We then determined downregulation of these six zymogens specifically in the exocrine pancreas of sauternes (sau(tb223)) larvae, carrying a mutation in the gene encoding delta-amino-levulinate synthase (ALAS2), the first enzyme in heme biosynthesis. We also found that ptf1a, a transcription factor regulating exocrine zymogens, is downregulated in both yquem/urod (-/-) and sau/alas2 (-/-) larvae. Further, hemin treatment rescues expression of ptf1a and these six zymogens in both yquem/urod (-/-) and sauternes/alas2 (-/-) larvae. Thus, it appears that heme deficiency downregulates ptf1a, which, in turn, leads to downregulation of exocrine zymogens. Our findings provide a better understanding of heme deficiency pathogenesis and

enhance our ability to diagnose and treat patients with porphyria or pancreatic diseases.
Descriptors: zebrafish, enzyme precursors genetics, heme metabolism, exocrine pancreas enzymology , peptide hydrolases genetics, 5 aminolevulinate synthetase genetics, 5 aminolevulinate synthetase metabolism, DNA primers metabolism, down regulation, enzymology, enzyme precursors metabolism, heme deficiency, hemin metabolism, hemin pharmacology , oligonucleotide array sequence analysis, exocrine pancreas metabolism, peptide hydrolases metabolism, phylogeny, transcription factors genetics and metabolism.

Wang, L., L. Wang, H.X. Zhang, J.H. Zhang, W.H. Chen, X.F. Ruan, and C. Xia (2006). **In vitro effects of recombinant zebrafish IFN on spring viremia of carp virus and infectious hematopoietic necrosis virus.** *Journal of Interferon and Cytokine Research* 26(4): 256-59. ISSN: 1079-9907.
Abstract: In order to evaluate the effects of fish recombinant interferon (rIFN) on fish pathogenic rhabdoviruses, the zebrafish *(Danio rerio*) IFN (DreIFN) allele B gene was cloned and expressed in Escherichia coli. In addition, the effects of recombinant DreIFN (rDreIFN) on spring viremia of carp virus (SVCV), infectious hematopoietic necrosis virus (IHNV), and vesicular stomatitis virus (VSV) were surveyed in fish and chicken cells. The mature peptide of DreIFN allele B gene encodes 163 amino acids. Residues 3 and 98 are a pair of cysteines that likely form an intrachain disulfide bridge. rDreIFN protein was detected as a band at 21.6 kDa by SDS-PAGE. The purified rDreIFN has anti-SVCV and anti-IHNV activity of 3 x 10(4) U/mg-10(7) U/mg. The results indicate that rDreIFN has higher activity against SVCV and IHNV on epithelioma papulosum cyprinid (EPC) than on grass carp (C. idellus) ovary (CO) cell lines and no activity against VSV on chick embryo fibroblasts (CEF).
Descriptors: zebrafish, infectious hematopoietic necrosis virus drug effects, interferon Type I, recombinant pharmacology, vesiculovirus drug effects, proteins pharmacology, amino acid sequence, carps, cell line, chick embryo, chickens, *Escherichia coli* genetics and metabolism, Type I interferon recombinant biosynthesis and genetics, molecular sequence data.

Wang, L.I., L. Wang, and C. Xia (2005). **Cloning and sequence analysis of the interferon gene from zebrafish, *Danio rerio*.** *Journal of Agricultural Biotechnology/Nongye Shengwu Jishu Xuebao* 13(1): 125-126. ISSN: 1006-1304.
Descriptors: zebrafish, interferon, nucleotide sequence, *Danio rerio*.

Wang, N., Y.H. Sun, J. Liu, G. Wu, J.G. Su, Y.P. Wang, and Z.Y. Zhu (2007). **Knock down of gfp and no tail expression in zebrafish embryo by in vivo-transcribed short hairpin RNA with T7 plasmid system.** *Journal of Biomedical Science* 14(6): 767-76. ISSN: 1021-7770.
NAL Call Number: R850.A1
Abstract: A short-hairpin RNA (shRNA) expression system, based on T7 RNA polymerase (T7RP) directed transcription machinery, has been developed and used to generate a knock down effect in zebrafish embryos by targeting green fluorescent protein (gfp) and no tail (ntl) mRNA. The vector pCMVT7R harboring T7RP driven by CMV promoter was introduced into zebrafish embryos and the germline transmitted transgenic individuals were screened out for subsequent RNAi application. The shRNA transcription vectors pT7shRNA were constructed and validated by in vivo transcription assay. When pT7shGFP vector was injected into the transgenic embryos stably expressing T7RP, gfp relative expression level showed a decrease of 68% by analysis of fluorescence real time RT-PCR. As a control, injection of chemical synthesized siRNA resulted in expression level of 40% lower than the control when the injection dose was as high as 2 microg/microl. More importantly, injection of pT7shNTL vector in zebrafish embryos expressing T7RP led to partial absence of endogenous ntl tran-

scripts in 30% of the injected embryos when detected by whole mount in situ hybridization. Herein, the T7 transcription system could be used to drive the expression of shRNA in zebrafish embryos and result in gene knock down effect, suggesting a potential role for its application in RNAi studies in zebrafish embryos.
Descriptors: zebrafish, GFP, short hairpin RNA, T7, knock down.

Wang, W.D., C.J. Huang, Y.F. Lu, J.P. Hsin, V.R. Prabhakar, C.F. Cheng, and S.P. Hwang (2006). **Heart-targeted overexpression of Nip3a in zebrafish embryos causes abnormal heart development and cardiac dysfunction.** *Biochemical and Biophysical Research Communications* 347(4): 979-87. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: We transiently expressed a proapoptotic protein, Nip3a, by a heart-specific BMP4 promoter in zebrafish embryos and generated two variants of embryos with abnormal heart phenotypes (A and B). Embryos with phenotype A heart defects showed hypoplastic or elongated ventricles, elongated or enlarged atriums with no normal cardiac looping resulting a significant longer SV-BA distance, and bradycardia at 48 h post-fertilization (hpf). Embryos with phenotype B heart defects showed an enlarged fluid-filled pericardium, severe hypoplasia, non-contracting ventricles, and elongated or enlarged slowly beating atriums with no normal looping. Histological sections further revealed the absence of a proper atrioventricular boundary and no endocardial cells lining this region in both 48- and 72-hpf Nip3a-overexpressing embryos, implicating defective endocardial cushion formation. These phenotypes are reminiscent of atrioventricular canal defects in humans. In addition, induced apoptotic myocardium cells were clustered in the presumptive atrioventricular boundary as well as in the adjacent ventricle and atrium of 48- and 72-hpf Nip3a-overexpressing embryos. Nip3a expression was readily detected in 80% epiboly BMP4-Nip3a-injected embryos, and defects in heart development were observed in both the linear heart tube and subsequent chamber formation stages. These results showed that myocyte apoptosis is a universal pathogenic factor for congenital heart failure using zebrafish as a model organism.
Descriptors: zebrafish, heart embryology, apoptosis physiology, bone morphogenetic proteins genetics and pharmacology, developmental gene expression regulation physiology, congenital heart defects genetics, congenital heart defects pathology, promoter regions genetics physiology, proteins biosynthesis.

Wang, Y., X. Fu, S. Gaiser, M. Koettgen, A. Kramer-Zucker, G. Walz, and T. Wegierski (2007). **OS-9 regulates the transit and polyubiquitination of TRPV4 in the endoplasmic reticulum**. *Journal of Biological Chemistry* 282(50): 36561-36570. ISSN: 0021-9258.
NAL Call Number: 381J824
Descriptors: zebrafish embryos, OS-9, protected, detrimental effects, trpV4 expression, in vivo, endoplasmic reticulum, variants.

Wang, Y. and W. Ge (2004). **Cloning of epidermal growth factor (EGF) and EGF receptor from the zebrafish ovary: evidence for EGF as a potential paracrine factor from the oocyte to regulate activin/follistatin system in the follicle cells.** *Biology of Reproduction* 71(3): 749-60. ISSN: 0006 3363.
NAL Call Number: QD415.F4
Abstract: In the present study, we cloned full-length cDNAs for epidermal growth factor (EGF), EGF receptor (EGFR), and three truncated forms of EGFR (EGFR15, 12, and 8) from the zebrafish ovary. Zebrafish EGF was predominantly expressed in the ovary and testis, while EGFR and its truncated forms were highly expressed in all tissues examined except the

liver. In the ovary, the expression of EGF seemed to be more abundant in the follicles of early stages, while EGFR had much higher expression levels at later stages. Interestingly, although EGF was expressed in both the follicle cells and oocytes, its expression level was significantly higher in the oocytes. However, the expression of EGFR was mainly restricted to the follicle cells with little expression in the oocytes. The unique spatial patterns of EGF and EGFR expression within the follicle suggest that EGF may serve as a messenger from the oocyte to signal the follicle cells. EGF strongly stimulated the expression of both activin beta A and beta B, while it suppressed basal and hCG-induced follistatin expression in cultured follicle cells. These results, together with the evidence that EGF was predominantly expressed in the oocytes whereas EGFR was expressed in the follicle cells, strongly suggest that EGF is likely a potential paracrine/juxtacrine factor from the oocytes to regulate the function of the follicle cells.

Descriptors: zebrafish, epidermal growth factor genetics, follistatin metabolism, inhibin beta subunits metabolism, ovarian follicle physiology, epidermal growth factor receptor genetics, amino acid sequence, cultured cells, molecular cloning, epidermal growth factor metabolism and pharmacology, gene expression, gonadotropins pharmacology, molecular sequence data, oocytes cytology and physiology, ovarian follicle cytology, paracrine communication drug effects and physiology, epidermal growth factor receptor metabolism, sexual maturation physiology, testis cytology and physiology.

Wang, Y. and W. Ge (2003). **Involvement of cyclic adenosine 3',5'-monophosphate in the differential regulation of activin betaA and betaB expression by gonadotropin in the zebrafish ovarian follicle cells.** *Endocrinology* 144(2): 491-9. ISSN: 0013 7227.

NAL Call Number: 448.8EN2

Abstract: Activin is a dimeric protein consisting of two similar but distinct beta-subunits, betaA and betaB. In our previous studies, both activin A (betaAbetaA) and activin B (betaBbetaB) have been demonstrated to stimulate oocyte maturation and promote oocyte maturational competence in the zebrafish. Follistatin, a specific activin-binding protein, can block both activin- and gonadotropin-induced final oocyte maturation in vitro, suggesting that activin is likely a downstream mediator of gonadotropin actions in the zebrafish ovary. In the present study, a full-length cDNA encoding zebrafish ovarian activin betaA was cloned and sequenced. The precursor of zebrafish activin betaA consists of 395 amino acids and its mature region exhibits about 78% homology with that of mammals. Using an in vitro primary culture of the ovarian follicle cells and semiquantitative RT-PCR assays, we examined the regulation of activin betaA and betaB expression by human chorionic gonadotropin (hCG) and its intracellular signal transduction mechanisms. hCG (15 IU/ml) increased the mRNA level of activin betaA-subunit; however, it significantly down-regulated the steady-state expression level of activin betaB in a time- and dose-dependent manner. The differential regulation of the two beta-subunits by hCG could be mimicked by 3-isobutyl-1-methylxanthine, forskolin, and dibutyryl-cAMP, suggesting involvement of the intracellular cAMP pathway. Interestingly, H89 (a specific inhibitor of protein kinase A, PKA) could effectively block hCG- and forskolin-stimulated activin betaA expression at 10 micro M, but it was unable to reverse the inhibitory effects of hCG and forskolin on betaB expression. This suggests that the hCG-stimulated activin betaA expression is dependent on the activation of the cAMP-PKA pathway, whereas the inhibitory effect of hCG on activin betaB expression is likely mediated by PKA-independent pathway(s).

Descriptors: zebrafish, cyclic AMP metabolism, gonadotropins pharmacology, inhibin beta subunits genetics, ovarian follicle physiology, 1 methyl 3 isobutylxanthine pharmacology, amino acid sequence, bucladesine pharmacology, cultured cells, molecular cloning, cyclic

AMP dependent protein kinases metabolism, complementary DNA, forskolin pharmacology, gene expression drug effects, gene expression physiology, molecular sequence data, ovarian follicle cytology , phosphodiesterase inhibitors pharmacology, reproducibility of results, reverse transcriptase PCR standards.

Wang, Y. and W. Ge (2003). **Spatial expression patterns of activin and its signaling system in the zebrafish ovarian follicle: evidence for paracrine action of activin on the oocytes.** *Biology of Reproduction* 69(6): 1998-2006. ISSN: 0006 3363.

NAL Call Number: QD415.F4

Abstract: We have previously demonstrated that activin is likely an ovarian mediator of pituitary gonadotropin(s) and local epidermal growth factor in their stimulating oocyte maturation and maturational competence in the zebrafish. However, the downstream events controlled by activin remain unknown. One possible mechanism is that activin may directly work on the oocytes to promote the development of oocyte maturational competence. To substantiate this hypothesis, we performed the present study to demonstrate the expression of the activin system in different compartments of zebrafish follicles, namely, the follicle cells and oocytes. The proteins examined include activin subunits (betaA and betaB), activin-binding protein (follistatin), activin type II receptors (type IIA and IIB), the type I activin receptor-like kinases (ALK1-like, ALK2-like, and ALK4-like), and the intracellular activin signaling molecules (Smad2, Smad3, Smad4, and Smad7). The results showed that the entire activin signaling system is expressed by the full-grown immature zebrafish oocytes (approximately 0.65 mm in diameter), including ALK4-like (ActRIB), ALK2-like (ActRIA), ActRIIA, ActRIIB, Smad2, Smad3, Smad4, and Smad7, therefore supporting our hypothesis that the oocytes are one of the direct targets of activin actions in the zebrafish ovary. In contrast, activin itself (betaA and betaB) and ALK1-like type I receptor are predominantly expressed in the follicle cells surrounding the oocytes. Interestingly, although follistatin is expressed in both the follicle cells and oocytes, its level of expression is significantly higher in the oocytes than the follicle cells, implying that follistatin may serve as a signal from the oocytes to modulate the activity of activin produced by the follicle cells. Taken together, the present study provides convincing evidence that although all members of the activin system are expressed in the whole follicle, they exhibit distinct spatial patterns of expression among different compartments of the follicle. It is likely that activin works directly on the oocytes in a paracrine manner to promote oocyte maturation and maturational competence. On the other hand, instead of being controlled passively by the follicle cells, the oocytes may actively participate in the regulation of follicle development by releasing various modulating molecules such as follistatin.

Descriptors: zebrafish, activins metabolism, oocytes metabolism, ovarian follicle metabolism, signal transduction, activin receptors, Type I activin receptors genetics and metabolism, activins genetics, DNA binding proteins genetics and genetics, proteins metabolism, follistatin genetics and metabolism, gene expression, inhibin beta subunits genetics and metabolism, PCR, RNA isolation and purification, Smad2, Smad3, Smad4 and Smad7 protein, trans activators genetics and metabolism.

Wang, Y., R. Takai, H. Yoshioka, and K. Shirabe (2006). **Characterization and expression of serotonin transporter genes in zebrafish.** *Tohoku Journal of Experimental Medicine, The* 208(3): 267-74. ISSN: 1349-3329.

Abstract: To understand the development of serotonergic neurons in vertebrates, we used zebrafish as a model system. In this study we cloned two cDNAs (complementary DNAs) coding for serotonin transporter (SERT) from the zebrafish, named serta and sertb. The serta

cDNA encodes a protein of 693 amino acids and showed high level of sequence identity with rat and human SERTs. In situ hybridization showed serta to be expressed in raphe nuclei, ventral posterior tuberculum and pineal organ. The expression of serta in raphe and ventral posterior tuberculum overlapped with the location of serotonin and expression of tryptophan hydroxylase, which is a key enzyme for serotonin synthesis. In the pineal organ serta is expressed in the cells in the vicinity of tryptophan hydroxylase-positive cells. We also cloned another zebrafish serotonin transporter, sertb, and found to be expressed in the medulla oblongata and in the inner nuclear layer of retina. The existence of two sert genes in the zebrafish genome indicates the gene was duplicated in the process of evolution as can be seen in other genes in the teleosts including zebrafish. The expression of the serta cDNA in cultured cells conferred a serotonin transport activity, thus indicating the validity of the cloned cDNA. We have established the expression system of zebrafish serotonin transporter in the cell culture in the present study, which is useful for the pharmacological analysis to determine the important residues for the interaction with serotonin and inhibitors. The expression system in the cell culture can be used to determine the effective concentration of inhibitors and addictive drugs. These information might be useful to evaluate the effect of those chemicals on serotonin neuron development and behavior of the animal.

Descriptors: zebrafish, developmental gene expression regulation, serotonin plasma membrane transport proteins metabolism, proteins metabolism, amino acid sequence, cell line, conserved sequence green fluorescent proteins metabolism, immunohistochemistry, in situ hybridization, molecular sequence data, phylogeny, pineal gland cytology, embryology, growth, development and metabolism, raphe nuclei cytology, embryology, growth, development and metabolism, retina cytology, embryology, growth, development and metabolism, reverse transcriptase PCR , rhombencephalon cytology, embryology, growth, development and metabolism, amino acid sequence homology, serotonin analysis, metabolism, serotonin plasma membrane transport proteins chemistry and genetics, tryptophan hydroxylase metabolism, proteins chemistry.

Wang, Y., A.O. Wong, and W. Ge (2003). **Cloning, regulation of messenger ribonucleic acid expression, and function of a new isoform of pituitary adenylate cyclase-activating polypeptide in the zebrafish ovary.** *Endocrinology* 144(11): 4799-810. ISSN: 0013 7227.

NAL Call Number: 448.8EN2

Abstract: Increasing evidence suggests that pituitary adenylate cyclase-activating polypeptide (PACAP) acts as a local factor in the ovary of mammals. In nonmammalian vertebrates, although the expression of PACAP has also been demonstrated in the ovary, the information on its functions and regulation is limited. In the present study, we identified a new type of PACAP, zebrafish (zf)PACAP38-2, from the zebrafish ovary. The precursor of GHRH-zfPACAP38-2 consists of 175 amino acids with only 64% homology with another type of zebrafish PACAP, zfPACAP38-1. RT-PCR analysis detected two messengers of zfPACAP38-2 in the zebrafish ovary. The short product was more abundant, and it encodes zfPACAP38-2 only, whereas the long form codes for both zfPACAP38-2 and GHRH. Using a primary culture of zebrafish follicle cells, we demonstrated that gonadotropin (human chorionic gonadotropin and goldfish pituitary extract) significantly stimulated zfPACAP38-2 expression within 2 h; however, the effect decreased to the control level after 8 h of treatment. The stimulation of zfPACAP38-2 expression by gonadotropin could be mimicked by cAMP analogs and forskolin but suppressed by H89 (10 mum), suggesting the involvement of the cAMP-protein kinase A signaling pathway. We also examined the expression of PACAP receptor VPAC2-R in the zebrafish ovary. Unlike zfPACAP38-2, which showed a trend of increase during follicle development, the expression of VPAC2-R mRNA in the follicles

showed no significant stage-dependent variation, and its expression in the follicle cells did not respond to gonadotropin treatment. Our studies further demonstrated that synthetic zfPACAP38-2 stimulated oocyte maturation and increased the expression of follistatin in zebrafish ovarian follicle cells. These results suggest that zfPACAP38-2 is a potential ovarian factor that mediates gonadotropin actions in paracrine/autocrine manners, and its functional roles are likely, to some extent, related to the ovarian activin/follistatin system.
Descriptors: zebrafish, molecular cloning, gene expression regulation, neuropeptides physiology, neurotransmitter agents genetics, ovary metabolism, messenger RNA metabolism, amino acid and base sequence genetics, cell aging drug effects, cultured cells, chorionic gonadotropin pharmacology, follistatin metabolism, gene expression drug effects, isomerism, molecular sequence data, neuropeptides genetics, oocytes drug effects and physiology, ovarian follicle cytology and physiology, ovary cytology, pituitary adenylate cyclase activating polypeptide, pituitary gland chemistry, vasoactive intestinal peptide receptors genetics and metabolism, vasoactive intestinal peptide receptors, Type II, time factors, tissue distribution, tissue extracts pharmacology.

Wang, Y., T. Zhong, L. Qian, Y. Dong, Q. Jiang, L. Tan, and H. Song (2005). **Wortmannin induces zebrafish cardia bifida through a mechanism independent of phosphoinositide 3-kinase and myosin light chain kinase.** *Biochemical and Biophysical Research Communications* 331(1): 303-8. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: Cardia bifida is an anomaly of the embryonic heart in which the bilateral myocardial rudiments fail to travel to the midline, resulting in the formation of two separate hearts in lateral positions. In zebrafish, eight loci responsible for the cardia bifida phenotype were identified in the large-scale genetic screen. Wortmannin has been reported to be a highly selective inhibitor of phosphoinositide 3-kinase and myosin light chain kinase activity. We provide the first evidence that wortmannin treatment of zebrafish embryos can induce cardia bifida in a dose-dependent manner and that wortmannin alters cardiac development between 6 and 16 h post-fertilization. In addition, we demonstrate that wortmannin induces zebrafish cardia bifida through a mechanism independent of phosphoinositide 3-kinase and myosin light chain kinase. Our findings may provide new insights into the cardiomyocyte function and disfunction.
Descriptors: zebrafish, androstadienes pharmacology, enzyme inhibitors pharmacology, heart drug effects and embryology, 1 phosphatidylinositol 3 kinase antagonists and inhibitors, abnormalities, drug effects, chemically induced congenital heart defects , myosin light chain kinase antagonists and inhibitors abnormalities.

Wang, Y.H., Y.H. Chen, Y.J. Lin, and H.J. Tsai (2006). **Spatiotemporal expression of zebrafish keratin 18 during early embryogenesis and the establishment of a keratin 18:RFP transgenic line.** *Gene Expression Patterns* 6(4): 335-9. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Zebrafish cytokeratin 18 (K18) is one of the type I keratin genes expressed the earliest after amputation of the zebrafish fin, but its spatiotemporal expression during early development is unclear. Whole-mount in situ hybridization revealed that k18 was a maternally inherited gene and that its expression is restricted to the single layer of enveloping cells on the surface of embryos during the gastrula stage. At later stages, K18 expression was detected in the epithelial cells, pronephric duct, digestive tract, dorsal aorta, and fins. By using microinjection, we generated the transgenic line Tg(k18(2.9):RFP), which carries an upstream 2.9-kb segment of k18 gene fused with a red fluorescent protein (RFP) reporter.

The spatiotemporal distributions of red fluorescent signal of Tg(k18(2.9):RFP) line correlated well with endogenous k18 transcripts detected by whole-mount in situ hybridization, indicating that this line is capable of recapitulating endogenous k18 expression patterns. We noticed that the red fluorescence appeared strongly in the dorsal, pectoral, pelvic, anal, and caudal fins when transgenic fish became adults. Interestingly, we also found that when F1 female from the Tg(k18(2.9):RFP) line were mated with wild-type males, 100% (326/326) of F2 offspring expressed red fluorescence at the one-cell stage. In contrast, when F1 male from the Tg(k18(2.9):RFP) line were mated with wild-type females, only 49.8% (138/277) of F2 embryos exhibited red fluorescence. On the basis of these findings, we suggest that the transcript of zebrafish K18 is inherited as a maternal effect. We believe that Tg(k18(2.9):RFP) fish should be an excellent experimental animal for studying the zygotic regulatory mechanism of k18.

Descriptors: zebrafish, developmental gene expression regulation, keratins genetics, genetically modified protein genetics, molecular cloning, DNA genetics, DNA isolation and purification embryonic development, reporter genes, in situ hybridization, keratin 18, microinjections, reverse transcriptase PCR, tissue distribution.

Wang, Y.H., Y.H. Chen, J.H. Lu, Y.J. Lin, M.Y. Chang, and H.J. Tsai (2006). **Epidermis-restricted expression of zebrafish cytokeratin II is controlled by a -141/+85 minimal promoter, and cassette -141/-111 is essential for driving the tissue specificity.** *Differentiation* 74(4): 186-94. ISSN: 0301 4681.

NAL Call Number: QH607.D5

Abstract: We isolated a 2.3 kb DNA segment from the upstream region of the zebrafish cytokeratin II (zfCKII) gene. Transgenic embryos, produced by using a series of 5' deletions linked to the red fluorescent protein (RFP) reporter, showed that the -141/+85 segment of zfCKII directed RFP expression in epidermal cells, whereas the -111/+85 segment did not. When -141/-111 was deleted from -355/+85 and microinjected into one-celled embryos, no fluorescence was observed at later stages, indicating that the -141/-111 segment is required for green fluorescent protein expression in epidermal cells. Furthermore, when a putative KLF-binding site at -119/-117 was mutated, RFP expression rates and intensities were reduced dramatically, although still observed, suggesting that -119/-117 within -141/-111 is a key cis-element for controlling epidermis-specific expression of the zfCKII gene. Finally, we generated a zebrafish transgenic line, Tg(zfCKII(2.3):RFP), which carries an upstream 2.3 kb regulatory region of the zfCKII gene fused with RFP. The expression pattern in the epidermal cells of Tg(zfCKII(2.3):RFP) fish recapitulated that of the endogenous gene. F2 embryos derived from Tg(zfCKII(2.3):RFP) males crossed with wild-type females revealed that the earliest onset of RFP expression was at the sphere stage, indicating that this transgenic approach can be used for studying zygotic expression of maternally inherited genes.

Descriptors: zebrafish, epidermis metabolism, gene expression regulation, keratin 2 metabolism, promoter regions genetics, metabolism, genetically modified, base sequence, epidermis cytology, reporter genes, keratin 2 genetics, molecular sequence data, recombinant fusion proteins metabolism and genetics, tissue distribution.

Wang, Y.X., L.X. Qian, Z. Yu, Q. Jiang, Y.X. Dong , X.F. Liu, X.Y. Yang, T.P. Zhong, and H.Y. Song (2005). **Requirements of myocyte-specific enhancer factor 2A in zebrafish cardiac contractility.** *Federation of European Biochemical Societies Journal* 579(21): 4843-50. ISSN: 1742 464X.

NAL Call Number: QP501.E8

Abstract: Myocyte-specific enhancer factor 2A (MEF2A) regulates a broad range of funda-

mental cellular processes including cell division, differentiation and death. Here, we tested the hypothesis that MEF2A is required in cardiac contractility employing zebrafish as a model organism. MEF2A is highly expressed in heart as well as somites during zebrafish embryogenesis. Knock-down of MEF2A in zebrafish impaires the cardiac contractility and results in sarcomere assembly defects. Dysregulation of cardiac genes in MEF2A morphants suggests that sarcomere assembly disturbances account for the cardiac contractile deficiency. Our studies suggested that MEF2A is essential in cardiac contractility.
Descriptors: zebrafish , DNA binding proteins metabolism, myocardial contraction physiology, myocardium metabolism, transcription factors metabolism, DNA binding proteins genetics, gene expression, mads domain proteins, microinjections, morphogenesis, myocardium ultrastructure, myogenic regulatory factors, antisense oligonucleotides, phenotype, promoter regions genetics, RNA splicing, recombinant fusion proteins metabolism and genetics, transcription factors genetics.

Wang YaJun and Ge Wei (2004). **Cloning of zebrafish ovarian P450c17 (CYP17, 17 alpha -hydroxylase/17, 20-lyase) and characterization of its expression in gonadal and extra-gonadal tissues**. *General and Comparative Endocrinology* 135(2): 241-249. ISSN: 0016 6480.
NAL Call Number: 444.8G28
Abstract: Cytochrome P450c17 (CYP17, 17 alpha -hydroxylase/17, 20-lyase) has been viewed as a critical enzyme for the biosynthesis of gonadal and adrenal steroids in vertebrates. A full-length cDNA coding for P450c17 has been cloned from the zebrafish ovary. It encodes 519 amino acids and shares high sequence identity with that of fathead minnow (92%), rainbow trout (74%), chicken (64%), rat (47%), and human (48%). Northern blot analysis demonstrated that it is predominantly expressed in the ovary and testis; however, a weak hybridization signal was also detected in the whole kidney of the zebrafish, suggesting a role for P450c17 in the steroidogenesis of both gonads and renal/interrenal tissues. We further examined the expression of P450c17 in the developing ovarian follicles. Northern analysis and RT-PCR revealed abundant expression of the enzyme in the follicles of all major stages including pre-vitellogenic, vitellogenic and post-vitellogenic full-grown follicles; however, unlike P450c17 in the rainbow trout and eel, the expression of zebrafish P450c17 did not show significant temporal difference among different developmental stages, similar to the finding in the fathead minnow. The constitutive nature of P450c17 expression during zebrafish follicle development may partly explain the poor transcriptional response of the enzyme to the treatments with gonadotropin or activin, both of which enhance oocyte maturation in this species. It is also of interest to note that when assayed by RT-PCR, the enzyme P450c17 also seemed to be expressed in several other non-gonadal tissues including the brain, gill, liver and intestine, and its expression did not show significant sexual dimorphism in the brain of adult zebrafish. The physiological relevance of these findings remains to be further elucidated. Copyright © 2008 CABI
Descriptors: zebrafish, complementary DNA, cytochrome P450, DNA cloning, enzyme activity, gene expression, gonads, kidneys, oocyte maturation, ovarian follicles, ovaries, steroidogenesis, *Danio rerio.*

Wang YaJun and Ge Wei (2004). **Developmental profiles of activin beta A, beta B, and follistatin expression in the zebrafish ovary: evidence for their differential roles during sexual maturation and ovulatory cycle**. *Biology of Reproduction* 71(6): 2056-2064. ISSN: 0006 3363.
NAL Call Number: QD415.F4
Abstract: Our recent experiments showed that gonadotropin(s) stimulated activin beta A and follistatin expression through the cAMP-PKA pathway but suppressed beta B via a

cAMP-dependent but PKA-independent pathway in cultured zebrafish follicle cells. Given that pituitary gonadotropins are the major hormones controlling the development and function of the ovary, the differential expression of activin beta A and beta B as well as follistatin in response to gonadotropin(s) raises an interesting question about the temporal expression patterns of these molecules in vivo during sexual maturation and ovulatory cycle. Three experiments were performed in the present study. In the first experiment using sexually immature zebrafish, we followed the expression of activin beta A, beta B, and follistatin at the whole ovary level during a 10-day period in which the ovary developed from the primary growth stage to the one with nearly full-grown follicles. Activin beta A expression was very low at the primary growth stage but significantly increased with the growth of the ovary, and its rise was accompanied by an increase in follistatin expression. In contrast, the expression of activin beta B could be easily detected in the ovary of all stages; however, it did not exhibit an obvious trend of variation during the development. The second experiment examined the stage-dependent expression of activin beta A, beta B, and follistatin at the follicle level in the adult mature zebrafish. The expression of activin beta A was again low in the follicles during the primary growth stage, but exhibited a phenomenal increase after the follicles entered vitellogenesis with the peak level reached at midvitellogenic stage; in contrast, activin beta B mRNA could be easily detected at all stages with a slight increase during follicle growth. The expression of follistatin, on the other hand, also increased significantly during vitellogenesis; however, its level dropped sharply after reaching the peak at the midvitellogenic stage. In the third experiment, we investigated the dynamic changes of the ovarian activin beta A, beta B, and follistatin expression during the daily ovulatory cycle. The expression of activin beta A and follistatin gradually increased from 1800 h onward and reached the peak level around 0400 h when the germinal vesicles had migrated to the periphery in the full-grown oocytes. In contrast, activin beta B expression steadily declined, although not statistically significant, during the same period, but increased sharply at 0700 h when mature oocytes started to appear in most of the ovaries collected. In conclusion, activin beta A and beta B exhibit distinct expression patterns during the development of the ovary and the daily ovarian cycle of the zebrafish. It seems that activin beta A is involved in promoting ovary and follicle growth, whereas activin beta B may have a tonic role throughout follicle development but becomes critical at the late stage of oocyte maturation and/or ovulation. Copyright © 2008 CABI
Descriptors: zebrafish, activins, c AMp, follistatin, germinal vesicle, gonadotropins, oocyte maturation, oocytes, ovarian follicles, ovaries, ovulation, sexual maturity, vitellogenesis, *Danio rerio*.

Wang, Y., C.C. Shupenko, L.F. Melo, and P.R. Strauss (2006). **DNA repair protein involved in heart and blood development**. *Molecular and Cellular Biology* 26(23): 9083-9093. ISSN: 0270 7306.
NAL Call Number: QH506.M664
Abstract: Apurinic/apyrimidinic endonuclease 1, a key enzyme in repairing abasic sites in DNA, is an embryonic lethal in mice. We are examining its role in embryogenesis in zebra fish. Zebra fish contain two genomic copies (zfAPEX1a and zfAPEX1b) with identical coding sequences. zfAPEX1b lacks introns. Recombinant protein (ZAP1) is highly homologous with and has the same enzymatic properties as its human orthologue. ZAP1 is highly expressed throughout development. Embryos microinjected with morpholino oligonucleotide (MO) targeting the translation start site die at approximately the midblastula transition (MBT) without apoptosis. They are rescued with mRNA for human wild-type APEX1 but not for APEX1 encoding endonuclease-defective protein. Rescued embryos develop dysmorphic hearts, pericardial edema, few erythrocytes, small eyes, and abnormal notochords. Although

the hearts in rescued embryos form defective loops ranging from no loop to one that is abnormally shaped, cardiac myosin (cmlc2) is present and contraction occurs. Embryos microinjected with MO targeting zfAPEX1a intron-exon junctions also pass the MBT with similar abnormalities. We conclude that AP endonuclease 1 is involved in both repairing DNA and regulating specific early stages of embryonic development. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, blood and lymphatics, transport and circulation, development, molecular genetics, cardiovascular system, transport and circulation, genome, organ development.

Wang Yong and F.C.C. Leung (2006). **Long inverted repeats in eukaryotic genomes: recombinogenic motifs determine genomic plasticity**. *Federation of European Biochemical Societies Letters* 580(5): 1277-1284. ISSN: 0014 5793.

NAL Call Number: QD415.F4

Abstract: Inverted repeats are unstable motifs in a genome, having a causal relation to fragment rearrangements and recombination events. We have investigated long inverted repeats (LIR) of >30 bp in length in eukaryotic genomes to assess their contribution to genome stability. An algorithm was first designed for searching for LIRs with <2 kb internal spacers and >85% identity (degree of homology between repeat copies of a LIR). There are much fewer LIRs in yeast, fruitfly, pufferfish and chicken than in *Caenorhabditis elegans*, zebrafish, frog and human. However, the high LIR frequencies do not necessarily imply high genome instability because of variant internal spacers and stem lengths and identities. From the collection of identified LIRs, we selected recombinogenic LIRs that had a short internal spacer and a high copy identity and were prone to induce high instability. We found that a relatively high proportion (5-9.8%) of the LIRs in *C. elegans*, zebrafish and frog were recombinogenic LIRs. In contrast, the proportions in human and mouse LIRs were quite low (0.4-1.1%) basically accounting for long internal spacers. We suggest that C. elegans, zebrafish and frog genomes are unstable in terms of the LIR frequency and the proportion of recombinogenic LIRs. For the other genomes, LIRs most likely have a minor impact. Copyright © 2008 CABI

Descriptors: zebrafish, free living nematodes, gene expression, genes, genetic stability, genome analysis, genomes, poultry, recombination, *Caenorhabditis elegans*, fishes , fowls, frogs, Tetraodontidae.

Wardle, F.C., D.T. Odom, G.W. Bell, B. Yuan, T.W. Danford, E.L. Wiellette, E. Herbolsheimer, H.L. Sive, R.A. Young, and J.C. Smith (2006). **Zebrafish promoter microarrays identify actively transcribed embryonic genes.** *Genome Biology* 7(8): R71. ISSN: 1465-6906.

NAL Call Number: QH431.G452

Abstract: We have designed a zebrafish genomic microarray to identify DNA-protein interactions in the proximal promoter regions of over 11,000 zebrafish genes. Using these microarrays, together with chromatin immunoprecipitation with an antibody directed against tri-methylated lysine 4 of Histone H3, we demonstrate the feasibility of this method in zebrafish. This approach will allow investigators to determine the genomic binding locations of DNA interacting proteins during development and expedite the assembly of the genetic networks that regulate embryogenesis.

Descriptors: zebrafish, developmental gene expression regulation, promoter regions genetics, chromatin immunoprecipitation, embryology, metabolism, genomics methods, histones genetics, oligonucleotide array sequence analysis, reverse transcriptase PCR.

Wargelius, A., H.C. Seo, L. Austbo, and A. Fjose (2003). **Retinal expression of zebrafish six3.1 and its regulation by Pax6.** *Biochemical and Biophysical Research Communications* 309(2): 475-81. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: Homologues of the homeobox genes sine oculis (so) and eyeless (ey) are important regulators of eye development in both vertebrates and invertebrates. A *Drosophila* paralogue of so, optix, is an orthologue of the vertebrate Six3 gene family. Our analysis of zebrafish six3.1 demonstrated retinal expression in two separate cell layers and the ciliary marginal zone. This pattern is consistent with the observations of Six3 in other vertebrates and indicates functional conservation. We studied the 5(') flanking region of six3.1 and showed that separate enhancing elements are required for expression at different stages of eye development. This analysis also revealed specific binding of zebrafish Pax6.1 protein to an element required for six3.1 expression in ganglion cells. Furthermore, an enhancement of six3.1 transcription by Pax6.1 was observed by co-injection experiments. These results provide evidence for a direct regulatory interaction between vertebrate Pax6 and Six3 genes in eye development.

Descriptors: zebrafish, developmental gene expression regulation drug effects, homeodomain proteins metabolism and pharmacology, nerve tissue proteins metabolism and genetics, retina embryology and metabolism, base sequence, eye proteins , regulator genes drug effects, molecular sequence data, paired box transcription factors, repressor proteins, sequence alignment.

Washietl, S., I.V. Hofacker, and P.F. Stadler (2005). **Fast and reliable prediction of noncoding RNAs**. *Proceedings of the National Academy of Sciences, USA* 102(7): 2454-2459. ISSN: 0027-8424.

NAL Call Number: 500N21P

Descriptors: zebrafish, base composition, computer programs, conserved sequence, databases, genes, methodology, nucleotide sequence, RNA, sampling, screens, secondary structure, specificity, thermodynamics, genomics, *Danio rerio*, Fugu, genetics and evolution.

Watanabe, M., M. Iwashita, M. Ishii, Y. Kurachi, A. Kawakami, S. Kondo, and N. Okada (2006). **Spot pattern of leopard *Danio* is caused by mutation in the zebrafish connexin41.8 gene.** *European Molecular Biology Organization Journal Reports* 7(9): 893-7. ISSN: 1469 221X.

NAL Call Number: QH506 .E463

Abstract: Leopard, a well-known zebrafish mutant that has a spotted skin pattern instead of stripes, is a model for the study of pigment patterning. To understand the mechanisms underlying stripe formation, as well as the spot variation observed in leopard, we sought to identify the gene responsible for this phenotype. Using positional cloning, we identified the leopard gene as an orthologue of the mammalian connexin 40 gene. A variety of different leopard alleles, such as leo(t1), leo(tq270) and leo(tw28), show different skin-pattern phenotypes. In this manuscript we show that the mutation in allele leo(t1) is a nonsense mutation, whereas alleles leo(tq270) and leo(tw28) contain the missense mutations I202F and I31F, respectively. Patch-clamp experiments of connexin hemichannels demonstrated that the I202F substitution in allele leo(tq270) disrupted the channel function of connexin41.8. These results demonstrate that mutations in this gene lead to a variety of leopard spot patterns.

Descriptors: mutant zebrafish, connexins genetics, spotted and leopard skin pattern, pig-

mentation genetics, alleles, body patterning genetics, connexins metabolism, HeLa cells, mutation, phenotype, variation genetics.

Wayne, N.L., M. Pandolfi, and D. Kozlowski (2006). **Development and use of transgenic zebrafish to study electrical activity from migrating gnrh neurons in the intact embryo.** *Biology of Reproduction*: 75. ISSN: 0006 3363.
NAL Call Number: QL876.B5
Descriptors: transgenic zebrafish, development, molecular genetics, nervous system: neural coordination , endocrine system, electrical activity, cell migration.

Webb, A.E. and D. Kimelman (2005). **Analysis of early epidermal development in zebrafish.** *Methods in Molecular Biology* 289: 137-46. ISSN: 1064 3745.
Abstract: The zebrafish (*Danio rerio)* is a member of the teleost (bony fish) lineage that diverged from the mammalian lineage 420 million years ago. Despite this ancient divergence, there are important similarities between the epidermis of fish and mammals. In addition, recent work suggests that the genes required for epidermal development in the zebrafish also are essential for this process in higher vertebrates, indicating that analysis of the zebrafish system will contribute to understanding mouse and human development. The zebrafish is a very useful genetic system, and many mutants with epidermal defects have been obtained in large-scale genetic screens. In addition, zebrafish embryos are transparent, develop externally, and can be collected in abundance. The aim of this chapter is to provide the researcher with basic protocols to examine gene expression, cell proliferation, and cell death in the developing zebrafish. Although only a few specific epidermal markers have been identified in the zebrafish thus far, we also provide a list of useful genes for epidermal analysis.
Descriptors: zebrafish, cell proliferation, epidermis cytology, developmental gene expression regulation genetics, cytology, immunohistochemistry, in situ hybridization.

Webb, S.E. and A.L. Miller (2003). **Imaging intercellular calcium waves during late epiboly in intact zebrafish embryos.** *Zygote* 11(2): 175-82. ISSN: 0967-1994.
NAL Call Number: QH491.Z94
Abstract: Through the injection of f-aequorin and the use of a photon imaging microscope, we have previously reported that a rhythmic series of intercellular Ca2+ waves circumnavigate zebrafish embryos over a 10 h period during gastrulation and axial segmentation. These waves first appear at about 65% epiboly and continue to arise every 5-10 min up to at least the 16-somite stage. In response to our publication, it was suggested that the waves may be an artefact caused by dechorionation of the embryos and would not be observed during the development of intact embryos (i.e. those with chorions). Here we demonstrate (again initially by aequorin imaging) that the rhythmic intercellular Ca2+ waves that traverse the blastoderm margin can also be observed in embryos that have an intact chorion. In addition, the appearance time, propagation pathway, velocity, duration and Ca2+ rise of the waves, as well as the interwave interval and the timing of wave onset, are approximately the same in both dechorionated embryos and those with an intact chorion. Furthermore, by loading intact embryos with Ca(2+)-green dextran at the single-cell stage and then using scanning confocal microscopy to obtain high-resolution images, we confirm the presence of circumferential Ca2+ waves and show that they pass through a population of deep cells located at the blastoderm margin. The confirmation of these pan-embryonic Ca2+ waves in zebrafish further corroborates our earlier suggestion that such waves might play a fundamental role in normal embryonic patterning during the gastrula period.

Descriptors: zebrafish embryos, calcium metabolism, aequorin metabolism, fluorescent dyes metabolism, scanning confocal microscopy, fluorescence microscopy, organic chemicals.

Wechselberger, C., C. Bianco, L. Strizzi, A.D. Ebert, N. Kenney, Y. Sun, and D.S. Salomon (2005). **Modulation of TGF-beta signaling by EGF-CFC proteins**. *Experimental Cell Research* 310(2): 249-255. ISSN: 0014 4827.

NAL Call Number: 442.8EX7

Abstract: Members of the transforming growth factor-beta (TGF-beta) family of ligands exhibit potent growth-suppressive and/or apoptosis-inducing effects on different types of cells. They perform essential roles in the elimination of damaged or abnormal cells from healthy tissues. On the other hand, TGF-beta s have also been shown to act as tumor-promoting cytokines in a number of malignancies that are capable of stimulating extracellular matrix production, cell migration, invasion, angiogenesis, and immune suppression. Dissecting the complex, multifaceted roles of different TGF-beta-related peptides especially during the development of pathological conditions and in carcinogenesis is an area of continuous research and development. The characterization of EGF-CFC proteins as essential co-receptors that contribute to the modulation of the physiological activities of some of the TGF-beta ligands will be beneficial for future medical research and the adaptation and possible readjustment of currently applied therapeutic regimes. (c) 2005 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, immune system, chemical coordination and homeostasis, tumor biology, cancer, neoplastic disease, pathology, immunology, apoptosis.

Wehman, A.M., W. Staub, J.R. Meyers, P.A. Raymond, and H. Baier (2005). **Genetic dissection of the zebrafish retinal stem-cell compartment.** *Developmental Biology* 281(1): 53-65. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: In a large-scale forward-genetic screen, we discovered that a limited number of genes are required for the regulation of retinal stem cells after embryogenesis in zebrafish. In 18 mutants out of almost 2000 F2 families screened, the eye undergoes normal embryonic development, but fails to continue growth from the ciliary marginal zone (CMZ), the post-embryonic stem-cell niche. Class I-A mutants (5 loci) display lower amounts of proliferation in the CMZ, while nearly all cells in the retina appear differentiated. Class I-B mutants (2 loci) have a reduced CMZ with a concomitant expansion in the retinal pigmented epithelium (RPE), suggesting a common post-embryonic stem cell is the source for these neighboring cell types. Class II encompasses three distinct types of mutants (11 loci) with expanded CMZ, in which the progenitor population is arrested in the cell cycle. We also show that in at least one combination, the reduced CMZ phenotype is genetically epistatic to the expanded CMZ phenotype, suggesting that Class I genes are more likely to affect the stem cells and Class II the progenitor cells. Finally, a comparative mapping analysis demonstrates that the new genes isolated do not correspond to genes previously implicated in stem-cell regulation. Our study suggests that embryonic and post-embryonic stem cells utilize separable genetic programs in the zebrafish retina.

Descriptors: mutant zebrafish, retina abnormalities, cytology, growth, development and physiology, stem cells physiology, cell shape, genetic screening, linkage genetics, phenotype, stem cells cytology, zebrafish growth and development.

Wei, D., J. Yao, X. Yang, L. Cheng, D. Lu, and J. Xue (2004). **Molecular cloning and expression of two closely related GTP-binding proteins from zebrafish.** *DNA Sequence* 15(4): 246-50. ISSN: 1042 5179.

NAL Call Number: QH435.D57

Abstract: Developmentally regulated GTP-binding proteins (DRGs) are a subclass of GTP-binding proteins that have been discovered recently. Here we report two zebrafish DRG cDNA clones closely related to human and mouse DRG genes. The two DRG sequences showed a high degree of similarity (55% identity, 72% similarity) at the amino acids level. Whole mount in situ hybridization revealed expression of zebrafish DRGs maternally, following the onset of zygotic transcription at the mid-blastula transition (MBT) and throughout embryonic. The expression of these two genes in different tissues follows a similar pattern, suggesting that they may serve a similar function.

Descriptors: zebrafish, GTP binding proteins genetics and metabolism, amino acid sequence, molecular cloning, complementary DNA, molecular sequence data, ovum metabolism, phylogeny.

Wei, S., J.M. Zhou, X. Chen, R.N. Shah, J. Liu, T.M. Orcutt, D. Traver, J.Y. Djeu, G.W. Litman, and J.A. Yoder (2007). **The zebrafish activating immune receptor Nitr9 signals via Dap12.** *ImmunoGenetics* 59(10): 813-21. ISSN: 0093-7711.

NAL Call Number: QR184.I4

Abstract: Both inhibitory and activating forms of natural killer (NK) cell receptors are found in mammals. The activating receptors play a direct role in the recognition of virally infected or transformed cells and transduce activating signals into the cell by partnering with an adaptor protein, which contains a cytoplasmic activation motif. Activating NK receptors encoded by the mammalian leukocyte receptor complex (e.g., killer cell immunoglobulin-like receptors) and the natural killer complex (e.g., Ly49s) partner with the adaptor protein DAP12, whereas NK receptors encoded in the CD94/NKG2 complex partner with the adaptor protein DAP10. Novel immune-type receptors (NITRs) found in bony fish share several common features with immunoglobulin-type NK receptors. Nitr9 is a putative activating receptor in zebrafish that induces cytotoxicity within the context of human NK cells. One isoform of Nitr9, Nitr9L, is shown here to preferentially partner with a zebrafish ortholog of Dap12. Cross-linking the Nitr9L-Dap12 complex results in activation of the phosphytidylinositol 3-kinase-->AKT-->extracellular signal-regulated kinase pathway suggesting that the DAP12-based activating pathway is conserved between bony fish and mammals.

Descriptors: zebrafish, kir receptors metabolism, immunology and genetics, signal transduction immunology, signal transducing adaptor proteins genetics, immunology and metabolism, amino acid sequence, cell line, molecular sequence data, phosphorylation, imunology, recombinant proteins immunology and metabolism, sequence alignment, proteins immunology.

Wei, X., Y. Luo, and D.R. Hyde (2006). **Molecular cloning of three zebrafish Lin7 genes and their expression patterns in the retina.** *Experimental Eye Research* 82(1): 122-31. ISSN: 0014 4835.

NAL Call Number: QP474

Abstract: The vertebrate retina develops from an undifferentiated sheet of neuroepithelial cells, whose differentiation requires the generation and maintenance of the correct cellular polarity. To examine the role of cell polarity in retinal development, we cloned three zebrafish lin7 genes (lin7a, lin7b, and lin7c), which each encodes a protein candidate that is required for generation/maintenance of neuroepithelial cell junctions. These three zebrafish Lin7

proteins share over 78% amino acid identity and contain both L27 and PDZ domains that are present in all Lin7 homologs. Immunoblots revealed that the Lin7b and Lin7c proteins were first expressed in the developing eye by 24hr postfertilization (hpf), while Lin7a was not detected in the eye until 72 hpf. At 33 hpf, the Lin7 proteins localized at, or slightly apical of, the actin-associated adherens junctions in the retinal neuroepithelium. This subcellular distribution required the expression of the Nok protein. In the absence of Nok, the Lin7 proteins failed to localize to either the ectopic adherens junctions or the cell membrane. At 4 days postfertilization, in situ hybridisation revealed that all three lin7 genes were expressed in both the ganglion cell layer and the bipolar cell region of the inner nuclear layer. The lin7a gene was also expressed in the amacrine and horizontal cell regions of the inner nuclear layer, while lin7c was also expressed in the outer nuclear layer. In the adult retina, where Lin7a is the predominant form expressed, the Lin7 proteins were localized to the outer and inner plexiform layers, the bipolar and horizontal cells of the inner nuclear layer, and the ganglion cells. These results suggest that the three zebrafish Lin7 proteins possess partially redundant, yet essential, roles in retinal development.

Descriptors: zebrafish, physiology, developmental gene expression regulation, retina embryology, cell polarity, molecular cloning, gene expression, guanylate cyclase genetics, immunoblotting, in situ hybridization, nerve tissue neuroepithelial cells chemistry, retina chemistry, retinal ganglion cells chemistry.

Wei, X., J. Zou, M. Takechi, S. Kawamura, and L. Li (2006). **Nok plays an essential role in maintaining the integrity of the outer nuclear layer in the zebrafish retina.** *Experimental Eye Research* 83(1): 31-44. ISSN: 0014 4835.

NAL Call Number: QP474

Abstract: Proper visual function of the vertebrate retina requires the maintenance of the integrity of the retinal outer nuclear layer (ONL), which is often affected in many blinding human retinal diseases. While the structural integrity of the ONL has long been considered to be maintained primarily through the outer limiting membrane (OLM), we have little knowledge on the development and maintenance of the OLM itself. Here, by analyzing the adhering properties of photoreceptors in zebrafish N-cad and nok mutants, we demonstrated for the first time that the nok gene is essential for the establishment and/or maintenance of the OLM. In addition, our results imply the possibility that Nok, Crumbs, and their associated proteins may constitute a type of photoreceptor-photoreceptor junctional complex that has not be described before. Thus, our study provides novel insights into the mechanisms by which the integrity of the ONL is maintained in the vertebrate retina.

Descriptors: N-cad and nok mutant zebrafish, eye proteins genetics, guanylate cyclase genetics, retina metabolism, cadherins genetics, cell adhesion genetics and movement genetics, immunohistochemistry methods, membrane proteins analysis, membrane mutation, phosphoproteins analysis, vertebrate photoreceptors metabolism, rosette formation.

Weidinger, G., J. Stebler, K. Slanchev, K. Dumstrei, C. Wise, R. Lovell Badge, C. Thisse, B. Thisse, and E. Raz (2003). **Dead end, a novel vertebrate germ plasm component, is required for zebrafish primordial germ cell migration and survival.** *Current Biology* 13(16): 1429-34. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: In most animals, primordial germ cell (PGC) specification and development depend on maternally provided cytoplasmic determinants that constitute the so-called germ plasm. Little is known about the role of germ plasm in vertebrate germ cell development, and its molecular mode of action remains elusive. While PGC specification in mammals occurs

via different mechanisms, several germ plasm components required for early PGC development in lower organisms are expressed in mammalian germ cells after their migration to the gonad and are involved in gametogenesis. Here we show that the RNA of dead end, encoding a novel putative RNA binding protein, is a component of the germ plasm in zebrafish and is specifically expressed in PGCs throughout embryogenesis; Dead End protein is localized to perinuclear germ granules within PGCs. Knockdown of dead end blocks confinement of PGCs to the deep blastoderm shortly after their specification and results in failure of PGCs to exhibit motile behavior and to actively migrate thereafter. PGCs subsequently die, while somatic development is not effected. We have identified dead end orthologs in other vertebrates including Xenopus, mouse, and chick, where they are expressed in germ plasm and germ-line cells, suggesting a role in germ-line development in these organisms as well.
Descriptors: zebrafish, germ cells metabolism, base sequence, cell movement and survival, molecular cloning, complementary DNA genetics, germ cells cytology, in situ hybridization, molecular sequence data, oligodeoxyribonucleotides, antisense genetics and pharmacology.

Weidinger, G., C.J. Thorpe, K. Wuennenberg-Stapleton, J. Ngai, and R.T. Moon (2005). **The Sp1-Related transcription Factors sp5 and sp5-like Act Downstream of Wnt/ beta -Catenin signaling in Mesoderm and Neuroectoderm Patterning**. *Current Biology* 15(6): 489-500. ISSN: 0960-9822.
NAL Call Number: QH301.C85
Descriptors: zebrafish, biological development, DNA, DNA microarrays, freshwater fish, gastrulation, gene expression, hindbrain, mesoderm , neuroectoderm, pattern formation, signal transduction, Sp1 protein, transcription factors, Wnt protein, catenin, *Danio rerio*, Sp5 protein, physical and computer methods and assays.

Weiser, D.C., U.J. Pyati, and D. Kimelman (2007). **Gravin regulates mesodermal cell behavior changes required for axis elongation during zebrafish gastrulation.** *Genes and Development* 21(12): 1559-71. ISSN: 0890-9369.
NAL Call Number: QH426.G46
Abstract: Convergent extension of the mesoderm is the major driving force of vertebrate gastrulation. During this process, mesodermal cells move toward the future dorsal side of the embryo, then radically change behavior as they initiate extension of the body axis. How cells make this transition in behavior is unknown. We have identified the scaffolding protein and tumor suppressor Gravin as a key regulator of this process in zebrafish embryos. We show that Gravin is required for the conversion of mesodermal cells from a highly migratory behavior to the medio-laterally intercalative behavior required for body axis extension. In the absence of Gravin, paraxial mesodermal cells fail to shut down the protrusive activity mediated by the Rho/ROCK/Myosin II pathway, resulting in embryos with severe extension defects. We propose that Gravin functions as an essential scaffold for regulatory proteins that suppress the migratory behavior of the mesoderm during gastrulation, and suggest that this function also explains how Gravin inhibits invasive behaviors in metastatic cells.
Descriptors: zebrafish, amino acid and base sequence, body patterning, cell movement and shape, gastrula cytology, developmental gene expression regulation, mesoderm cytology, molecular sequence data, myosin Type II metabolism, messenger RNA genetics and metabolism, amino acid sequence homology, Rhoa GTP binding protein metabolism.

Wen, K.K. and P.A. Rubenstein (2003). **Biochemical consequences of the cardiofunk (R177H) mutation in yeast actin**. *Journal of Biological Chemistry* 278(48): 48386-48394. ISSN: 0021-9258.

NAL Call Number: 381J824
Descriptors: zebrafish, actin, adenosinetriphosphatase, defects, freshwater fish, heart, mutations, nucleotides, polymerization, pyrene, tropomyosin, Yeasts, *Danio rerio*, *Saccharomyces cerevisiae*, cardiofunk, budding yeast , cardiogenesis, fungal genetics, stock assessment and management.

Wen, Z.L. (2007). **Zebrafish rbc (SQLZL) mutant gene encodes a novel transcription regulator essential for erythrocyte development**. *NeuroSignals* 15(1): 50. ISSN: 1424 862X.
NAL Call Number: QP517.C45B56
Descriptors: zebrafish, nervous system: neural coordination, development, molecular genetics, blood and lymphatics, transport and circulation, positional cloning, genetic techniques, cell development, binding domain, transcription modulation.

White, R.J., Q. Nie, A.D. Lander, and T.F. Schilling (2007). **Complex regulation of cyp26a1 creates a robust retinoic acid gradient in the zebrafish embryo.** *Public Library of Science Biology* 5(11): E304. ISSN: 1544-9173.
NAL Call Number: QH301.P56
Abstract: Positional identities along the anterior-posterior axis of the vertebrate nervous system are assigned during gastrulation by multiple posteriorizing signals, including retinoic acid (RA), fibroblast growth factors (Fgfs), and Wnts. Experimental evidence has suggested that RA, which is produced in paraxial mesoderm posterior to the hindbrain by aldehyde dehydrogenase 1a2 (aldh1a2/raldh2), forms a posterior-to-anterior gradient across the hindbrain field, and provides the positional information that specifies the locations and fates of rhombomeres. Recently, alternative models have been proposed in which RA plays only a permissive role, signaling wherever it is not degraded. Here we use a combination of experimental and modeling tools to address the role of RA in providing long-range positional cues in the zebrafish hindbrain. Using cell transplantation and implantation of RA-coated beads into RA-deficient zebrafish embryos, we demonstrate that RA can directly convey graded positional information over long distances. We also show that expression of Cyp26a1, the major RA-degrading enzyme during gastrulation, is under complex feedback and feedforward control by RA and Fgf signaling. The predicted consequence of such control is that RA gradients will be both robust to fluctuations in RA synthesis and adaptive to changes in embryo length during gastrulation. Such control also provides an explanation for the fact that loss of an endogenous RA gradient can be compensated for by RA that is provided in a spatially uniform manner.
Descriptors: zebrafish, body patterning genetics, central nervous system embryology, cytochrome P450 enzyme system genetics and metabolism, enzymology, developmental gene expression regulation, tretinoin metabolism, Fgf genetics and metabolism, signal transduction.

Whitehead, G.G., S. Makino, C.L. Lien, and M.T. Keating (2005). **Fgf20 is essential for initiating zebrafish fin regeneration.** *Science* 310(5756): 1957-60. ISSN: 0036-8075.
NAL Call Number: 470Sci2
Abstract: Epimorphic regeneration requires the presence or creation of pluripotent cells capable of reproducing lost organs. Zebrafish fin regeneration is mediated by the creation of blastema cells. Here, we characterize the devoid of blastema (dob) mutant that fails fin regeneration during initial steps, forms abnormal regeneration epithelium, and does not form blastema. This mutation has no impact on embryonic survival. Dob results from an fgf20a null mutation, Y148S. Fgf20a is expressed during initiation of fin regeneration at the epithe-

lial-mesenchymal boundary and later overlaps with the blastema marker msxb. Thus, fgf20a has a regeneration-specific requirement, initiating fin regeneration, and controlling blastema formation.

Descriptors: zebrafish, Fgf physiology, regeneration physiology, extremities, homeodomain proteins biosynthesis, mesoderm, mutation, regeneration genetics, temperature, wound healing, proteins biosynthesis.

Whitlock, K.E. (2004). **Making scents: development and function of the olfactory sensory system**. G. Zhiyuan and V. Korzh *Molecular Aspects of Fish and Marine Biology: Fish Development and Genetics: the Zebrafish and Medaka Models,* Vol. 2, World Scientific Publishing Co. Pte. Ltd.: Singapore, Singapore, p. 216-260. ISBN: <41 ISBN>.

NAL Call Number: QL638.C94 F57

Abstract: The olfactory sensory system is a spectacular sensory system holding all the mysteries of the biological world. The olfactory organ arises as a highly orchestrated interaction between the placodally derived tissues forming the sensory epithelium and the neural crest derived cells that will form the structural elements encasing the olfactory epithelium. Once formed, the sensory neurons must forge axonal connections with the developing olfactory bulbs and maintain the fidelity of these connections throughout life. This is a particular challenge in the olfactory system for the sensory neurons undergo constant regeneration throughout life. The olfactory sensory neurons express odorant receptors in order to interact with the world. Recently it has been shown that the genes encoding the olfactory receptors number around 100 in fishes and 1000 in mammals, making up a large part of their respective genomes. Thus the developing sensory neurons must choose to express a given receptor type and maintain the representation of the receptors in a constantly regenerating epithelium. Fish have long been a classic model for the study of neuroethology; we can now couple this knowledge with cellular and molecular tools to unravel the development of the amazing olfactory sensory system using the world's largest group of vertebrate animals, the fishes. Copyright © 2008 CABI

Descriptors: zebrafish, animal models, gene expression, genes, neurons, neurophysiology, receptors, signal transduction, smell, *Danio rerio*, olfactory sensory system.

Whitlock, K.E., N. Illing, N.J. Brideau, K.M. Smith, and S. Twomey (2006). **Development of GnRH cells: setting the stage for puberty**. *Molecular and Cellular Endocrinology* 254: 39-50. ISSN: 0303 7207.

NAL Call Number: QP187.A1M6

Abstract: Cells containing gonadotropin-releasing hormone (GnRH) are essential not only for reproduction but also for neuromodulatory functions in the adult animal. A variety of studies have hinted at multiple origins for GnRH-containing cells in the developing embryo. We have shown, using zebrafish as a model system, that GnRH cells originate from precursors lying outside the olfactory placode: the region of the anterior pituitary gives rise to hypothalamic GnRH cells and the cranial neural crest gives rise to the GnRH cells of the terminal nerve and midbrain. Cells of both the forming anterior pituitary and cranial neural crest are closely apposed to the precursors of the olfactory epithelium during early development. Disruption of kallmann gene function results in loss of the hypothalamic but not the terminal nerve GnRH cells during early development. The GnRH proteins are expressed early in development and this expression is mirrored by the onset of GnRH receptor (GnRH-R) expression during early development. Thus the signaling of the GnRH neuronal circuitry is set up early in development laying the foundation for the GnRH network that is activated at puberty leading to reproductive function in the mature animal. (c) 2006 Pub-

lished by Elsevier Ireland Ltd. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, molecular genetics, endocrine system, chemical coordination and homeostasis, puberty, reproduction, Kallmann gene.

Wibrand, K. (2003). ***Identification of genes expressed in the primordial germ cells of zebrafish by characterisation of maternal transcripts*** . Dissertation, Sars international centre for marine molecular biology: 115 p.
Descriptors: zebrafish, clones, cytology, DNA, developmental stages, embryonic development, freshwater fish, gene expression, genes, histones, hybridization, *Danio rerio*, genetics and evolution.

Wickstrom, E., K.A. Urtishak, M. Choob, X. Tian, N. Sternheim, L.M. Cross, A. Rubinstein, and S.A. Farber (2004). **Downregulation of gene expression with negatively charged peptide nucleic acids (PNAs) in zebrafish embryos.** *Methods in Cell Biology* 77: 137-58. ISSN: 0091-679X.
NAL Call Number: 442P92
Abstract: We found that negatively charged, highly soluble PNA analogs with alternating phosphonates (HypNA-pPNAs) are effective and specific antisense agents in zebrafish embryos, showing comparable potency and greater specificity against chordin, ntl and uroD. In addition, we successfully phenocopied a dharma mutant that had not been found susceptible to MO knockdown. Both MO and HypNA-pPNAs against a tumor suppressor gene induced comparable upregulation of p53, illustrating similar effects on transcription profiles. HypNA-pPNAs are therefore a valuable alternative for reverse genetic studies, enabling the targeting of previously inaccessible genes in zebrafish or validating newly identified orthologs, and perhaps for reverse genetic studies in other organisms.
Descriptors: zebrafish, down regulation, peptide nucleic acids pharmacology, anions chemistry and pharmacology, drug effects, developmental gene expression regulation drug effects, glycohomeodomain intercellular signaling peptides, antisense oligonucleotides chemistry and pharmacology, peptide nucleic acids chemistry, T-box domain proteins genetics.

Wiellette, E., Y. Grinblat, M. Austen, E. Hirsinger, A. Amsterdam, C. Walker, M. Westerfield, and H. Sive (2004). **Combined haploid and insertional mutation screen in the zebrafish.** *Genesis* 40(4): 231-40. ISSN: 1526-954X.
NAL Call Number: QH426.D32
Abstract: To identify genes required for development of the brain and somites, we performed a pilot screen of gynogenetic haploid zebrafish embryos produced from mothers mutagenized by viral insertion. We describe an efficient method to identify new mutations and the affected gene. In addition, we report the results of a small-scale screen that identified five genes required for brain development, including novel alleles of nagie oko, pou5f1, ribosomal protein L36, and n-cadherin, as well as a novel allele of the laminin g1 gene that is required for normal skeletal muscle fiber organization and somite patterning.
Descriptors: zebrafish, haploidy, insertional mutagenesis methods, mutation, alleles, body patterning, brain embryology, molecular cloning, guanylate cyclase genetics, phenotype, somites.

Wienholds, E., W.P. Kloosterman, E. Miska, E. Alvarez Saavedra, E. Berezikov, E. de Bruijn, H.R. Horvitz, S. Kauppinen, and R.H. Plasterk (2005). **MicroRNA expression in zebrafish embryonic development.** *Science* 309(5732): 310-1. ISSN: 0036-8075.
NAL Call Number: 470Sci2

Abstract: MicroRNAs (miRNAs) are small noncoding RNAs, about 21 nucleotides in length, that can regulate gene expression by base-pairing to partially complementary mRNAs. Regulation by miRNAs can play essential roles in embryonic development. We determined the temporal and spatial expression patterns of 115 conserved vertebrate miRNAs in zebrafish embryos by microarrays and by in situ hybridizations, using locked-nucleic acid-modified oligonucleotide probes. Most miRNAs were expressed in a highly tissue-specific manner during segmentation and later stages, but not early in development, which suggests that their role is not in tissue fate establishment but in differentiation or maintenance of tissue identity.

Descriptors: zebrafish, metabolism, gene expression, microRNAs genetics and metabolism, Northern blotting, embryonic development, in situ hybridization, multigene family, oligonucleotide array sequence analysis, oligonucleotide probes, organ specificity, time factors.

Wienholds, E., M.J. Koudijs, F.J. van Eeden, E. Cuppen, and R.H. Plasterk (2003). **The microRNA-producing enzyme Dicer1 is essential for zebrafish development.** *Nature Genetics* 35(3): 217-8. ISSN: 1061-4036.

NAL Call Number: QH426.N37

Abstract: MicroRNAs (miRNAs) are produced by the Dicer1 enzyme; the role of Dicer1 in vertebrate development is unknown. Here we report target-selected inactivation of the dicer1 gene in zebrafish. We observed an initial build-up of miRNA levels, produced by maternal Dicer1, in homozygous dicer1 mutants, but miRNA accumulation stopped after a few days. This resulted in developmental arrest around day 10. These results indicate that miRNA-producing Dicer1 is essential for vertebrate development.

Descriptors: zebrafish, endoribonucleases physiology, microRNAs biosynthesis, RNA helicases physiology, dead box RNA helicases, endoribonucleases genetics, gene silencing, mutation, RNA helicases genetics.

Wienholds, E. and R.H. Plasterk (2004). **Target-selected gene inactivation in zebrafish.** *Methods in Cell Biology* 77: 69-90. ISSN: 0091-679X.

NAL Call Number: 442P92

Descriptors: zebrafish , gene silencing, gene targeting methods, DNA mutational analysis methods, gene library, mutagenesis genetics.

Wienholds, E., F. van Eeden, M. Kosters, J. Mudde, R.H. Plasterk, and E. Cuppen (2003). **Efficient target-selected mutagenesis in zebrafish.** *Genome Research* 13(12): 2700-7. ISSN: 1088-9051.

NAL Call Number: QP606.D46P34

Abstract: One of the most powerful methods available to assign function to a gene is to inactivate or knockout the gene. Recently,we described the first target-selected knockout in zebrafish. Here,we report on the further improvements of this procedure,resulting in a highly efficient and easy method to do target-selected mutagenesis in zebrafish. A library of 4608 ENU-mutagenized F1 animals was generated and kept as a living stock. The DNA of these animals was screened for mutations in 16 genes by use of CEL-I-mediated heteroduplex cleavage (TILLING) and subsequent resequencing. In total,255 mutations were identified,of which 14 resulted in a premature stop codon,7 in a splice donor/acceptor site mutation,and 119 in an amino acid change. By this method,we potentially knocked out 13 different genes in a few months time. Furthermore,we show that TILLING can be used to detect the full spectrum of ENU-induced mutations in a vertebrate genome with the presence of many naturally occurring polymorphisms.

Descriptors: zebrafish, site directed mutagenesis procedure, genetic crosses, DNA primers chemical synthesis, drug administration schedule, ethyl methanesulfonate pharmacology, gene library, nucleic acid amplification techniques, nucleic acid heteroduplexes metabolism, plant proteins metabolism, point mutation.

Wilbanks, A.M., G.B. Fralish, M.L. Kirby, L.S. Barak, Y.X. Li, and M.G. Caron (2004). **Beta-arrestin 2 regulates zebrafish development through the Hedgehog signaling pathway.** *Science* 306(5705): 2264-67. ISSN: 0036 8075.
NAL Call Number: 470Sci2
Abstract: [beta]-arrestins are multifunctional proteins that act as scaffolds and transducers of intracellular signals from heptahelical transmembrane-spanning receptors (7TMR). Hedgehog (Hh) signaling, which uses the putative 7TMR, Smoothened, is established as a fundamental pathway in development, and unregulated Hh signaling is associated with certain malignancies. Here, we show that the functional knockdown of [beta]-arrestin 2 in zebrafish embryos recapitulates the many phenotypes of Hh pathway mutants. Expression of wild-type [beta]-arrestin 2, or constitutive activation of the Hh pathway downstream of Smoothened, rescues the phenotypes caused by [beta]-arrestin 2 deficiency. These results suggest that a functional interaction between [beta]-arrestin 2 and Smoothened may be critical to regulate Hh signaling in zebrafish development.
Descriptors: zebrafish, development, Hedgehog signaling pathway, mutant , phenotype, Beta-arrestin 2.

Willer, G.B., V.M. Lee, R.G. Gregg, and B.A. Link (2005). **Analysis of the zebrafish perplexed mutation reveals tissue-specific roles for de novo pyrimidine synthesis during development.** *Genetics* 170(4): 1827-37. ISSN: 0016-6731.
NAL Call Number: 442.8G28
Abstract: The zebrafish perplexed mutation disrupts cell proliferation and differentiation during retinal development. In addition, growth and morphogenesis of the tectum, jaw, and pectoral fins are also affected. Positional cloning was used to identify a mutation in the carbamoyl-phosphate synthetase2-aspartate transcarbamylase-dihydroorotase (cad) gene as possibly causative of the perplexed mutation and this was confirmed by gene knockdown and pyrimidine rescue experiments. CAD is required for de novo biosynthesis of pyrimidines that are required for DNA, RNA, and UDP-dependent protein glycosylation. Developmental studies of several vertebrate species showed high levels of cad expression in tissues where mutant phenotypes were observed. Confocal time-lapse analysis of perplexed retinal cells in vivo showed a near doubling of the cell cycle period length. We also compared the perplexed mutation with mutations that affect either DNA synthesis or UDP-dependent protein glycosylation. Cumulatively, our results suggest an essential role for CAD in facilitating proliferation and differentiation events in a tissue-specific manner during vertebrate development. Both de novo DNA synthesis and UDP-dependent protein glycosylation are important for the perplexed phenotypes.
Descriptors: zebrafish, cell cycle mutation, pyrimidines biosynthesis, aspartate carbamoyltransferase genetics and metabolism, carbamoyl phosphate synthase glutamine hydrolyzing genetics and metabolism, dihydroorotase genetics and metabolism, confocal microscopy, video microscopy.

Wingert, R.A., R. Selleck, J. Yu, H.D. Song, Z. Chen, A. Song, Y. Zhou, B. Thisse, C. Thisse, A.P. McMahon, and A.J. Davidson (2007). **The cdx genes and retinoic acid control the positioning and segmentation of the zebrafish pronephros.** *Public Library of Science Genetics*

3(10): 1922-38. ISSN: 1553-7390.

Abstract: Kidney function depends on the nephron, which comprises a blood filter, a tubule that is subdivided into functionally distinct segments, and a collecting duct. How these regions arise during development is poorly understood. The zebrafish pronephros consists of two linear nephrons that develop from the intermediate mesoderm along the length of the trunk. Here we show that, contrary to current dogma, these nephrons possess multiple proximal and distal tubule domains that resemble the organization of the mammalian nephron. We examined whether pronephric segmentation is mediated by retinoic acid (RA) and the caudal (cdx) transcription factors, which are known regulators of segmental identity during development. Inhibition of RA signaling resulted in a loss of the proximal segments and an expansion of the distal segments, while exogenous RA treatment induced proximal segment fates at the expense of distal fates. Loss of cdx function caused abrogation of distal segments, a posterior shift in the position of the pronephros, and alterations in the expression boundaries of raldh2 and cyp26a1, which encode enzymes that synthesize and degrade RA, respectively. These results suggest that the cdx genes act to localize the activity of RA along the axis, thereby determining where the pronephros forms. Consistent with this, the pronephric-positioning defect and the loss of distal tubule fate were rescued in embryos doubly-deficient for cdx and RA. These findings reveal a novel link between the RA and cdx pathways and provide a model for how pronephric nephrons are segmented and positioned along the embryonic axis.

Descriptors: zebrafish, gene expression regulation, developmental gene expression regulation, kidney embryology, tretinoin metabolism, molecular cloning, homeodomain proteins metabolism, in situ hybridization, mice, mutation, phenotype, tertiary protein structure, transcription factors metabolism.

Wingert, R.A., J.L. Galloway, B. Barut, H. Foott, P. Fraenkel, J.L. Axe, G.J. Weber, K. Dooley, A.J. Davidson, B. Schmidt, B.H. Paw, G.C. Shaw, P. Kingsley, J. Palis, H. Schubert, O. Chen, J. Kaplan, and L.I. Zon (2005). **Deficiency of glutaredoxin 5 reveals Fe-S clusters are required for vertebrate haem synthesis**. *Nature* 436(7053): 1035-1039. ISSN: 0028-0836.

NAL Call Number: 472N21

Abstract: Iron is required to produce haem and iron - sulphur (Fe - S) clusters, processes thought to occur independently(1,2). Here we show that the hypochromic anaemia in shiraz (sir) zebrafish mutants is caused by deficiency of glutaredoxin 5 (grx5), a gene required in yeast for Fe - S cluster assembly. We found that grx5 was expressed in erythroid cells of zebrafish and mice. Zebrafish grx5 rescued the assembly of Delta grx5 yeast Fe - S, showing that the biochemical function of grx5 is evolutionarily conserved. In contrast to yeast, vertebrates use iron regulatory protein 1 (IRP1) to sense intracellular iron and regulate mRNA stability or the translation of iron metabolism genes(1,2). We found that loss of Fe - S cluster assembly in sir animals activated IRP1 and blocked haem biosynthesis catalysed by aminolaevulinate synthase 2 (ALAS2). Overexpression of ALAS2 RNA without the 50 iron response element that binds IRP1 rescued sir embryos, whereas overexpression of ALAS2 including the iron response element did not. Further, antisense knockdown of IRP1 restored sir embryo haemoglobin synthesis. These findings uncover a connection between haem biosynthesis and Fe - S clusters, indicating that haemoglobin production in the differentiating red cell is regulated through Fe - S cluster assembly. Copyright©Thomson Reuters 2009

Descriptors: shiraz mutant zebrafish, blood and lymphatics, transport and circulation, molecular genetics, hypochromic anemia, blood and lymphatic disease, genetics.

Winkler, C., H. Elmasri, B. Klamt, J.N. Volff, and M. Gessler (2003). **Characterization of hey bHLH genes in teleost fish**. *Development Genes and Evolution* 213(11): 541-553. ISSN: 0949-944X.

NAL Call Number: QL951.R68

Descriptors: zebrafish, blood vessels, cell fate, duplication, embryogenesis, embryonic development, evolution, freshwater fish, genes, mesoderm, mutants, overexpression, phylogenetics, phylogeny, transcription, *Danio rerio*, *Takifugu rubripes*, *Tetraodon nigroviridis*.

Winkler, C., M. Schafer, J. Duschl, M. Schartl, and J.N. Volff (2003). **Functional divergence of two zebrafish midkine growth factors following fish-specific gene duplication.** *Genome Research* 13(6a): 1067-81. ISSN: 1088-9051.

NAL Call Number: QP606.D46P34

Abstract: In mammals, the unique midkine (mdk) gene encodes a secreted heparin-binding growth factor with neurotrophic activity. Here, we show the presence of two functional mdk genes named mdka and mdkb in zebrafish and rainbow trout. Both midkine proteins are clearly different from the related pleiotrophin, which was also identified in zebrafish and other fishes. Zebrafish mdka and mdkb genes map to linkage groups LG7 and LG25, respectively, both presenting synteny to human chromosome 11, in which the unique human ortholog mdk is located. At least four other genes unique in mammals are also present as duplicates on LG7 and LG25. Phylogenetic and divergence analyses suggested that LG7/LG25 paralogs including mdka and mdkb have been formed at approximately the same time, early during the evolution of the fish lineage. Hence, zebrafish and rainbow trout mdka and mdkb might have been generated by an ancient block duplication, and might be remnants of the proposed fish-specific whole-genome duplication. In contrast to the ubiquitous expression of their mammalian counterpart, zebrafish mdka and mdkb are expressed in spatially restricted, mostly nonoverlapping patterns during embryonic development and strongly in distinct domains in the adult brain. Ectopic ubiquitous expression of both mdk genes in early zebrafish embryos caused completely distinct effects on neural crest and floorplate development. These data indicate that mdka and mdkb underwent functional divergence after duplication. This provides an outstanding model to analyze the molecular mechanisms that lead to differences in pathways regulating the formation of homologous embryonic structures in different vertebrates.

Descriptors: zebrafish, carrier, molecular evolution, gene duplication, nerve growth factors physiology, amino acid sequence, brain chemistry genetics, carrier proteins biosynthesis, carrier cytokines genetics, gene expression profiling, gene expression regulation genetics, developmental gene expression regulation genetics, molecular sequence data, nerve growth factors genetics, *Oncorhynchus mykiss* genetics, phylogeny, nucleic acid sequence homology.

Winkler, C., C. Eggert, D. Gradl, G. Meister, M. Giegerich, D. Wedlich, B. Laggerbauer, and U. Fischer (2005). **Reduced U snRNP assembly causes motor axon degeneration in an animal model for spinal muscular atrophy**. *Genes and Development* 19(19): 2320-2330. ISSN: 0890 9369.

NAL Call Number: QH426.G46

Abstract: Spinal muscular atrophy (SMA) is a motoneuron disease caused by reduced levels of survival motoneuron (SMN) protein. Previous studies have assigned SMN to uridine-rich small nuclear ribonucleoprotein particle (U snRNP) assembly, splicing, transcription, and RNA localization. Here, we have used gene silencing to assess. the effect of SMN protein deficiency on U snRNP metabolism in living cells and organisms. In HeLa cells, we show that reduction of SMN to levels found in SMA patients impairs U snRNP assembly. In

line with this, induced silencing of SMN expression in Xenopus laevis or zebrafish arrested embryonic development. Under less severe knock-down conditions, zebrafish embryos proceeded through development yet exhibited dramatic SMA-like motor axon degeneration. The same was observed after silencing two other essential factors in the U snRNP assembly pathway, Gemin2 and pIC1n. Importantly, the injection of purified U snRNPs into either SMN- or Gemin2-deficient embryos of Xenopus and zebrafish prevented developmental arrest and motoneuron degeneration, respectively. These findings suggest that motoneuron degeneration in SMA patients is a direct consequence of impaired production of U snRNPs. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, molecular genetics, muscular system, movement and support, nervous system, neural coordination, spinal muscular atrophy, nervous system disease, muscle disease, pathology, genetics.

Wirtz, K.W.A. (2006). **Phospholipid transfer proteins in perspective**. *Federation of European Biochemical Societies Letters* 580(23): 5436-5441. ISSN: 0014 5793.
NAL Call Number: QD415.F4
Abstract: Since their discovery and subsequent purification from mammalian tissues more than 30 years ago an impressive number of studies have been carried out to characterize and elucidate the biological functions of phosphatidylcholine transfer protein (PC-TP), phosphatidylinositol transfer protein (PI-TP) and non-specific lipid transfer protein, more commonly known as sterol carrier protein 2 (SCP-2). Here I will present information to show that these soluble, low-molecular weight proteins constitute domain structures in StArR-related lipid transfer (START) proteins (i.e. PC-TP), in retinal degeneration protein, type B (RdgB)-related PI-TPs (e.g. Din RdgB, Nir2, Nir3) and in peroxisomal P-oxidation enzyme-related SCP-2 (i.e. 3-oxoacyl-CoA thiolase, also denoted as SCP-X and the 80-kDa D-bifunctional protein). Further I will summarize the most recent studies pertaining to the physiological function of these soluble phospholipid transfer proteins in metazoa. (c) 2006 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics, cell biology, phospholipid.

Wolff, C., S. Roy, K.E. Lewis, H. Schauerte, G. Joerg Rauch, A. Kirn, C. Weiler, R. Geisler, P. Haffter, and P.W. Ingham (2004). **Iguana encodes a novel zinc-finger protein with coiled-coil domains essential for Hedgehog signal transduction in the zebrafish embryo.** *Genes and Development* 18(13): 1565-76. ISSN: 0890-9369.
NAL Call Number: QH426.G46
Abstract: Signaling by lipid-modified secreted glycoproteins of the Hedgehog family play fundamental roles during pattern formation in animal development and in humans; dysfunction of Hedgehog pathway components is frequently associated with a variety of congenital abnormalities and cancer. Transcriptional regulation of Hedgehog target genes is mediated by members of the Gli zinc-finger transcription factors. The relative nuclear concentrations of Gli activator (Gli(act)) and repressor (Gli(rep)) forms, together with their nucleocytoplasmic trafficking, appear to be critical determinants for target gene expression. Whereas such stringent controls of Gli activity are critical in ensuring appropriate levels of pathway activation, the mechanisms by which these processes are regulated remain inadequately understood. Here, using genetic analysis, we show that the zebrafish iguana gene product acts downstream of the Smoothened protein to modulate Gli activity in the somites of the developing embryo. Positional cloning reveals that iguana encodes the zebrafish ortholog of Dzip1, a novel zinc-finger/coiled-coil domain protein that we show can shuttle between the cytoplasm and

nucleus in a manner correlated with Hedgehog pathway activity.
Descriptors: zebrafish, carrier proteins metabolism, *Drosophila* proteins, signal transduction, trans activators metabolism, proteins metabolism, zinc fingers, carrier cyclic AMP dependent protein kinases metabolism developmental gene expression regulation, Hedgehog proteins, molecular sequence data, mutation, oncogene proteins metabolism and genetics, tertiary protein structure, G protein Coupled receptors metabolism, repressor proteins metabolism and genetics, trans activators genetics, transcription factors genetics and metabolism.

Woltering, J.M. and A.J. Durston (2006). **The zebrafish hoxDb cluster has been reduced to a single microRNA.** *Nature Genetics* 38(6): 601-2. ISSN: 1061-4036.
NAL Call Number: QH426.N37
Descriptors: zebrafish, homeobox genes, microRNAs genetics, multigene family.

Wong, A.C. and A.L. VanEenennaam (2006). **Evaluation of dual site-specific recombination systems in *danio rerio* for the biological containment of transgenic fish**. *Transgenic Research* 15(1): 125. ISSN: 0962-8819.
NAL Call Number: QH442.6T74
Descriptors: transgenic zebrafish, molecular genetics, molecular biophysics.

Wood, A.W. and C. Duan (2003). **Targeted gene knockdown reveals an important role for insulin-like growth factor binding protein-2 in zebrafish embryonic development**. *Integrative and Comparative Biology* 43(6): 1012. ISSN: 1540 7063.
NAL Call Number: QL1.I67
Descriptors: zebrafish, cardiovascular system, transport and circulation, blood and lymphatics, methods and techniques, skeletal system , movement and support, digestive system, ingestion and assimilation, sense organs, sensory reception, in situ hybridization, histology and cytology techniques, genetic techniques, targeted gene knockdown, genetic techniques, embryonic development, cranial vasculature, tissue specific expression patterns.

Wood, A.W., P.J. Schlueter, and C. Duan (2005). **Targeted knockdown of insulin-like growth factor binding protein-2 disrupts cardiovascular development in zebrafish embryos.** *Molecular Endocrinology* 19(4): 1024-34. ISSN: 0888-8809.
NAL Call Number: QP187.3M64M6
Abstract: IGF binding protein-2 (IGFBP-2) is an evolutionarily conserved protein that binds IGFs and modulates their biological activities. Although the actions of IGFBP-2 have been well studied in vitro, we have a poor understanding of its in vivo functions, particularly during early development. Using the transparent zebrafish embryo as a model, we show that IGFBP-2 mRNA is expressed in lens epithelium and cranial boundary regions during early embryonic development and becomes localized to the liver by the completion of embryogenesis. Targeted knock-down of IGFBP-2 by antisense morpholino-modified oligonucleotides resulted in delayed development, reduced body growth, reduced IGF-I mRNA levels, and disruptions to cardiovascular development, including reduced blood cell number, reduced blood circulation, cardiac dysfunction, and brain ventricle edema. Detailed examination of vascular tissues, using a stable transgenic line of zebrafish expressing green fluorescent protein in vascular endothelial cells, revealed specific angiogenic (vessel sprouting) defects in IGFBP-2 knockdown embryos, with effects being localized in regions associated with IGFBP-2 mRNA expression. These findings suggest that IGFBP-2 is required for general embryonic development and growth and plays a local role in regulating vascular development in a model vertebrate organism.

Descriptors: zebrafish, cardiovascular system embryology, insulin like growth factor binding protein 2 physiology, cardiovascular abnormalities genetics, down regulation, abnormalities, metabolism, embryonic development, gene expression drug effects, gene targeting, hematopoiesis genetics and physiology, insulin like growth factor I and 2 genetics, oligodeoxyribonucleotides, antisense genetics and pharmacology, messenger RNA analysis and metabolism.

Wood, J.D., J.A. Landers, M. Bingley, C.J. McDermott, V. Thomas McArthur, L.J. Gleadall, P.J. Shaw, and V.T. Cunliffe (2006). **The microtubule-severing protein Spastin is essential for axon outgrowth in the zebrafish embryo.** *Human Molecular Genetics* 15(18): 2763-71. ISSN: 0964-6906.

NAL Call Number: RB155.5.H87

Abstract: Hereditary spastic paraplegia (HSP) is a collection of neurological disorders characterized by developmental failure or degeneration of motor axons in the corticospinal tract and progressive lower limb spasticity. SPG4 mutations are the most common cause of autosomal dominant HSP and Spastin (the SPG4 gene product) is a microtubule severing protein that shares homology with katanin, the microtubule severing activity of which promotes axon growth in cultured neurons. Given the sequence and functional similarity between spastin and katanin, we hypothesized that spastin promotes the dynamic disassembly and remodelling of microtubules required for robust, properly directed motor axon outgrowth. To investigate this hypothesis, we cloned the zebrafish spg4 orthologue and used morpholino antisense oligonucleotides directed against the translation start site and the intron 7-8 splice donor site to knock down spastin function in the developing zebrafish embryo. Reduced spg4 function caused dramatic defects in motor axon outgrowth without affecting the events driving the initial specification of motor neurones. Other neuronal subtypes also exhibited a requirement for spg4 function, since spg4 knock down caused both widespread defects in neuronal connectivity and extensive CNS-specific apoptosis. Our results reveal a critical requirement for spastin to promote axonal outgrowth during embryonic development, and they validate the zebrafish embryo as a novel model system to dissect the pathogenetic mechanisms underlying HSP. Taken together with other recent studies, our findings suggest that axon outgrowth defects may be a common feature of childhood SPG3A and SPG4 cases.

Descriptors: zebrafish , adenosine triphosphatases and axons metabolism, adenosine triphosphatases deficiency and genetics, base sequence, molecular cloning, complementary DNA genetics, microtubules metabolism, molecular sequence data, motor neurons metabolism, oligodeoxyribonucleotides, antisense genetics and pharmacology, hereditary spastic paraplegia genetics and metabolism, synapses metabolism, proteins deficiency.

Woods, I.G., D.A. Lyons, M.G. Voas, H.M. Pogoda, and W.S. Talbot (2006). **NSF is essential for organization of myelinated axons in zebrafish.** *Current Biology* 16(7): 636-48. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: BACKGROUND: Myelinated axons are essential for rapid conduction of action potentials in the vertebrate nervous system. Of particular importance are the nodes of Ranvier, sites of voltage-gated sodium channel clustering that allow action potentials to be propagated along myelinated axons by saltatory conduction. Despite their critical role in the function of myelinated axons, little is known about the mechanisms that organize the nodes of Ranvier. RESULTS: Starting with a forward genetic screen in zebrafish, we have identified an essential requirement for nsf (N-ethylmaleimide sensitive factor) in the organization of myelinated axons. Previous work has shown that NSF is essential for membrane

fusion in eukaryotes and has a critical role in vesicle fusion at chemical synapses. Zebrafish nsf mutants are paralyzed and have impaired response to light, reflecting disrupted nsf function in synaptic transmission and neural activity. In addition, nsf mutants exhibit defects in Myelin basic protein expression and in localization of sodium channel proteins at nodes of Ranvier. Analysis of chimeric larvae indicates that nsf functions autonomously in neurons, such that sodium channel clusters are evident in wild-type neurons transplanted into the nsf mutant hosts. Through pharmacological analyses, we show that neural activity and function of chemical synapses are not required for sodium channel clustering and myelination in the larval nervous system. CONCLUSIONS: Zebrafish nsf mutants provide a novel vertebrate system to investigate Nsf function in vivo. Our results reveal a previously unknown role for nsf, independent of its function in synaptic vesicle fusion, in the formation of the nodes of Ranvier in the vertebrate nervous system.

Descriptors: zebrafish larva, N ethylmaleimide sensitive, Ranvier's nodes ultrastructure and metabolism, action potentials physiology, cell death physiology, chimera metabolism, genetic markers, hair cells physiology, larva anatomy, metabolism, histology and genetics, movement physiology, mutation, myelin basic proteins genetics, N ethylmaleimide sensitive phenotype, messenger RNA metabolism, sodium channels physiology, synaptic transmission physiology.

Woods, I.G. and W.S. Talbot (2005). **The you gene encodes an EGF-CUB protein essential for Hedgehog signaling in zebrafish.** *Public Library of Science Biology* 3(3): E66. ISSN: 1544-9173.

NAL Call Number: QH301.P56

Abstract: Hedgehog signaling is required for many aspects of development in vertebrates and invertebrates. Misregulation of the Hedgehog pathway causes developmental abnormalities and has been implicated in certain types of cancer. Large-scale genetic screens in zebrafish have identified a group of mutations, termed you-class mutations, that share common defects in somite shape and in most cases disrupt Hedgehog signaling. These mutant embryos exhibit U-shaped somites characteristic of defects in slow muscle development. In addition, Hedgehog pathway mutations disrupt spinal cord patterning. We report the positional cloning of you, one of the original you-class mutations, and show that it is required for Hedgehog signaling in the development of slow muscle and in the specification of ventral fates in the spinal cord. The you gene encodes a novel protein with conserved EGF and CUB domains and a secretory pathway signal sequence. Epistasis experiments support an extracellular role for You upstream of the Hedgehog response mechanism. Analysis of chimeras indicates that you mutant cells can appropriately respond to Hedgehog signaling in a wild-type environment. Additional chimera analysis indicates that wild-type you gene function is not required in axial Hedgehog-producing cells, suggesting that You is essential for transport or stability of Hedgehog signals in the extracellular environment. Our positional cloning and functional studies demonstrate that You is a novel extracellular component of the Hedgehog pathway in vertebrates.

Descriptors: zebrafish, extracellular matrix trans activators physiology, essential genes, Hedgehog proteins, molecular sequence data.

Woods, I.G., C. Wilson, B. Friedlander, P. Chang, D.K. Reyes, R. Nix, P.D. Kelly, F. Chu, J.H. Postlethwait, and W.S. Talbot (2005). **The zebrafish gene map defines ancestral vertebrate chromosomes.** *Genome Research* 15(9): 1307-14. ISSN: 1088-9051.

NAL Call Number: QP606.D46P34

Abstract: Genetic screens in zebrafish *(Danio rerio)* have identified mutations that define the roles of hundreds of essential vertebrate genes. Genetic maps can link mutant phenotype

with gene sequence by providing candidate genes for mutations and polymorphic genetic markers useful in positional cloning projects. Here we report a zebrafish genetic map comprising 4073 polymorphic markers, with more than twice the number of coding sequences localized in previously reported zebrafish genetic maps. We use this map in comparative studies to identify numerous regions of synteny conserved among the genomes of zebrafish, Tetraodon, and human. In addition, we use our map to analyze gene duplication in the zebrafish and Tetraodon genomes. Current evidence suggests that a whole-genome duplication occurred in the teleost lineage after it split from the tetrapod lineage, and that only a subset of the duplicates have been retained in modern teleost genomes. It has been proposed that differential retention of duplicate genes may have facilitated the isolation of nascent species formed during the vast radiation of teleosts. We find that different duplicated genes have been retained in zebrafish and Tetraodon, although similar numbers of duplicates remain in both genomes. Finally, we use comparative mapping data to address the proposal that the common ancestor of vertebrates had a genome consisting of 12 chromosomes. In a three-way comparison between the genomes of zebrafish, Tetraodon, and human, our analysis delineates the gene content for 11 of these 12 proposed ancestral chromosomes.
Descriptors: zebrafish, evolution, vertebrates genetics, chromosome mapping and genetics, expressed sequence tags, gene duplication, karyotyping, linkage genetics, mutation, genetic polymorphism, tetraodontiformes classification and genetics, zebrafish classification.

Wright, D., R.K. Butlin, and O. Carlborg (2006). **Epistatic regulation of behavioural and morphological traits in the zebrafish (*Danio rerio*).** *Behavior Genetics* 36(6): 914-22. ISSN: 0001 8244.
NAL Call Number: QH301.B45
Abstract: There is currently a lack of studies examining epistasis in general, and specifically for behavioural traits of evolutionary significance. The advent of more efficient analytical methods for exploring epistasis in QTL studies removes the computational restraint on this type of analysis and suggests that performing further analyses of existing datasets may reveal a more complete picture of the genetic architecture of the traits. Here we report the results from an epistatic QTL analysis of an F2 cross between a wild population and a standard laboratory strain of zebrafish. This further analysis was performed using a simultaneous search to identify epistatically interacting QTL affecting behavioural and morphological traits and uncovered several novel epistatic interactions that reached either genome-wide or suggestive significance levels as determined by a randomisation testing approach. These results provide novel insight into the genetic architecture of the regulation of behavioural as well as morphological phenotypes and call for more studies of epistasis for this group of traits.
Descriptors: zebrafish, behavior, quantitative trait loci, genetic crosses, DNA genetics, DNA isolation and purification, genotype, genetic models, phenotype.

Wright, D., R. Nakamichi, J. Krause, and R.K. Butlin (2006). **Quantitative trait loci (QTL) analysis of behavioral and morphological differentiation between wild and laboratory zebrafish (*Danio rerio*).** *Behavior Genetics* 36(2): 271-84. ISSN: 0001 8244.
NAL Call Number: QH301.B45
Abstract: The zebrafish is an important model organism for neuro-anatomy and developmental genetics. It also offers opportunities for investigating the functional and evolutionary genetics of behaviour but these have yet to be exploited. The ecology of anti-predator behaviour has been widely studied in fish and has been shown to vary among populations and between wild and domesticated (laboratory) fish. Here, we utilise the strong behavioural differences present between a wild-derived strain of fish from Bangladesh and the laboratory

strain AB. In total, 184 F2 fish were generated and tested for shoaling tendency and willingness to approach an unfamiliar object ('boldness'). Our results indicate the existence of QTL for boldness on chromosomes 9 and 16 and suggest another genomic region that influences anti-predator behaviour on chromosome 21. QTL for growth rate, weight and fat content, all of which are elevated in laboratory fish, were detected on chromosome 23. These initial results confirm the potential for QTL mapping of behavioural traits in zebrafish and also for dissecting the consequences of selection during domestication.

Descriptors: zebrafish, laboratory genetics, wild types genetics, behavior physiology, quantitative trait loci genetics, arousal genetics, chromosome mapping, genetic crosses, genetic models, species specificity, zebrafish anatomy and histology, Bangladesh.

Wu, K.M., C.J. Huang, S.P. Hwang, and Y.S. Chang (2006). **Molecular cloning, expression and characterization of the zebrafish bram1 gene, a BMP receptor-associated molecule.** *Journal of Biomedical Science* 13(3): 345-55. ISSN: 1021-7770.

NAL Call Number: R850.A1

Abstract: We have identified a cDNA clone encoding BMP receptor-associated molecule 1 (BRAM1) from the zebrafish expressed sequence tag (EST) database. The 2606 bp full-length bram1 cDNA was cloned, and further confirmed by nucleotide sequencing. The zebrafish sequence encodes a protein of 195 amino acids with an evolutionarily conserved MYND domain, which displays approximately approximately 98% homology with human and mouse BRAM1, and approximately approximately 64% homology with C. elegans BRA-1 and BRA-2. The bram1 gene, composed of five exons and four introns, spans approximately approximately 14 kb on linkage group 14 of the zebrafish genome. RT-PCR and whole mount in situ hybridization analyses disclosed that zebrafish BRAM1 is a maternal factor. The protein interacts directly with zebrafish BMP Receptor type IA, as observed from GST-pull down and co-immunoprecipitation assays. Furthermore, cotransfection of zebrafish BRAM1 with the corresponding BMP receptor resulted in down-regulation of BMP-mediated signaling. Our results collectively indicate that BRAM1 plays a biological role during zebrafish development.

Descriptors: zebrafish, bone morphogenetic proteins metabolism, carrier proteins chemistry and genetics, 3t3 cells, amino acid and base sequence, COS cells, *Caenorhabditis elegans* , *Cercopithecus aethiops*, molecular cloning, expressed sequence tags, mice, molecular sequence data, amino acid sequence homology.

Wu, S., B.A. Adams, E.A. Fradinger, and N.M. Sherwood (2006). **Role of two genes encoding PACAP in early brain development in zebrafish.** *Annals of the New York Academy of Sciences* 1070: 602-21. ISSN: 0077 8923.

NAL Call Number: 500 N484

Abstract: To study the role of pituitary adenylate cyclase-activating polypeptide (PACAP) in early brain development, we examined PACAP and its receptors for first expression and then separately knocked down the two forms of PACAP in zebrafish where development is rapid and observable. We injected morpholinos (antisense oligonucleotides) into fertilized eggs to block PACAP. Morphological changes in the brain were observed in embryos at 27 h post fertilization (hpf). Using in situ hybridization of early brain marker genes, we found that the most striking effects were an increase in pax2.1 expression in eye stalks associated with absence of either form of PACAP or an increase in eng2 and fgf8 in the midbrain-hindbrain boundary after loss of PACAP2. These marker genes are among the earliest factors in the formation of the midbrain-hindbrain boundary, an early organizing center. We suggest that PACAP is a target gene with feedback inhibition on pax2.1, eng2, or fgf8 in specific brain

areas. In the hindbrain, the absence of either form of PACAP had little effect, as shown by expression of ephA4 and meis1.1. During midbrain development, our evidence suggests that PACAP1 can activate mbx. In both the diencephalon and/or forebrain, lack of PACAP1 or PACAP2 led to an increase in fgf8, again suggesting a suppressive effect of PACAP during development on these important genes that help to define cells in the forebrain. The early expression of transcripts for PACAP and its receptors by 0.5-6 hpf make both PACAP1 and PACAP2 candidates for factors that influence brain development.
Descriptors: zebrafish, brain embryology and metabolism, pituitary adenylate cyclase activating polypeptide genetics, biological markers, eye embryology and metabolism, developmental gene expression regulation, pituitary adenylate cyclase activating polypeptide receptors metabolism and genetics, time factors, genetic transcription genetics.

Wu, W., X. Liu, M. Xu, J.R. Peng, and R. Setiono (2005). **A hybrid SOM-SVM approach for the zebrafish gene expression analysis.** *Genomics, Proteomics and Bioinformatics* 3(2): 84-93. ISSN: 1672-0229.
NAL Call Number: Q447
Abstract: Microarray technology can be employed to quantitatively measure the expression of thousands of genes in a single experiment. It has become one of the main tools for global gene expression analysis in molecular biology research in recent years. The large amount of expression data generated by this technology makes the study of certain complex biological problems possible, and machine learning methods are expected to play a crucial role in the analysis process. In this paper, we present our results from integrating the self-organizing map (SOM) and the support vector machine (SVM) for the analysis of the various functions of zebrafish genes based on their expression. The most distinctive characteristic of our zebrafish gene expression is that the number of samples of different classes is imbalanced. We discuss how SOM can be used as a data-filtering tool to improve the classification performance of the SVM on this data set.
Descriptors: zebrafish, computational biology methods, gene expression profiling methods, gene expression regulation genetics, multigene family genetics, zebrafish classification.

Wu, X., P. Wang, C.A. Brown, C.A. Zilinski, and M.M. Matzuk (2003). **Zygote Arrest 1 (Zar1) is an evolutionarily conserved gene expressed in vertebrate ovaries**. *Biology of Reproduction* 69(3): 861-867. ISSN: 0006-3363.
NAL Call Number: QD415.F4
Descriptors: zebrafish, amino acid sequence, DNA, embryogenesis, embryonic development, evolution, gene expression, nucleotide sequence, ovaries, *Danio rerio*, *Fugu rubripes*, vertebrata, *Xenopus laevis*, African clawed frog , Zar1 gene, amino acid sequence prediction, cDNA, chromosome 14, man, mice, rats, genetics and evolution.

Wu, Y., G. Zhang, Q. Xiong, F. Luo, C. Cui, W. Hu, Y. Yu, J. Su, A. Xu, and Z. Zhu (2006). **Integration of double-fluorescence expression vectors into zebrafish genome for the selection of site-directed knockout/knockin.** *Marine Biotechnology* 8(3): 304-11. ISSN: 1436-2228.
NAL Call Number: TP248.27.M37M37
Abstract: Production of zebrafish by modifying endogenous growth hormone (GH) gene through homologous recombination is described here. We first constructed the targeting vectors pGHT1.7k and pGHT2.8k, which were used for the knockout/knockin of the endogenous GH gene of zebrafish, and injected these two vectors into the embryos of zebrafish. Overall, the rate of targeted integration with the characteristic of germ line transmission in zebrafish was 1.7 x 10(-6). In one experimental patch, the integrating efficiency

of pGHT2.8k was higher than that of pGHT1.7k, but the lethal effect of pGHT2.8k was stronger than that of pGHT1.7k. The clones with the correct integration of target genes were identified by a simple screening procedure based on green fluorescent protein (GFP) and RFP dual selection, which corresponded to homologous recombination and random insertion, respectively. The potential homologous recombination zebrafish was further bred to produce a heterozygous F1 generation, selected based on the presence of GFP. The potential targeted integration of exogenous GH genes into a zebrafish genome at the P0 generation was further verified by polymerase chain reaction and Southern blot analysis. Approximately 2.5% of potential founder knockout and knockin zebrafish had the characteristic of germ line transmission. In this study, we developed an efficient method for producing the targeted gene modification in zebrafish for future studies on genetic modifications and gene functions using this model organism.

Descriptors: zebrafish larva, gene deletion, site directed green fluorescent mutagenesis methods, genetically modified genetics, gene expression regulation, genome genetics.

Wu, Y.L. and Z. Gong (2004). **A novel zebrafish kelchlike gene klhl and its human ortholog KLHL display conserved expression patterns in skeletal and cardiac muscles.** *Gene* 338(1): 75-83. ISSN: 0378-1119.

NAL Call Number: QH442.A1G4

Abstract: In this study, a novel gene, kelchlike (klhl) was identified in zebrafish by whole-mount in situ hybridization screen for important genes involved in embryogenesis. A full-length klhl cDNA was cloned and characterized. We found that klhl was a member of the kelch-repeat superfamily, containing two evolutionary conserved domains--broad-complex, tramtrack, bric-a-brac/poxvirus and zinc finger (BTB/POZ) domain, and kelch motif. Database mining revealed the presence of putative orthologs of klhl in human, mouse, rat, and pufferfish. klhl was determined to map to zebrafish linkage group (LG) 13 and was found to be syntenic with the proposed orthologs of klhl in human, mouse, and rat. In an effort to elucidate the function of klhl, klhl expression was investigated by Northern blot analysis and in situ hybridization. klhl is specifically expressed in the fast skeletal and cardiac muscle. Northern blot analyses show that the human ortholog, KLHL, is also specifically expressed in the skeletal muscles and heart. In silico analyses of rat expressed sequence tag (EST) clones corresponding to rat Klhl ortholog also indicate that its expression is also restricted to rat muscle tissues, suggesting a conserved role of klhl in vertebrates. The expression pattern of klhl, as well as the presence of the kelch repeats indicates a possible role for Klhl in the organization of striated muscle cytoarchitecture.

Descriptors: zebrafish, carrier gene expression profiling, skeletal muscle metabolism, myocardium metabolism, amino acid sequence, molecular cloning, complementary DNA chemistry and genetics, embryonic development, developmental gene expression regulation, in situ hybridization, microfilament molecular sequence data, radiation hybrid mapping, sequence alignment, DNA sequence analysis, amino acid sequence homology.

Wu, Y.P., Q. Xiong, G.X. Zhang, and A.L. Xu (2004). **The research progress of zebrafish gene engineering**. *Acta Genetica Sinica* 31(10): 1167-74. ISSN: 0379-4172.

Abstract: The research progress of zebrafish gene engineering is reviewed, in including the recent explosion status of transgenic zebrafish lines, transgeneic zebrafish in targeted screens, the development of transgenic technology in zebrafish as well as the current hotspots and future perspective of zebrafish gene engineering. By now the zebrafish gene engineering, nuclear transplantation and chromosome set manipulation technologies have been established in China. With firm foundation of zebrafish cytogenetics and embryology, we will

obtain gene engineering zebrafish with gene targeting integration and promote the advancement and development of biotechnology in applied research field in near future.
Descriptors: zebrafish, genetic engineering, genetically modified, gene targeting, China.
Language of Text: Chinese.
Notes: A review.

Xiang, F., J.X. Zou, F.J. Deng, S.Y. Liu, and Y. Sui (2004). **The molecular taxonomy and phylogeny of zebrafish (*Danio rerio*) based on The Cyt- b partial sequences.** *Chinese Journal of Zoology* 39(5): 13-18. ISSN: 0250 3263.
Abstract: The taxonomy and phylogenic relationships of zebrafish were investigated by the cyt- b gene partial DNA sequence analysis of mitochondria. 426 bp of cyt- b from *Danio rerio* (long fin) , *D .rerio* (short fin), *D . rerio frankei*, *Gambusia affinis* and *Epinephelus awoara* , respectively, were cloned and sequenced. Combined with the homologous sequences of *Megalobrama amblycephala , Oryzias latipes , Ctenopharyngodon idella* and *Etroplus maculates* from Genebank, the aligment shows that the mean base composition for partial cyt- b is 26.7% adenine, 26.1% cytosine, 16.6% guanine, and 30.5% thymine and that the third position of the codon have a base bias (G% is only 6.4%) . The sequence similarity of *D . rerio* (long fin) , *D . rerio* (short fin) and *D . rerio frankei* is above 99% . Phylogenetic trees were constructed with Neighbor Joining method. Those results collectively indicated that *D . rerio frankei* might be clustered with *D . rerio* (long fin) first, then with *D. rerio* (short fin) . The results support the opinion of Frankel and David that *D. rerio frankei* belongs to a subspecies of *D. rerio* and is not an individual species.
Descriptors: zebrafish, *Danio rerio*, cytochromes, cytochrome B, molecular genetics and partial DNA sequences, molecular taxonomy and phylogenetic relationships indication, phylogeny.
Language of Text: Chinese.

Xiang, W. and C.H. Siu (2003). **Cloning of complete cDNA for two l1cam homologues in zebrafish.** *Molecular and Cellular Proteomics* 2(9): 742. ISSN: 1535 9476.
Descriptors: zebrafish, development, nervous system, neural coordination, crash syndrome, genetic disease, nervous system disease, cloning, genetic techniques.

Xie, J., S.L. Bessling, T.K. Cooper, H.C. Dietz, A.S. McCallion, and S. Fisher (2007). **Manipulating mitotic recombination in the zebrafish embryo through RecQ helicases.** *Genetics* 176(2): 1339-42. ISSN: 0016-6731.
NAL Call Number: 442.8G28
Abstract: RecQ DNA helicases resolve Rad-51-mediated recombination and suppress aberrant homologous recombination. RecQ gene loss is associated with cancer susceptibility and increased mitotic recombination. We have developed an in vivo assay based on a zebrafish pigment mutant for suppression of RecQ activity, and demonstrate that zebrafish RecQ genes have conserved function in suppressing mitotic recombination.
Descriptors: zebrafish embryos, physiology , mitosis, RecQ helicases genetics, genetic recombination, amino acid sequence, bloom syndrome genetics, molecular sequence data, mutation, RNA genetics, sequence alignment, amino acid sequence homology, genetic transcription, Werner syndrome genetics.

Xie, X., M. Kamal, and E.S. Lander (2006). **A family of conserved noncoding elements derived from an ancient transposable element**. *Proceedings of the National Academy of Sciences, USA* 103(31): 11659-11664. ISSN: 0027-8424.

NAL Call Number: 500N21P
Descriptors: zebrafish, evolution, fossils, freshwater fish, genomes, living fossils, radiation, transposons, *Danio rerio*, palaeontology.

Xu, C., G. Wu, Y. Zohar, and S.J. Du (2003). **Analysis of myostatin gene structure, expression and function in zebrafish.** *Journal of Experimental Biology* 206(22): 4067-79. ISSN: 0022 0949.
NAL Call Number: 442.8B77
Abstract: Myostatin is a member of the TGF-beta family that functions as a negative regulator of skeletal muscle development and growth in mammals. Recently, Myostatin has also been identified in fish; however, its role in fish muscle development and growth remains unknown. We have reported here the isolation and characterization of myostatin genomic gene from zebrafish and analysis of its expression in zebrafish embryos, larvae and adult skeletal muscles. Our data showed that myostatin was weakly expressed in early stage zebrafish embryos, and strongly expressed in swimming larvae, juvenile and skeletal muscles of adult zebrafish. Transient expression analysis revealed that the 1.2 kb zebrafish myostatin 5' flanking sequence could direct green fluorescent protein (GFP) expression predominantly in muscle cells, suggesting that the myostatin 5' flanking sequence contained regulatory elements required for muscle expression. To determine the biological function of Myostatin in fish, we generated a transgenic line that overexpresses the Myostatin prodomain in zebrafish skeletal muscles using a muscle-specific promoter. The Myostatin prodomain could act as a dominant negative and inhibit Myostatin function in skeletal muscles. Transgenic zebrafish expressing the Myostatin prodomain exhibited no significant change in myogenic gene expression and differentiation of slow and fast muscle cells at their embryonic stage. The transgenic fish, however, exhibited an increased number of myofibers in skeletal muscles, but no significant difference in fiber size. Together, these data demonstrate that Myostatin plays an inhibitory role in hyperplastic muscle growth in zebrafish.
Descriptors: zebrafish, gene expression, skeletal muscle metabolism, transforming growth factor beta genetics, 5' flanking region genetics, genetically modified, base sequence, body weight, chromosome mapping, DNA primers, metabolism, green fluorescent proteins, in situ hybridization, larva metabolism, luminescent proteins, microinjections, molecular sequence data, skeletal muscle physiology, plasmids genetics, tertiary protein structure physiology, reverse transcriptase PCR, DNA sequence analysis, transgenes, myostatin.

Xu, J., B.P. Srinivas, S.Y. Tay, A. Mak, X. Yu, S.G. Lee, H. Yang, K.R. Govindarajan, B. Leong, G. Bourque, S. Mathavan, and S. Roy (2006). **Genomewide expression profiling in the zebrafish embryo identifies target genes regulated by Hedgehog signaling during vertebrate development.** *Genetics* 174(2): 735-52. ISSN: 0016-6731.
NAL Call Number: 442.8G28
Abstract: Hedgehog proteins play critical roles in organizing the embryonic development of animals, largely through modulation of target gene expression. Little is currently known, however, about the kinds and numbers of genes whose expression is controlled, directly or indirectly, by Hedgehog activity. Using techniques to globally repress or activate Hedgehog signaling in zebrafish embryos followed by microarray-based expression profiling, we have discovered a cohort of genes whose expression responds significantly to loss or gain of Hedgehog function. We have confirmed the Hedgehog responsiveness of a representative set of these genes with whole-mount in situ hybridization as well as real time PCR. In addition, we show that the consensus Gli-binding motif is enriched within the putative regulatory elements of a sizeable proportion of genes that showed positive regulation in our assay, indicating that their expression is directly induced by Hedgehog. Finally, we provide

evidence that the Hedgehog-dependent spatially restricted transcription of one such gene, nkx2.9, is indeed mediated by Gli1 through a single Gli recognition site located within an evolutionarily conserved enhancer fragment. Taken together, this study represents the first comprehensive survey of target genes regulated by the Hedgehog pathway during vertebrate development. Our data also demonstrate for the first time the functionality of the Gli-binding motif in the control of Hedgehog signaling-induced gene expression in the zebrafish embryo.

Descriptors: zebrafish, gene expression profiling, developmental gene expression regulation physiology, genome physiology, Hedgehog, signal transduction genetics, base sequence, molecular sequence data, oncogene proteins chemistry and physiology, trans activators chemistry and physiology.

Xu, P., S. Wang, L. Liu, E. Peatman, B. Somridhivej, J. Thimmapuram, G. Gong, and Z. Liu (2006). **Channel catfish BAC-end sequences for marker development and assessment of syntenic conservation with other fish species**. *Animal Genetics* 37(4): 321-326. ISSN: 0268 9146.

Online: http://www.blackwell-synergy.com/doi/pdf/10.1111/j.1365-2052.2006.01453.x

NAL Call Number: QH506.B356

Abstract: In the present study, 25 195 BAC ends for channel catfish (Ictalurus punctatus) were sequenced, generating 20 366 clean BAC-end sequences (BES), with an average read length of 557 bp after trimming. A total of 11 414 601 bp were generated, representing approximately 1.2% of the catfish genome. Based on this survey, the catfish genome was found to be highly AT-rich, with 60.7% A+T and 39.3% G+C. Approximately 12% of the catfish genome consisted of dispersed repetitive elements, with the Tc1/mariner transposons making up the largest percentage by base pair (4.57%). Microsatellites were detected in 17.5% of BES. Catfish BACs were anchored to the zebrafish and Tetraodon genome sequences by BLASTN, generating 16% and 8.2% significant hits (E < e-5) respectively. A total of 1074 and 773 significant hits were unique to the zebrafish and Tetraodon genomes, respectively, of which 417 and 406, respectively, were identified as known genes in other species, providing a major genome resource for comparative genomic mapping. Copyright © 2008 CABI

Descriptors: zebrafish, bacterial artificial chromosomes, gene mapping, genetic markers, genome analysis, genomes, microsatellites, nucleotide sequences, *Ictalurus punctatus.*

Xue, L. and K. Qian (2003). **Cloning and sequence analysis of Hoxa-11a gene from zebrafish (*Danio rerio*)**. *Journal of Zhejiang University (Agriculture and Life Sciences)* 29(4): 433-438. ISSN: 1008-9209.

Descriptors: zebrafish, alanine, amino acid sequence, amphibiotic species, DNA, freshwater fish, genes, nucleotide sequence, PCR, serine, Anura, *Danio rerio*, genetics and evolution.

Yamakoshi, K. and N. Shimoda (2003). **De novo DNA methylation at the CpG island of the zebrafish no tail gene.** *Genesis* 37(4): 195-202. ISSN: 1526-954X.

NAL Call Number: QH426.D32

Descriptors: zebrafish, CpG islands genetics, DNA methylation, notochord anatomy and histology, T-box domain tail anatomy and histology, chromatin metabolism, fertilization physiology, gene expression regulation physiology, notochord metabolism, T-box domain proteins metabolism, tail metabolism.

Yamakoshi, K. and N. Shimoda (2003). **PCR-based cloning of an intronless zebrafish no tail gene.** *Biochemical and Biophysical Research Communications* 306(2): 598-602. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: The zebrafish no tail gene (ntl) is indispensable for the formation of the notochord and tail structure. Here, we show the presence of an intronless ntl gene in zebrafish, which we designated cryptail (ctl). ctl could not be found in any zebrafish genomic resources examined and was only just cloned by a PCR-based approach that relied on its lack of introns and homology to ntl. The amplifiable region of ctl was confined to the transcribed region of ntl. ctl thus appeared to have been generated by reverse transcription of ntl mRNA, like a processed pseudogene. ctl was very polymorphic even in individual fish, but had no missense mutations. This may suggest that ctl has a physiological function in zebrafish.
Descriptors: zebrafish, introns, T-box domain molecular cloning, genetic models, mutation, PCR, genetic and single nucleotide polymorphism, genetic transcription.

Yamakoshi, K., Y. Shishido, and N. Shimoda (2005). **Generation of aberrant transcripts of and free DNA ends in zebrafish no tail gene.** *Marine Biotechnology* 7(3): 163-72. ISSN: 1436-2228.
NAL Call Number: TP248.27.M37M37
Abstract: The zebrafish no tail gene (ntl) is indispensable for the formation of the notochord and the tail structure. In a wild-type zebrafish population, we occasionally observed adult zebrafish with a narrow or no tailfin. This led us to examine the hypothesis that the activity of ntl was somehow genetically unstable. Here we present two findings regarding the gene. First, approximately 3% of ntl transcripts were aberrant; most of them carried deletions at various positions. Second, free, DNA double-stranded ends (DSEs) were formed at an AT dinucleotide repeat in ntl. DSEs were also generated in another zebrafish gene, noggin2 (nog2). DSEs in ntl and nog2 had common characteristics, which suggested that the AT repeats in these genes elicited DSEs by blocking progression of the replication.
Descriptors: zebrafish, alternative splicing genetics, DNA chemistry, phenotype, messenger RNA genetics, T-box domain proteins genetics, base sequence, DNA mutational analysis, DNA primers, complementary DNA genetics, dinucleotide repeats genetics , gene components, molecular sequence data, DNA sequence analysis.

Yamanoue, Y., M. Miya, J.G. Inoue, K. Matsuura, and M. Nishida (2006). **The mitochondrial genome of spotted green pufferfish *Tetraodon nigroviridis* (Teleostei: Tetraodontiformes) and divergence time estimation among model organisms in fishes**. *Genes and Genetic Systems* 81(1): 29-39. ISSN: 1341 7568.
NAL Call Number: QH426.G462
Abstract: We determined the whole mitochondrial genome sequence for spotted green pufferfish, *Tetraodon nigroviridis* (Teleostei: Tetraodontiformes). The genome (16,488 bp) contained 37 genes (two ribosomal RNA genes, 22 transfer RNA genes, and 13 protein-coding genes) plus control region as found in other vertebrates, with the gene order identical to that of typical vertebrates. The sequence was used to estimate phylogenetic relationships and divergence times among major lineages of fishes, including representative model organisms in fishes. We employed partitioned Bayesian approaches for these two analyses using two datasets that comprised concatenated amino acid sequences from 12 protein-coding genes (excluding the ND6 gene) and concatenated nucleotide sequences from the 12 protein-coding genes (without 3rd codon positions), 22 transfer RNA genes, and two ribosomal RNA genes. The resultant trees from the two datasets were well resolved and largely congruent with those from previous studies, with spotted green pufferfish being placed in a reasonable phylogenetic position. The approximate divergence times between spotted green pufferfish and

model organisms in fishes were 85 million years ago (MYA) vs. torafugu, 183 MYA vs. three-spined stickleback, 191 MYA vs. medaka, and 324 MYA vs. zebrafish, all of which were about twice as old as the divergence times estimated by their earliest occurrences in fossil records. Copyright © 2008 CABI

Descriptors: zebrafish, amino acid sequences, genes, genomes, mitochondrial DNA, nucleotide sequences, phylogeny, RNA, fishes, Tetraodontiformes.

Yamashita, S., C. Miyagi, T. Fukada, N. Kagara, Y.S. Che, and T. Hirano (2004). **Zinc transporter LIVI controls epithelial-mesenchymal transition in zebrafish gastrula organizer.** *Nature* 429(6989): 298-302. ISSN: 0028-0836.

NAL Call Number: 472 N21

Abstract: Vertebrate gastrulation is a critical step in the establishment of body plan. During gastrulation, epithelial-mesenchymal transition (EMT) occurs. EMT is one of the central events of embryonic development, organ and tissue regeneration, and cancer metastasis. Signal transducers and activators of transcription (STATs) mediate biological actions such as cell proliferation, differentiation and survival in response to cytokines and growth factors, in a variety of biological processes. STATs are also important in EMT during gastrulation, organogenesis, wound healing and cancer progression. We previously showed that STAT3 is activated in the organizer during zebrafish gastrulation and its activity is essential for gastrulation movements. The requirement for STAT3 is cell-autonomous for the anterior migration of gastrula organizer cells, and non-cell-autonomous for the convergence of neighbouring cells. The molecular mechanisms of STAT's action in EMT, however, are unknown. Here we identify LIV1, a breast-cancer-associated zinc transporter protein, as a downstream target of STAT3 that is essential and sufficient for STAT3's cell-autonomous role in the EMT of zebrafish gastrula organizer cells. Furthermore, we demonstrate that LIV1 is essential for the nuclear localization of zinc-finger protein Snail, a master regulator of EMT. These results establish a molecular link between STAT3, LIV1 and Snail in EMT.

Descriptors: zebrafish, carrier proteins metabolism, cell differentiation, epithelial cells and gastrula cytology, mesoderm cytology, organizers, embryonic cytology, cell nucleus active transport, cell line, DNA binding proteins genetics, embryology, metabolism, epithelial cells metabolism, gastrula metabolism, mesoderm metabolism, mice, molecular sequence data, morphogenesis, organizers, embryonic embryology, organizers, embryonic metabolism, STAT3 transcription factor, trans activators metabolism, transcription factors metabolism.

Yamauchi, H., Y. Hotta, M. Konishi, A. Miyake, A. Kawahara, and N. Itoh (2006). **Fgf21 is essential for haematopoiesis in zebrafish.** *European Molecular Biology Organization Journal Reports* 7(6): 649-54. ISSN: 1469 221X.

NAL Call Number: QH506 .E463

Abstract: Fibroblast growth factors (Fgfs) function as key secreted signalling molecules in many developmental events. The zebrafish is a powerful model system for the investigation of embryonic vertebrate haematopoiesis. Although the effects of Fgf signalling on haematopoiesis in vitro have been reported, the functions of Fgf signalling in haematopoiesis in vivo remain to be explained. We identified Fgf21 in zebrafish embryos. Fgf21-knockdown zebrafish embryos lacked erythroid and myeloid cells but not blood vessels and lymphoid cells. The knockdown embryos had haemangioblasts and haematopoietic stem cells. However, the knockdown embryos had significantly fewer myeloid and erythroid progenitor cells. In contrast, Fgf21 had no significant effect on cell proliferation and apoptosis in the intermediate cell mass. These results indicate that Fgf21 is a newly identified factor essential for the determination of myelo-erythroid progenitor cell fate in vivo.

Descriptors: zebrafish, Fgf physiology, hematopoiesis, chromosomes, cytology, physiology, erythroid progenitor cells physiology, Fgf genetics, hematopoietic stem cells physiology, antisense oligonucleotides pharmacology, phylogeny.

Yan, C.H. and K.M. Chan (2004). **Cloning of zebrafish metallothionein gene and characterization of its gene promoter region in HepG2 cell line.** *Biochimica Et Biophysica Acta* 1679(1): 47-58. ISSN: 0006 3002.
NAL Call Number: 381B522
Descriptors: zebrafish, metallothionein genetics, promoter regions genetics, amino acid and base sequence, cell line, molecular cloning, DNA primers, electrophoretic mobility shift assay, molecular sequence data, amino acid sequence homology, nucleic acid sequence homology.

Yan, W., L. Ma, C.A. Zilinski, and M.M. Matzuk (2004). **Identification and characterization of evolutionarily conserved pufferfish, zebrafish, and frog orthologs of GASZ.** *Biology of Reproduction* 70(6): 1619-25. ISSN: 0006 3363.
NAL Call Number: QD415.F4
Abstract: We previously identified Gasz (a germ cell-specific gene encoding a protein containing four ankyrin repeats, a sterile-alpha motif, and a basic leucine zipper) in six mammalian species. Here, we report GASZ orthologs in pufferfish (*Fugu rubripes*), zebrafish (*Danio verio*), and frog (*Xenopus laevis*). Sequences of the three Gasz cDNAs were determined by database mining and 5'- and 3'-rapid amplification of cDNA ends (RACE) followed by sequencing. The three orthologous vertebrate genes encode proteins structurally similar to mammalian GASZ and contain the characteristic four ankyrin repeats (ANKs) and sterile-alpha motif (SAM). Their ANK and SAM domains share 55- 74% and 38-55% amino acid identity with those in human GASZ, respectively. Similar to human and mouse Gasz genes, pufferfish Gasz is composed of 13 exons, spanning approximately 12 kilobases, and flanked by Cftr at its 5'-end and Wnt2 at its 3'-end. Northern and Western blot analyses detect frog Gasz expression only in testis and ovary. In situ hybridization and immunohistochemical analyses show that frog Gasz mRNA and protein expression is confined to pachytene spermatocytes in the testis and to oocytes in the ovary. In frog oocytes, GASZ protein appears to localize to a cytoplasmic structure resembling the Balbiani body, a postulated mRNA transport organizer in the cytoplasm. The high evolutionary conservation and germ cell specificity suggest that GASZ plays an essential role in gametogenesis. The data presented here are important for future studies of the physiological roles of GASZ using fish and amphibians as animal models.
Descriptors: zebrafish, signal transducing adaptor *Takifugu* genetics, *Xenopus laevis* genetics, signal transducing adaptor proteins chemistry, amino acid sequence, ankyrin repeat, base sequence, complementary DNA genetics, molecular evolution, gene expression, leucine zippers genetics, molecular sequence data, oogenesis genetics, ovary metabolism, messenger RNA genetics and metabolism, amino acid sequence homology, nucleic acid sequence homology, species specificity, spermatogenesis genetics, testis metabolism, *Xenopus* proteins genetics.

Yang, C.T., A.E. Hindes, K.A. Hultman, and S.L. Johnson (2007). **Mutations in gfpt1 and skiv2l2 cause distinct stage-specific defects in larval melanocyte regeneration in zebrafish.** *Public Library of Science Genetics* 3(6): E88. ISSN: 1553-7390.
Abstract: The establishment of a single cell type regeneration paradigm in the zebrafish provides an opportunity to investigate the genetic mechanisms specific to regeneration processes.

We previously demonstrated that regeneration melanocytes arise from cell division of the otherwise quiescent melanocyte precursors following larval melanocyte ablation with a small molecule, MoTP. The ease of ablating melanocytes by MoTP allows us to conduct a forward genetic screen for mechanisms specific to regeneration from such precursors or stem cells. Here, we reported the identification of two mutants, eartha(j23e1) and julie(j24e1) from a melanocyte ablation screen. Both mutants develop normal larval melanocytes, but upon melanocyte ablation, each mutation results in a distinct stage-specific defect in melanocyte regeneration. Positional cloning reveals that the eartha(j23e1) mutation is a nonsense mutation in gfpt1 (glutamine:fructose-6-phosphate aminotransferase 1), the rate-limiting enzyme in glucosamine-6-phosphate biosynthesis. Our analyses reveal that a mutation in gfpt1 specifically affects melanocyte differentiation (marked by melanin production) at a late stage during regeneration and that gfpt1 acts cell autonomously in melanocytes to promote ontogenetic melanocyte darkening. We identified that the julie(j24e1) mutation is a splice-site mutation in skiv2l2 (superkiller viralicidic activity 2-like 2), a predicted DEAD-box RNA helicase. Our in situ analysis reveals that the mutation in skiv2l2 causes defects in cell proliferation, suggesting that skiv2l2 plays a role in regulating melanoblast proliferation during early stages of melanocyte regeneration. This finding is consistent with previously described role for cell division during larval melanocyte regeneration. The analyses of these mutants reveal their stage-specific roles in melanocyte regeneration. Interestingly, these mutants identify regeneration-specific functions not only in early stages of the regeneration process, but also in late stages of differentiation of the regenerating melanocyte. We suggest that mechanisms of regeneration identified in this mutant screen may reveal fundamental differences between the mechanisms that establish differentiated cells during embryogenesis, and those involved in larval or adult growth.

Descriptors: mutant zebrafish, glutamine fructose 6 phosphate transaminase isomerizing genetics, melanocytes enzymology, RNA helicases genetics, regeneration genetics, cell differentiation genetics, melanocytes cytology and physiology, molecular sequence data.

Yang, H.S., W.J. Wu, and S.W. Lou (2005). **Identification of essential genes involved in cortisol prereceptor pathway in zebrafish**. *Journal of the Fisheries Society of Taiwan* 32(1): 108. ISSN: 0379-4180.

NAL Call Number: SH135.T3

Descriptors: zebrafish, adrenal cortex, aldosterone, corticoids, genes, glucocorticoid receptors, glucocorticoids, homeostasis, hormones, hydrocortisone, mineralocorticoid receptors, receptors, signal transduction, steroid hormones, steroids, *Danio rerio*, physiology, biochemistry, biophysics.

Yang, H.W., J.L. Kutok, N.H. Lee, H.Y. Piao, C.D. Fletcher, J.P. Kanki, and A.T. Look (2004). **Targeted expression of human MYCN selectively causes pancreatic neuroendocrine tumors in transgenic zebrafish.** *Cancer Research* 64(20): 7256-62. ISSN: 0008 5472.

Abstract: The zebrafish model organism has been used extensively for studies of genetic pathways in development, indicating its potential applicability to cancer. Here we show that targeted expression of MYCN in cells of the pancreatic islet induces neuroendocrine carcinoma. Four transgenic fish developed abdominal tumors between 4 and 6 months of age, and histologic analysis revealed lobulated arrangements of neoplastic cells with expression of the MYCN transgene. The tumors also expressed insulin mRNA, and pancreatic exocrine cells and ducts were identified within the neoplasms, indicating a pancreatic origin for the tumor. Transmission electron microscopy revealed cytoplasmic, endocrine-dense core granules, analogous to those found in human neuroendocrine tumors. Our studies establish

a zebrafish transgenic model of pancreatic neuroendocrine carcinoma, setting the stage to evaluate molecular pathways downstream of MYCN in this vertebrate forward genetic model system.

Descriptors: zebrafish, neuroectodermal tumors genetics, nuclear oncogene pancreatic neoplasms genetics, genetically modified, islet cell carcinoma genetics and metabolism, insulin biosynthesis and genetics, Islets of Langerhans pathology, neuroectodermal tumors metabolism, nuclear proteins biosynthesis, oncogene proteins biosynthesis, pancreatic neoplasms metabolism, promoter regions genetics, messenger RNA biosynthesis and genetics, transgenes.

Yang, X.Y., J.H. Yao, L. Cheng, D.W. Wei, D.R. Lu, and J.L. Xue (2003). **Whole amount in situ hybridization and transgene via microinjection in zebrafish**. *Journal of Experimental Biology [Shi Yan Sheng Wu Xue Bao]* 36(3): 243-7. ISSN: 0022-0949.

NAL Call Number: 442.8B77

Abstract: Zebrafish is new model organism to study development of the vertebrate. In this report, we chose a fragment of gene GATA-1 which specifically expressed in zebrafish hematopoiesis system as probe to carry on whole amount in situ hybridization. Then we transported reporter gene driven by GATA-1 promoter into the embryos via microinjection approach and observed the spatial and temporal expression pattern of GFP. Our results demonstrated that efficient and reliable technology of whole amount in situ hybridization and microinjection in zebrafish was established.

Descriptors: zebrafish, genetically modified genetics, developmental gene expression regulation, transcription factors genetics, complementary DNA genetics, gene transfer techniques, reporter in situ hybridization, microinjections, promoter regions genetics, recombinant trans proteins genetics.

Language of Text: Chinese.

Yang, X.Y., J.H. Yao, L. Cheng, D.W. Wei, J.L. Xue, and D.R. Lu (2003). **Molecular cloning and expression of a smooth muscle-specific gene SM22alpha in zebrafish.** *Biochemical and Biophysical Research Communications* 312(3): 741-6. ISSN: 0006 291X.

NAL Call Number: 442.8B5236

Abstract: SM22alpha is a kind of 22-kDa protein which is exclusively expressed in smooth muscle containing tissues of the vertebrates. Here we report molecular cloning of a novel zebrafish SM22alpha gene. The full length of zebrafish SM22alpha cDNA is 1296bp and it encodes a polypeptide of 201 amino acids which shares 69.2%, 69.7%, 69.2%, 67.2%, and 61.2% overall identity with human, mouse, rat, chicken, and bovine SM22alpha, respectively. Characterization of zebrafish SM22alpha genomic sequence reveals that it spans 7.7kb and contains five exons and four introns. The expression pattern of SM22alpha in zebrafish embryonic development is studied by whole-mount in situ hybridization. Strong expression is observed in vascular, gut, swim bladder, branchial arches, and fin epidermis. Furthermore, we carry out gene knock-down by antisense morpholino oligonucleotide, which results in disappearance of yolk extension, caudal fin aberrance, and deficiency of circulation system in zebrafish embryo. Cross-section of SM22alpha-deficient embryo suggests that SM22alpha may play roles in smooth muscle cell morphology transform.

Descriptors: zebrafish, developmental gene expression regulation physiology, microfilament proteins metabolism and genetics, muscle proteins metabolism and genetics, amino acid sequence, cattle, chickens, molecular cloning, gene expression profiling, in situ hybridization, mice, microfilament proteins chemistry, molecular sequence data, muscle proteins chemistry,

smooth muscle embryology and metabolism, antisense oligonucleotides genetics, organ specificity, rats, amino acid sequence homology, species specificity.

Yang, Z., H. Jiang, T. Chachainasakul, S. Gong, X.W. Yang, N. Heintz, and S. Lin (2006). **Modified bacterial artificial chromosomes for zebrafish transgenesis.** *Methods* 39(3): 183-8. ISSN: 0882-4010.
NAL Call Number: QP519.7.M47
Abstract: Transgenesis using bacterial artificial chromosomes (BAC) offers greater fidelity in directing desirable expression of foreign genes. Application of this technology in the optically transparent zebrafish with fluorescent protein reporters enables unparalleled visual analysis of regulation of gene expression in a living organism. Here we describe a streamlined procedure of direct selecting multiple BAC clones based on public sequence databases followed by rapid modification with GFP or RFP for transgenic analysis in zebrafish. Experimental procedures for BAC DNA preparation, microinjection of zebrafish embryos and screening of transgenic zebrafish carrying GFP/RFP modified BAC clones are detailed.
Descriptors: zebrafish, genetically modified genetics, artificial bacterial chromosomes genetics, genetic engineering methods, *Escherichia coli* genetics, gene expression regulation, genes, bacterial, reporter genes, green fluorescent proteins analysis, plasmids genetics, rec a recombinases genetics, recombinant fusion proteins analysis, genetic recombination.

YangJing, F., W. Dong, Y.X. Cao, and S.Z. Wang (2005). **Reduction of Shh gene expression and development of the lower jaw in zebrafish embryos exposed to TCDD**. *Zoological Research* 26(5): 506-512. ISSN: 0254 5853.
NAL Call Number: QL1.T85
Abstract: 2, 3, 7, 8-tetrachlorodibenzo-p -dioxin (TCDD) is one of the most toxic environmental pollutants. The goal of this study is to understand the cause of the short lower jaw induced by TCDD in the zebrafish embryos. Embryos are exposed to TCDD (0 - 1. 0 mu g/L) at 24 h post fertilization (24 hpf) until the time of observation and studied by gene knockdown, immunohistochemistry staining and in situ hybridization. The results show that TCDD treatment leads to decreases in the size of the lower jaw and reduced Shh gene expression. This effect can be blocked by injection of Morpholino antisense oligos against AhR2, the potential TCDD receptor. We also find Cyclopamine, a Shh inhibitor, caused short lower jaw and decreased cell proliferation in the zebrafish embryos. These results suggest that TCDD might inhibit the expression of Shh through AhR and lead to decreased cell proliferation and short lower jaw in zebrafish embryos. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, toxicology, skeletal system, movement and support, molecular genetics, in situ hybridization, histology and cytology techniques, genetic techniques, immunohistochemistry staining, immunologic techniques, gene knockout, cell proliferation.
Language of Text: Chinese.

Yao, J., L. Wang, L. Chen, S. Zhang, Q. Zhao, W. Jia, and J. Xue (2006). **Cloning and developmental expression of the DEC1 ortholog gene in zebrafish.** *Gene Expression Patterns* 6(8): 919-27. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: DEC1/STRA13/SHARP2 is a transcription factor of the bHLH family that has been suggested to play key roles in mammalian cell differentiation, the cell cycle and circadian regulation. However, the function of the DEC1 gene during embryogenesis is not well understood. In the present study, we cloned a zebrafish ortholog of the human DEC1 gene and analyzed its expression during development of zebrafish. The predicted protein encoded

by zebrafish DEC1 consists of 403 amino acids, and shares 59%, 60% and 59% identity in overall amino acid sequence with human DEC1, mouse STRA13 and rat SHARP2, respectively. Zebrafish DEC1 contains a bHLH domain exhibiting 97% identity with that of the mammalian ortholog. During zebrafish embryogenesis, DEC1 is expressed in a strong ubiquitous manner before early segmentation. At 15-72 hpf, DEC1 shows a specific and dynamic expression in the developing eyes, somites, pineal gland (epiphysis), heart, brain, spinal cord, notochord, pronephric duct, common cardinal vein and blood cells. In older zebrafish, DEC1 also is expressed in multiple tissues including the brain, eye, gut, liver and pancreas. Our data provide evidence that expression of DEC1 is evolutionally conserved in zebrafish.
Descriptors: zebrafish, basic helix loop helix transcription factors genetics and metabolism, developmental gene expression regulation, homeodomain proteins metabolism, amino acid and base sequence, molecular cloning, computational biology methods, complementary DNA isolation and purification molecular sequence data , tissue distribution.

Yasuda, S., M.Y. Liu, Y.S. Yang, R. Snow, S. Takahashi, and M.C. Liu (2006). **Identification of novel hydroxysteroid-sulfating cytosolic SULTs, SULT2 ST2 and SULT2 ST3, from zebrafish: cloning, expression, characterization, and developmental expression.** *Archives of Biochemistry and Biophysics* 455(1): 1-9. ISSN: 0003 9861.
NAL Call Number: 381 Ar2
Abstract: By searching the expressed sequence tag database, two zebrafish cDNAs encoding putative cytosolic sulfotransferases (SULTs) were identified. Sequence analysis indicated that these two zebrafish SULTs belong to the cytosolic SULT2 gene family. The recombinant form of these two novel zebrafish SULTs, designated SULT2 ST2 and SULT2 ST3, were expressed using the pGEX-2TK glutathione S-transferase (GST) gene fusion system and purified from transformed BL21 (DE3) Escherichia coli cells. Purified GST-fusion protein form of SULT2 ST2 and SULT2 ST3 exhibited strong sulfating activities toward dehydroepiandrosterone (DHEA) and corticosterone, respectively, among various endogenous compounds tested as substrates. Both enzymes displayed pH optima at approximately 6.5. Kinetic constants of the two enzymes, as well as the GST-fusion protein form of the previously identified SULT2 ST1, with DHEA and corticosterone as substrates were determined. Developmental stage-dependent expression experiments revealed distinct patterns of expression of SULT2 ST2 and SULT2 ST3, as well as the previously identified SULT2 ST1, during embryonic development and throughout the larval stage onto maturity.
Descriptors: zebrafish, hydroxysteroids metabolism, sulfates metabolism, sulfotransferases genetics, amino acid sequence, molecular cloning, corticosterone metabolism, cytosol enzymology, complementary DNA chemistry, complementary DNA genetics, dehydroepiandrosterone metabolism, polyacrylamide gel electrophoresis, developmental gene expression regulation, enzymologic gene expression regulation, hydrogen ion concentration, isoenzymes genetics and metabolism, molecular sequence data, phylogeny, messenger RNA genetics and metabolism, recombinant proteins isolation, purification and metabolism, reverse transcriptase PCR, DNA sequence analysis, amino acid sequence homology, sulfotransferases isolation and purification, sulfotransferases metabolism.

Yazawa, R., I. Hirono, and T. Aoki (2005). **Characterization of promoter activities of four different Japanese flounder promoters in transgenic zebrafish**. *Marine Biotechnology* 7(6): 625-633. ISSN: 1436 2228.
NAL Call Number: TP248.27.M37M37
Abstract: An important consideration in transgenic research is the choice of promoter for regulating the expression of a foreign gene. In this study several tissue-specific and induc-

ible promoters derived from Japanese flounder *Paralichthys olivaceus* were identified, and their promoter activity was examined in transgenic zebrafish. The 5' flanking regions of the Japanese flounder complement component C3, gelatinase B, keratin, and tumor necrosis factor (TNF) genes were linked to green fluorescence protein (GFP) as a reporter gene. The promoter regulatory constructs were introduced into fertilized zebrafish eggs. As a result we obtained several stable transgenic zebrafish that displayed green fluorescence in different tissues. Complement component C3 promoter regulated GFP expression in liver, and gelatinase B promoter regulated it in the pectoral fin and gills. Keratin promoter regulated GFP expression in skin and liver. TNF gene promoter regulated GFP expression in the pharynx and heart. TNF promoter had lipoplysaccharide-inducible activity, such that when transgenic embryos were immersed lipopolysaccharide, GFP expression increased in the epithelial tissues. These 4 promoters regulated the expression of GFP in different patterns in transgenic zebrafish. Copyright © 2008 CABI
Descriptors: zebrafish, complement, embryos, gene expression, genes, genetic regulation, keratin, lipopolysaccharides, nucleotide sequences, promoters, reporter genes, tumor necrosis factor, *Danio rerio*, *Paralichthys olivaceus.*

Yazawa, R., I. Hirono, and T. Aoki (2006). **Transgenic zebrafish expressing chicken lysozyme show resistance against bacterial diseases**. *Transgenic Research* 15(3): 385-391. ISSN: 0962 8819.
NAL Call Number: QH442.6T74
Abstract: We established a transgenic zebrafish strain expressing chicken lysozyme gene under the control of the Japanese flounder keratin gene promoter, and investigated its resistance to a pathogenic bacterial infection. To generate the lysozyme transgenic construct, Japanese flounder keratin promoter was linked to both the hen egg white (HEW) lyoszyme gene and green fluorescence protein (GFP) gene used as a selection marker for the transgenic strains, in a recombinant plasmid. The recombinant plasmid was microinjected into fertilized zebrafish eggs. In F2 transgenic zebrafish, GFP expression was strong in the epithelial tissues, liver and gill from the embryonic stage to the adult stage. The expressions of HEW lysozyme and GFP mRNA were confirmed in the liver and skin by RT-PCR. Western blot analysis showed that both HEW lysozyme and GFP were present in protein extracts from the liver of transgenic zebrafish, but not in protein extracts from the muscle. The lytic activity of protein extracts from the liver (assessed by a lysoplate assay using Micrococcus lysodeikticus as a substrate) was 1.75 times higher in F2 transgenic zebrafish than in the wild type. In a challenge experiment, 65% of the F2 transgenic fish survived an infection of Flavobacterium columnare and 60% survived an infection of Edwardsiella tarda, whereas 100% of the control fish were killed by both pathogens. However, the survival rates of the transgenic fish were not significantly higher when higher concentrations of bacteria were used. Copyright © 2008 CABI
Descriptors: transgenic zebrafish, bacterial diseases and resistance, genes, genetic resistance, gills, immune response, liver, lysozyme, promoters, *Danio rerio*, *Edwardsiella tarda*, fishes, *Flavobacterium.*

Yazawa, R., I. Hirono, T. Ohira, and T. Aoki (2005). **Induction of Japanese flounder tnf promoter activity by lipopolysaccharide in zebrafish embryo.** *Marine Biotechnology* 7(3): 231-5. ISSN: 1436-2228.
NAL Call Number: TP248.27.M37M37
Abstract: A recombinant plasmid containing the 2381-bp promoter region of Japanese flounder tumor necrosis factor (TNF) and green fluorescence protein (GFP) was introduced into zebrafish fertilized eggs by microinjection. GFP was expressed in 2 transgenic zebrafish lines in the heart and around the pharynx under unstressed condition. When embryos were

exposed to lipopolysaccharide (LPS), GFP was expressed in the whole zebrafish embryonic surface, and Western blot analysis also showed that the level of the expressed GFP protein in zebrafish embryo was higher at an LPS concentration of 140 microg/ml than at LPS concentrations of 0 and 70 microg/ml. Stimulation with either concanavalin A or phorbol myristate acetate induced weak GFP expression, but stimulation with both of them induced strong expression similar to that induced by LPS. GFP expression peaked 1 hour after stimulation, then gradually decreased. These results indicate that transcription regulated by the Japanese flounder TNF promoter could be under the control of the LPS-recognition system in zebrafish embryos.

Descriptors: zebrafish and flounder genetics, gene expression regulation drug effects, lipopolysaccharides pharmacology, promoter regions genetics, tumor necrosis factors metabolism, genetically modified, Western blotting, concanavalin a pharmacology, DNA primers, polyacrylamide gel electrophoresis, metabolism, gene expression regulation genetics, gene transfer techniques, green fluorescent proteins metabolism, lipopolysaccharides metabolism, plasmids genetics, reverse transcriptase PCR, tetradecanoylphorbol acetate pharmacology, tumor necrosis factors genetics.

Yee, N.S., W. Gong, Y. Huang, K. Lorent, A.C. Dolan, R.J. Maraia, and M. Pack (2007). **Mutation of RNA Pol III subunit rpc2/polr3b Leads to Deficiency of Subunit Rpc11 and disrupts zebrafish digestive development.** *Public Library of Science Biology* 5(11): E312. ISSN: 1544-9173.

NAL Call Number: QH301.P56

Abstract: The role of RNA polymerase III (Pol III) in developing vertebrates has not been examined. Here, we identify a causative mutation of the second largest Pol III subunit, polr3b, that disrupts digestive organ development in zebrafish slim jim (slj) mutants. The slj mutation is a splice-site substitution that causes deletion of a conserved tract of 41 amino acids in the Polr3b protein. Structural considerations predict that the slj Pol3rb deletion might impair its interaction with Polr3k, the ortholog of an essential yeast Pol III subunit, Rpc11, which promotes RNA cleavage and Pol III recycling. We engineered Schizosaccharomyces pombe to carry an Rpc2 deletion comparable to the slj mutation and found that the Pol III recovered from this rpc2-delta yeast had markedly reduced levels of Rpc11p. Remarkably, overexpression of cDNA encoding the zebrafish rpc11 ortholog, polr3k, rescued the exocrine defects in slj mutants, indicating that the slj phenotype is due to deficiency of Rpc11. These data show that functional interactions between Pol III subunits have been conserved during eukaryotic evolution and support the utility of zebrafish as a model vertebrate for analysis of Pol III function.

Descriptors: mutation zebrafish, digestive system growth and development, developmental gene expression regulation, larva enzymology, RNA polymerase III genetics, cell proliferation drug effects, digestive system drug effects, digestive system enzymology, larva drug effects, larva growth and development, mutation, antisense oligonucleotides pharmacology, RNA polymerase III metabolism.

Yeh, F.L., L.Y. Hsu, B.A. Lin, C.F.L.I.C. Chen, S.H. Tsai, and T. Hsu (2006). **Cloning of zebrafish (*Danio rerio*) heat shock factor 2 (HSF2) and similar patterns of HSF2 and HSF1 mRNA expression in brain tissues.** *Biochimie* 88(12): 1983-8. ISSN: 0300 9084.

NAL Call Number: 383S01

Abstract: The transcriptional activation of heat shock protein (HSP) genes is initiated by the binding of heat shock factors (HSFs) to heat shock elements (HSEs) located at the promotor regions. Multiple HSFs exist in larger eukaryotic organisms in order to sense various

types of stress signals. Here we report the cloning of zebrafish (*Danio rerio*) HSF2 (zHSF2) cDNA (GenBank accession number AF412833) that has an open reading frame of 1470 nucleotides, encoding a polypeptide of 489 amino acids. Domain architecture analysis of the deduced zHSF2 sequence revealed the presence of a DNA-binding domain at the N-terminal end, an adjacent oligomerization domain and a vertebrate heat shock transcription factor domain. Amino acid alignment showed a 70% sequence identity between zHSF2 and human or mouse HSF2, while only a 45% identity was found between zHSF1a and zHSF2. Recombinant zHSF2 bound with a very high specificity to HSEs arranged as inverted arrays of 5'-nGAAn-3', as replacing one GAA with GTA almost abolished the formation of HSE-binding complex. Similar patterns of zHSF1a and zHSF2 mRNA expression in the brain regions of developing zebrafish were detected by whole mount in situ hybridization and paraffin sectioning, suggesting that most of the two HSF gene activities were controlled by a common mechanism during the embryonic development of zebrafish. The levels of both zHSF1a and zHSF2 mRNA in zebrafish tissues were moderately increased after heat stress.
Descriptors: zebrafish, brain metabolism, transcription factors genetics, amino acid sequence, brain embryology, electrophoretic mobility shift assay, gene expression profiling, developmental gene expression regulation, heat shock heat shock proteins metabolism and genetics, in situ hybridization, molecular sequence data, messenger RNA genetics and metabolism, recombinant proteins metabolism and genetics, reverse transcriptase PCR, sequence alignment, transcription factors metabolism.

Yeh, F.L., S.Y. Wang, L.Y. Hsu, D.Y. Wang, and T. Hsu (2004). **Cloning of the mismatch recognition protein MSH2 from zebrafish *(Danio rerio*) and its developmental stage-dependent mRNA expression.** *Biochimica Et Biophysica Acta* 1680(2): 129-36. ISSN: 0006 3002.
NAL Call Number: 381B522
Abstract: Eukaryotic mismatch repair of simple base mispairs and small insertion-deletion loops is activated by the binding of a heterodimeric complex composed of MutS homolog 2(MSH2) and MSH6. Here we report the cloning of zebrafish (*Danio rerio)* MSH2 (zMSH2) cDNA that has an open reading frame of 2811 nucleotides encoding a polypeptide of 936 amino acids. The deduced amino acid sequence of zMSH2 shares a 69% identity to both human and mouse MSH2. The zMSH2 protein contains a putative tyrosine-42 mismatch-contacting residue located at the N-terminal mismatch recognition region and four C-terminal ATP-binding consensus sequences conserved among MutS homologs. The 105-kDa recombinant zMSH2 bound apparently stronger to a G-T heteroduplex than to a homoduplex probe as shown by a gel shift assay. A preferential expression of both zMSH2 and zMSH6 mRNA in early embryos was found by Northern blot analysis. Whole mount in situ hybridization revealed a major expression of zMSH2 in different regions of the brain, including eyes, telencephalon, and the fourth ventricle in 12- to 48-h-old embryos. The production of zMSH2 mRNA gradually decreased in more mature 60- to 120-h-old zebrafish, reflecting a positive correlation between the amount of proliferating cells and MSH gene expression.
Descriptors: zebrafish, base pair mismatch, DNA binding proteins genetics, developmental gene expression regulation, proto oncogene proteins genetics, adenosine triphosphate metabolism, amino acid and base sequence, binding sites, Northern blotting, molecular cloning, consensus sequence, DNA binding proteins genetics, proteins metabolism, electrophoretic mobility shift assay, in situ hybridization, molecular sequence data, muts homolog 2 protein, open reading frames, proto oncogene proteins metabolism, messenger RNA genetics and metabolism, recombinant proteins isolation, purification, metabolism and genetics, amino acid sequence homology.

Yeh, F.L., H.L. Yan, S.Y. Wang, T.Y. Jung, and T. Hsu (2003). **Molecular cloning of zebrafish (*Danio rerio*) MutS homolog 6(MSH6) and noncoordinate expression of MSH6 gene activity and G-T mismatch binding proteins in zebrafish larvae.** *Journal of Experimental Zoology. Part A, Comparative Experimental Biology* 297(2): 118-29. ISSN: 1548-8969.
NAL Call Number: QL1.J854
Abstract: Eukaryotic MutS homolog 6(MSH6) is a DNA mismatch recognition protein associated with mismatch repair of simple base-base mispairs and small insertion-deletion loops. As replication or recombination errors generated during embryonic development of living organisms should be efficiently corrected to maintain the integrity of genetic materials, we attempted to study MSH6 gene expression in developing zebrafish (*Danio rerio*) and the influence of MSH6 expression on the production of mismatch binding factors. A full-length cDNA encoding zebrafish MSH6 (zMSH6) was first obtained by rapid amplification of cDNA ends (RACE). The deduced amino acid sequence of zMSH6 shares 57% and 56% identity with human and mouse MSH6, respectively. The 190-kDa recombinant zMSH6 containing 1,369 amino acids bound preferentially to a heteroduplex than to a homoduplex DNA. Northern blot and semiquantitative RT-PCR analysis detected apparent levels of zMSH6 mRNA expression in 12 and 36-hr-old zebrafish embryos, while this expression in 84-hr-old larvae was dramatically reduced to 23% of that in 12-hr-old embryos when beta-actin mRNA was constitutively synthesized. Incubation of G-T and G-G heteroduplex probes with 12 to 60-hr-old zebrafish extracts produced predominantly high-shifting binding complexes with very similar band intensity. Although low in zMSH6 mRNA production, the extracts of 84-hr-old larvae interacted significantly stronger than the embryonic extracts with both G-T and G-G mispairs, producing high and low-shifting complexes. Heteroduplex-recognition proteins in 108-hr-old larvae gave a similar pattern of mismatch binding. The intensities of G-T complexes produced by 84 and 108-hr-old zebrafish extracts were 2.5 to 3-fold higher than those of G-G complexes. Our data indicate that the production of efficient MSH6-independent binding factors, particularly G-T-specific recognition proteins, is upregulated in zebrafish at the larval stage when MSH6 gene activity is downregulated.
Descriptors: zebrafish, base pair mismatch, molecular cloning, DNA repair, DNA binding proteins genetics, proteins biosynthesis, developmental gene expression regulation, amino acid and base sequence, Northern blotting, complementary DNA, down regulation, larva growth and development, molecular sequence data, messenger RNA analysis, reverse transcriptase PCR, up regulation.

Yelick, P.C. and R.C. Albertson (2005). **Independent roles revealed for fgf8 signaling in left-right patterning of the visceral organs and craniofacial skeleton**. *Federation of American Societies for Experimental Biology Journal* 19(5, Suppl. S, Part 2): A1362-A1363. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: Laterality is fundamental to the vertebrate body plan, which exhibits essential left-right (LR) asymmetries in developing brain and visceral organs. Here weinvestigate the role of fgf8 signaling in LR patterning of the zebrafish embryo. We find that fgf8 is required early in development for morphogenesis of Kupffer's vesicle (KV), a ciliated structure necessary for proper LR orientationof the heart and gut. We also demonstrate that fgf8 organizes the LR axis during craniofacial development. When fgf8 signaling is reduced and/or absent, embryos exhibit asymmetric cranial neural crest (CNC) segmentation, and consistently biased asymmetric pharyngeal arch cartilage and bone formation. Finally, we demonstrate that fgf8 deficient laterality defects in the head and gut are uncoupled from one another. These results make important contributions toward understanding vertebrate laterality. First, we show that fgf8 signaling is required for one of the earliest stages (i.e., KV morphogenesis)

of LR asymmetricvisceral organ development in zebrafish. Second, our results suggest an inherent handedness to craniofacial development, and offer the zebrafish as a new model for studying the etiology of craniofacial asymmetry defects. Finally, we present the first molecular evidence that distinct mechanisms regulate development of the LR axis in the head and body. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, skeletal system, movement and support, molecular genetics, digestive system, ingestion and assimilation, endocrine system, chemical coordination and homeostasis.

Yeo, G.H., F.S.H. Cheah, E.W. Jabs, and S.S. Chong (2007). **Zebrafish twist1 is expressed in craniofacial, vertebral, and renal precursors**. *Development Genes and Evolution* 217(11-12): 783-789. ISSN: 0949-944X.
NAL Call Number: QL951.R68
Descriptors: zebrafish, twist1, craniofacial, vertebral, renal, qene structure, nucleotide.

Yergeau, D.A., C.N. Cornell, S.K. Parker, Y. Zhou, and H.W.I. Detrich (2005). **Bloodthirsty, an RBCC/TRIM gene required for erythropoiesis in zebrafish**. *Developmental Biology* 283(1): 97-112. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The Antarctic icefishes (family Channichthyidae, suborder Notothenioidei) constitute the only vertebrate taxon that fails to produce red blood cells. These fishes can be paired with closely related, but erythrocyte-producing, notothenioids to discover erythropoietic genes via representational difference analysis. Using a B30.2-domain-encoding DNA probe so derived from the hematopoietic kidney (pronephros) of a red-blooded Antarctic rockcod, *Notothenia coriiceps*, we discovered a related, novel gene, bloodthirsty (bty), that encoded a 547-residue protein that contains sequential RING finger, B Box, coiled-coil, and B30.2 domains. bty mRNA was expressed by the pronephric kidney of *N. coriiceps* at a steady-state level 10-fold greater than that found in the kidney of the icefish *Chaenocephalus aceratus*. To test the function of bty, we cloned the orthologous zebrafish gene from a kidney cDNA library. Whole-mount in situ hybridization of zebrafish embryos showed that bty mRNA was present throughout development and, after the mid-blastula transition, was expressed in the head and in or near the site of primitive erythropoiesis in the tail just prior to red cell production. One- to four-cell embryos injected with two distinct antisense morpholino oligonucleotides (MOs) targeted to the 5'-end of the bty mRNA failed to develop red cells, whereas embryos injected with 4- and 5-bp mismatch control MOs produced wild-type quantities of erythrocytes. The morphant phenotype was rescued by co-injection of synthetic bty mRNA containing an artificial 5'-untranslated region (UTR) with the antisense MO that bound the 5'-UTR of the wild-type bty transcript. Furthermore, the expression of genes that mark terminal erythroid differentiation was greatly reduced in the antisense-MO-treated embryos. We conclude that bty is likely to play a role in differentiation of the committed red cell progenitor. Copyright © 2008 CABI
Descriptors: zebrafish, complementary DNA, DNA cloning, embryonic development, embryos, erythropoiesis, gene expression, genes, messenger RNA, *Danio rerio.*

Yin, D., Y. Gu, Y. Li, X. Wang, and Q. Zhao (2006). **Pentachlorophenol treatment in vivo elevates point mutation rate in zebrafish p53 gene.** *Mutation Research* 609(1): 92-101. ISSN: 0027-5107.
NAL Call Number: QH431.M8
Abstract: Pentachlorophenol (PCP), a probable human carcinogen, has been heavily used

as an aseptic and a biocide throughout the world, and is widely present in the environment. Recent survey in Germany revealed that the average PCP amount in the urine of general German populations was 1.04 microg/L while the peak concentration could reach up to 19.1 microg/L. PCP was reported to cause DNA damage, but whether it can be involved in inducing point mutations in genome is unknown. To determine the genotoxicity of PCP on vertebrate, we first performed acute toxicity test on zebrafish for the effect of PCP exposure. The LC50 values of zebrafish exposed to PCP at 24, 48, 72 and 96 h were determined to be 0.196, 0.130, 0.130 and 0.130 mg/L, respectively. We then treated zebrafish with PCP for 10 days at 0 (control), 0.5, 5 and 50 microg/L, respectively, to determine whether PCP could be involved in inducing point mutations. Employing denaturing high-performance liquid chromatography analysis and DNA sequencing, we demonstrated that exposure of PCP to zebrafish at a concentration as low as 5 microg/L for 10 days elevates point mutation rate in p53 gene in liver cells. This is the first direct evidence revealing that PCP can elevate point mutation rate in the vertebrate genomes. The result implies PCP might be involved in carcinogenesis by elevating point mutation rate in the somatic genomes.
Descriptors: zebrafish, pentachlorophenol toxicity, point mutation drug effects, tumor suppressor protein p53 genetics, acute toxicity tests, base sequence, high pressure liquid chromatography methods, DNA chemistry, genetics and mutational analysis, drug dose response relationship, fish insecticides toxicity, LC50 liver drug effects and metabolism, molecular sequence data, point mutation genetics, single nucleotide polymorphism genetics, transition temperature.

Yin, V.P., J. Thomson, R. Thummel, D.R. Hyde, S.M. Hammond, and K.D. Poss (2008). **Fgf-dependent depletion of microRNA-133 promotes appendage regeneration in zebrafish**. *Genes & Development* 22(6): 728-733. ISSN: 0890-9369.
NAL Call Number: QH426.G46
Descriptors: zebrafish, regulation, miRNAs, fin regeneration, mir-133, fibroblast growth factor (FGF), receptor inhibition.

Ying, S.Y. and S.L. Lin (2005). **MicroRNA: fine-tunes the function of genes in zebrafish.** *Biochemical and Biophysical Research Communications* 335(1): 1-4. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: MicroRNAs (miRNAs), 18-25 nt single-stranded RNAs, act as regulators in fine-tuning gene function. The absence of miRNAs is not life-threatening in early embryonic development of Dicer-knockout zebrafish and mice, which may account for genetic expression of various traits as a result of miRNA complexity in higher animals during natural evolution. The Pol-II-mediated intronic miRNA is a useful tool to validate the function of computer-predicated miRNAs in zebrafish and mice.
Descriptors: zebrafish, microRNAs metabolism, proteins metabolism and genetics, evolution, introns genetics, microRNAs genetics.

Yoda, H., A. Momoi, C.V. Esguerra, D. Meyer, W. Driever, H. Kondoh, and M. Furutani Seiki (2003). **An expression pattern screen for genes involved in the induction of the posterior nervous system of zebrafish.** *Differentiation* 71(2): 152-62. ISSN: 0301 4681.
NAL Call Number: QH607.D5
Abstract: The posterior nervous system, including the hindbrain and the spinal cord, has been shown to be formed by the transformation of neural plate of anterior character by signals derived from non-axial mesoderm. Although secreted factors, such as fibroblast growth factors (FGFs), Wnts, retinoic acid (RA) and Nodal, have been proposed to be the

posteriorizing factors, the mechanism how neural tissue of posterior character is induced and subsequently specified along the anteroposterior axis remains elusive. To identify intercellular signaling molecules responsible for posteriorization of the neural plate as well as to find molecules induced intracellularly by the posteriorizing signal in the caudal neural plate, we screened by in situ hybridization for genes specifically expressed in posterior tissues, including the posterior neural plate and non-axial mesoderm when posteriorization of the neural plate takes place. From a subtracted library differentiating anterior versus posterior neural plate, 420 cDNA clones were tested, out of which 76 cDNA fragments showed expression restricted to the posterior tissue. These clones turned out to represent 32 different genes, including one novel secreted factor and one transmembrane protein. Seven genes were induced by non-axial mesodermal implants and bFGF beads, suggesting that these are among the early-response genes of the posteriorizing signal. Thus, our approach employing cDNA subtraction and subsequent expression pattern screening allows us to clone candidate genes involved in a novel signaling pathway contributing to the formation of the posterior nervous system.
Descriptors: zebrafish, embryonic induction physiology, gene expression profiling, nervous system embryology, body patterning physiology, gene library.

Yoder, J.A., T.M. Orcutt, D. Traver, and G.W. Litman (2007). **Structural characteristics of zebrafish orthologs of adaptor molecules that associate with transmembrane immune receptors.** *Gene* 401(1-2): 154-64. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: Transmembrane bound receptors comprised of extracellular immunoglobulin (Ig) or lectin domains play integral roles in a large number of immune functions including inhibitory and activating responses. The function of many of the activating receptors requires a physical interaction with an adaptor protein possessing a cytoplasmic regulatory motif. The partnering of an activating receptor with an adaptor protein relies on complementary charged residues in the two transmembrane domains. The mammalian natural killer (NK) and Fc receptors (FcR) represent two of many receptor families, which possess activating receptors that partner with adaptor proteins for signaling. Zebrafish represent a powerful experimental model for understanding developmental regulation at early stages of embryogenesis and for efficiently generating transgenic animals. In an effort to understand developmental aspects of immune receptor function, we have accessed the partially annotated zebrafish genome to identify six different adaptor molecules: Dap10, Dap12, Cd3zeta, Cd3zeta-like, FcRgamma and FcRgamma-like that are homologous to those effecting immune function in mammals. Their genomic organizations have been characterized, cDNA transcripts have been recovered, phylogenetic relationships have been defined and their cell lineage-specific expression patterns have been established.
Descriptors: zebrafish, signal transducing adaptor proteins chemistry and metabolism, immunologic receptors chemistry and metabolism, signal transducing adaptor amino acid sequence, cD3 antigens genetics and metabolism, cell lineage, cell membrane immunology and metabolism, molecular cloning, complementary DNA developmental gene expression regulation, genome, natural killer cells immunology, molecular sequence data, phylogeny, tertiary protein structure, IGG receptors immunology, immunologic receptors genetics, amino acid sequence homology.

Yoo, K.W., C.H. Kim, H.C. Park, S.H. Kim, H.S. Kim, S.K. Hong, S. Han, M. Rhee, and T.L. Huh (2003). **Characterization and expression of a presomitic mesoderm-specific mespo gene in zebrafish.** *Development Genes and Evolution* 213(4): 203-6. ISSN: 0949 944X.

NAL Call Number: QL951.R68
Abstract: A complete zebrafish mespo cDNA encoding a protein of 131 amino acids with a bHLH domain in the C-terminal has been isolated. The bHLH domain of zebrafish Mespo is highly similar to those in the mouse, chick and *Xenopus*, sharing 82.4%, 80.4% and 74.5% amino acid identity, respectively. At 50% epiboly, the zebrafish mespo is first detected in the marginal zone of the blastoderm but excluding the prospective shield. Subsequently, mespo expression is intensified in the involuting mesoderm at 60% epiboly, and then restricted to the presomitic mesoderm (PSM) at 95% epiboly. At the 1-somite stage, mespo expression becomes reduced in the most rostral PSM. During segmentation, mespo expression is gradually downregulated at the most rostral segmental plate where cells are being coalesced to form somites. In spadetail mutant embryos, most of mespo-expressing cells were missing.
Descriptors: zebrafish, developmental gene expression regulation, mesoderm physiology, transcription factors genetics, amino acid sequence, basic helix loop helix transcription factors extremities embryology, helix loop helix motifs, mesoderm cytology, molecular sequence data, mutation, amino acid sequence homology, T-box domain trans activators genetics, transcription factors metabolism, *Xenopus* proteins genetics.

Yoo, K.W., E.H. Kim, S.H. Jung, M. Rhee, B.K. Koo, K.J. Yoon, Y.Y. Kong, and C.H. Kim (2006). **Snx5, as a Mind bomb-binding protein, is expressed in hematopoietic and endothelial precursor cells in zebrafish.** *Federation of European Biochemical Societies Journal* 580(18): 4409-16. ISSN: 1742 464X.
NAL Call Number: QP501.E8
Abstract: Notch signaling has an evolutionarily conserved function for cell fate determination and stem cell maintenance. Previously, we identified a novel component of the Notch signaling pathway in zebrafish, mind bomb, which encodes an E3 ubiquitin ligase essential for Notch signal activation. Further studies showed that Mind bomb(-/-) mouse embryos exhibited pan-Notch phenotypes in various tissues, suggesting that Mind bomb function is conserved in mammals. Therefore we sought to understand the various molecular partners of Mind bomb using yeast two-hybrid screening. In this search we identified Sorting nexin 5 (Snx5) as a novel interacting partner of Mind bomb. Furthermore we demonstrated that Snx5 colocalizes with Mind bomb in early endosomal compartments, suggesting that Snx5 is important for Mind bomb trafficking. In addition, we identified zebrafish orthologue of Snx5 and showed that snx5 is predominantly expressed in hematopoietic and endothelial precursor cells in zebrafish. We also found defects in hematopoiesis and blood vessel development in snx5 morpholino-injected embryos. Taken together, we show that Snx5, a novel interacting partner of Mind bomb, may have an essential role for cell fate determination in early development.
Descriptors: zebrafish, carrier proteins metabolism, endothelial cells metabolism, hematopoietic stem cells metabolism, ubiquitin protein ligases metabolism, proteins metabolism, carrier proteins antagonists and inhibitors, carrier proteins chemistry, endothelial cells cytology, immunoprecipitation, mice, physiologic neovascularization, stem cells metabolism, two hybrid system techniques, ubiquitin protein ligases chemistry, vesicular transport proteins, proteins antagonists, inhibitors and chemistry.

Yoong, S., B. O'Connell, A. Soanes, M.O. Crowhurst, G.J. Lieschke, and A.C. Ward (2007). **Characterization of the zebrafish matrix metalloproteinase 9 gene and its developmental expression pattern.** *Gene Expression Patterns* 7(1-2): 39-46. ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: Members of the matrix metalloproteinase (MMP) family are important for the

remodeling of the extracellular matrix in a number of biological processes including a variety of immune responses. Two members of the family, MMP2 and MMP9, are highly expressed in specific myeloid cell populations in which they play a role in the innate immune response. To further expand the repertoire of molecular reagents available to study zebrafish myeloid cell development, the matrix metalloproteinase 9 (mmp9) gene from this organism has been identified and characterized. The encoded protein is 680 amino acids with high homology to known MMP9 proteins, particularly those of other teleost fish. Maternal transcripts of mmp9 are deposited in the oocyte and dispersed throughout the early embryo. These are replaced by specific zygotic transcripts in the notochord from 12h post fertilization (hpf) and also transiently in the anterior mesoderm from 14 to 16h post fertilization. From 24h post fertilization, mmp9 expression was detected in a population of circulating white blood cells that are distinct from macrophages, and which migrate to the site of trauma, and so likely represent zebrafish heterophils. In the adult, mmp9 expression was most prominent in the splenic cords, a site occupied by mature myeloid cells in other species. These results suggest that mmp9 will serve as a useful marker of mature myeloid cells in the zebrafish.
Descriptors: zebrafish, matrix metalloproteinase 9 genetics, amino acid and base sequence, molecular cloning, DNA genetics, developmental gene expression regulation, enzymologic gene expression regulation, in situ hybridization, molecular sequence data, myelopoiesis genetics, phylogeny, reverse transcriptase PCR.

Yoshida, T. and M. Mishina (2003). **Neuron-specific gene manipulations to transparent zebrafish embryos.** *Methods in Cell Science* 25(1-2): 15-23. ISSN: 1381-5741.
NAL Call Number: QH573.T52
Abstract: To investigate the molecular basis of neural network formation, we introduced a novel double-cassette vector approach for visualizing and manipulating neuronal development in living zebrafish embryos. Two genes are physically linked in the double-cassette vector system, which ensures co-expression of an effector-protein and an EGFP-reporter in the same neuron. By generating transgenic enhanced green fluorescent protein (EGFP) expressing zebrafish lines, we first established that EGFP under control of either the olfactory marker protein (OMP) gene promoter or the nicotinic acetylcholine receptor beta3 (nAChR-beta3) gene promoter, directed strong EGFP expression to the olfactory sensory neurons and the retinal ganglion cells (RGCs), respectively. These transgenic lines allowed the visualization of the development of the entire olfactory sensory neurons and RGCs in vivo. By injection of vectors with EGFP under control of either the OMP or the nAChRbeta3 gene promoter, we followed the development of individual olfactory sensory neurons and RGCs. The double-cassette expression vector strategy enabled us to clarify the roles of protein kinase A (PKA) and glycogen synthase kinase-3beta (GSK-3beta) in the development of olfactory sensory neurons and RGCs. The combination of visualization and neuron-specific gene manipulation provides a powerful reverse genetic in vivo approach for the study of genes of interest in neural differentiation, axonal pathfinding, and synaptogenesis.
Descriptors: zebrafish, cyclic AMP dependent protein kinases metabolism, glycogen synthase kinase 3 metabolism, afferent neurons enzymology, olfactory pathways enzymology , retinal ganglion cells enzymology, molecular cloning, anatomy and histology, green fluorescent, luminescent proteins metabolism, nerve tissue afferent neurons cytology, olfactory marker protein pathways cytology, promoter regions genetics, nicotinic receptors genetics, retinal ganglion cells cytology.

Yoshimura, Y., M. Tani, N. Okino, H. Iida, and M. Ito (2004). **Molecular cloning and functional analysis of zebrafish neutral ceramidase.** *Journal of Biological Chemistry* 279(42): 44012-22. ISSN: 0021-9258.

NAL Call Number: 381J824

Abstract: Almost all observations on the functions of neutral ceramidase have been carried out at cellular levels but not at an individual level. Here, we report the molecular cloning of zebrafish neutral ceramidase (znCD) and its functional analysis during embryogenesis. We isolated a cDNA clone encoding znCD by 5' and 3' rapid amplification of cDNA ends-PCR. It possessed an open reading frame of 2,229 base pairs encoding 743 amino acids. A possible signal/anchor sequence near the N terminus and four potential O-glycosylation and eight potential N-glycosylation sites were found in the putative sequence. The enzyme activity at neutral pH increased markedly after transformation of Chinese hamster CHOP and zebrafish BRF41 cells with the cDNA. The overexpressed enzyme was found to be distributed in endoplasmic reticulum/Golgi compartments as well as the plasma membranes. The antisense morpholino oligonucleotide (AMO), which was designed based on the sequence of znCD mRNA, successfully blocked the translation of znCD in a wheat germ in vitro translation system. The knockdown of znCD with AMO led to an increase in the number of zebrafish embryos with severe morphological and cellular abnormalities such as abnormal morphogenesis in the head and tail, pericardiac edema, defect of blood cell circulation, and an increase of apoptotic cells, especially in the head and neural tube regions, at 36 h post-fertilization. The ceramide level in AMO-injected embryos increased significantly compared with that in control embryos. Simultaneous injection of both AMO and synthetic znCD mRNA into one-cell-stage embryos rescued znCD activity and blood cell circulation. These results indicate that znCD is essential for the metabolism of ceramide and the early development of zebrafish.

Descriptors: zebrafish, amidohydrolases genetics, metabolism and chemistry, amino acid and base sequence, CHO cells, cell line, molecular cloning, cricetinae, DNA primers, molecular sequence data, recombinant proteins metabolism, sequence alignment, amino acid sequence homology.

Young, S.R., C. Mumaw, J.A. Marrs, and D.G. Skalnik (2006). **Antisense targeting of CXXC finger protein 1 inhibits genomic cytosine methylation and primitive hematopoiesis in zebrafish.** *Journal of Biological Chemistry* 281(48): 37034-44. ISSN: 0021-9258.

NAL Call Number: 381J824

Abstract: CXXC finger protein 1 (CFP1) binds to unmethylated CpG dinucleotides and is a component of the Set1 histone methyltransferase complex. Mice lacking CFP1 suffer a peri-implantation lethal phenotype, and CFP1-deficient embryonic stem cells are viable but unable to differentiate and exhibit a 60-80% decrease in genomic cytosine methylation. A zebrafish homolog of CFP1 has been identified, is approximately 70% similar to murine CFP1, and is widely expressed during development. Zebrafish embryos treated with a zCFP1 antisense morpholino oligonucleotide had little or no circulating red blood cells and exhibited abnormal yolk sac morphology at 48 h post-fertilization. Many of the antisense-treated zebrafish also exhibited cardiac edema, and 14% were dead at 24 h post-fertilization. Morphant zebrafish also exhibited elevated levels of apoptosis, particularly in the intermediate cell mass, the site of primitive erythropoiesis, as well as aberrations in vascular development. Genomic DNA isolated from morphant embryos exhibited a 60% reduction of global genomic cytosine methylation. A similar phenotype was observed with an independent zCFP1 antisense morpholino oligonucleotide, but not following injection of an unrelated control oligonucleotide. The morphant phenotype was rescued when mRNA

encoding murine CFP1 was co-injected with the antisense oligonucleotide. Genomic data base analysis reveals the presence of a second version of zebrafish CFP1 (zCFP1b). However, the morphant phenotype observed following specific depletion of zCFP1 indicates that these related genes have nonredundant functions controlling normal zebrafish hematopoiesis and epigenetic regulation. These findings establish the importance of CFP1 during postgastrulation development.

Descriptors: zebrafish, DNA methylation, DNA binding proteins genetics, hematopoiesis physiology, antisense oligonucleotides pharmacology, trans activators genetics and pharmacology and pharmacology, amino acid motifs and sequence, apoptosis, cytosine, DNA binding, drug dose response relationship, epigenesis, genetic, developmental gene expression regulation, molecular sequence data, oligonucleotides chemistry.

Yu, D., P. Zhang, and X. Zhan (2005). **Cortactin mediated morphogenic cell movements during zebrafish *(Danio rerio*) gastrulation.** *Science in China. Series C, Life Sciences* 48(6): 601-07. ISSN: 1006-9305.

Abstract: Cell migration is essential to direct embryonic cells to specific sites at which their developmental fates are ultimately determined. However, the mechanism by which cell motility is regulated in embryonic development is largely unknown. Cortactin, a filamentous actin binding protein, is an activator of Arp2/3 complex in the nucleation of actin cytoskeleton at the cell leading edge and acts directly on the machinery of cell motility. To determine whether cortactin and Arp2/3 mediated actin assembly plays a role in the morphogenic cell movements during the early development of zebrafish, we initiated a study of cortactin expression in zebrafish embryos at gastrulating stages when massive cell migrations occur. Western blot analysis using a cortactin specific monoclonal antibody demonstrated that cortactin protein is abundantly present in embryos at the most early developmental stages. Immunostaining of whole-mounted embryo showed that cortactin immunoreactivity was associated with the embryonic shield, predominantly at the dorsal side of the embryos during gastrulation. In addition, cortactin was detected in the convergent cells of the epiblast and hypoblast, and later in the central nervous system. Immunofluorescent staining with cortactin and Arp3 antibodies also revealed that cortactin and Arp2/3 complex colocalized at the periphery and many patches associated with the cell-to-cell junction in motile embryonic cells. Therefore, our data suggest that cortactin and Arp2/3 mediated actin polymerization is implicated in the cell movement during gastrulation and perhaps the development of the central neural system as well.

Descriptors: zebrafish, cell movement, cortactin biosynthesis, genetics and physiology, developmental gene expression regulation, Western blotting methods, central nervous system embryology, gastrula cytology and physiology, sequence homology, amino acid.

Yu, H.H., C. Houart, and C.B. Moens (2004). **Cloning and embryonic expression of zebrafish neuropilin genes.** *Gene Expression Patterns* 4(4): 371-8. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Neuropilin (Nrp), a cell surface receptor for class 3 semaphorins and for certain heparin forms of vascular endothelial growth factors, functions in many biological processes including axon guidance, neural cell migration and angiogenesis in the development of the nervous system and the cardiovascular system. To understand the role of neuropilins in zebrafish embryogenesis, we have cloned three zebrafish neuropilin homologues, nrp1b, nrp2a and nrp2b. Based on synteny, zebrafish nrp1b and the previously cloned nrp1a are orthologous to human nrp1, and zebrafish nrp2a and 2b orthologous to human nrp2. We have characterized the expression patterns of these four zebrafish neuropilin genes in wild

type embryos from the beginning of somitogenesis to 48 h post-fertilization. Zebrafish nrp1a is expressed in the neural tube including telencephalon, epithalamus, cells along the axonal trajectory of the posterior commissure and the medial longitudinal fascicle, hindbrain neurons, vagus motor neurons and spinal motoneurons. Zebrafish nrp1b is expressed in the nose, the cranial neural crest cell (NCC) derived tissue underlying the hypothalamus, endothelial precursors and the trunk and tail vasculature. Zebrafish nrp2a is expressed in telencephalon, anterior pituitary, oculomotor and trochlear motor neurons, cells along the supra-optic and posterior commissures, hindbrain rhombomere 1, hindbrain neurons, cranial NCCs and sclerotome. Zebrafish nrp2b is expressed in telencephalon, thalamus, hypothalamus, epiphysis, cells along the anterior and posterior commissures, post-optic and supra-optic commissures and the olfactory axonal trajectory, hindbrain neurons, cranial NCCs, somites and spinal cord neurons.
Descriptors: zebrafish, developmental gene expression regulation physiology, neuropilins genetics and biosynthesis, base sequence, molecular cloning, developmental gene expression regulation genetics, molecular sequence data, organ specificity genetics and physiology, tertiary protein structure genetics and physiology.

Zhang, D.C., Y.Q. Shao, Y.Q. Huang, and S.G. Jiang (2005). **Cloning, characterization and expression analysis of interleukin-10 from the zebrafish (*Danio rerio*).** *Journal of Biochemistry and Molecular Biology* 38(5): 571-76. ISSN: 1225-8687.
Abstract: Cytokines are proteins produced by many different cells of the immune system and play a significant role in initiating and regulating the inflammatory process. In this research, an important cytokine, interleukin-10 (IL-10) gene, has been identified and characterized from zebrafish (*Danio rerio*) genome database. Zebrafish IL-10 is located within a 2690 bp fragment and contains five exons and four introns, sharing the same organization with mammalian IL-10 genes. An open reading frame of 543 bp was found to encode a putative 180 amino acid protein with a signal peptide of 22 amino acids, which shares 29.7-80.9 % homology with amino acid sequences of other known IL-10. The signature motif of IL-10 is also conserved in zebrafish IL-10. The predicted transcript was finally confirmed by sequencing of cDNA clones. Multi-tissue reverse transcriptase PCR (RT-PCR) was performed to examine the tissue distribution and expression regulation of this gene in seven organs of normal and lipopolysaccharide (LPS) stimulation zebrafish. The results demonstrated that this gene was expressed slightly in normal kidney, gill and gut, no expression was detected in other four tissues. The expression was clearly upregulated after LPS stimulation. Using the ideal zebrafish model, further study of IL-10 characterization and function may provide insight on the understanding of the innate immune system.
Descriptors: zebrafish, interleukin 10 genetics and metabolism, amino acid and base sequence, molecular cloning, interleukin 10 classification, lipopolysaccharides metabolism, molecular sequence data, phylogeny, sequence alignment, tissue distribution, proteins classification.

Zhang, J., S. Bai, C. Tanase, H. Nagase, and M.P.J. Sarras (2003). **The expression of tissue inhibitor of metalloproteinase 2 (TIMP-2) is required for normal development of zebrafish embryos.** *Development Genes and Evolution* 213(8): 382-9. ISSN: 0949 944X.
NAL Call Number: QL951.R68
Abstract: MMP activities are controlled by a combination of proteolytic pro-enzyme activation steps and inhibition by endogenous inhibitors like alpha2-macroglobulin and the tissue inhibitors of metalloproteinases (TIMPs). TIMPs are the key inhibitors in tissue. The expression of both MMPs and TIMPs is controlled during tissue remodeling to maintain

a balance in the turnover of extracellular matrix. Disruption of this balance may result in a broad spectrum of diseases. Additionally, TIMP-2 has been reported to have growth factor activities. To further study the function of TIMP-2 in development, we utilized zebrafish as an experimental model system. We have successfully isolated a TIMP-2 homologue from zebrafish (zTIMP-2). This zebrafish TIMP-2 showed high similarity to human TIMP-2 with all critical features conserved. Whole-mount in situ analysis showed that zTIMP-2 was expressed as early as the one-cell stage indicating a maternal origin. This expression continued through later stages of development. RT-PCR analysis confirmed the early expression pattern from the 16-cell stage through blastula, gastrula and 24-h stages. In addition, at the protein level, immunoreactive zTIMP-2 was detected using antibody against recombinant human TIMP-2. RFP-reporter analysis indicated that TIMP-2 can be secreted into the extracellular space where ECM is forming. Functional studies showed that the balance of TIMP-2 expression is important to normal development as reflected by the fact that both blockage of TIMP-2 translation using antisense morpholino oligonculeotides or increased translation of TIMP-2 using a mRNA microinjection approach resulted in abnormal zebrafish development. This is in contrast to murine knockout studies that indicate that TIMP-2 does not have a major role in mouse embryogenesis.

Descriptors: zebrafish embryos, tissue inhibitor of metalloproteinase 2 biosynthesis, amino acid sequence, molecular cloning, physiology, models, molecular sequence data, sequence alignment, tissue inhibitor of metalloproteinase 2 isolation and purification.

Zhang, J., S. Bai, X. Zhang, H. Nagase, and M.P.J. Sarras (2003). **The expression of gelatinase A (MMP-2) is required for normal development of zebrafish embryos.** *Development Genes and Evolution* 213(9): 456-63. ISSN: 0949 944X.

NAL Call Number: QL951.R68

Abstract: Gelatinase A, also called matrix metalloproteinase 2 (MMP-2), belongs to the matrix metalloproteinase (MMP) family. MMP-2 cleaves type IV collagen, denatured collagen (gelatin), and other extracellular matrix (ECM) components. MMP-2 has been reported to be involved in a number of biological and pathological processes, but previous studies have not indicated that its expression is essential for early embryogenesis. In the current study, we have utilized zebrafish as a developmental model to study the role of MMP-2 during embryogenesis. We have successfully isolated a zebrafish MMP-2 (zMMP-2) homologue showing over 80% identity and over 90% similarity to its human counterpart. In situ analysis showed that zMMP-2 was expressed as early as the one-cell stage implying a maternal origin during oogenesis, and embryos continued to express zMMP-2 through at least the 72-h stage of development. RT-PCR analysis confirmed the in situ expression pattern and gelatin zymography indicated that a metalloproteinase with the same gel mobility as vertebrate MMP-2 was present in zebrafish embryos. Injection of zMMP-2 antisense morpholino oligonucleotides into 1- to 4-cell embryos resulted in a truncated axis, monitored through 72 h of development indicating that this metalloproteinase plays an important role in zebrafish embryogenesis. Monpholino-induced alterations in development began to be observed at 12 h of embryogenesis based on morphological and axis marker studies. The results obtained in zebrafish are in contrast to murine knockout studies that indicate that MMP-2 does not have a major role in mouse embryogenesis.

Descriptors: zebrafish, matrix metalloproteinase 2 genetics, amino acid sequence, enzymology, matrix metalloproteinase 2 biosynthesis, molecular sequence data, messenger RNA metabolism.

Zhang, J., S. Bai, X. Zhang, H. Nagase, and M.P.J. Sarras (2003). **The expression of novel membrane-type matrix metalloproteinase isoforms is required for normal development of zebrafish embryos.** *Matrix Biology* 22(3): 279-93. ISSN: 0945-053X.

NAL Call Number: QP552.C6

Abstract: Matrix metalloproteinases (MMPs) play important roles in the turnover of components of extracellular matrix (ECM) and in the processing of active and latent-signaling molecules bound to the ECM or associated with the cell surface. Through such actions, MMPs regulate a variety of cellular and developmental processes. Membrane-type matrix metalloproteinases (MT-MMPs) are of particular importance because they function in the immediate pericellular environment that modulates both cell-cell and cell-ECM interactions. In this study, we utilized zebrafish as a developmental model to study the role of MT-MMPs during early embryogenesis. We successfully isolated two isoforms of a MT-MMP homologue that are structurally similar to MT1-MMP. They have been named zebrafish MT-MMPalpha and beta. Zebrafish MT-MMPbeta is unique among vertebrate MT-MMPs in that it contains an Arg-Glu-Asp (RED) multiple-repeat motif in its linker region. Whole mount in situ analysis, RT-PCR, immunofluorescence, reporter analysis, Western blot analysis, and zymography indicated that MT-MMPalpha and beta were expressed through at least the first 72 h of development and that this expression was targeted to the cell surface. Functional studies using injection of either mRNA or morpholino antisense oligonucleotides resulted in a truncation of the cranial to caudal axis as monitored through 72 h post fertilization, indicating that zebrafish MT-MMPalpha and beta had an important role in embryonic development. Axis markers indicated that these effects likely involved processes occurring later than 10 h of embryogenesis.

Descriptors: zebrafish embryo development, metalloendopeptidases metabolism, amino acid and base sequence, cell membrane enzymology, complementary DNA genetics, developmental gene expression regulation, enzymologic gene expression regulation, reporter genes, in situ hybridization, isoenzymes genetics and metabolism, matrix metalloproteinases, membrane associated, metalloendopeptidases genetics, molecular sequence data, amino acid sequence homology, species specificity.

Zhang, L., H. Zhou, Y. Su, Z. Sun, H. Zhang, L. Zhang, Y. Zhang, Y. Ning, Y.G. Chen, and A. Meng (2004). **Zebrafish Dpr2 inhibits mesoderm induction by promoting degradation of nodal receptors.** *Science* 306(5693): 114-7. ISSN: 0036-8075.

NAL Call Number: 470Sci2

Abstract: Nodal proteins, members of the transforming growth factor-beta (TGFbeta) superfamily, have been identified as key endogenous mesoderm inducers in vertebrates. Precise control of Nodal signaling is essential for normal development of embryos. Here, we report that zebrafish dapper2 (dpr2) is expressed in mesoderm precursors during early embryogenesis and is positively regulated by Nodal signals. In vivo functional studies in zebrafish suggest that Dpr2 suppresses mesoderm induction activities of Nodal signaling. Dpr2 is localized in late endosomes, binds to the TGFbeta receptors ALK5 and ALK4, and accelerates lysosomal degradation of these receptors.

Descriptors: zebrafish, Type I activin receptors and metabolism, embryonic induction, mesoderm physiology, transforming growth factor beta receptors metabolism, proteins metabolism, amino acid sequence, cell line, embryology, endosomes metabolism, fluorescent antibody technique, gene expression profiling, developmental gene expression regulation, in situ hybridization, intracellular signaling peptides and proteins, lysosomes metabolism, molecular sequence data, mutation, antisense oligonucleotides, signal transduction, transforming growth factor beta genetics and metabolism.

Zhang, L.F., Y.H. Gui, T. Zhong, Y.X. Wang, L.X. Qian, Y.X. Dong, Q. Jiang, S.N. Sun, and H.Y. Song (2007). **Effect of external retinoic acid on Tbx1 gene during zebrafish embryogenesis.** *Chinese Journal of Pediatrics* 45(4): 267-71. ISSN: 1008-8830.

Abstract: OBJECTIVE: DiGeorge/del22q11 syndrome is one of the most common genetic causes of outflow tract and aortic arch defects in human. DiGeorge/del22q11 is thought to involve an embryonic defect restricted to the pharyngeal arches and the corresponding pharyngeal pouches. Previous studies have evidenced that retinoic acid (RA) signaling is definitely indispensable for the development of the pharyngeal arches. Tbx1, one of the T-box containing genes, is proved to be the most attractive candidate gene for DiGeorge/del22q11 syndrome. However, the interaction between RA and Tbx1 has not been fully investigated. Exploring the interaction will contribute to discover the molecular pathways disrupted in DiGeorge/del22q11 syndrome, and will also be essential for understanding genetic basis for congenital heart disease. It now seems possible that genes and molecular pathways disrupted in DiGeorge syndrome will also account for some isolated cases of congenital heart disease. Accordingly, the present study aimed to extensively study the effects of external RA on the cardiac development and Tbx1 expression during zebrafish embryogenesis. METHODS: The chemical genetics approach was applied by treating zebrafish embryos with 5 x 10(-8) mol/L RA and 10(-7) mol/L RA at 12.5 hour post fertilization (hpf). The expression patterns of Tbx1 were monitored by whole-mount in situ hybridization and quantitative real-time RT-PCR, respectively. RESULTS: The zebrafish embryos treated with 5 x 10(-8) mol/L RA and 10(-7) mol/L RA for 1.5 h at 12.5 hpf exhibited selective defects of abnormal heart tube. The results of whole-mount in situ hybridization with Tbx1 RNA probe showed that Tbx1 was expressed in cardiac region, pharyngeal arches and otic vesicle during zebrafish embryogenesis. RA treatment led to a distinct spatio-temporal expression pattern for Tbx1 from that in wild type embryo. The real-time PCR analysis showed that Tbx1 expression levels were markedly reduced by RA treatment. Tbx1 expression in the pharyngeal arches and heart were obviously down regulated compared to the wild type embryos. In contrast to 5 x 10(-8) mol/L RA-treated groups, 10(-7) mol/L RA caused a more severe effect on the Tbx1 expression level. CONCLUSION: These results suggested that there was a genetic link between RA and Tbx1 during development of zebrafish embryo. RA could produce an altered Tbx1 expression pattern in zebrafish. RA may regulate the Tbx1 expression in a dose-dependant manner. RA could represent a major epigenetic factor to cause abnormal expression of Tbx1, secondarily, disrupt the pharyngeal arch and heart development.

Descriptors: zebrafish embryos, retinoic acid, genetics, aortic arch defects, pharyngeal arch.

Language of Text: Chinese.

Zhang, S.H., J.H. Yao, H.D. Song, L. Wang, and J.L. Xue (2006). **Cloning, expression and functional study of translation elongation factor 2 (EF-2) in zebrafish.** *International Journal of Developmental Biology, The* 50(4): 399-403. ISSN: 0214-6282.

Abstract: We have identified translation elongation factor 2 (EF-2) in zebrafish (GenBank Accession No. AAQ91234). Analysis of the DNA sequence of zebrafish EF-2 shows that the 2826 bp cDNA spans an open reading frame between nucleotide 55 to 2631 and encodes a protein of 858 amino acids. Zebrafish EF-2 protein shares 92%, 93%, 93% and 92% identity with the corresponding amino acid sequence in human, mouse, Chinese hamster and Gallus EF-2, respectively. Whole-mount in situ hybridization showed that zebrafish EF-2 was a developmentally regulated gene and might play important roles during the early development of zebrafish embryos. Therefore, we further studied the function of EF-2 during early embryogenesis. Using morpholino antisense oligo knockdown assays, anti-MO injected embryos were found to display abnormal development. The yolk balls were larger than

normal and the melanophores spreading on their bodies became fewer. Furthermore, their tails were incurvate and their lenses were much smaller than those of the normal embryos. However the EF-2 overexpression data showed that extra EF-2 protein had no obvious effect on zebrafish embryonic development.

Descriptors: zebrafish, developmental gene expression regulation physiology, peptide elongation factor 2 biosynthesis and genetics, amino acid sequence, molecular cloning, in situ hybridization, molecular sequence data, oligodeoxyribonucleotides pharmacology, antisense oligonucleotides pharmacology, peptide elongation factor 2 deficiency and physiology.

Zhang, Y.T., A.D. Lander, and Q. Nie (2007). **Computational analysis of BMP gradients in dorsal-ventral patterning of the zebrafish embryo.** *Journal of Theoretical Biology* 248(4): 579-89. ISSN: 0022-5193.

NAL Call Number: 442.8J8223

Abstract: The genetic network controlling early dorsal-ventral (DV) patterning has been extensively studied and modeled in the fruit fly Drosophila. This patterning is driven by signals coming from bone morphogenetic proteins (BMPs), and regulated by interactions of BMPs with secreted factors such as the antagonist short gastrulation (Sog). Experimental studies suggest that the DV patterning of vertebrates is controlled by a similar network of BMPs and antagonists (such as Chordin, a homologue of Sog), but differences exist in how the two systems are organized, and a quantitative comparison of pattern formation in them has not been made. Here, we develop a computational model in three dimensions of the zebrafish embryo and use it to study molecular interactions in the formation of BMP morphogen gradients in early DV patterning. Simulation results are presented on the dynamics BMP gradient formation, the cooperative action of two feedback loops from BMP signaling to BMP and Chordin synthesis, and pattern sensitivity with respect to BMP and Chordin dosage. Computational analysis shows that, unlike the case in Drosophila, synergy of the two feedback loops in the zygotic control of BMP and Chordin expression, along with early initiation of localized Chordin expression, is critical for establishment and maintenance of a stable and appropriate BMP gradient in the zebrafish embryo.

Descriptors: zebrafish embryos, body patterning physiology, bone morphogenetic proteins metabolism, bone morphogenetic genetics and physiology, computational biology methods, metabolism, developmental gene expression regulation, glycoglycoproteins metabolism and genetics, intercellular signaling peptides genetics and metabolism, morphogenesis physiology, nucleolus organizer region ultrastructure.

Zhang, Y., J.R. Yeh, A. Mara, R. Ju, J.F. Hines, P. Cirone, H.L. Griesbach, I. Schneider, D.C. Slusarski, S.A. Holley, and C.M. Crews (2006). **A chemical and genetic approach to the mode of action of fumagillin**. *Chemistry and Biology* 13(9): 1001-1009. ISSN: 1074 5521.

NAL Call Number: QP501

Abstract: Previous mode of action studies identified methionine aminopeptidase 2 (MetAP-2) as the target of the antiangiogenic natural product fumagillin and its drug candidate analog, TNP-470. We report here that TNP-470-mediated MetAP-2 inhibition blocks noncanonical Wnt signaling, which plays a critical role in development, cell differentiation, and tumorigenesis. Consistent with this finding, antisense MetAP-2 morpholino oligonucleotide injection in zebrafish embryos phenocopies gastrulation defects seen in noncanonical Wnt5 loss-of-function zebrafish mutants. MetAP-2 inhibition or depletion blocks signaling downstream of the Wnt receptor Frizzled, but upstream of Calmodulin-dependent Kinase II, RhoA, and c-Jun N-terminal Kinase. Moreover, we demonstrate that TNP-470 does not block the canonical Wnt/beta-catenin pathway. Thus, TNP-470 selectively regulates nonca-

nonical over canonical Wnt signaling and provides a unique means to explore and dissect the biological systems mediated by these pathways. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, pharmacology, molecular genetics, enzymology, biochemistry, cell differentiation.

Zhao, C., X. He, C. Tian, and A. Meng (2006). **Two GC-rich boxes in huC promoter play distinct roles in controlling its neuronal specific expression in zebrafish embryos.** *Biochemical and Biophysical Research Communications* 342(1): 214-20. ISSN: 0006 291X.
NAL Call Number: 442.8B5236
Abstract: HuC, a vertebrate ortholog of *Drosophila elav* gene, encodes an RNA binding protein and is involved in early neurogenesis. Zebrafish huC is expressed in distinct neurons, including Rohon-Beard (RB) sensory neurons, interneurons and motoneurons, during primary neurogenesis, and in all neurons later during secondary neurogenesis. In this study, we identify two GC-rich box elements, proximal GC (p-GC) box from -172 to -149 and distal GC (d-GC) box from -218 to -208, in zebrafish huC promoter. Using transgenic approach, we demonstrate that deletion of the p-GC box from the promoter results in loss of expression of the reporter GFP in neurons while deletion of the d-GC box leads to GFP expression only in dorsal RB sensory neurons. These results suggest that the p-GC box alone confers transcriptional activity of huC promoter in primary RB neurons and the d-GC is required for huC transcription in the full spectrum of spinal cord neurons. Further studies are needed to identify specific Sp1-like transcription factors that bind to these GC boxes and activate huC transcription.
Descriptors: zebrafish, GC rich sequence genetics, hu paraneoplastic encephalomyelitis antigens genetics and metabolism, neurons metabolism, promoter regions genetics, genetically modified, embryology, developmental gene expression regulation, reporter molecular sequence data, organ specificity, sp1 transcription factor metabolism.

Zhao, J., Y. Cao, C. Zhao, J. Postlethwait, and A. Meng (2003). **An sp1-like transcription factor spr2 acts downstream of fgf signaling to mediate mesoderm induction.** *European Molecular Biology Organization Journal* 22(22): 6078-6088. ISSN: 0261 4189.
NAL Call Number: QH506.E46
Descriptors: zebrafish, development, molecular genetics: biochemistry and molecular biophysics, gene knockout, genetic techniques, laboratory techniques, early gastrulation, embryogenesis, immediate early response, late blastulation.

Zhao, Q., B. Dobbs McAuliffe, and E. Linney (2005). **Expression of cyp26b1 during zebrafish early development.** *Gene Expression Patterns* 5(3): 363-9 . ISSN: 1567-133X.
NAL Call Number: QH450.B73
Abstract: We have cloned the zebrafish ortholog of the mammalian cyp26b1 gene. The predicted zebrafish cyp26b1 protein shares greater than 73% identity with mammalian homologues. cDNA transfection assays showed that like human cyp26b1, zebrafish cyp26b1 is involved in limiting the activity of retinoic acid. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of embryonic RNAs suggested that no maternal cyp26b1 message is detectable. Zygotic cyp26b1 message could be detected at 75% epiboly by RT-PCR and localized to presumptive rhombomere 3 and rhombomere 4 at the early two-somite (2S) stage (10.5 hpf: hour post fertilization) by whole mount in situ hybridization. As development proceeds expression expands anteriorly to include rhombomere 2 at the 10S stage (14hpf). By 14S (16hpf) expression in the hindbrain has also expanded posteriorly and encompasses rhombomere 2 through rhombomere 6. At later stages, 24 through 48 hpf,

additional expression was found in the eyes, diencephalon, midbrain-hindbrain boundary, cerebellum, pectoral fin and the pharyngeal arch primordia.
Descriptors: zebrafish, cytochrome P450 enzyme system biosynthesis, metabolism and genetics, proteins biosynthesis, amino acid and base sequence, cerebellum enzymology, molecular cloning, cytochrome P450 enzyme system chemistry, diencephalon enzymology, gene expression, in situ hybridization, molecular sequence data, reverse transcriptase PCR , rhombencephalon enzymology, genetic transcription drug effects, tretinoin physiology.

Zhao, Y., Q. Pan Hammarstrom, Z. Zhao, and L. Hammarstrom (2005). **Identification of the activation-induced cytidine deaminase gene from zebrafish: an evolutionary analysis.** *Developmental and Comparative Immunology* 29(1): 61-71. ISSN: 0145 305X.
NAL Call Number: QR180.D4
Descriptors: zebrafish, cytidine deaminase metabolism, molecular evolution, metabolism, amino acid and base sequence, molecular cloning, conserved sequence, cytidine deaminase genetics, dogs , immunoglobulin class switching, molecular sequence data, *Pan troglodytes*, phylogeny , amino acid sequence homology.

Zhao, Y., Z. Yang, J.K. Phelan, D.A. Wheeler, S. Lin, and E.R. McCabe (2006). **Zebrafish DAX1 is required for development of the interrenal organ, the adrenal cortex equivalent.** *Molecular Endocrinology* 20(11): 2630-40. ISSN: 0888-8809.
NAL Call Number: QP187.3M64M6
Abstract: Mutations in the human nuclear receptor, DAX1, cause X-linked adrenal hypoplasia congenita (AHC). We report the isolation and characterization of a DAX1 homolog, dax1, in zebrafish. The dax1 cDNA encodes a protein of 264 amino acids, including the conserved carboxy-terminal ligand binding-like motif; but the amino-terminal region lacks the unusual repeats of the DNA binding-like domain in mammals. Genomic sequence analysis indicates that the dax1 gene structure is conserved also. Whole-mount in situ hybridization revealed the onset of dax1 expression in the developing hypothalamus at approximately 26 h post fertilization (hpf). Later, at about 28 hpf, a novel expression domain for dax1 appeared in the trunk. This bilateral dax1-expressing structure was located immediately above the yolk sac, between the otic vesicle and the pronephros. Interestingly, weak and transient expression of dax1 was observed in the interrenal glands (adrenal cortical equivalents) at approximately 31 hpf. This gene was also expressed in the liver after 3 dpf in the zebrafish larvae. Disruption of dax1 function by morpholino oligonucleotides (MO) down-regulated expression of steroidogenic genes, cyp11a and star, and led to severe phenotypes similar to ff1b (SF1) MO-injected embryos. Injection of dax1 MO did not affect ff1b expression, whereas ff1b MO abolished dax1 expression in the interrenal organ. Based on these results, we propose that dax1 is the mammalian DAX1 ortholog, functions downstream of ff1b in the regulatory cascades, and is required for normal development and function of the zebrafish interrenal organ.
Descriptors: zebrafish, adrenal cortex embryology, DNA binding, interrenal gland embryology, retinoic acid receptors physiology, amino acid and base sequence, DNA binding proteins genetics, proteins antagonists and inhibitors, DNA binding proteins genetics and metabolism, embryonic development physiology, molecular sequence data, antisense oligonucleotides pharmacology, phylogeny, retinoic acid receptors antagonists, inhibitors and metabolism, repressor proteins antagonists, inhibitors and metabolism, amino acid sequence homology, transcription factors physiology, water electrolyte balance physiology.

(2004). ***Fish Development and Genetics: The Zebrafish and Medaka Models***, Vol. 2, World Scientific Publishing Co. Pte. Ltd.: Singapore, Singapore, 675 p. ISBN: 9812388214.
NAL Call Number: QL638.C94 F57
Abstract: The zebrafish is the most important fish model in developmental and genetic analyses. This book contains 19 review articles covering a broad spectrum of topics, from development to genetic tools. The contents range from early development, the role of maternal factors and gastrulation, to tissue differentiation and organogenesis, such as development of the organizer, notochord, floor plate, nervous system, somites, muscle, skeleton and endoderm. The genetic tools cover morpholino knock-down, transgenics, fish cloning, transposons and genome evolution. The book also includes two chapters on genome mapping and embryonic stem cells in medaka, another important model fish. Summarizing the state-of-the-art studies of the zebrafish model and focusing on the molecular aspects of development, this book is a valuable reference for students learning the basic aspects of the zebrafish model and for research seeking resources in zebrafish research. Copyright © 2008 CABI
Descriptors: zebrafish, cloning, models for biological development, embryonic development, embryos, gene mapping, genetics, genomes, maternal effects, morphogenesis, muscles, nervous system, organogenesis, reviews, skeletal development, transposable elements, *Danio rerio*, *Oryzias latipes*.

Zhong, H., X. Wu, H. Huang, Q. Fan, Z. Zhu, and S. Lin (2006). **Vertebrate MAX-1 is required for vascular patterning in zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 103(45): 16800-5. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: During embryogenesis, stereotypic vascular patterning requires guidance cues from neighboring tissues. However, key molecules involved in this process still remain largely elusive. Here, we report molecular cloning, expression, and functional studies of zebrafish max-1, a homolog of *Caenorhabditis elegans* max-1 that has been implicated in motor neuron axon guidance. During early embryonic development, zebrafish max-1 is specifically expressed in subsets of neuronal tissues, epithelial cells, and developing somites through which vascular endothelial cells migrate from large ventral axial vessels to form stereotypic intersegmental blood vessels (ISV). Blocking zebrafish max-1 mRNA splicing by morpholino injection led to aberrant ISV patterning, which could be rescued by injection of either *C. elegans* or zebrafish max-1 mRNA. Analysis of motor neurons in the same region showed normal neuronal axon pathfinding. Further studies suggested that the ISV defect caused by max-1 knockdown could be partially rescued by overexpression of ephrinb3 and that max-1 was involved in mediating membrane localization of ephrin proteins, which have been shown to provide guidance cues for endothelial cell migration. Our findings therefore suggest that max-1, acting upstream of the ephrin pathway, is critically required in vascular patterning in vertebrate species.
Descriptors: zebrafish, basic helix loop helix leucine zipper transcription factors genetics, blood vessels embryology, genetically modified, base sequence, *Caenorhabditis elegans* genetics and embryology, ephrin b3 genetics, developmental gene expression regulation, gene targeting, in situ hybridization, molecular sequence data, antisense oligoribonucleotides genetics, messenger RNA genetics.

Zhong, H., Z. Zhu, and Q. Fan (2004). **Dynamic expression pattern of zebrafish bfabp (fabp7) during early embryogenesis**. *Chinese Science Bulletin* 49(4): 419-420. ISSN: 1001-6538.
Descriptors: zebrafish, embryonic development, freshwater fish, gene expression, *Danio rerio*, genetics and evolution.

Zhong, T.P. (2005). **Zebrafish genetics and formation of embryonic vasculature.** *Current Topics in Developmental Biology* 71: 53-81. ISSN: 0070 2153.
NAL Call Number: QL951.C8
Abstract: The embryonic vasculature develops in a conserved manner in all vertebrates. Endothelial progenitor cells differentiate from mesodermal cells, then migrate and assemble into the dorsal aorta and the cardinal vein. This primitive circulatory loop undergoes sprouting and branching via a two-step navigation mechanism to form the trunk vascular network. Various studies using several model systems have uncovered a number of signaling mechanisms that regulate these complex processes. A genetic approach in zebrafish has led to identification of mutations and molecules that are responsible for specification of endothelial progenitor cells, differentiation of arterial and venous cells, and patterning of the dorsal aorta and intersegmental vessels. These studies highlight the unique utilities and benefits of the zebrafish system for studying development of embryonic blood vessels.
Descriptors: zebrafish, genetics, mutations, endothelial, progenitor, cells, arterial differentiation, venous cells, dorsal aorta, intersegmental vessels.

Zhou, F., G. Zhao, J.D. Liu, J. Chen, X.Y. Zhang , and Q.X. Yu (2004). **Advanced methods of preparing pachytene bivalents and high resolution multiple bands of zebrafish (*Danio rerio*).** *Acta Genetica Sinica* 31(5): 474-9. ISSN: 0379 4172.
Abstract: The pachytene bivalents with high-resolution multiple G bands of zebrafish were obtained after the treatment with alkaline hypotonic solution and high concentration of chloroform fixative solution. When comparing six group chromosomes from different pachytene specimens, the characteristic and the number of bands were well matched. In order to systematize this technique and get stable result, we summarize the preparation procedure of the zebrafish bivalents. The 6-month-old to one-year-old zebrafish whose spermary appears ivory-white and opaque, is good material. The whole testis should be treated with hypotonic solution for 1.5 approximately 2 h at room temperature. Then, the testes were fixed for 20 min in high chloroform fixative solution (chloroform: methanol: acetic acid, 3: 6:1), and fixed in Carnoy's solution (methanol: acetic acid, 3:1) for two times. In addition, with the treatment of restriction endonuclease Alu I directed in situ nick translation, we successfully obtained well-resolved restrictive endonuclease banding of zebrafish bivalents, which was considered as G-like band patterns. The aging of the specimen is also important factor, should let them dry at room temperature for one week. The application of these methods in cytogenetics research of zebrafish and other fish can be expected. Construction of the steady technique system to prepare high resolution banding bivalents and idiogram of zebrafish is the basement to found stable and accurate framework for physical map.
Descriptors: zebrafish, chromosome banding, physical chromosome mapping techniques, seasons.
Language of Text: Chinese.

Zhou, W., L. Saint Amant, H. Hirata, W.W. Cui, S.M. Sprague, and J.Y. Kuwada (2006). **Non-sense mutations in the dihydropyridine receptor beta1 gene, CACNB1, paralyze zebrafish relaxed mutants.** *Cell Calcium* 39(3): 227-36. ISSN: 0143 4160.
NAL Call Number: QP772.V53C4
Abstract: Contractions by skeletal muscle require proper excitation-contraction (EC) coupling, whereby depolarization of the muscle membrane leads to an increase in cytosolic Ca(2+) and contraction. Changes in membrane voltage are detected by dihydropyridine receptors (DHPR) that directly interact with and activate ryanodine receptors to release Ca(2+) from the sarcoplasmic reticulum into the cytosol. A genetic screen for motility

mutations isolated a new allele of the immotile zebrafish mutant relaxed. Muscles in relaxed embryos do not contract in response to potassium chloride (KCl) thus appear unresponsive to membrane depolarization, but do contract when stimulated by caffeine, an agonist of ryanodine receptors. This suggests that relaxed mutant muscles are defective in EC coupling. Indeed, immunohistochemical analysis demonstrated that mutants lack DHPRs in skeletal muscles. The mutant phenotype results from non-sense mutations in the zebrafish CACNB1 gene that encodes the DHPR beta1 subunit. The zebrafish CACNB1 gene is expressed in skeletal muscles, PNS and CNS. Electrophysiological recordings showed no obvious abnormalities in the motor output of relaxed mutants, presumably due to redundancy provided by other beta subunits. The structural and functional homology of CACNB1 in zebrafish and mammals, suggests that zebrafish can be useful for studying EC coupling and potential neuronal function of CACNB1.

Descriptors: zebrafish, l type calcium channels genetics, nonsense codon, ryanodine receptor calcium release channel genetics, amino acid sequence, molecular sequence data, muscle contraction physiology, skeletal muscle physiology, nervous system metabolism, paralysis genetics, sequence alignment.

Zhou, W., K. Shirabe, and J.Y. Kuwada (2006). **Molecular cloning and expression of two small leucine-rich proteoglycan (SLRP) genes, dspg3l and optcl, in zebrafish.** *Gene Expression Patterns* 6(5): 482-8. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: Epiphycan (DSPG3) and opticin are two class III small leucine-rich proteoglycans (SLRP). We isolated two zebrafish cDNAs, dspg3l and optcl, that encode proteins homologous to epiphycan and opticin in other vertebrates. Like epiphycans in other species, dspg3l is exclusively expressed in the developing notochord and cartilage. optcl is expressed transiently in the developing nervous system, eyes and somites much like opticin. The zebrafish dspg3l and optcl genes are located in linkage group 4 and 11, respectively. The genomic locations for both genes in zebrafish are syntenic with the genomic locations of dspg3 and opticin (optc) in human and mouse. Synteny and the expression patterns of these genes suggest that the dspg3l and optcl are the orthologs to the mammalian dspg3 and optc genes, respectively.

Descriptors: zebrafish , proteoglycans genetics, amino acid and base sequence, molecular cloning, DNA primers, molecular sequence data, proteoglycans chemistry, amino acid sequence homology, proteins chemistry.

Zhu, H., D. Traver, A.J. Davidson, A. Dibiase, C. Thisse, B. Thisse, S. Nimer, and L.I. Zon (2005). **Regulation of the lmo2 promoter during hematopoietic and vascular development in zebrafish.** *Developmental Biology* 281(2): 256-69. ISSN: 0012 1606.

NAL Call Number: 442.8D49

Abstract: The Lmo2 transcription factor, a T-cell oncoprotein, is required for both hematopoiesis and angiogenesis. To investigate the fate of lmo2-expressing cells and the transcriptional regulation of lmo2 in vivo, we generated stable transgenic zebrafish that express green fluorescent protein (EGFP) or DsRed under the control of an lmo2 promoter. A 2.5-kb fragment contains the cis-regulatory elements required to recapitulate endogenous lmo2 expression in embryonic hematopoietic and vascular tissues. We further characterized embryonic Lmo2+ cells through transplantation into vlad tepes (vlt), an erythropoietic mutant. These Lmo2+ primitive wave donor cells differentiated into circulating hematopoietic cells and extended the life span of vlt recipients, but did not demonstrate long-term repopulation of the erythroid lineage. Promoter analysis identified a 174-bp proximal promoter that was sufficient to recapitulate lmo2 expression. This element contains critical

ETS-binding sites conserved between zebrafish and pufferfish. Furthermore, we show that ets1 is coexpressed with lmo2, and overexpression experiments indicate that ets1 can activate the lmo2 promoter through this element. Our studies elucidate the transcriptional regulation of this key transcription factor, and provide a transgenic system for the functional analysis of blood and blood vessels in zebrafish.

Descriptors: zebrafish, DNA binding proteins genetics and biosynthesis, hematopoietic system embryology, metalloproteins biosynthesis, physiologic neovascularization physiology, genetically modified, base sequence, blood vessels embryology, blood vessels metabolism, cell differentiation, DNA binding proteins genetics, erythrocytes metabolism and physiology, hematopoietic stem cells physiology, cytology and metabolism, metallomolecular sequence data, promoter regions genetics, proto oncogene protein C ETS 1 biosynthesis, proteins.

Zhu, H. and L.I. Zon (2004). **Use of the Dsred fluorescent reporter in zebrafish.** *Methods in Cell Biology* 76: 3-12. ISSN: 0091-679X.

NAL Call Number: 442P92

Abstract: Green fluorescent protein (GFP) is firmly established as a fluorescent reporter for the imaging of specific tissues in zebrafish. The employment of other reporters such as DsRed in transgenic zebrafish has made multicolored labeling experiments possible. To date, several DsRed transgenic lines have been generated for lineage labeling, transplantation assays, and commercial applications. Advances in multicolored labeling experiments will depend on the implementation of newly engineered reporters and fusion proteins, as well as on innovative experiments that exploit the power of direct visualization.

Descriptors: zebrafish, luminescent staining and labeling methods, genetically modified, cell transplantation methods, green fluorescent, luminescent proteins chemistry, recombinant fusion proteins chemistry and genetics.

Zhu, S., L. Liu, V. Korzh, Z. Gong, and B.C. Low (2006). **RhoA acts downstream of Wnt5 and Wnt11 to regulate convergence and extension movements by involving effectors Rho kinase and Diaphanous: use of zebrafish as an in vivo model for GTPase signaling.** *Cellular Signalling* 18(3): 359-72. ISSN: 0898 6568.

NAL Call Number: QH604.2.C47

Abstract: Gastrulation shapes the early embryos by forming three germ layers, ectoderm, mesoderm and endoderm. In vertebrates, this process requires massive cell rearrangement including convergence and extension (CE) movements that involve narrowing and lengthening of embryonic tissues as well as cell elongation. Such polarization and movements require precise reorganization and regulation of the cytoskeleton network and cell adhesion. Rho small GTPases are key regulators for dynamic actin cytoskeleton. However, the signaling mechanisms underlying their functions in CE remain to be further elucidated. We have cloned the zebrafish *Danio rerio* rhoA and by capitalizing on the specific functional knockdown using morpholinos against rhoA and the availability of CE mutants defective in Wnt signaling, we showed that rhoA morphants were reminiscent to noncanonical wnt mutants with serious disruption in CE movements. Injection of rhoA mRNA effectively rescued such defects in wnt5 and wnt11 mutants. Furthermore, CE defects in rhoA knockdown or wnt mutants can be suppressed through functional bypass after ectopic expression of the two mammalian Rho effectors, the Rho kinase and Diaphanous (mDia). These results provide the first evidence that the RhoA in vivo acts downstream of Wnt5 and Wnt11 to effect, without affecting cell fates, on the CE movements in zebrafish embryos. Significantly, it elicits such effect via both effectors, Rho kinase and Dia. These findings also support the versatility of the zebrafish as a model to further investigate the roles of various classes of small GTPases in

regulating cell dynamics in vivo.
Descriptors: zebrafish, GTP phosphohydrolases physiology, protein serine threonine kinases physiology, proto oncogene, Wnt proteins physiology, amino acid sequence, cell movement, physiology, gastrula physiology, developmental gene expression regulation physiology, in situ hybridization, intracellular signaling peptides and proteins , molecular sequence data, mutation, proto oncogene reverse transcriptase PCR, signal transduction, Wnt proteins genetics, proteins analysis, RhoA GTP binding protein physiology, analysis and genetics.

Zhu, Y., J.W. Stiller, M.P. Shaner, A. Baldini, J.L. Scemama, and A.A. Capehart (2004). **Cloning of somatolactin alpha and beta cDNAs in zebrafish and phylogenetic analysis of two distinct somatolactin subtypes in fish.** *Journal of Endocrinology* 182(3): 509-18. ISSN: 0022-0795.
Abstract: Somatolactin (SL) is a pituitary hormone belonging to the growth hormone/prolactin superfamily, with recognizable homologues in all fish taxa examined to date. Although sequences from most fish share reasonably high sequence identity, several more highly divergent SLs have been reported. Goldfish SL and a second SL protein found in rainbow trout (rtSLP) are remarkably different from each other and also dissimilar to other SLs. It has been unclear whether rtSLP is a recent paralogue restricted to rainbow trout, or reflects a more ancient duplication of the SL gene, and whether it is related to the goldfish sequence. Here we report the cloning of two different zebrafish SL cDNAs, which share only 57.5% nucleotide and 47.7% deduced amino acid identities. One copy, designated zebrafish SLalpha (zfSLalpha), displays a typical range of sequence similarity to most other SLs. The other copy, zebrafish SLbeta (zfSLbeta), shows low identity to most other SLs; surprisingly, it is most similar to the divergent SL sequence from goldfish. The mRNAs of zfSLalpha and zfSLbeta were expressed specifically in two distinct regions of the pars intermedia in zebrafish. Cells expressing zfSLalpha are located at the posterior pars intermedia, bordering the neurohypophysis, whereas zfSLbeta is expressed in the anterior part of the pars intermedia, bordering the pars distalis. Phylogenetic analyses indicate that zfSLbeta, goldfish SL and rtSLP all belong to the SL hormone family; however, along with the genes from eel and catfish, these divergent sequences form a group that is clearly distinct from all other SLs. These results suggest the presence of two distinct SL families, SLalpha and SLbeta, which may trace back to a teleost genome duplication prior to divergence of the cyprinids and salmonids.
Descriptors: zebrafish, molecular cloning, complementary DNA, glycopituitary gland metabolism, pituitary hormones genetics, amino acid and base sequence, Cyprinidae genetics, fish proteins, gene expression, in situ hybridization methods, molecular sequence data, phylogeny, protein isoforms genetics, Salmonidae genetics.

Zilinski, C.A., R. Shah, M.E. Lane, and M. Jamrich (2005). **Modulation of zebrafish pitx3 expression in the primordia of the pituitary, lens, olfactory epithelium and cranial ganglia by Hedgehog and nodal signaling.** *Genesis* 41(1): 33-40. ISSN: 1526-954X.
NAL Call Number: QH426.D32
Abstract: In this article we report the isolation of a novel zebrafish gene, pitx3, which plays an important role in the formation of several placode-derived structures. In wildtype embryos, pitx3 is first expressed in a crescent-shaped area in the anterior end of the embryo. At later stages, the primordia of the anterior pituitary, the lens, the olfactory sensory epithelium, and cranial ganglia express this gene. Pitx3 is not expressed in the more posterior preplacodal region that gives rise to the epibranchial, otic, and lateral line placodes. The dynamics of pitx3 in the anterior region of wildtype embryos suggests that pitx3 expression marks a common step in the formation of the pituitary, lens, olfactory placode as well as the

trigeminal placode. Analysis of pitx3 expression in mutants lacking the hedgehog or nodal function demonstrates the differential dependence of pitx3 expression in these structures on nodal and hedgehog signaling. While the lens and trigeminal placodes express pitx3 in the absence of hedgehog and nodal signaling, there is no expression of pitx3 in the anteriormost ectoderm adjacent to the neural plate from which the anterior pituitary would derive. In mutants with impaired hedgehog signaling, the lens placode frequently extends into more anterior ventral regions of the embryo.

Descriptors: zebrafish, ganglia embryology, homeodomain proteins metabolism, crystalline lens embryology, olfactory mucosa embryology, anterior pituitary gland embryology, trans activators metabolism, transforming growth factor beta metabolism, amino acid motifs, amino acid sequence embryonic development, ganglia cytology, ganglia metabolism, developmental gene expression regulation, head, Hedgehog proteins, homeodomain proteins chemistry, in situ hybridization, crystalline lens cytology and metabolism, molecular sequence data, morphogenesis, mutation, olfactory mucosa cytology and metabolism, phylogeny, anterior pituitary gland cytology and metabolism, tertiary protein structure, amino acid sequence homology, trans activators genetics, transforming growth factor beta genetics.

Zimov, S. and S. Yazulla (2004). **Localization of vanilloid receptor 1 (TRPV1/VR1)-like immunoreactivity in goldfish and zebrafish retinas: restriction to photoreceptor synaptic ribbons.** *Journal of Neurocytology* 33(4): 441-52. ISSN: 0030-4864.

Abstract: The vanilloid receptor type 1 (TRPV1/VR1) is a non-specific calcium-permeable ionotropic cation channel expressed in the peripheral sensory system as well as in the central nervous system. An endogenous ligand for TRPV1 is arachidonoyl ethanolamide (anandamide), which also activates the metabotropic cannabinoid receptor 1 (CB1). Previous studies in this laboratory reported CB1 receptors and CB1-mediated effects on voltage-gated currents in goldfish cones and bipolar cells. In this study, we show TRPV1-like-immunoreactivity (TRPV1-L-IR) by immunoblot analysis of goldfish retina and rat brain homogenates with a guinea pig polyclonal antibody against the C-terminus of the rat TRPV1. Light-level immunocytochemistry showed restriction of the guinea pig-TRPV1 antibody to a very narrow band in the outer plexiform layer in goldfish and zebrafish retinas. However, no immunoreactivity was detected using rabbit-polyclonal antibodies against the C or N-termini of the rat TRPV1. Pre and post-embedding electron microscopy (EM) immunocytochemistry revealed that TRPV1-L-IR (using the guinea pig antibody) was restricted to synaptic ribbons of all cones and many rods, but never was observed at the synaptic ribbons of bipolar cells. While pre-embedded tissue showed diffuse label associated only with photoreceptor-synaptic ribbons, analysis of post-embedded tissue showed label tightly restricted to synaptic ribbons of all cones and many rods. Oblique sections showed that immunogold particles were confined to the outer electron dense region of the ribbons, with few or no gold particles in the ribbon core or associated with tethers or vesicles. Although TRPV1-L-IR described here, does not necessarily represent TRPV1 antigen associated with synaptic ribbons, these data provide an unequivocal label with which to study the functional dynamics of ribbon formation and degradation in teleost photoreceptors.

Descriptors: zebrafish, goldfish anatomy and histology, ion channels analysis, photoreceptors chemistry and cytology, retina chemistry and cytology, synapses ultrastructure, ion channels genetics, rats, TRPV cation channels.

Zinkevich, N.S., D.V. Bosenko, B.A. Link, and E.V. Semina (2005). **Identification and ocular expression of zebrafish laminin alpha 1 gene**. *Investigative Ophthalmology & Visual Science* 46(Suppl. S): 3122. ISSN: 0146 0404.

Abstract: Purpose: To report a sequence and expression of zebrafish laminin alpha 1. Laminins are components of all basement membranes and have well demonstrated roles in diverse developmental processes including eye formation. Methods: Sequences homologous to mammalian laminin alpha 1 were identified by sequence comparison using BLASTN/X engines and zebrafish genome database available at Sanger Institute. Oligonucleotides were designed and used to amplify overlapping fragments of zebrafish laminin alpha 1 gene from embryonic cDNA. Expression was studied by RT-PCR and in situ hybridization. Results: A 9-kb cDNA contig containing putative laminin alpha 1 reading frame has been identified. The zlama1 contig demonstrated overall 63% identity at amino acid level with the human laminin alpha 1 sequence with identity level varying from 43% to 90% for some regions. RT-PCR experiments identified zlama1 transcript in zebrafish embryos starting from one-cell stage with the most abundant level observed in 1418 hour embryos. Expression diminished in 72-hour embyos and remained low at later stages.In adult fish, low levels of zlama1 were detected in eye but not other tissues. The zlama1 transcript localized to the lens during eye development by in situ hybridization. Conclusions: A new zebra fish gene homologous to laminin alpha 1 has been identified. The zlama1 is expressed during lens development and in adult eye. The role of this gene in embryonic development will be investigated by morpholino-mediated knockdown experiments and mutant analysis. Copyright©Thomson Reuters 2009
Descriptors: zebrafish eye development, molecular genetics, sense organs, sensory reception, embryonic development.

Zinkevich, N.S., R.C. Tyler, D.V. Bosenko, G.B. Willer, R.G. Gregg, E.V. Semina, and B.A. Link (2004). **A new zebrafish mutant with arrested lens development**. *Investigative Ophthalmology & Visual Science* 45(Suppl. 2): U132. ISSN: 0146 0404.

Abstract: Purpose: The zebrafish has received considerable attention as a valuable modelfor genetic studies of vertebrate development. In order to reveal genes essential for lens development, we screened chemically mutagenized zebrafish for related phenotypes and identified a mutation resulting in arrest of lens and anterior segment development, a69. Identification of the gene responsible for a69may reveal new factors/pathways involved in development of the lens and anterior segment of the eye.Methods, Morphological and histological analysis of zebrafish embryos was performed using standard techniques. Screening of candidate genes was performed bydirect sequencing of gene-specific PCR products. Recombinant linkage mapping was performed with simple sequence length polymorphic markers from Research Genetics.Results: The zebrafish a69 mutant is characterized by a malformed eye with a small and often irregular shaped pupil. Another visible defect is a shortened body length. Histological analysis revealed abnormal lens development with onset around 32 hpf. At this stage in normal fish, the developing lens is fully separated from the corneal ectoderm and contains a layer of epithelial cells at the anterior side and differentiating lens fibers. In a69 mutants, the lens cavity is filled with disorganized cells that eventually degenerate. Retinal development also mildly disturbed as photoreceptor markers are found in the innerretina. This is most likely to be a secondary anomaly. Some variability in morphological phenotype was observed depending on genetic background - body length was affected in 98% of ocular mutants in the original "AB" background, while only similar to 50% displayed shortened body length in "TU" outcross. Ocularphenotype also varied from mild alterations in the pupil shape and size to severe microphthalmia. Because of the similarity of this phenotype to mouse aphakia and dysgenetic lens, we screened zebrafish pitx3 and foxe3 gene sequences in a69 fish. No mutations have been identified. A genome-wide search for the a69 gene is now underway. Conclusions: From these studies, we conclude that the a69 gene plays an essential role during early stages of lens development and most likely represents anew factor. Identification of the

a69 gene will extend our knowledge of factorsand mechanisms of vertebrate lens development and facilitate screens for phenotype modifying genes. Copyright©Thomson Reuters 2009

Descriptors: mutant zebrafish, nervous system, neural coordination, development, molecular genetics, sense organs, sensory reception, microphthalmia, nervous system disease, eye disease, genetics, linkage mapping, genetic techniques, histological analysis, histology and cytology techniques, retinal development, A69.

Zirger, J.M., C.E. Beattie, D.B. McKay, and R.T. Boyd (2003). **Cloning and expression of zebrafish neuronal nicotinic acetylcholine receptors.** *Gene Expression Patterns* 3(6): 747-54. ISSN: 1567-133X.

NAL Call Number: QH450.B73

Abstract: We propose to use the zebrafish (*Danio rerio*) as a vertebrate model to study the role of neuronal nicotinic acetylcholine receptors (nAChR) in development. As a first step toward using zebrafish as a model, we cloned three zebrafish cDNAs with a high degree of sequence similarity to nAChR beta3, alpha2 and alpha7 subunits expressed in other species. RT-PCR was used to show that the beta3 and alpha2 subunit RNAs were present in zebrafish embryos only 2-5hours post-fertilization (hpf) while alpha7 subunit RNA was not detected until 8hpf, supporting the differential regulation of nAChRs during development. In situ hybridization was used to localize zebrafish beta3, alpha2, and alpha7 RNA expression. nAChR binding techniques were used to detect the early expression of two high-affinity [3H]-epibatidine binding sites in 2 days post-fertilization (dpf) zebrafish embryos with IC(50) values of 28.6pM and 29.7nM and in 5dpf embryos with IC(50) values of 28.4pM and 8.9nM. These studies are consistent with the involvement of neuronal nAChRs in early zebrafish development.

Descriptors: zebrafish, nicotinic receptors genetics, heterocyclic bicyclo compounds metabolism, molecular cloning, gene expression, neurons, nicotinic agonists metabolism, protein subunits genetics and metabolism, pyridines metabolism, messenger RNA analysis and metabolism, nicotinic receptors, reverse transcriptase PCR.

Ziv, L. and Y. Gothilf (2006). **Period2 expression pattern and its role in the development of the pineal circadian clock in zebrafish.** *Chronobiology International* 23(1-2): 101-12. ISSN: 0742 0528.

NAL Call Number: QP84.6.C47

Abstract: In zebrafish, pineal arylalkylamine-N-acetyltransferase (zfaanat2) mRNA expression begins at 22 h post-fertilization (hpf), and the clock-controlled rhythm of its transcript begins on the third day of development. Here we describe the role of light and of the clock gene, period2 (zper2) in the development of this rhythm. In 1-day-old zebrafish embryos, zper2 expression is transiently up-regulated by light in the pineal gland and, to a lesser extent, in other areas of the brain. Expression of zper2 that was not affected by light occurred in the olfactory placode and lactotroph cells of the pituitary primordium. Circadian analysis of pineal zfaanat2 mRNA expression indicated that light exposure is required for proper development of the circadian clock-controlled rhythmic expression of this gene. Knockdown of zPER2 using antisense technology abolished the effect of light on development of the zfaanat2 rhythm in the pineal gland, corroborating the role of zper2 in light entrainment of the circadian oscillator in zebrafish. Further analysis of zper2 expression at earlier stages of development revealed that light exposure at the blastula to mid-segmentation stages also caused a transient increase in zper2 expression. At mid-segmentation, before pineal differentiation, light-induced zper2 expression was enhanced in pineal progenitor cells. Thus, a

possible role for early photoreception and light-induced zper2 expression in the development of clock-controlled rhythms remains to be investigated.
Descriptors: zebrafish, eye proteins biosynthesis, eye, developmental gene expression regulation, pineal gland embryology, proteins biosynthesis, arylalkylamine N acetyltransferase metabolism, circadian rhythm, kinetics, light, photoreceptors physiology, pituitary gland metabolism, time factors.

Ziv, L., A. Tovin, D. Strasser, and Y. Gothilf (2007). **Spectral sensitivity of melatonin suppression in the zebrafish pineal gland.** *Experimental Eye Research* 84(1): 92-9. ISSN: 0014 4835.
NAL Call Number: QP474
Abstract: The pineal gland of the zebrafish (*Danio rerio*) is a clock-containing photoreceptive organ. Superfused pineal glands kept in darkness display rhythmic melatonin production that lasts for days, with high melatonin levels during the night and low levels during the day. Nocturnal light, however, evokes an acute suppression of melatonin synthesis in the photoreceptor cells. Towards characterizing zebrafish pineal photopigment that is involved in the acute melatonin suppression we have measured the spectral sensitivity of melatonin-suppression response in superfused pineal glands. The effect of 2 h light exposure of seven wavelengths (lambdaavg 408, 460, 512, 560, 608, 660 and 697+/-10-15 nm) at multiple irradiances (10(7)-10(14) photons/cm2/s) was determined, and an action spectrum was plotted. The resultant action spectrum provides evidence for the involvement of multiple photopigments in melatonin suppression. The most efficient melatonin-suppression response was achieved by exposure to light of around 512 nm; however, another peak of lower irradiance sensitivity was observed in the middle to long wavelengths. Opsins-specific RT-PCR analysis confirmed the expression of exo-rhodopsin and visual red-sensitive opsin in the pineal gland, while other zebrafish visual opsins as well as VA and VAL opsins were not detected. Dartnall monograms for exo-rhodopsin and visual red-sensitive opsin account for most but not all of the spectral sensitivity features. Therefore, additional pineal photopigments may contribute to the melatonin-suppression response in the pineal gland.
Descriptors: zebrafish, light, melatonin biosynthesis, pineal gland radiation effects and metabolism, circadian rhythm physiology, gene expression regulation radiation effects, opsin genetics and metabolism, organ culture techniques methods, photic stimulation methods, reverse transcriptase PCR methods.

Zoidl, G., R. Bruzzone, S. Weickert, M. Kremer, C. Zoidl, G. Mitropoulou, M. Srinivas, D.C. Spray, and R. Dermietzel (2004). **Molecular cloning and functional expression of zfCx52.6: a novel connexin with hemichannel-forming properties expressed in horizontal cells of the zebrafish retina.** *Journal of Biological Chemistry* 279(4): 2913-21. ISSN: 0021-9258.
NAL Call Number: 381J824
Abstract: Gap junction-mediated electrical coupling contributes to synchronous oscillatory activities of neurons, and considerable progress has been made in defining the molecular composition of gap junction channels. In particular, cloning and functional expression of gap junction proteins (connexins (Cx)) from zebrafish retina have shown that this part of the brain possesses a high degree of connexin diversity that may account for differential functional properties of electrical synapses. Here, we report the cloning and functional characterization of a new connexin, designated zebrafish Cx52.6 (zfCx52.6). This connexin shows little similarity to known connexins from fish and higher vertebrates. By combining in situ hybridization with Laser Capture Microdissection and RT-PCR, we found that this novel fish connexin is expressed in horizontal cells in the inner nuclear layer of the retina. Functional expression of zfCx52.6 in neuroblastoma cells and Xenopus oocytes led to functional gap

junctional channels and, in single oocytes, induced large non-junctional membrane currents indicative of the formation of hemichannels, which were inhibited in reversible fashion by raising extracellular Ca(2+) concentrations.
Descriptors: zebrafish, connexins genetics, retina metabolism, amino acid sequence, molecular cloning, connexins metabolism, gap junctions metabolism, molecular sequence data, sequence alignment, *Xenopus.*

Zou, J., F. Beermann, J. Wang, K. Kawakami, and X. Wei (2006). **The Fugu tyrp1 promoter directs specific GFP expression in zebrafish: tools to study the RPE and the neural crest-derived melanophores.** *Pigment Cell Research* 19(6): 615-27. ISSN: 0893-5785.
Abstract: In vertebrates, pigment cells account for a small percentage of the total cell population and they intermingle with other cell types. This makes it difficult to isolate them for analyzes of their functions in the context of development. To alleviate such difficulty, we generated two stable transgenic zebrafish lines (pt101 and pt102) that express green fluorescent protein (GFP) in melanophores under the control of the 1 kb Fugu tyrp1 promoter. In pt101, GFP is expressed in both retinal pigment epithelium (RPE) cells and the neural crest-derived melanophores (NCDM), whereas in pt102, GFP is predominately expressed in the NCDM. Our results indicate that the Fugu tyrp1 promoter can direct transgene expression in a cell-type-specific manner in zebrafish. In addition, our findings provide evidence supporting differential regulations of melanin-synthesizing genes in RPE cells and the NCDM in zebrafish. Utilizing the varying GFP expression levels in these fish, we have isolated melanophores via flow cytometry and revealed the capability of sorting the NCDM from RPE cells as well. Thus, these transgenic lines are useful tools to study melanophores in zebrafish.
Descriptors: zebrafish embryos, green fluorescent melanophores metabolism, neural crest embryology, oxidoreductases genetics, pigment epithelium of eye embryology, promoter regions genetics, genetically modified, cell differentiation genetics, cell line and lineage genetics, embryology methods, developmental gene expression regulation physiology, melanophores cytology, molecular biology methods, neural crest cytology and metabolism, pigment epithelium of eye cytology and metabolism, *Takifugu*, transgenes genetics.

Zou, S.M., X.Y. Jiang, Z.Z. He, J. Yuan, X.N. Yuan, and S.F. Li (2007). **Hox gene clusters in blunt snout bream, *Megalobrama amblycephala* and comparison with those of zebrafish, fugu and medaka genomes.** *Gene* 400(1-2): 60-70. ISSN: 0378-1119.
NAL Call Number: QH442.A1G4
Abstract: Hox genes encode transcription factors that play a key role in specifying the body plan in metazoans and are therefore essential in explaining patterns of evolutionary diversity. While each Hox cluster contains the same genes among the different mammalian species, this does not happen in ray-finned fish, in which both the number and organization of Hox genes and even Hox clusters are variables. Here we reveal the organization of Hox genes loci in blunt snout bream. Forty-nine Hox genes including a pseudogene A9b in total have been found in seven clusters as follows: 8 Hox genes in the Aa cluster; 5 in Ab; 10 in Ba; 4 in Bb; 11 in Ca; 4 in Cb; and 7 in Da. In terms of gene content, clusters organization and sequence similarities of putative amino acids, blunt snout bream is more closely related to zebrafish than to fugu and medaka. In contrast to the situation in fugu and medaka, both blunt snout bream and zebrafish have duplicated HoxC cluster but only a single copy of the HoxD cluster. The result implies that the loss of the second HoxD cluster might be a shared feature of the Ostariophysi, to which zebrafish and blunt snout bream both belong. Phylogenetic analysis bases on the paralogous genes from twin clusters supports the duplication-first model, i.e., four original clusters may have duplicated in an event before the divergence of

the blunt snout bream-plus-zebrafish lineage and the fugu-plus-medaka lineage. Additionally, the relationship between the decrease of GC level and the loss of conservation and function of one of the paralogous genes from twin clusters is discussed.
Descriptors: zebrafish, Cyprinidae genetics, base sequence, molecular evolution, gene duplication, homeobox genes, molecular sequence data, multigene family, *Oryzias*, phylogeny, nucleic acid sequence homology, *Takifugu.*

Ecology

Bila, D.M., A.F. Montalvao, A.C. Silva, and M. Dezotti (2005). **Ozonation of a landfill leachate: evaluation of toxicity removal and biodegradability improvement**. *Journal of Hazardous Materials* 117(2/3): 235-242. ISSN: 0304 3894.
NAL Call Number: T55.3.H3J6
Abstract: This work shows an evaluation of treatments for the leachate produced at the Gramacho Municipal Landfill in Rio de Janeiro state, Brazil. This leachate has very peculiar characteristics, with a high salinity level and very low biodegradability (BOD5/COD of 0.05). A sequence of processes was employed in the treatment of this leachate. Initially, a physicochemical treatment was used, while the second stage consisted of application of ozone to improve the biodegradability of the leachate. The final stage comprised a biological treatment. The physical-chemical treatment led to COD and DOC removal levels of 40 and 25%, respectively, with the use of Al2(SO4)3. The sequence of treatments proposed brought good results, with an increase in the BOD5/COD ratio from 0.05 to 0.3 after ozonation. The toxicity tests performed using *Brachydanio rerio* and *Poecilia vivipara* showed that the toxicity of the leachate had hardly been reduced by ozonation. These results are in agreement with the fact that, despite the higher BOD5/COD ratio, the biological process did not present a good performance. The total average removal levels of COD and DOC achieved using the combined treatment were 73 and 63%, respectively, for an ozone dose of 3.0 g L-1 by the leachate. Copyright © 2008 CABI
Descriptors: zebrafish, biochemical oxygen demand, biodegradation, biological treatment, chemical oxygen demand, landfill leachates, removal, salinity, toxicity, *Danio rerio*, *Poecilia vivipara*.

Cargouet, M., D. Perdiz, and Y. Levi (2007). **Evaluation of the estrogenic potential of river and treated waters in the Paris area (France) using in vivo and in vitro assays**. *Ecotoxicology and Environmental Safety* 67(1): 149-156. ISSN: 0147-6513.
NAL Call Number: QH545.A1E29
Descriptors: zebrafish, assay, byproducts, chlorination, disinfection, drinking water, drinking water, ELISA, ecotoxicology, environmental factors, fish, freshwater fish, pollution indicators, population dynamics, reproduction, rivers, sex hormones, side effects, surface water, surface water, toxicity tests, treated water, water pollution effects, water pollution, water treatment, endocrine disruptors, estrogens, *Danio rerio*.

Chen Jiangning , H. Yu, Y. Liu, W. Jiang, J. Jiang, J.f. Zhang, and Z. Hua (2004). **Ecotoxicological evaluation of 4-aminobiphenyl using a test battery**. *Ecotoxicology and Environmental Safety* 58(1): 104-109. ISSN: 0147 6513.
NAL Call Number: QH545.A1E29
Abstract: Experimental bioassays are currently used in ecotoxicology and environmental toxicology to provide information for risk assessment evaluation of new chemicals and to investigate their effects and mechanisms of action; in addition, ecotoxicological models are used for the detection, control, and monitoring of the presence of pollutants in the environment. As a single bioassay will never provide a full picture of the quality of the environment, a representative, cost-effective, and quantitative test battery should be developed. In this study, the effects of 4-aminobiphenyl (4-ABP) were investigated using a battery of ecotoxicological model systems, including immobilization of Daphnia magna, development of zebrafish embryos, and inhibition of mammalian cell proliferation. The growth inhibition effects of 4-ABP were assessed on mouse connective tissue fibroblast cells (L929 cells) and human hepatocelluar carcinoma cells (HepG2 cells) by using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-trazolium bromide reduction

assay. The results reveal that 4-aminobiphenyl is toxic for aquatic organisms and mammalian cells. The system most sensitive to 4-aminobiphenyl is D. magna immobilization, followed by development of zebrafish embryos, and inhibition of cell proliferation. L929 and HepG2 cell growth inhibition bioassays show low sensitivity. These findings indicate that a single model for the possible harmful effect of 4-ABP has its limitations; only a test battery, composed of bioassays on different species, can provide an accurate assessment of the action of 4-ABP in the whole environment. Copyright 2003 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, toxicology, 3 (4,5-dimethylthiazol-2-yl)-2,5-diphenyl 2H trazolium bromide reduction assay, quantitative test battery, ficroblast cells.

Cheng, S.H. and J. Cheng (2005). **Carbon nanotubes delay slightly the hatching time of zebrafish embryos.** *Abstracts of Papers American Chemical Society* 229(Part 1): U911. ISSN: 0065 7727.
NAL Call Number: 381Am33Pa
Descriptors: zebrafish, development, marine ecology, environmental sciences, electron microscopy, hatching time.

Cortemeglia, C. and T.L. Beitinger (2006). **Susceptibility of transgenic and wildtype zebra danios, *Danio rerio*, to predation**. *Environmental Biology of Fishes* 76(1): 93-100. ISSN: 0378-1909.
NAL Call Number: TD169.E54
Descriptors: zebrafish, color, genotypes, predation, predator prey interactions, *Danio rerio*, *gambusia affinis*, *micropterus salmoides*, Largemouth bass, Mosquitofish.

Diekmann, M. and R. Nagel (2005). **Different survival rates in zebrafish (*Danio rerio*) from different origins**. *Journal of Applied Ichthyology* 21(5): 451-454. ISSN: 0175-8659.
NAL Call Number: QL614.244
Descriptors: zebrafish, domestic wastes, fry, pollution effects, recirculating systems, survival, *Danio rerio*.

Dugatkin, L.A., M.A. McCall, R.G. Gregg, A. Cavanaugh, C. Christensen, and M. Unseld (2005). **Zebrafish (*Danio rerio*) exhibit individual differences in risk-taking behavior during predator inspection.** *Ethology Ecology and Evolution* 17(1): 77-81. ISSN: 0394 9370.
NAL Call Number: QL750.E82
Abstract: Risk-taking behavior associated with predator inspection has been described, in a number of fish species. As a first step in parsing out the contribution of genes and environment to risk-taking behavior, the predator inspection behavior of zebrafish was examined. Predator inspection behavior of seventy males was measured once a day for five consecutive days and their mean daily inspection score - a measure of risk-taking tendencies - was recorded. Zebrafish did not habituate. to, the presence of a predator and significant individual differences. in risk-taking behavior were uncovered.
Descriptors: zebrafish, *Danio rerio*, behavioral variation, predator inspection, exploratory behavior, behavioral variation in male risk taking, defensive behavior, predators.

Engeszer, R.E., L.B. Patterson, A.A. Rao, and D.M. Parichy (2007). **Zebrafish in the wild: a review of natural history and new notes from the field.** *Zebrafish* 4(1): 21-40. ISSN: 1545 8547.
Abstract: The zebrafish, *Danio rerio*, has emerged as a major model organism for biomedical research, yet little is known about its natural history. We review the literature pertaining to the geographic range, biotic and abiotic habitats, and life cycle of the zebrafish. We also report our own field study to document several aspects of zebrafish natural history across sites in northeast India. We found zebrafish particularly abundant in silt-bottomed, well vegetated pools and rice

paddies adjacent to slow moving streams at a range of elevations. We further identified co-occurring fishes likely to be zebrafish competitors and predators. Finally, we present observations that indicate substantial habitat degradation and loss, and suggest guidelines for documenting and preserving natural zebrafish populations.
Descriptors: zebrafish, natural history, biotic, abiotic, habitat, rice paddies, India.

Engeszer, R.E., G. Wang, M.J. Ryan, and D.M. Parichy (2008). **Sex-specific perceptual spaces for a vertebrate basal social aggregative behavior**. *Proceedings of the National Academy of Sciences, USA* 105(3): 929-933. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, shoaling patterns, sex-specific behavior, stripe pattern.

Fabian, N.J., L.B. Albright, G. Gerlach, H.S. Fisher, and G.G. Rosenthal (2007). **Humic acid interferes with species recognition in zebrafish (*Danio rerio*).** *Journal of Chemical Ecology* 33(11): 2090-6. ISSN: 0098-0331.
NAL Call Number: QD415.A1 J6
Abstract: Few studies have addressed how chemosensation may be impaired by chemical alterations of the environment and anthropogenic disturbance. Humic acid (HA) is a pervasive, naturally occurring organic derivative found in aquatic and terrestrial environments; human activity, however, can lead to elevated levels of HA. Recent studies suggest that environments that contain high levels of HA may hinder chemical communication. We tested the ability of adult zebrafish (*Danio rerio)* to discriminate between conspecific and heterospecific urinary chemical cues found in the presence and absence of HA. We show that high humic acid levels (200 mg/l) can impair the ability to differentiate conspecifics from heterospecifics. We also found that zebrafish prefer untreated water over HA-treated water. These findings suggest that, in addition to human-produced synthetic compounds, changes in the abundance of naturally occurring substances may also negatively impact natural behaviors in aquatic species by disturbing the sensory environment.
Descriptors: zebrafish, humic acid, species recognition, chemosensation, pollution, synthetic compounds.

Fraysse, B., R. Mons, and J. Garric (2006). **Development of a zebrafish 4-day embryo-larval bioassay to assess toxicity of chemicals.** *Ecotoxicology and Environmental Safety* 63(2): 253-67. ISSN: 0147 6513.
NAL Call Number: QH545.A1E29
Abstract: A 4-day embryo-larval zebrafish test, from blastula stage to hatching included, was developed. The observations of embryo developmental were made at different development stages, for which morphological, physiological, and behavioral endpoints were selected and quantified for unexposed and exposed embryos. The sensitivity and the ability of these endpoints to inform about mode of action (MoA) were established in testing three model toxicants with well-known toxic effects (propranolol, malathion, cadmium). Lethal, sublethal (heart rate/edema, spontaneous movements, and hatching rate/time disturbance), and teratogenic effects were detected for all the studied compounds. This bioassay allows characterization of impairments at different biological levels: neuromuscular, physiological, morphological, and behavioral, and brings useful information about the toxic MoA of the chemicals on nontarget organisms. In this sense to answers the chemical industries and international organization (EMEA) requirements for the environmental risk assessment of new chemicals and pharmaceuticals.
Descriptors: zebrafish, embryo, drug effects, toxicity tests methods, biological assay, cadmium

toxicity, embryonic development drug effects, heart rate drug effects , malathion toxicity, propranolol toxicity, tail drug effects, tail growth and development.

Gerlach, G. (2006). **Pheromonal regulation of reproductive success in female zebrafish: female suppression and male enhancement**. *Animal Behaviour* 72(5): 1119-1124. ISSN: 0003-3472.
NAL Call Number: 410 B77
Descriptors: zebrafish, breeding success, competition, competitors, dominance, eggs, females, fish eggs, freshwater fish, injuries, males, mating, pheromones, progeny, reproduction, reproductive behavior, sexual selection, *Danio rerio*, Teleostei.

Gerlach, G., A. Hodgins Davis, B. MacDonald, and R.C. Hannah (2007). **Benefits of kin association: related and familiar zebrafish larvae (*Danio rerio*) show improved growth.** *Behavioral Ecology and Sociobiology* 61(11): 1765-70. ISSN: 0340 5443.
NAL Call Number: QL751.B4
Abstract: Zebrafish (Danio rerio) larvae prefer the olfactory cues of kin to non-kin. We examined the potential benefits of kin preference by comparing growth rate, shoaling, and aggressive behavior in juvenile zebrafish housed in groups of either familiar kin or unfamiliar non-kin. Over an observation period of 5 days, the animals grew 33% more in kin groups; however, neither shoaling nor the frequency of aggressive interactions was different in groups of related versus unrelated individuals. Shoaling behavior increased with increasing observation time and increasing age, while aggressive behavior remained the same. We conclude that associating with kin probably creates a less stressful environment that allows for higher growth rates, which can lead to higher direct fitness based on increased survival and earlier reproduction. Kin recognition leading to kin-structured groups may therefore be under positive selection.
Descriptors: zebrafish, kin recognition, kin selection , *Danio rerio*, aggression, shoaling, growth rates, internet resource.

Hallare, A., K. Nagel, H. Kohler, and R. Triebskorn (2006). **Comparative embryotoxicity and proteotoxicity of three carrier solvents to zebrafish (*Danio rerio*) embryos.** *Ecotoxicology and Environmental Safety* 63(3): 378-88. ISSN: 0147 6513.
NAL Call Number: QH545.A1E29
Abstract: The present study examines the effects of ethanol (ETOH), dimethyl sulfoxide (DMSO), and acetone on zebrafish embryos and the implications of the observed results On the use of these solvents to zebrafish early life stage tests. The embryos were exposed to different concentrations (0.0, 0.0001, 0.001, 0.01, 0.1, 0.05, 1, 1.5, and 2.0% v/v) of the respective solvents by diluting reagent-grade solvent with reconstituted water [DIN 38415-6-Suborganismische Testverfahren (Gruppe T) Tell 6: Giftigkeit gegenuber Fischen. Deutsches institute fur Normung e.V]. The following endpoints were investigated (mortality, hatching rate, abnormalities, heart rate, and hsp 70 induction). No effect on survival was recorded for both acetone and DMSO even up to the highest concentration. On the other hand, embryos exposed to 1.5% and 2.0% ethanol showed a significant reduction in Survival rate. No developmental defects occurred with any of the solvents at the 0.1% concentration. However, starting with 1.0%, weak to very pronounced abnormalities (weak pigmentation, edema, crooked bodies, eye defect, tail defect, reduced heartbeat, and abnormal hatching) were observed depending on the solvent type and the concentration used. Ethanol has been shown to be the most embryotoxic solvent while DMSO and acetone have comparably lesser effects. Heat shock protein 70 was induced by all solvents but at different concentration ranges. DMSO has been shown to be the most potent inducer of stress proteins. Based oil the study, the chemicals tested here may be used as carrier solvents in the zebrafish embryo assay at levels below 1.5, 1.5, and 1% v/v for acetone, DMSO, and ethanol,

respectively. For stress protein analysis of the exposed embryos, however, the solvent levels should be below 0.1%., 0.01%, and 1.5%, respectively. Additional and separate investigations utilizing other biomarkers should be carried out to further validate the suitability Of using these solvents in a typical zebrafish embryo assay.

Descriptors: zebrafish, toxicology , embryotoxicity, proteotoxicity, ethanol, dimethyl sulfoxide, acetone, survival.

Hallare, A.V., T. Kosmehl, T. Schulze, H. Hollert, H.R. Kohler, and R. Triebskorn (2005). **Assessing contamination levels of Laguna Lake sediments (Philippines) using a contact assay with zebrafish (*Danio rerio*) embryos.** *Science of the Total Environment* 347(1-3): 254-71. ISSN: 0048 9697.

NAL Call Number: RA565.S365

Abstract: The present study investigated the suitability of a sediment contact assay using zebrafish (*Danio rerio*) embryos to evaluate the degree of lake sediment contamination. As end-points, developmental parameters (mortality, abnormality, heart rate, and hatching rate) as well as stress protein responses (hsp 70 levels) in the developing embryos were recorded during a 96-h exposure. Fertilized zebrafish eggs were exposed to both the whole as well as organic extract concentrations prepared from collected sediments from 5 sites along Laguna Lake, Philippines. Compared to whole sediment exposure, more severe embryotoxic and teratogenic responses were elicited in embryos exposed to organic extracts. However, since whole sediment-exposed embryos also revealed significant developmental defects, this exposure phase served as the more realistic exposure scenario in our study. Weak to strong upregulation of hsp 70 levels was also registered among embryos exposed to both whole sediments and organic extracts. The observed embryotoxic and proteotoxic responses by zebrafish embryos to Laguna Lake sediment exposures were discussed in relation to the analyzed contaminants in the sediments (heavy metals, PAHs (perylene), solvent). Overall, the present study points out that the sediment contact assay with zebrafish embryos offers a practicable and highly sensitive bioassay for the general assessment of sediment toxicity.

Descriptors: zebrafish , geologic sediments, HSP70 heat shock proteins metabolism, water pollutants, lake sediment, chemical toxicity, carbon analysis, abnormalities, environmental monitoring, fresh water, heavy metals analysis, aromatic polycyclic hydrocarbons analysis, survival rate, chemical water pollutants analysis, zebrafish abnormalities, metabolism, Philippines.

Henry, T.B., F.M. Menn, J.T. Fleming, J. Wilgus, R.N. Compton, and G.S. Sayler (2007). **Attributing effects of aqueous C60 nano-aggregates to tetrahydrofuran decomposition products in larval zebrafish by assessment of gene expression.** *Environmental Health Perspectives* 115(7): 1059-65. ISSN: 0091 6765.

NAL Call Number: RA565.A1E54

Abstract: BACKGROUND: C(60) is a highly insoluble nanoparticle that can form colloidal suspended aggregates in water, which may lead to environmental exposure in aquatic organisms. Previous research has indicated toxicity from C(60) aggregate; however, effects could be because of tetrahydrofuran (THF) vehicle used to prepare aggregates. OBJECTIVE: Our goal was to investigate changes in survival and gene expression in larval zebrafish Danio rerio after exposure to aggregates of C(60) prepared by two methods: a) stirring and sonication of C(60) in water (C(60)-water); and b) suspension of C(60) in THF followed by rotovaping, resuspension in water, and sparging with nitrogen gas (THF-C(60)). RESULTS: Survival of larval zebrafish was reduced in THF-C(60) and THF-water but not in C(60)-water. The greatest differences in gene expression were observed in fish exposed to THF-C(60) and most (182) of these genes were similarly expressed in fish exposed to THF-water. Significant up-regulation (3- to 7-fold) of genes

involved in controlling oxidative damage was observed after exposure to THF-C(60) and THF-water. Analyses of THF-C(60) and THF-water by gas chromatography-mass spectrometry did not detect THF but found THF oxidation products gamma-butyrolactone and tetrahydro-2-furanol. Toxicity of gamma-butyrolactone (72-hr lethal concentration predicted to kill 50% was 47 ppm) indicated effects in THF treatments can result from gamma-butyrolactone toxicity. CONCLUSION: This research is the first to link toxic effects directly to a THF degradation product (gamma-butyrolactone) rather than to C(60) and may explain toxicity attributed to C(60) in other investigations.

Descriptors: zebrafish, fullerenes toxicity, furans toxicity, gene expression drug effects, larva drug effects, furans metabolism, larva metabolism, nanoparticles, oligonucleotide array sequence analysis, water.

Notes: The present work was first presented at the meeting "Overcoming Obstacles to Effective Research Design in Nanotoxicology" held 24-26 April 2006 in Cambridge, Massachusetts, USA.

Incardona, J.P., M.G. Carls, H. Teraoka, C.A. Sloan, T.K. Collier, and N.L. Scholz (2005). **Aryl hydrocarbon receptor-independent toxicity of weathered crude oil during fish development**. *Environmental Health Perspectives* 113(12): 1755-1762. ISSN: 0091 6765.

NAL Call Number: RA565.A1E54

Abstract: Polycyclic aromatic hydrocarbons (PAHs), derived largely from fossil fuels and their combustion, are pervasive contaminants in rivers, lakes and nearshore marine habitats. Studies after the "Exxon Valdez" oil spill in Prince William Sound, Alaska, USA, demonstrated that fish embryos exposed to low levels of PAHs in weathered crude oil develop a syndrome of oedema and craniofacial and body axis defects. Although mechanisms leading to these defects are poorly understood, it is widely held that PAH toxicity is linked to aryl hydrocarbon receptor (AhR) binding and cytochrome P450 1A (CYP1A) induction. Using zebrafish (*Danio rerio*) embryos, it was shown that the weathered crude oil syndrome was distinct from the well-characterized AhR-dependent effects of dioxin toxicity. Blockade of AhR pathway components with antisense morpholino oligonucleotides demonstrated that the key developmental defects induced by weathered crude oil exposure were mediated by low molecular weight tricyclic PAHs through AhR-independent disruption of cardiovascular function and morphogenesis. These findings have multiple implications for the assessment of PAH impacts on coastal habitats. Copyright © 2008 CABI

Descriptors: zebrafish, abnormalities, aromatic hydrocarbons, biological development, cardiovascular system, embryos, fuel oils, morphogenesis, oedema, oil spills, polycyclic hydrocarbons, toxic substances, weathered crude oil, toxicity, transcription factors, *Danio rerio*, fishes.

Le Page, Y., M. Scholze, O. Kah, and F. Pakdel (2006). **Assessment of xenoestrogens using three distinct estrogen receptors and the zebrafish brain aromatase gene in a highly responsive glial cell system.** *Environmental Health Perspectives* 114(5): 752-8. ISSN: 0091 6765.

NAL Call Number: RA565.A1E54

Abstract: The brain cytochrome P450 aromatase (Aro-B) in zebrafish is expressed in radial glial cells and is strongly stimulated by estrogens (E2); thus, it can be used in vivo as a biomarker of xenoestrogen effects on the central nervous system. By quantitative real-time polymerase chain reaction, we first confirmed that the expression of Aro-B gene is robustly stimulated in juvenile zebrafish exposed to several xenoestrogens. To investigate the impact of environmental estrogenic chemicals on distinct estrogen receptor (ER) activity, we developed a glial cell-based assay using Aro-B as the target gene. To this end, the ER-negative glial cell line U251-MG was transfected with the three zebrafish ER subtypes and the Aro-B promoter linked to a luciferase reporter

gene. E2 treatment of U251-MG glial cells cotransfected with zebrafish ER-alpha and the Aro-B promoter-luciferase reporter resulted in a 60- to 80-fold stimulation of luciferase activity. The detection limit was <0.05 nM, and the EC50 (median effective concentration) was 1.4 nM. Interestingly, in this glial cell context, maximal induction achieved with the Aro-B reporter was three times greater than that observed with a classical estrogen-response-element reporter gene (ERE-tk-Luc). Dose-response analyses with ethynylestradiol (EE2), estrone (E1), alpha-zeralenol, and genistein showed that estrogenic potency of these agents markedly differed depending on the ER subtype in the assay. Moreover, the combination of these agents showed an additive effect according to the concept of concentration addition. This confirmed that the combined additive effect of the xenoestrogens leads to an enhancement of the estrogenic potency, even when each single agent might be present at low effect concentrations. In conclusion, we demonstrate that our bioassay provides a fast, reliable, sensitive, and efficient test for evaluating estrogenic potency of endocrine disruptors on ER subtypes in a glial context.

Descriptors: zebrafish, radial glial cells, aromatase genetics, brain enzymology, estrogens toxicity, neuroglia drug effects, estrogen receptors genetics, xenobiotics toxicity, base sequence, cell line, DNA primers, drug dose response relationship, site directed mutagenesis neuroglia cytology, neuroglia enzymology, neuroglia metabolism.

Marks, C., T.N. West, B. Bagatto, and F.B.G. Moore (2005). **Developmental environment alters conditional aggression in zebrafish.** *Copeia*(4): 901-908. ISSN: 0045 8511.

NAL Call Number: QL1

Abstract: The developmental environment influences a wide variety of phenotypic traits in the adults of many vertebrates (i.e., developmental plasticity). In this study, we test to see if developmental environment (EDEV) interacts with the adult behavioral environment (EBEHAV) in determining behavioral phenotypes. We reared Zebrafish (Danio rerio) from eggs in either continuously hypoxic or normoxic conditions. We then tested aggression and avoidance (i.e., hiding) levels of fish from each developmental treatment in both environments. Developmental environment was a significant source of variation in avoidance behavior while the stimulus environment did not influence avoidance. Without a period of acclimation we found that EBEHAV and an EDEV X EBEHAV interaction were both significant sources of variation. However, when the fish were allowed to physiologically acclimate to the environment for 16 h, aggression level was highest for fish tested in the environment in which they developed. In that case the EDEV X EBEHAV interaction was the only significant source of variation. These results demonstrate that a more complete understanding of phenotypic response can be gained by incorporating environmental conditions across multiple time scales.

Descriptors: zebrafish, *Danio rerio,* development, developmental environment, influence on adult aggression and avoidance behaviors, phenotype, gases.

Matz, C.J., R.G. Treble, and P.H. Krone (2007). **Accumulation and elimination of cadmium in larval stage zebrafish following acute exposure.** *Ecotoxicology and Environmental Safety* 66(1): 44-8. ISSN: 0147 6513.

NAL Call Number: QH545.A1E29

Abstract: A number of recent studies have examined the impact of acute cadmium exposure on early zebrafish development at the morphological, cellular, and molecular levels. However, no information on the accumulation and elimination of cadmium during early life stages of zebrafish development has been available. Here we have quantified cadmium accumulation in larval zebrafish (*Danio rerio*) by graphite furnace atomic absorption spectroscopy following short-term acute exposure and recovery periods. Zebrafish (80 h postfertilization) were exposed to various concentrations of cadmium (0.2, 1.0, 5.0, 25, 125 microM) for 3 h. Cadmium accumulation

in larvae increased with exposure concentration. After exposure at 5.0, 25, and 125 microM cadmium, the fish were allowed to recover in freshwater for 0, 12, or 24 h. Cadmium content did not show a statistically significant decrease over the recovery period when exposed to 5.0 or 25 microM cadmium, whereas significant losses over the recovery period were observed following 125 microM exposure. These results suggest that the larval zebrafish decrease total cadmium body burden only following relatively high short-term acutely toxic exposures.
Descriptors: zebrafish growth and development, cadmium metabolism, cadmium toxicity, larva drug effects, larva metabolism, toxicity tests .

Mercy, A. (2004). **Status of standardized breeding and propagation technology of indigenous ornamental fishes of the Western Ghats of India**. *World Aquaculture* 35(4): 40-43. ISSN: 1041-5602.
NAL Call Number: SH1.W62
Descriptors: zebrafish, aquaculture development, aquaculture techniques, aquarium culture, endemic species, feeding behavior, freshwater aquaculture or geographical distribution, habitat, inland water environment, ornamental fish, rare species, river valleys, rivers, sexual maturity, sexual reproduction, species diversity, tropical fish, *Danio malabaricus*, *Danio rerio*, *Garra mullya*, *melanostigma*, *Nemacheilus triangularis*, *Puntius conchonius*, *Puntius filamentosus*.

Micael, J., M.A. Reis Henriques, A.P. Carvalho, and M.M. Santos (2007). **Genotoxic effects of binary mixtures of xenoandrogens (tributyltin, triphenyltin) and a xenoestrogen (ethinylestradiol) in a partial life-cycle test with zebrafish (*Danio rerio*).** *Environment International* 33(8): 1035-9. ISSN: 0160 4120.
NAL Call Number: TD169.E54
Abstract: A partial life-cycle test with the model fish *Danio rerio* was performed in order to evaluate the genotoxic potential of binary mixtures of xenoandrogenic (tributyltin--TBT; triphenyltin--TPT) and an estrogenic compound (ethinylestradiol--EE2). Five days post-fertilisation larvae were diet-exposed to environmental relevant concentrations of TBT and TPT (25 ng/g-100 ng/g), and water-exposed to ethinylestradiol (3.5 ng/L) for a four-month period; binary mixtures of TBT plus EE2 and TPT plus EE2 were run in parallel. The erythrocytic nuclear abnormalities (ENA) assay in circulating erythrocytes was used to evaluate genotoxicity in the end of the four-month exposure period. A significant increase ($p<0.05$, Kruskall-Wallis non-parametric ANOVA) in ENA frequency, in comparison with control animals, was observed in those animals exposed to TBT and TPT (the highest doses only), and to EE2 and the binary mixtures, although neither synergistic nor additive effects of the tested compounds were evident. Overall, the results clearly indicate that chronic exposure to low levels of TBT, TPT, EE2 and binary mixtures of TBT plus EE2 and TPT plus EE2 are genotoxic to zebrafish, which may suggest that wild fish populations may be under increased DNA damage in areas contaminated by these endocrine disrupting chemicals.
Descriptors: zebrafish, genotoxic effects, mixture, xenoandrogens, tributyltin, triphenyltin, xenestrogen, ethinylestradiol.

Mills, L.J. and C. Chichester (2005). **Review of evidence: are endocrine-disrupting chemicals in the aquatic environment impacting fish populations?** *Science of the Total Environment* 343(1/3): 1-34. ISSN: 0048 9697.
NAL Call Number: RA565.S365
Abstract: In this paper, evidence from the current literature is presented that addresses either of two questions: (1) do EDCs in the aquatic environment have the potential to impact the reproductive health and survival of various fish species, and (2) are EDCs in the aquatic environ-

ment actually impacting the reproductive health and sustainability of indigenous populations of fish? Overall, data from laboratory experiments support the hypothesis that EDCs in the aquatic environment can impact the reproductive health of various fish species, but evidence that EDCs in the aquatic environment are actually impacting the reproductive health and sustainability of indigenous fish populations is less convincing. The scarcity of evidence linking impacts of environmental EDCs with changes in reproductive success of indigenous fish populations may reflect a critical need for a dependable method or indicator to assess reproduction of fish in situ. In addition, more studies that investigate whether fish populations routinely exposed to EDCs in situ are experiencing changes in population structure are needed. Linking endocrine disruption and reproductive impairment with an ecologically relevant impact on the sustainability of real fish populations remains, with few exceptions, an open challenge. Copyright © 2008 CABI
Descriptors: zebrafish, aquatic environment, chemicals, disruption, endocrine system, environmental impact, estradiol, estriol, estrone, methoxychlor, methyltestosterone, phenol, phthalates, polluted water, population structure, reproduction, reproductive performance, reviews, sewage effluent, survival, sustainability, testosterone, toxicity, water pollution, water quality, carp, *Danio rerio*, fishes, goldfish, *Oryzias latipes*, *Pimephales promelas*, *Poecilia reticulata*, rainbow trout.

Moretz, J.A., E.P. Martins, and B.D. Robison (2007). **The effects of early and adult social environment on zebrafish (*Danio rerio*) behavior.** *Environmental Biology of Fishes* 80(1): 91-101. ISSN: 0378 1909.
NAL Call Number: TD169.E54
Abstract: Because early social experience can have a profound effect on later mate and social choices, the availability of options and decisions made early in development can have major effects on adult behavior. Herein, we use strain differences among zebrafish, *Danio rerio*, as an experimental tool to test the effects of social experience on behavior. By manipulating the strain composition of groups in which the subject fish are housed at different ages, we tested (1) whether mixing with dissimilar individuals influenced subsequent behavior, (2) whether prolonged mixing during the juvenile stage had a more pronounced effect than a shorter period of mixing during adulthood and (3) whether mixing had a lasting effect after animals were resorted into groups of same strain animals. We found that social experience had a profound impact on social behavior. Both Nadia and TM1 individuals engaged in more frequent biting after having been in mixed strain groups compared to pure strain groups. This was true of groups mixed as juveniles, as well as adults, indicating that this response was not dependent on exposure during a critical developmental period. Also, TM1 fish (but not Nadia) having recently been housed in mixed-strain groups were more willing to leave the immediate vicinity of a shoal than were TM1 fish raised in pure strain groups. This change was more pronounced in groups mixed as juveniles than as adults. In addition, the observed changes persisted after mixed groups were separated into pure strain groups for a month. Other behavioral measures including Activity Level, Predator Response and Stress Recovery were unaffected by previous social experience.
Descriptors: zebrafish, aggression , *Danio rerio*, social-experience, boldness.

Moretz, J.A., E.P. Martins, and B.D. Robison (2007). **Behavioral syndromes and the evolution of correlated behavior in zebrafish**. *Behavioral Ecology* 18(3): 556-562. ISSN: 1045-2249.
NAL Call Number: QL750
Descriptors: zebrafish, aggression, aggressive behavior, biting, domestication, ecosystem disturbance, evolution, freshwater fish, interspecific relationships, personality, predators, schooling behavior, sex differences, social behavior, *Danio rerio*.

Nash, J.P., D.E. Kime, L.T.M.v.d. Ven, P.W. Wester, F. Brion, G. Maack, P. Stahlschmidt Allner, and C.R. Tyler (2004). **Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish**. *Environmental Health Perspectives* 112(17): 1725-1733. ISSN: 0091 6765.

NAL Call Number: RA565.A1E54

Abstract: Heightened concern over endocrine-disrupting chemicals is driven by the hypothesis that they could reduce reproductive success and affect wildlife populations, but there is little evidence for this expectation. The pharmaceutical ethynylestradiol (EE2) is a potent endocrine modulator and is present in the aquatic environment at biologically active concentrations. To investigate impacts on reproductive success and mechanisms of disruption, we exposed breeding populations (n=12) of zebrafish (*Danio rerio*) over multiple generations to environmentally relevant concentrations of EE2. Life-long exposure to 5 ng/L EE2 in the F1 generation caused a 56% reduction in fecundity and complete population failure with no fertilization. Conversely, the same level of exposure for up to 40 days in mature adults in the parental F0 generation had no impact on reproductive success. Infertility in the F1 generation after life-long exposure to 5 ng/L EE2 was due to disturbed sexual differentiation, with males having no functional testes and either undifferentiated or intersex gonads. These F1 males also showed a reduced vitellogenic response when compared with F0 males, indicating an acclimation to EE2 exposure. Depuration studies found only a partial recovery in reproductive capacity after 5 months. Significantly, even though the F1 males lacked functional testes, they showed male-pattern reproductive behaviour, inducing the spawning act and competing with healthy males to disrupt fertilization. Endocrine disruption is therefore likely to affect breeding dynamics and reproductive success in group-spawning fish. Our findings raise major concerns about the population-level impacts for wildlife of long-term exposure to low concentrations of oestrogenic endocrine disruptors. Copyright © 2008 CABI

Descriptors: zebrafish, exposure, fecundity, infertility, male animals, reproductive behavior, reproductive performance, sex differentiation, testes, water pollution, *Danio rerio*.

Njiwa, J.R., P. Muller, and R. Klein (2004). **Binary mixture of DDT and Arochlor1254: effects on sperm release by Danio rerio.** *Ecotoxicology and Environmental Safety* 58(2): 211-9. ISSN: 0147 6513.

NAL Call Number: QH545.A1E29

Abstract: A long-term toxicity test with zebrafish was carried out with different concentrations of DDT, Arochlor1254 (A54), and their 1:1 mixture under flow-through conditions. By collecting and counting the number of sperm released during separate mating events we observed that gametes are released asynchronously. Sperms are released in the form of sperm trails laid on the nest surface; subsequently active spermatozoa leave the trails and move in the water for several minutes. Sperm trails consist of bands of viscous material in which sperm are embedded. The water samples for the estimation of sperm presence were collected gradually within 180 min after 24 h, 2 weeks, 1 month, and 2 months of exposure. It was established that the reductions in count, activity of sperm, and average life span of sperm trails were significant ($P<0.05$) at the tested concentrations, 5 and 50 microg/L after 1 month and 2 weeks of exposure to each chemical tested and to their mixture, respectively. In conclusion, this study demonstrates that DDT and A54 could react synergically and increase the alteration in sperm release and activity as well as the life span of their trails.

Descriptors: zebrafish, aroclor 1254 pharmacology, ddt pharmacology, fertilization drug effects, sperm count veterinary, spermatozoa drug effects, aroclor 1254 toxicity, ddt toxicity, drug dose response relationship, drug combinations, drug synergism, estrogens pharmacology, estrogens toxicity, half life, sexual behavior drug effects, sperm motility drug effects, spermatozoa physiology,

time factors, chemical water pollutants pharmacology, chemical water pollutants toxicity, xenobiotics.

Peichel, C.L. (2004). **Social behavior: how do fish find their shoal mate?** *Current Biology* 14(13): R503-R504. ISSN: 0960-9822.
NAL Call Number: QH301.C85
Descriptors: zebrafish, animal morphology, coloration, experience, morphology, pattern recognition, pigments, schooling behavior, shoals, social behavior, *Danio rerio*.

Rodgher, S., E.L.G. Espindola, O. Rocha, R. Fracacio, R.H.G. Pereira, and M.H.S. Rodrigues (2005). **Limnological and ecotoxicological studies in the cascade of reservoirs in the Tiete River (Sao Paulo, Brazil)**. *Brazilian Journal of Biology* 65(4): 697-710. ISSN: 1519 6984.
NAL Call Number: QH301.B73
Abstract: An evaluation was made on the quality of samples of water and sediment collected from a series of reservoirs in the Tiete River (Sao Paulo, Brazil), based on limnological and ecotoxicological analyses. The samples were collected during two periods (February and July 2000) from 15 sampling stations. Acute toxicity bioassays were performed using the test organism Daphnia similis, while chronic bioassays were carried out with Ceriodaphnia dubia and Danio rerio larvae. The water samples were analysed for total nutrients, total suspended matter and total cadmium, chromium, copper and zinc concentrations, while the sediment samples were examined for organic matter, granulometry and potentially bioavailable metals (cadmium, chromium, copper and zinc). The results obtained for the limnological variable revealed differences in the water quality, with high contribution of nutrients and metals for Tiete and Piracicaba rivers, besides the incorporation and sedimentation, consequently causing a reduction of materials in Barra Bonita reservoir, thus promoting the improvement of the water quality in the other reservoirs. The toxicity bioassays revealed acute toxicity for Daphnia similis only in the reservoirs located below Barra Bonita dam. On the other hand, chronic toxicity for Ceriodaphnia dubia and acute for Danio rerio showed a different pattern, decreasing in magnitude from Barra Bonita to Tres Irimaos, demonstrating an environmental degradation gradient in the reservoirs. Copyright © 2008 CABI
Descriptors: zebrafish, bioassays, bioavailability, cadmium, chromium, copper, dams, heavy metals, limnology, nutrient availability, organic matter, polluted water, reservoirs, river water, sediment, suspended solids, toxicity, water quality, zinc, *CerioDaphnia dubia*, *Danio rerio*, *Daphnia*, Brazil.
Language of Text: Portuguese.

Ruhl, N. and S.P. McRobert (2005). **The effect of sexand shoal size on shoaling behaviour in *Danio rerio***. *Journal of Fish Biology* 67(5): 1318-1326. ISSN: 0022 1112.
NAL Call Number: QL614.J68
Abstract: Male and female zebra fish *Danio rerio* were given choices of shoals that differed in sex and size. Male zebra fish preferred to associate with female shoals over male shoals, but had no preference when given a choice between a mixed-sex shoal and either a male or female shoal. Female zebra fish showed no significant preference when given a choice between male and females shoals, nor between mixed-sex shoals and either male or female shoals. When given choices between shoals of differing size, females preferred to associate with the larger shoal, whether or not they were composed of males or females. Males, however, had no preference for larger shoals over smaller shoals, whether or not they were composed of males or females. These results showed that male zebra fish were capable of distinguishing between males and females solely on the basis of visual cues. Furthermore, these results demonstrated a significant difference between the shoal-

ing choices of male and female zebra fish, which may indicate a difference in the function of shoaling for the two sexes. Copyright © 2008 CABI
Descriptors: zebrafish, behavior, sex differences, social behavior, *Danio rerio*.

Sato-Maeda, M., M. Obinata, and W. Shouji (2005). **Position adjustment of zebrafish cap primary motoneuron in spinal cord may be influenced by sema3a2**. *Zoological Science* 22(12): 1472. ISSN: 0289 0003.
NAL Call Number: 442P92
Descriptors: zebrafish, nervous system, neural coordination, freshwater ecology, ecology , environmental sciences, motoneuron, position adjustment.

Segner, H., K. Caroll, M. Fenske, C.R. Janssen, G. Maack, D. Pascoe, C. Schafers, G.F. Vandenbergh, M. Watts, and A. Wenzel (2003). **Identification of endocrine-disrupting effects in aquatic vertebrates and invertebrates: report from the European IDEA project**. *Ecotoxicology and Environmental Safety* 54(3): 302-314. ISSN: 0147-6513.
NAL Call Number: QH545.A1E29
Descriptors: zebrafish , aquatic organisms, behavior, bioassays, biological development, breeding success, cell differentiation, endocrine system, exposure, fertilization, fish, fish, hatching, invertebrates, life cycle analysis, model studies , pollution effects, reproduction, safety, sexual reproduction, spawning, stages, testing procedures, toxicity testing, toxicity tests, vitellogenesis, xenobiotics, *Chironomus riparius*, *Danio rerio*, *Eisenia foetida*, *gammarus pulex*, *Hyalella azteca*, *Hydra vulgaris*, *Lymnaea stagnalis*, Bisphenol A, endocrine disruptors, estrogenic activity, Ethynylestradiol, IDEA project, Octylphenol, Swamp lymnaea.

Segner, H., J.M. Navas, C. Schafers, and A. Wenzel (2003). **Potencies of estrogenic compounds in in vitro screening assays and in life cycle tests with zebrafish in vivo.** *Ecotoxicology and Environmental Safety* 54(3): 315-22. ISSN: 0147 6513.
NAL Call Number: QH545.A1E29
Abstract: The objective of this study was to compare the estrogenic potency of environmental estrogens at two testing tiers: at the initial level of in vitro screening assays, and at the level of definitive fish reproduction tests in vivo. The in vitro tests included a recombinant yeast estrogen receptor (ER) assay, a competitive radioreceptor assay using the hepatic ER of carp (Cyprinus carpio), and assays on vitellogenin induction in cultured hepatocytes of rainbow trout (Oncorhynchus mykiss) and carp. In vivo, full life cycle tests with zebrafish (Danio rerio) were performed, using fertilization success as estrogen-sensitive reproductive endpoint. The test compounds included the natural estrogen 17beta-estradiol (E2) (only applied in the in vitro assays); the synthetic estrogen ethynylestradiol (EE2); and two xenoestrogens, 4-tert-octylphenol (OP) and bisphenol A (BPA). Among the in vitro assays, differences were observed in the relative ranking of the test substances, and in the absolute sensitivity (EC50 values), although the interassay differences of EC50 values were within one order of magnitude. The in vivo activity of the test compounds was not accurately predicted by the in vitro assays, with respect to neither sensitivity nor ranking. The in vitro assays tended to overestimate the relative potency of the xenoestrogens; i.e. the ratio between the activity of the reference compound, EE2, and that of the test compound. The best prediction of the in vivo fish test results was obtained from the recombinant yeast assay.
Descriptors: zebrafish, estrogens adverse effects, life cycle stages, reproduction drug effects, chemical water pollutants adverse effects, xenobiotics adverse effects, carps, cell culture techniques, hepatocytes, lethal dose 50, oncorhynchus mykiss, reference values.

Spence, R., M.K. Fatema, M. Reichard, K.A. Huq, M.A. Wahab, Z.F. Ahmed, and C. Smith (2006). **The distribution and habitat preferences of the zebrafish in Bangladesh**. *Journal of Fish Biology* 69(5): 1435-1448. ISSN: 0022-1112.
NAL Call Number: QL614.J68
Descriptors: zebrafish, aquatic plants, biological surveys, ecological distribution, flood plains, habitat preferences, habitat selection, lakes, ponds, river discharge, vegetation, vertical distribution, water column, *Danio rerio*, *Oryza sativa*.

Spence, R. and C. Smith (2006). **Mating preference of female zebrafish, *Danio rerio*, in relation to male dominance.** *Behavioral Ecology* 17(5): 779-783. ISSN: 1045 2249.
NAL Call Number: QL750
Abstract: Mating success tends to be skewed toward dominant males, though female mate preferences may not always correlate with male dominance. In this study, we investigated the mating preferences of female zebrafish, *Danio rerio*, in the absence of male-male competition. We paired females sequentially with males of known dominance rank, using a nested, repeated measures design, with egg production as a measure of female mate preference. We predicted that females would spawn more frequently and produce larger clutches when paired with males of higher dominance rank. We found significant differences among females in the size of clutches produced and among males in the size of clutches received, but these differences were independent of male dominance rank. Male body size was not related to either dominance rank or clutch size received. These results indicate that females vary clutch size in relation to the males with which they are paired but that they do not favor dominant males. Thus, male competition may normally override female mate preference in zebrafish.
Descriptors: zebrafish, *Danio rerio*, pair formation, mate preference, females, relationship with male dominance, social hierarchy.

Spence, R. and C. Smith (2007). **The role of early learning in determining shoaling preferences based on visual cues in the zebrafish, *Danio rerio.*** *Ethology* 113(1): 62-7. ISSN: 0179 1613.
NAL Call Number: QL750.E74
Abstract: Social preferences for specific colour patterns learned in early development, termed imprinting, may influence social and mating decisions. Imprinting has been demonstrated in both birds and fish and has been proposed to play a role in speciation. We raised wild-type zebrafish, *Danio rerio*, either with individuals of the same colour pattern, an alternative colour pattern (leopard), or a closely related, unpatterned species, *D. albolineatus*. We also cross-reared individual 'leopard' *D. rerio* and *D. albolineatus* with wild-type *D. rerio.* We tested the prediction that *Danio* would prefer to shoal with fish of the colour pattern with which they had been raised, irrespective of their own appearance. Rearing condition affected shoaling preferences between *D. rerio* and *D. albolineatus*, with individuals of both colour patterns preferring to associate with shoals of the colour pattern with which they had been raised. The more subtle distinction between the wild-type and 'leopard' *D. rerio* colour patterns did not elicit a shoaling preference. Thus, zebrafish exhibit shoaling preferences based on visual cues, the effect being stronger when patterns are more distinct. There is a strong learned component to these preferences, although the extent to which they may influence mating decisions is unclear.
Descriptors: zebrafish, color pattern, imprinting, shoaling, behavior, color pattern preference, visual cues, learned behavior.

Tao Liao, Shiwei Jin, F.X. Yang, Yang Hui, and Ying Xu (2006). **An enzyme-linked immunosorbent assay for rare minnow (*Gobiocypris rarus*) vitellogenin and comparison of vitellogenin responses in rare minnow and zebrafish (*Danio rerio*).** *Science of the Total Environment* 364(1-

3): 284-94. ISSN: 0048-9697.
NAL Call Number: RA565.S365
Abstract: A competitive enzyme-linked immunosorbent assay (ELISA) was developed to determine vitellogenin (Vtg) in rare minnow (*Gobiocypris rarus*) based on the separation and purification of rare minnow Vtg (r-Vtg) as well as the production of polyclonal antibody against r-Vtg in rabbits. Three different ELISAs for measuring r-Vtg were then compared: (1) indirect ELISA with the antibody against carp *(Cyprinus carpio*) Vtg (c-Vtg) (IC-ELISA); (2) competitive ELISA with the antibody against c-Vtg, and using r-Vtg for coating the plates and preparing standard curve (CC-ELISA); (3) competitive ELISA with the antibody against r-Vtg, and using r-Vtg for coating the plates and preparing standard curve (CR-ELISA). The result showed that the homologous CR-ELISA was the most sensitive among the three assays for quantifying r-Vtg. The sensitivities to 17alpha-ethinylestradiol (EE(2)) of rare minnow and zebrafish (Danio rerio) were compared upon the establishment of homologous competitive ELISA. The lowest observed effect concentrations (LOECs) to induce Vtg were found to be 0.8 ng EE(2) l(-1) for rare minnow and 4 ng EE(2) l(-1) for zebrafish respectively. Afterwards, CR-ELISA was applied to measure Vtg concentration in whole body homogenate (WBH) of juvenile rare minnow fed by three diets (tubifex from wastewater treatment plant, Artemia nauplii and commercial pellet food), and the agreements between bioassay and GC-MS analysis demonstrated that rare minnow was a sensitive fish model for assessing estrogenic effects of endocrine disrupting compounds in aquatic environment.
Descriptors: zebrafish, Cyprinidae physiology, ELISA (ELISA) methods, vitellogenesis physiology, vitellogenins metabolism, animal feed analysis, environmental monitoring methods, estradiol pharmacology, estradiol congeners analysis, estrogens analysis, hormone antagonists analysis, sensitivity and specificity, species specificity, vitellogenesis drug effects, vitellogenins analysis, vitellogenins immunology, chemical water pollutants administration and dosage, chemical water pollutants analysis, endocrine disrupters.

Tiedeken, J.A. and J.S. Ramsdell (2007). **Embryonic exposure to domoic acid increases the susceptibility of zebrafish larvae to the chemical convulsant pentylenetetrazole.** *Environmental Health Perspectives* 115(11): 1547-52. ISSN: 0091 6765.
NAL Call Number: RA565.A1E54
Abstract: BACKGROUND: Domoic acid (DA) is a neurotoxin produced by diatoms of the genus *Pseudo-nitzschia* that targets the limbic system to induce tonic-clonic seizures and memory impairment. In utero DA exposure of mice leads to a reduction in seizure threshold to subsequent DA exposures in mid-postnatal life, and similar studies have shown neurotoxic effects in rats that were delayed until adolescence. OBJECTIVE: We used in ovo microinjection of zebrafish (*Danio rerio*) to characterize the effect of embryonic exposure of DA on seizure-inducing agents later in life as an alternative species model to screen environmental contaminants that might induce a fetal-originating adult disease. METHODS: Embryos were microinjected within hours of fertilization to DA concentrations ranging from 0.12 to 1.26 ng/mg egg weight. Seven days later, the larval animals were characterized for sensitivity to the chemical convulsant pentylenetetrazole (PTZ), an agent that is well-defined in laboratory rodents and, more recently, in zebrafish. RESULTS: In ovo DA exposure, most significantly at 0.4 ng/mg, reduces the latency time until first PTZ seizure in larval fish and increases the severity of seizures as determined by seizure stage and movement parameters. The interaction between in ovo DA exposure and PTZ caused seizure behaviors to individually asymptomatic doses of PTZ (1.0 and 1.25 mM) and DA (0.13 and 0.22 ng/mg). CONCLUSION: These studies demonstrate that in ovo exposure to DA reduces the threshold to chemically induced seizures in larval fish and increases the severity of seizure behavior in a manner that is consistent with in utero studies of laboratory rodents.

Descriptors: zebrafish, embryo, exposure, domoic acid, susceptibility, zebrafish larvae, chemical convulsant, pentylenetetrazole.

Tsujimura, T., A. Chinen, and S. Kawamura (2005). **Identification of a lcr-like distal regulatory region for multiple green-sensitive opsin genes in zebrafish**. *Zoological Science* 22(12): 1511. ISSN: 0289 0003.
NAL Call Number: 442P92
Descriptors: zebrafish, freshwater ecology, ecology, environmental sciences, molecular genetics.

Tyminska, A., H. Mazur-Marzec, M. Graczyk, and M. Plinski (2005). **Effect of two different Nodularia spumigena extracts on *Danio rerio* egg mortality and hatching time**. *Oceanological and Hydrobiological Studies* 34(Suppl. 3): 149-159. ISSN: 1730-413X.
Descriptors: zebrafish, abnormalities, algae, algal blooms, amino acids, biological damage, freshwater fish, hatching, peptides, *Danio rerio*, *Nodularia spumigena*.

Van den Belt, K., R. Verheyen, and H. Witters (2003). **Comparison of vitellogenin responses in zebrafish and rainbow trout following exposure to environmental estrogens.** *Ecotoxicology and Environmental Safety* 56(2): 271-81. ISSN: 0147 6513.
NAL Call Number: QH545.A1E29
Abstract: In this comparative study, the suitability of the commonly used in vivo biomarker for estrogenicity, vitellogenin (VTG), upon waterborne exposure to known environmental estrogens is evaluated in both male zebrafish (*Danio rerio*) and juvenile rainbow trout (*Oncorhynchus mykiss*). The results from initial experiments in which both species were exposed to 4-tert-octylphenol (OP) or 17 alpha-ethynylestradiol under semistatic conditions for 3 weeks demonstrated a difference in species sensitivity for OP exposure. Additional dose-response studies (semistatic, 3 weeks) with 4-nonylphenol (20, 100, and 500 microg/L), bisphenol A (40, 200, and 1000 microg/L), dibutylphthalate (40, 200, and 1000 microg/L DBP), and 17beta-estradiol (20 and 100 ng/L E2) were conducted. All these compounds, except for DBP, were found to be estrogenic to both fish species. The results demonstrated a difference in species sensitivity for NP with the zebrafish being about 5 times less sensitive. For the other compounds tested, no indications for a difference in species sensitivity was found. The results from this study demonstrated that both fish species can be used for the detection of VTG as biomarker for estrogenicity, taken into the potential interspecies differences in sensitivity which might be important for the evaluation of fish population effects.
Descriptors: zebrafish, environmental exposure, estrogens toxicity, ethinyl estradiol toxicity, *Oncorhynchus mykiss* physiology, phenols toxicity, surface active agents toxicity, vitellogenins biosynthesis.

Van den Belt, K., R. Verheyen, and H. Witters (2003). **Effects of 17alpha-ethynylestradiol in a partial life-cycle test with zebrafish (*Danio rerio*): effects on growth, gonads and female reproductive success.** *Science of the Total Environment* 309(1-3): 127-37. ISSN: 0048-9697.
NAL Call Number: RA565.S365
Abstract: In this study, the impact of an exposure of zebrafish embryos (*Danio rerio)* until 3 months post fertilization to 17alpha-ethynylestradiol (EE2: 0; 0,1; 1; 10; 25 ng/l) was evaluated for growth and development, gonad development and body vitellogenin (VTG) content. After a recovery period of 5 months, the female reproductive success was evaluated. The results demonstrated a significant reduction in total body length, body weight, whole body Ca and P content and an increase in morphological abnormalities for fish exposed to 25 ng/l EE2 as a function of exposure time. An increase in total body VTG content was observed for fish exposed for a period

of 3 months down to levels of 1 ng/l EE2. At the age of 3 months, a dose-dependent increase of the number of fish with no macroscopic recognizable gonads was observed (up to 100% at 25 ng/l EE2). After a recovery period on clean tap water for 5 months, all fish had developed either ovaries or testis with a gonadosomatic index not different from control fish and the sex ratio was similar in EE2 treatment groups and controls. Nevertheless, a reduced number of spawning females and a reduced egg production were found for the female fish exposed to 10 or 25 ng/l EE2 for 3 months and which were allowed to recover for 5 months. Although the underlying mechanism could not be elucidated, these findings did indicate that the reproduction potential of fish populations might be disturbed by a long-term exposure to EE2 (> or =10 ng/l) from fertilization until sexual maturity.
Descriptors: zebrafish, environmental exposure, estradiol congeners adverse effects, ethinyl estradiol adverse effects, fertilization drug effects, gonads growth and development, sexual maturation drug effects, drug dose response relationship, gonads drug effects, life cycle stages, population dynamics, vitellogenins analysis.

Virta, V.C. and M.S. Cooper (2005). **Ontogeny and phylogeny of the yolk extension in cypriniform fishes**. *Integrative and Comparative Biology* 45(6): 1206. ISSN: 1540 7063.
NAL Call Number: QL1.I67
Descriptors: zebrafish, development, population studies, marine ecology, environmental sciences, evolution and adaptation, phylogeny, ontogeny, gastrulation evolution, yolk syncytial layer morphogenesis.

Witten, P.E., A. Huysseune, and B.K. Hall (2003). **A cellular bone and mononucleated osteoclasts in advanced teleosts: new characters or adaptations resulting from heterochrony?** *Integrative and Comparative Biology* 43(6): 916. ISSN: 1540 7063.
NAL Call Number: QL1.I67
Descriptors: zebrafish, metabolism, skeletal system, movement and support, cell biology, marine ecology, environmental sciences, evolution and adaptation, bone resorption, mineral metabolism, heterochrony , bone metabolic activity, stable mineral rich marine environment, skeletal features.

Wright, D., L.B. Rimmer, V.L. Pritchard, J. Krause, and R.K. Butlin (2003). **Inter and intra-population variation in shoaling and boldness in the zebrafish (*Danio rerio*).** *Naturwissenschaften* 90(8): 374-377. ISSN: 0028 1042.
NAL Call Number: 474 N213
Abstract: Population differences in anti-predator behaviour have been demonstrated in several species, although less is known about the genetic basis of these traits. To determine the extent of genetic differences in boldness (defined as exploration of a novel object) and shoaling within and between zebrafish (Danio rerio) populations, and to examine the genetic basis of shoaling behaviour in general, we carried out a study that involved laboratory-raised fish derived from four wild-caught populations. Controlling for differences in rearing environment, significant inter-population differences were found in boldness but not shoaling. A larger shoaling experiment was also performed using one of the populations as the basis of a North Carolina type II breeding design (174 fish in total) to estimate heritability of shoaling tendency. A narrow-sense heritability estimate of 0.40 was obtained, with no apparent dominance effects.
Descriptors: zebrafish, *Danio rerio*, behavioral genetics variation, boldness and shoaling variation, inter and intrapopulation variation, genetics relations, behavior , boldness, schooling, shoaling, Bangladesh, Nepal.

Zhiyi Rong and Haowen Yin (2004). **A method for genotoxicity detection using random amplified polymorphism DNA with *Danio rerio***. *Ecotoxicology and Environmental Safety* 58(1): 96-103. ISSN: 0147 6513.

NAL Call Number: QH545.A1E29

Abstract: The increasing presence of genotoxic pollutants in the aquatic environment has resulted in the development of quick monitoring methods. We applied random amplified polymorphism DNA (RAPD) method to evaluate the toxic effects of genotoxic chemicals. In 22 normal wide-type D. rerio (zebrafish), 78 amplified bands were obtained after PCR using the primer set, in which the frequency of emergence of more than 60% is 21. Four stable bands were observed in the amplified product, which appeared in all normal test samples. The zebrafish RAPD fingerprinting database was constructed based on data obtained. The RAPD pattern from zebrafish exposed to genotoxic chemicals, such as cyclophosphamide in dilution series ranging from 0.1 to 10 mg/litre and dimethoate in dilution series ranging from 1.0 to 100 mg/litre, displayed some changes in polymorphism band patterns, including the loss of stable bands. A distinct distance between the band patterns of exposed zebrafish and the control samples was also observed via the cluster method. The result derived from numerical analysis is more sensitive to the result obtained from stable band analysis because it can reveal the distance between the band pattern of the zebrafish exposed to 0.05 mg cyclophosphamide/litre and the control sample. These results proved the accuracy and the sensitivity of the two analyses of genotoxicity effect based on RAPD fingerprinting research.
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Descriptors: zebrafish, *Danio rerio*, cluster analysis, contaminants, cyclophosphamide, dimethoate, DNA, DNA fingerprinting, genetic distance, genetic markers, genetic polymorphism, genotoxicity, methodology, nontarget organisms, pesticide residues, pollutants, PCR, random amplified polymorphic DNA, techniques, toxic substances.

Zoller, U. (2006). **Estuarine and coastal zone marine pollution by the nonionic alkylphenol ethoxylates endocrine disrupters: is there a potential ecotoxicological problem?** *Environment International* 32(2): 269-272. ISSN: 0160 4120.

NAL Call Number: TD169.E54

Abstract: The nonionic biodegradation-resistant ("hard") alkylphenol ethoxylate (APEO) surfactants and their degradation products are known endocrine disrupting chemicals (EDCs). We report here the findings concerning the APEOs concentrations and homologic distribution profiles in Israel's estuarine and coastal zone seawater to serve as a case study. The concentrations in sewage-containing rivers, estuaries and 50-60-m offshore sea (Mediterranean) water were found to be 12.5-75.1, 4.2-25.0 and 0.9-2.6 micro g/L, respectively. The corresponding homologic distribution profiles were found to be within the range of 1-10% each, somewhat skewing, as expected, towards the more toxic shorter-chain ethoxylates. Egg production by zebrafish, exposed to 75, 25 and 10 micro g/L of a typical industrial APEOs was reduced up to 89.6%, 84.7% and 76.9%, respectively, between the 8th and 28th days of exposure. Apparently, there is a potential APEOs-related ecotoxicological health risk problem.
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Descriptors: zebrafish, biodegradation, coastal areas, egg production, estuaries, exposure, marine environment, nonionic surfactants, polluted water, rivers, sea water, sewage effluent, water pollution, water quality, *Danio rerio*.

Notes: Special issue:Marine pollution and ecotoxicology.

Zon, L.I. (2006). **An amazing week**. *BioTechniques* 40(2): 131. ISSN: 0736 6205.
NAL Call Number: RB37.1B56
Descriptors: zebrafish, freshwater ecology, environmental sciences, molecular genetics, molecular biophysics, genome.

Hematology

Auer, R.L., S. Riaz, and F.E. Cotter (2007). **The 13q and 11q B-cell chronic lymphocytic leukaemia-associated regions derive from a common ancestral region in the zebrafish.** *British Journal of Haematology* 137(5): 443-53. ISSN: 0007 1048.

Abstract: Loss of the long arm of chromosomes 11 and 13 is the most consistent cytogenetic abnormalities for patients with B-cell chronic lymphocytic leukaemia (B-CLL). They suggest the presence of as yet unidentified tumour suppressor genes within well-defined minimal-deleted regions (MinDRs). We have identified 38 orthologues of the human genes in MinDRs in zebrafish cDNA and syntenic regions for the human deletions in the zebrafish genome. One region on chromosome 9 in the zebrafish genome is of potential interest. Within chromosome 9, five genes and two microRNAs were identified with shared synteny to the MinDRs in B-CLL (two genes to human chromosome 11, three to human chromosome 13 and two chromosome 13 microRNAs). The critical region on zebrafish chromosome 9 maps to the MinDR for both human chromosomes, suggesting a common ancestry for B-CLL tumour suppressor genes. Target-selected mutagenesis to identify zebrafish mutants with knock-outs of genes in this region will allow analysis of their in vivo potential for lymphoproliferation and may define causative genes for B-CLL within human chromosomes 11q and 13q. Our study provides an explanation for involvement of both 11q and 13q in B-CLL and the potential to develop animal models for this common lymphoproliferative disorder.

Descriptors: zebrafish, chickens, chromosomes, tumor suppressor genes, B-cell chronic leukemia genetics, synteny, amino acid motifs, chromosome mapping, human chromosomes pair 11 and pair 13, gene deletion, genetic markers, genome, microRNAs, sequence homology.

Bahary, N., K. Goishi, C. Stuckenholz, G. Weber, J. Leblanc, C.A. Schafer, S.S. Berman, M. Klagsbrun, and L.I. Zon (2007). **Duplicate VegfA genes and orthologues of the KDR receptor tyrosine kinase family mediate vascular development in the zebrafish.** *Blood* 110(10): 3627-36. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Vascular endothelial growth factor A (VEGFA) and the type III receptor tyrosine kinase receptors (RTKs) are both required for the differentiation of endothelial cells (vasculogenesis) and for the sprouting of new capillaries (angiogenesis). We have isolated a duplicated zebrafish VegfA locus, termed VegfAb, and a duplicate RTK locus with homology to KDR/FLK1 (named Kdrb). Morpholino-disrupted VegfAb embryos develop a normal circulatory system until approximately 2 to 3 days after fertilization (dpf), when defects in angiogenesis permit blood to extravasate into many tissues. Unlike the VegfAa(121) and VegfAa(165) isoforms, the VegfAb isoforms VegfAb(171) and VegfAb(210) are not normally secreted when expressed in mammalian tissue culture cells. The Kdrb locus encodes a 1361-amino acid transmembrane receptor with strong homology to mammalian KDR. Combined knockdown of both RTKs leads to defects in vascular development, suggesting that they cooperate in mediating the vascular effects of VegfA in zebrafish development. Both VegfAa and VegfAb can individually bind and promote phosphorylation of both Flk1 (Kdra) and Kdrb proteins in vitro. Taken together, our data support a model in the zebrafish, in which duplicated VegfA and multiple type III RTKs mediate vascular development.

Descriptors: zebrafish, VegfA genes, orthologues, Kdr receptor, tyrosine kinase, vascular development.

Baron, M.H., S. Willey, K. Sahr, H. Zhang, K. Balbi, M.A. Dyer, and M. Adlam (2004). **Induction and patterning of pre-hemato-vascular mesoderm by the mouse Mix homeodomain protein.** *Blood* 104(11, Part 1): 163A. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Members of the *Xenopus* and zebrafish Mix/Bix family of paired class homeodomain proteins play determining roles in both mesoderm and endoderm development and are induced by members of the TGFbeta/BMP family of signaling molecules. A single Mix gene has been identified in mouse, humans and chick. Prior to gastrulation, the mouse Mix (mMix) gene is expressed in the visceral endoderm and later in the primitive streak and nascent mesoderm, where it overlaps, in part, with T. Mix expression in ES-derived embryoid bodies is early and transient, overlapping partially with Flk1 activation around the time of formation of hemangioblasts. Both mMix mRNA and protein are found in a FACS-sorted population of T + Flk1 + cells from ES cell-derived embryoid bodies (EBs) which contains hemangiablasts. A complex embryonic lethal phenotype has been reported for Mix deficient embryos, including defects in allantoic (vascular) and cardiogenesis. Mesoderm forms in these embryos but is not patterned properly. Embryonic lethality occurs around E10.5-11, presumably as a result of the cardiovascular defects. We have generated inducible ES cell lines in which expression of Mix protein is responsive to doxycycline. Ectopic expression of Mix in EBs results in premature, enhanced expression of hemangioblast, angioblast and hematopoietic stem cell markers (mRNA and FACS analyses) and increased formation of stem/progenitor cells in clonogenic assays in methylcellulose. Together, the expression analyses, knockout phenotype, and gain-of-function studies in ES cells suggest that mMix functions early in induction and Patterning of mesoderm, including formation of hematopoietic and endothelial lineages. Potential mMix targetgenes are being identified by microarray analyses of the inducible Mix ES lines. To examine mMix activities in vivo, we have generated null and conditionalmMix knockout mice from several independently targeted ES cell lines. Analysis of these animals is in progress. Like *Xenopus* Mix. 1, mouse Mix may represent an important connection between the TGFbeta/BMP pathway and hematopoietic/vascular development in the embryo.
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Descriptors: zebrafish embryos, cardiovascular system, transport and circulation, blood lymphatics, molecular genetics, biochemistry, molecular biophysics, fluorescence activated cell sorting , spectrum analysis techniques, cytology techniques.

Burns, C.E. and L.I. Zon (2004). **Notch signaling is necessary and sufficient for runx 1-dependent stem cell induction in the embryonic aorta-gonad-mesonephros (agm) region**. *Blood* 104(11, Part 2): 127B. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Vertebrate hematopoiesis can be divided into two embryonic phases: a short primitive wave predominantly generating erythrocytes and a definitive (fetal/adult) wave producing long-term hematopoietic stem cells (HSCs). The definitive wave occurs in the embryonic aorta-gonad-mesonephros (AGM) region through the asymmetric induction of HSCs from the ventral, but not dorsal, aortic endothelial wall. Since Notch signaling is critical for orchestrating a variety of developmental cell fate choices from invertebrates to humans and has been implicated in affecting the differentiation of some hematopoietic lineages, we analyzedwhether the Notch pathway regulates definitive HSC induction in vivo. The zebrafish mutant mindbomb harbors a mutation in an essential E3 ligase that ubiquitylates Delta, which in turn allows the Notch intercellular domain to be released and activate downstream target gene transcription. Thus, in the absence of Mindbomb function Notch signaling does not occur. We found that although mindbomb mutants show normal primitive hematopoiesis, definitive c-myb and runx1 HSC expression is lacking. Since embryos injected with synthetic morpholinos designed to inhibit proper splicing of

runx1 RNA (runx morphants) show the samehematopoietic phenotype as mindbomb mutants, we next addressed the epistatic relationship between notch and runx1 using classic gain-of-function and loss-of-function analyses. In runx1 morphants expression of a notch receptor, notch3, and a delta ligand, deltaC, in the developing dorsal aorta was normal. Moreover, injection of runx1 RNA rescued HSCs in the AGM of mindbomb mutants. Together, these results suggest that Runx1 functions downstream of Notch in promoting HSC fate. We next analyzed whether a constitutively activated form of Notch(NICD) is sufficient for HSC specification in the AGM using an inducible binary transgenic system. Zebrafish carrying the heat-shock promoter driving the activator gal4 were mated to animals carrying 6 gal4 -responsive tandem upstream activating sequences (UAS) driving NICD. At the 10 somite-stage the embryos were heat-shocked at 37 degrees C for I hour to activate NICD throughout the double transgenic animals. Surprisingly, expression of both HSC markers, c-myb and runx1, were expanded from their normal restricted domain in the ventral endothelium to the entire circumference of the dorsal aorta. Most interestingly,the presence of ectopic c-myb and runx1 transcripts were observed in the developing post-cardinal vein, a vessel that normally does not produce HSCs. Thesedata imply that activation of the Notch pathway generates increased numbers of HSCs in vivo. When runx1 RNA is injected into wild-type embryos a similar expansion of c-myb transcripts is seen throughout the entire dorsal aorta and post-cardinal vein, further indicating that Runx1 functions downstream of Notch in HSC induction. In summary, discovery of the molecular programs essential and sufficient for fetal/adult hematopoietic ontogeny will lead to a further understanding of the physiologic and pathologic processes regulating stem cell homeostasis and translate into more effective therapies for blood disorders. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, cardiovascular system, molecular genetics, development, endocrine system, reproductive system, hematologic disorder, Notch signaling, stem cell induction, downstream transcription.

Camaschella, C., A. Campanella, L. De Falco, L. Boschetto, R. Merlini, L. Silvestri, S. Levi, and A. Iolascon (2007). **The human counterpart of zebrafish shiraz shows sideroblastic-like microcytic anemia and iron overload.** *Blood* 110(4): 1353-8. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Inherited microcytic-hypochromic anemias in rodents and zebrafish suggest the existence of corresponding human disorders. The zebrafish mutant shiraz has severe anemia and is embryonically lethal because of glutaredoxin 5 (GRLX5) deletion, insufficient biogenesis of mitochondrial iron-sulfur (Fe/S) clusters, and deregulated iron-regulatory protein 1 (IRP1) activity. This leads to stabilization of transferrin receptor 1 (TfR) RNA, repression of ferritin, and ALA-synthase 2 (ALAS2) translation with impaired heme synthesis. We report the first case of GLRX5 deficiency in a middle-aged anemic male with iron overload and a low number of ringed sideroblasts. Anemia was worsened by blood transfusions but partially reversed by iron chelation. The patient had a homozygous (c.294A>G) mutation that interferes with intron 1 splicing and drastically reduces GLRX5 RNA. As in shiraz, aconitase and H-ferritin levels were low and TfR level was high in the patient's cells, compatible with increased IRP1 binding. Based on the biochemical and clinical phenotype, we hypothesize that IRP2, less degraded by low heme, contributes to the repression of the erythroblasts ferritin and ALAS2, increasing mitochondrial iron. Iron chelation, redistributing iron to the cytosol, might relieve IRP2 excess, improving heme synthesis and anemia. GLRX5 function is highly conserved, but at variance with zebrafish, its defect in humans leads to anemia and iron overload.

Descriptors: zebrafish mutant shiraz, sideroblastic anemia genetics, iron overload, mutation genetics, oxidoreductases genetics, aconitate hydratase, sideroblastic anemia pathology, Western

blotting, glutaredoxin 5 (GRLX5) deletion, cultured cells, ELISA, immunoprecipitation, middle aged , mitochondria enzymology, oxidoreductases metabolism, reverse transcriptase PCR.

Chen, E., S. Hermanson, and S.C. Ekker (2004). **Syndecan-2 is essential for angiogenic sprouting during zebrafish development.** *Blood* 103(5): 1710-9. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: We used a morpholino-based gene-targeting screen to identify a novel protein essential for vascular development using the zebrafish, Danio rerio. We show that syndecan-2, a cell-surface heparan sulfate proteoglycan, is essential for angiogenic sprouting during embryogenesis. The vascular function of syndecan-2 is likely conserved, as zebrafish and mouse syndecan-2 show similar expression patterns around major trunk vessels, and human syndecan-2 can restore angiogenic sprouting in syndecan-2 morphants. In contrast, forced expression of a truncated form of syndecan-2 results in embryos with defects in angiogenesis, indicating that the highly conserved cytoplasmic tail is important for the vascular function of syndecan-2. We further show that vascular endothelial growth factor (VEGF) and syndecan-2 genetically interact in vivo using both gain-of-function and loss-of-function studies in zebrafish. VEGF-mediated ectopic signaling is compromised in syndecan-2 morphants, and ectopic syndecan-2 potentiates ectopic VEGF signaling. Syndecan-2 as a novel angiogenic factor is a potential candidate for use in the development of angiogenesis-based therapies.
Descriptors: zebrafish, developmental gene expression regulation, syndecan-2 , membrane glycoproteins biosynthesis and physiology, physiologic neovascularization, proteoglycans biosynthesis and physiology, 5' untranslated regions, amino acid and base sequence, chromosome mapping, molecular cloning, cytoplasm metabolism, green fluorescent proteins, heparan sulfate proteoglycans chemistry, in situ hybridization, luminescent proteins metabolism, mice, molecular sequence data, phenotype, phylogeny, protein binding, messenger RNA metabolism, signal transduction, syndecan 2, vascular endothelialgrowth factor A metabolism, proteins.

Chen, E., J.D. Larson, and S.C. Ekker (2006). **Functional analysis of zebrafish microfibril-associated glycoprotein-1 (Magp1) in vivo reveals roles for microfibrils in vascular development and function.** *Blood* 107(11): 4364-74. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: Mutations in fibrillin-1 (FBN1) result in Marfan syndrome, demonstrating a critical requirement for microfibrils in vessel structure and function. However, the identity and function of many microfibril-associated molecules essential for vascular development and function have yet to be characterized. In our morpholino-based screen for members of the secretome required for vascular development, we identified a key player in microfibril formation in zebrafish embryogenesis. Microfibril-associated glycoprotein-1 (MAGP1) is a conserved protein found in mammalian and zebrafish microfibrils. Expression of magp1 mRNA is detected in microfibril-producing cells. Analysis of a functional Magp1-mRFP fusion protein reveals localization along the midline and in the vasculature during embryogenesis. Underexpression and overexpression analyses demonstrate that specific Magp1 protein levels are critical for vascular development. Integrin function is compromised in magp1 morphant embryos, suggesting that reduced integrin-matrix interaction is the main mechanism for the vascular defects in magp1 morphants. We further show that Magp1 and fibrillin-1 interact in vivo. This study implicates MAGP1 as a key player in microfibril formation and integrity during development. The essential role for MAGP1 in vascular morphogenesis and function also supports a wide range of clinical applications, including therapeutic targets in vascular disease and cardiovascular tissue engineering.
Descriptors: zebrafish embryology, blood vessels growth and development and physiology, contractile, extracellular matrix, microfibrils physiology, contractile proteins analysis and genetics,

extracellular matrix proteins analysis and genetics, integrins metabolism, microfibrils chemistry, microfilament proteins metabolism, morphogenesis, messenger RNA analysis.

Craven, S.E., D. French, W. Ye, F. de Sauvage, and A. Rosenthal (2005). **Loss of Hspa9b in zebrafish recapitulates the ineffective hematopoiesis of the myelodysplastic syndrome.** *Blood* 105(9): 3528-34. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: Myelodysplastic syndrome (MDS) comprises a heterogeneous group of often fatal hematopoietic stem cell disorders for which neither curative nor standard treatment exists. The complex karyotypes and multistep nature of MDS have severely restricted the identification of causative genetic mutations and thus limited insight into new and more effective therapies. Here we describe a zebrafish mutant crimsonless (crs) with a developmental blood defect that closely recapitulates the ineffective hematopoiesis of MDS including anemia, dysplasia, increased blood cell apoptosis, and multilineage cytopenia. By positional cloning, rescue, and morpholino knock-down experiments, we demonstrate that crs encodes a conserved mitochondrial matrix chaperone HSPA9B containing a glycine-to-glutamate substitution within the substrate-binding domain. This mutation compromises mitochondrial function, producing oxidative stress and apoptosis distinctly in blood cells. Thus, we identify an essential role for Hspa9b in hematopoiesis and implicate both loss of HSPA9B specifically and mitochondrial dysfunction generally in the pathogenesis of the MDS.
Descriptors: zebrafish, HSP70 heat shock , genetics and physiology, hematopoiesis genetics, myelodysplastic syndromes blood, apoptosis genetics, blood cells pathology, mitochondria pathology, mitochondrial proteins genetics and physiology, research models, missense mutation, myelodysplastic syndromes etiology and pathology, oxidative stress genetics.

Davidson, A.J., P. Ernst, Y. Wang, M.P.S. Dekens, P.D. Kingsley, J. Palis, S.J. Korsmeyer, G.Q. Daley, and L.I. Zon (2003). **Caudal-like genes act upstream of Hox genes to induce hematopoietic progenitors in zebrafish and mouse embryonic stem cells.** *Blood* 102(11): 166a. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: The zebrafish mutant kugelig (kgg) is caused by a defect in the caudal -like homeobox gene cdx4 and has an early deficit in scl-expressing hematopoietic precursors. kgg mutants exhibit severe anemia and altered expression of multiple hox genes. To investigate the role of the cdx-hox pathway during early blood specification we overexpressed a number of different hox genes in kgg mutants. Overexpression of hoxb7a and hoxa9a rescued embryonic erythropoiesis to near wild-type levels. In contrast, hoxb6b rescued poorly, whereas hoxb4 and hoxb8a failed to rescue. Overexpression of scl, which induces ectopic blood development in wild-type embryos, also failed to restore erythropoiesis in kgg mutants. These results indicate that the specification of blood by scl requires the cdx-hox pathway and suggests that specific hox genes are required to make mesoderm competent to form blood. In mammals, three cdx paralogues have been identified (cdx1, cdx2, and cdx4). Expression of the zebrafish orthologue of cdx2 overlaps with cdx4 during gastrulation and early somite stages. Morpholino-mediated 'knock-down' of cdx2 had little effect on embryonic erythropoiesis. However, when cdx2 morpholinos were injected into kgg mutants, the resulting embryos displayed a complete block in erythroid development. These findings indicate that cdx2 and cdx4 have redundant activities for blood development. Overexpression of cdx4 during zebrafish development induces ectopic erythroid cells near the midline of the embryo. Similarly, overexpression of Cdx4 in mouse ES cells results in an expansion of hematopoietic progenitors including a 13-fold increase in CFU-GEMM. In addition, Cdx4 induced widespread changes in Hox expression levels, including a 30-fold induction of HoxB4. Taken together, our data suggests that the cdx-hox pathway is necessary and sufficient for hematopoietic stem cell for-

mation during vertebrate embryogenesis. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, molecular genetics, cdx Hox pathway, embryogenesis, erythropoiesis, mutant kugelig, hematopoietic precursors.

Davidson, A.J., Y. Wang, G.Q. Daley, and L.I. Zon (2004). **Inhibition of retinoic acid signaling by the cdx-Hox pathway is essential for blood cell formation during embryogenesis.** *Blood* 104(11, Part 1): 42A. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: The zebrafish mutant kugelig (kgg) is caused by a defect in the caudal-relatedhomeobox gene cdx4 and has a deficit in both 'primitive' and 'definitive' hematopoietic stem cells. The embryonic anemia in the mutants can be rescued by overexpressing hox genes such as hoxb7a and hoxa9a, but not hoxb8a. This suggests that specific hox genes are required to make mesoderm competent to form blood. To further explore this, we undertook a microarray analysis to identify differentially expressed genes in kgg mutants and wild-type embryos. We found that raldh2, an enzyme required for retinoic acid (RA) production, is overexpressed in kgg mutants during the early stages of blood formation. This data led us to hypothesize that RA may act to suppress blood formation and that the cdx-hox pathway functions to limit RA production, thereby permitting blood formation to occur. To test this, we treated wildtype zebrafish embryos with RA and found that they became severely anemic. Treating kgg embryos with DEAB, a chemical that blocks raldh2 activity, restored hematopoiesis in kgg mutants. Expression of hoxa9a was not rescued in these treated embryos, indicating that RA acts downstream of the hox genes. DEAB also induced an expansion of erythroid cells in wild-type embryos, thus supporting the notion that the levels of RA during development are a critical determinant for blood formation. By performing a time-course rescue experiment, we determined that DEAB is effective when scl+ hematopoietic progenitors are first formed from mesoderm, suggesting that RAacts upstream of the blood-inducer scl. In support of this, SCL overexpression rescues GATA-1 expression in embryos treated with RA. We next looked at the effects of DEAB and RA on the formation of mouse hematopoietic progenitors arising from ES cell-derived embryoid bodies (EBs). Addition of DEAB to EBs between days 2 to 3 of development resulted in a 5-8 fold increase in 'primitive' erythroid colonies (CFU-Ep), analogous to our results in zebrafish embryos. In contrast, RA treatment caused a general inhibition in the growth of all colonytypes. Taken together, these results suggest a new model in which suppressionof RA by the cdx-hox pathway is necessary for yolk sac hematopoiesis to occur. This model provides an explanation for how hox genes control the spatiotemporal formation of hematopoietic tissue during organogenesis and may shed new light on the pathogenesis of leukemias involving translocations of the cdx, hox,and retinoic acid receptors. Copyright©Thomson Reuters 2009
Descriptors: zebrafish mutant kugelig, blood and lymphatics, molecular genetics, development, leukemia, neoplastic disease, pathology, hematopoiesis, embryonic anemia, microarray analysis, embryogenesis, organogenesis, cdx Hox pathway, blood formation.

Day, K., N. Krishnegowda, and P. Jagadeeswaran (2004). **Knockdown of prothrombin in zebrafish.** *Blood Cells, Molecules and Diseases* 32(1): 191-8. ISSN: 1079 9796.
NAL Call Number: RC633.A1
Abstract: Thrombin is a serine protease generated from its zymogen, prothrombin, and plays a central role in the coagulation cascade. It is also important for mammalian development. The zebrafish has now been established as an excellent genetic model for studies on mammalian hemostasis and development. In this report, we used prothrombin-specific antisense morpholinos to knock down the levels of prothrombin to characterize the effects of prothrombin deficiency

in the zebrafish embryo. Prothrombin morpholino-injected zebrafish embryos yielded an early phenotype exhibiting severe abnormalities that later showed occasional bleeding. In a second late phenotype, the embryos had no observable morphological abnormalities in early stages, but showed occasional bleeding at later stages. These phenotypes resembled characteristics shown by prothrombin knockout mice. Laser-induced vascular injury on some of the normal appearing phenotypic larvae showed a prolonged time to occlusion, and recombinant zebrafish prothrombin injected into these larvae restored a normal time to occlusion thus showing the specificity of the morpholino effect. The system developed here should be useful for investigation of the role of thrombin in vertebrate development.

Descriptors: zebrafish larvae, oligodeoxyribonucleotides, antisense genetics, prothrombin genetics, prothrombin physiology, abnormalities etiology and genetics, hemorrhage etiology and genetics, hemostasis, in situ hybridization, animal models, phenotype, vertebrates growth and development, proteins genetics and physiology.

De Domenico, I., M.B. Vaughn, D. Yoon, J.P. Kushner, D.M. Ward, and J. Kaplan (2007). **Zebrafish as a model for defining the functional impact of mammalian ferroportin mutations.** *Blood* 110(10): 3780-3. ISSN: 0006-4971.

NAL Call Number: RB145.A1B57

Abstract: The term hemochromatosis represents a group of inherited disorders leading to iron overload. Mutations in HFE, HJV, and TfR2 cause autosomal-recessive forms of hemochromatosis. Mutations in ferroportin, however, result in dominantly inherited iron overload. Some mutations (H32R and N174I) in ferroportin lead to macrophage iron loading, while others (NI44H) lead to hepatocyte iron loading. Expression of H32R or N174I ferroportin cDNA in zebrafish leads to severe iron-limited erythropoiesis. Expression of wild-type ferroportin or hepcidin-resistant ferroportin (N144H) does not affect erythropoiesis. Zebrafish provides a facile way of identifying which ferroportin mutants may lead to macrophage iron loading.

Descriptors: zebrafish ferroportin mutants, hemochromatosis, macrophage iron overload, erythropoiesis, model.

Eckfeldt, C.E., E.M. Mendenhall, S.C. Ekker, and C.M. Verfaillie (2004). **Functional analysis of the differential gene expression profile of human hsc using a functional genomics screen in the zebrarish.** *Blood* 104(11, Part 1): 42A. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Self-renewal and lineage differentiation of hematopoietic stem cells (HSC) is likely regulated by a combination of intrinsic and extrinsic signals. At present these signals are poorly understood. Recently, numerous groups have identified the expressed gene profile of HSC in an attempt to identify novel genes that regulate HSC fate decisions. Likewise, our group compared the expressed gene profiles of CD34(+)CD33(-)CD38(-)Rho(lo)c-kit(+) (Rho(lo)) cells, enriched in primitive progenitors and presumed human HSC, compared to CD34(+)CD33(-) CD38(-)Rho(hi)! (Rho(hi)) cells, depleted of such cells, fromumbilical cord blood (UCB) and bone marrow (BM) to identify conserved signaling pathways active in these ontogenically distinct populations using the Afftymetrix (TM) HG-U133 GeneChip (R) set. We identified a putative molecular signature for human HSC containing 286 genes, expressed more highly in Rho(lo) or Rho(hi) cells from both cell sources using a $p<0.05$ and fold change of 1.5 cutoff. To assess the role of this series of genes in a high-throughput fashion, we developed an in vivo functional genomics screen in the zebrafish that allows for the determination of hematopoietic function of the differentially expressed genes. Candidate gene expression was knocked down by injecting morpholino antisense oligonucleotides (MO) into 1-4 cell embryos from GATA 1:DsRed transgenic zebrafish that have red fluorescent GATA1(+) blood cells and blood production was

scored by fluorescence microscopy at 30 and48 hours post-fertilization. Of the 286 differentially expressed genes, 128 were deemed too universal or likely non-specific for hematopoiesis, and were not targeted. These included histones, HLA-antigens, hemoglobin genes, and genesencoding proteins involved in general cellular metabolism. Of the remaining 158, MO were designed for 70 (44%), no zebrafish ortholog could be identified for 72 (46%), inadequate sequence information was available to design morpholinos for 9 (6%) and multiple zebrafish orthologs were identified for 7 (4%). Fluorescence microscopy of GATA1:DsRed fish has revealed a reproducible reductionin GATA1(+) blood cell number for 13 of 55 MO-targeted genes analyzed to date, giving a 24% frequency for hematopoietic phenotypes in the screen, that compares very favorably with the noted 0.5-1% frequency of hematopoietic genes identified in ENU mutagenesis screens that mutate genes in a near randomfashion. The functionally-validated genes identified thus far include known genes that lack a known hematopoietic function such as SPRY1, CRTAP, IRAK3 and UCP2, as well as genes that currently lack a functional annotation such as C12orf2, DKFZp564D137, MGC15875 and FLJ21269. Hematopoietic phenotypes are being further characterized by whole-mount in situ hybridization for hematopoietic genes and rescue of hematopoietic phenotypes by co-injection of overexpression vectors in the zebrafish. In addition, we are confirming the role of the genesin mammalian hematopoiesis using overexpression and knockdown studies in murine and human repopulating HSC. This is the first description of a high-throughput functional genomics screen in the zebrafish to functionally validate differentially expressed genes, an essential step in obtaining meaningful functional data from global gene profiling studies. Copyright©Thomson Reuters 2009

Descriptors: zebrafish orthologs, circulation, molecular genetics, cell biology, in situ hybridization, genetic techniques, fluorescence microscopy, functional genomics screen, Afftymetrix HG U133 genechip, gene expression profile.

Evans, T., S. Gupta, and L.M. McReynolds (2004). **New animal models reveal stage-specific hematopoietic functions for the bmp signaling pathway.** *Blood* 104(11, Part 1): 41A-42A. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Genetic analyses in mouse and zebrafish demonstrate that a conserved BMP signaling pathway is essential for normal development of the embryonic vascular andhematopoietic systems. However, it has not been clear which aspects of hemato-vascular development are regulated by the pathway beyond the initial specification of embryonic mesoderm. We present new animal models for studying BMP signaling at specific stages of both embryonic and adult hematopoiesis. First, wedeveloped approaches to modulate BMP signaling conditionally at specific stages of development in transgenic zebrafish. For this purpose we adapted the Cre-lox system to express a dominant-negative BMP receptor in cells derived from specific progenitor populations, with subsequent analysis of hematopoietic andvascular development using GFP reporter strains of fish. For example, we findthat PIMP signaling is required in LMO2+ cells for the normal development of both hematopoietic and vascular systems. Yolk sac vasculature and trunk angiogenesis are both disrupted, and primitive erythropoiesis is also impaired, whenthese cells express the mutant receptor. In contrast, BMP signaling is apparently no longer required in primitive erythroid cells once they have activated expression of the GATA-I gene. In a second approach, we studied hematopoiesis in zebrafish mutants for Smad5, which is an intracellular mediator of BMP signaling. For example, homozygote piggytail (pgy) mutant embryos live until day 3-5, yet the fish have little to no blood and no circulation. Surprisingly, we find that key hematopoietic regulatory genes including GATA-1, GATA-2, SCL, and LMO2 are expressed in the pgy mutant fish, but that their patterns of expression are altered. Thus, the progenitor cells initiate a hematopoietic program but fail to differentiate. We also established adult pgy fish that

carry a GATA I::GFP transgene, to facilitate the analysis of adult-stage blood cell profiles using flow cytometry. Blood cell populations were monitored under normal conditions and following phenylhydrazine-induced hemolytic anemia in wild-type and pgy/+ fish. Preliminary results indicate that pgy/+ adult fish are able tomount an erythropoietic response to hemolytic anemia. However, the kinetics are altered, specifically in the transition from the proerythroblast to the polychromatophilic erythroblast stage. In summary, we present data using novel animal models disrupted for normal BMP signaling, and demonstrate roles for thispathway at specific stages of differentiation for both embryonic and adult hematopoiesis. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, mice, cardiovascular system, transport and circulation, molecular genetics, development, hematopoiesis, erythropoiesis , hemolytic anemia , lymphatic disease, chemically induced, genetic analysis and techniques, angiogenesis, BMP signalingpathway, hemato vascular development.

Feng, H., D.M. Langenau, J.A. Madge, A. Quinkertz, A. Gutierrez, D.S. Neuberg, J.P. Kanki, and A.T. Look (2007). **Heat-shock induction of T-cell lymphoma/leukaemia in conditional Cre/lox-regulated transgenic zebrafish.** *British Journal of Haematology* 138(2): 169-75. ISSN: 0007 1048.
Abstract: The zebrafish is an ideal vertebrate model system to investigate the complex genetic basis of cancer because it has the capacity for in vivo tumour-cell imaging and forward genetic screens, and the molecular mechanisms regulating malignancy are remarkably conserved when compared with human. Our laboratory has previously generated transgenic zebrafish models that overexpress the mouse c-Myc gene fused to enhanced green fluorescent protein (EGFP) and develop T-cell acute lymphoblastic leukaemia (T-ALL) that recapitulates the human disease both molecularly and pathologically. Our previous models have been limited by disease onset prior to sexual maturity and by the low disease penetrance when conditional transgenic embryos are injected with Cre RNA. Here, we report a novel system in which compound transgenic fish expressed both Cre controlled by the heat-shock promoter and a rag2-promoter-regulated lox-dsRED2-lox-EGFP-mMyc cassette rag2-LDL-EMyc in developing T cells. After heat-shock treatment at 3 d postfertilisation (dpf) for 45 min at 37 degrees C, 81% of compound transgenic fish developed T-lymphoblastic lymphoma (T-LBL, mean latency 120 +/- 43 (standard deviation) days of life), which rapidly progressed to T-ALL. Heat-shock-regulated transgenic technology in zebrafish provides the missing link necessary to exploit the powerful genetic capacity of this organism to probe the multi-step molecular pathogenesis of leukaemia.
Descriptors: transgenic zebrafish, HSP70 heat shock leukemia,T cell, acute genetics, lymphoma,T cell genetics, genetically modified, disease models, fluorescent dyes, genes, Myc genetics , green fluorescent proteins, heterozygote, neoplasm promoter regions genetics, proto oncogene proteins C Myc genetics, temperature, transgenes genetics.

Galloway, J.L., C. Thisse, Y. Zhou, R. Beltre, B. Thisse, and L.I. Zon (2004). **Conversion of erythropoiesis to myelopoiesis in Gata1-deficient zebrafish embryos.** *Blood* 104(11, Part 1): 759A. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: Primitive in vertebrates initiates in the andyields nucleated erythrocytes and macrophages that later enter circulation. In zebrafish, a group of blood cells contained within the axial vein make up the intermediate cell mass (ICM), the teleost equivalent of the mammalian yolk sac. To identify novel genes involved in hematopoiesis, a high-throughput wholeembryo in situ hybridization screen was performed. Examination of the expression pattern of 3700 clones from an adult zebrafish hematopoietic cDNA library discovered 24 genes with expression in the blood during development. Each stage of hematopoiesis was defined by a subset of genes, provid-

ing a molecular signature of blood cell maturation, from hematopoietic progenitors to terminally differentiated erythrocytes. By using antisense morpholinos to the transcription factors gata1 and gata2, we were able to dissect the regulation of these 24genes. Examination of gene expression in Gata1, Gata2, and Gata1/Gata2-deficient animals revealed that most erythroid genes are dependent upon Gata factorsfor expression. Surprisingly, three novel genes, expressed in hematopoietic progenitors, do not require Gata factors for their expression demonstrating that some erythroid genes are regulated in a Gata-independent manner. During our analysis, we also found persistent ectopic expression of the myeloid transcription factor, PU.1, in the ICM cells and a subsequent expansion of mpo expressing granulocytes and L-plastin expressing macrophages. By utilizing gata1-GFP transgenic zebrafish, we were able to isolate blood cells by flow cytometry andexamine their morphology. We discovered that blood cells from the Gata1-deficient animals exhibited features characteristic of myeloid cells when compared to wild-type blood cells. By confocal microscopy, we detected some blood cellsin the ICM of Gata1-deficient embryos that co-express globin and PU.1. while blood cells of wild-type embryos never co-express these markers at this stage.These observations demonstrate that in the absence of Gata I the presumptive erythroid progenitors have transformed into the myeloid lineage, and that a major cell fate alteration has occurred. Ultimately, our studies have molecularly defined blood development by gene expression, and illustrated that Gata1 governs lineage fate decisions of hematopoietic progenitors in the developing embryo. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, development, blood and lymphatics, erythropoiesis, myelopoiesis, intermediate cell mass, GATA 1, hematopoiesis, embryonic yolk sac.

Galloway, J.L., R.A. Wingert, C. Thisse, B. Thisse, and L.I. Zon (2005). **GATA-independent regulation of red cell specific gene expression.** *Blood* 106(11, Part 1): 494A. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: During vertebrate embryonic hematopoiesis, the first blood cells can be identified by expression of the transcription factor genes scl and GATA2, followed by expression of GATA 1, a gene required for the erythroid lineage. A high-throughput in situ hybridization screen in zebrafish analyzed the expression pattern of 3700 clones from a hematopoietic cDNA library and discovered 24 genes with expression in the blood. Examination of gene expression in Biklf, GATA1, GATA2, and GATA1/GATA2-deficient animals revealed that most blood genes are dependent upon GATA factors for expression rather than the Kruppel-like transcription factor Biklf. Three novel genes, expressed specifically in crythroid precursors, did not require GATA factors for their expression, demonstrating that some blood genes are regulated in a GATA-independent manner. These three genes were kelch repeat protein (kelch repeats have been implicated in diverse cellular functions from actin binding to sequestering transcriptions factors), kiaa0650, which contains an SMC-hinge domain, and testhymin, which has no known structural motifs. By using combinations of antisense morpholmos to the known hematopoietic genes biklf, GATA 1, GATA2, and set, we were able to examine the regulation of these novel genes in double and triple knockdown embryos. While expression of kelch-repeat protein was lost in the absence of GATA I and Biklf, expression of testhymin and kiaa0650 was maintained in GATA1/GATA2/Biklf-deficient embryos, suggesting that these similarly expressed genes are differentially regulated. As with GATA 1, kiaa0650 and kelch-repeat protein required Scl for their expression in the early hematopoietic mesoderm while testhynnin did not. Furthermore, loss of Set and GATA2 did not completely ablate testhymin expression, suggesting that this gene is induced by factors upstream or parallel to Set and GATA2. Taken together, our zebrafish studies establish a regulation of gene expression by a developmental hierarchy of specific transcription factors that act in combination during blood cell maturation. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryology, blood and lymphatics, development, molecular genetics,

high throughput in situ hybridization, histology and cytology techniques, genetic techniques, hematopoiesis, complementary DNA library, blood cell maturation, GATA 1 and 2.

Gu, Y., P. Jin, L. Zhang, X. Zhao, X. Gao, Y. Ning, A. Meng, and Y. Chen (2006). **Functional analysis of mutations in the kinase domain of the tgf-beta receptor ALK1 reveals different mechanisms for induction of hereditary hemorrhagic telangiectasia**. *Blood* 107(5): 1951-1954. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Genetic studies in mouse and zebrafish have established the importance of activin receptor-like kinase 1 (ALK1) in formation and remodeling of blood vessels. Single-allele mutations in the ALK1 gene have been linked to the human type 2 hereditary hemorrhagic telangiectasia (HHT2). However, how these ALK1 mutations contribute to this disorder remains unclear. To explore the mechanism underlying effect of the HHT-related ALK1 mutations on receptor activity, we generated 11 such mutants and investigated their signaling activities using reporter assay in mammalian cells and examined their effect on zebrafish embryogenesis. Here we show that some of the HHT2-related mutations generate a dominant-negative effect whereas the others give rise to a null phenotype via loss of protein expression or receptor activity. These data indicate that loss-of-function mutations in a single allele of the ALK1 locus are sufficient to contribute to defects in maintaining endothelial integrity. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, mice, development, molecular genetics, enzymology, cardiovascular system, type 2 hereditary hemorrhagic telanglectasia, vascular disease, genetic disease, blood and lymphatic disease, pathology, genetics, etiology, embryogenesis.

Hanaoka, R., S. Katayama, I.B. Dawid, and A. Kawahara (2006). **Characterization of the heme synthesis enzyme coproporphyrinogen oxidase (CPO) in zebrafish erythrogenesis.** *Genes to Cells* 11(3): 293-303. ISSN: 1356-9597.

NAL Call Number: QH441.5

Abstract: Hemoglobin consists of heme and globin proteins and is essential for oxygen transport in all vertebrates. Although biochemical features of heme synthesis enzymes have been well characterized, the function of these enzymes in early embryogenesis is not fully understood. We found that the sixth heme synthesis enzyme, coproporphyrinogen oxidase (CPO), is predominantly expressed in the intermediate cell mass (ICM) that is a major site of zebrafish primitive hematopoiesis. Knockdown of zebrafish CPO using anti-sense morpholinos (CPO-MO) leads to a significant suppression of hemoglobin production without apparent reduction of blood cells. Injection of human CPO RNA, but not a mutant CPO RNA that is similar to a mutant responsible for a hereditary coproporphyria (HCP), restores hemoglobin production in the CPO-MO-injected embryos. Furthermore, expression of CPO in the ICM is severely suppressed in both vlad tepes/gata1 mutants and in biklf-MO-injected embryos. In contrast, over-expression of biklf and gata1 significantly induces ectopic CPO expression. The function of CPO in heme biosynthesis is apparently conserved between zebrafish and human, suggesting that CPO-MO-injected zebrafish embryos might be a useful in vivo assay system to measure the biological activity of human CPO mutations.

Descriptors: zebrafish embryos, coproporphyrinogen oxidase metabolism, erythropoiesis, developmental gene expression regulation, metabolism, amino acid sequence, blood cells metabolism, coproporphyrinogen oxidase antagonists and inhibitors, embryo cytology, embryo drug effects and metabolism, GATA1 transcription factor chemistry, genetics and metabolism, hematopoiesis, hemoglobins metabolism, in situ hybridization, kruppel like transcription factors chemistry, genetics and metabolism, microinjections, molecular sequence data, antisense oligonucleotides

genetics and pharmacology, amino acid sequence homology, proteins chemistry, genetics and metabolism.

Hsia, N. and L.I. Zon (2005). **Transcriptional regulation of hematopoietic stem cell development in zebrafish.** *Experimental Hematology* 33(9): 1007-14. ISSN: 0301 472X.
NAL Call Number: RB145.E97
Abstract: The zebrafish (*Danio rerio*) is a well-established vertebrate model for studying hematopoiesis. The major advantages of this system include robust experimental techniques in both genetics and embryology, which have been utilized to model many aspects of human development and disease. Although much is known about the transcription factors involved in the terminal differentiation of peripheral blood lineages, little is known about the development and maintenance of the hematopoietic stem cell (HSC). This review will focus on the current knowledge of the transcriptional regulation of the HSC in the context of the zebrafish. Future studies using new technologies in the zebrafish model will enhance our understanding of the molecular networks regulating HSC pluripotency and differentiation.
Descriptors: zebrafish embryos, developmental gene expression regulation, hematopoiesis genetics, hematopoietic stem cells cytology, transcription factors physiology, embryonic development genetics, hematopoiesis physiology, hematopoietic stem cells physiology, pluripotent stem cells cytology and physiology.

Hsu, K., D. Traver, J.L. Kutok, A. Hagen, T.X. Liu, B.H. Paw, J. Rhodes, J.N. Berman, L.I. Zon, J.P. Kanki, and A.T. Look (2004). **The pu.1 promoter drives myeloid gene expression in zebrafish.** *Blood* 104(5): 1291-7. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: PU.1 is a member of the Ets family of transcription factors and plays an essential role in the development of both myeloid and lymphoid cells. To examine zebrafish pu.1 (zpu.1) expression in subpopulations of blood cells during zebrafish development, we linked a 9-kb zebrafish genomic fragment upstream of the zpu.1 initiator codon to green fluorescent protein (GFP) and microinjected this construct to generate stable transgenic lines. GFP-positive fluorescent myeloid precursors were observed migrating from the anterolateral mesoderm in living embryos from 16 to 28 hours after fertilization (hpf) in a pattern that overlaps the expression pattern of endogenous zpu.1 mRNA. Analysis of larval histologic sections revealed GFP-expressing hematopoietic cells in the developing zebrafish kidney. Flow cytometric analysis of cells from adult whole kidney marrow revealed 2 discrete subpopulations of GFP-positive cells, which after cell sorting exhibited either myeloid or early lymphoid morphology. Thus, the zebrafish zpu.1 promoter fragment used here is capable of driving reporter gene expression in subsets of embryonic and adult hematopoietic cells. These transgenic lines will be useful to dissect the cellular and molecular control of myeloid cell differentiation, and this promoter fragment may prove useful in the development of zebrafish models of acute myeloid leukemia.
Descriptors: zebrafish larvae, hematopoiesis physiology, myeloid cells physiology, promoter regions genetics physiology, proto oncogene trans activators genetics, genetically modified, developmental gene expression regulation, reporter genes, green fluorescent proteins, kidney embryology and physiology, luminescent zpu.1 promoter fragment.

Hsu, K., D. Traver, J.L. Kutok, A. Hagen, T.X. Liu, B.H. Paw, J. Rhodes, J. Berman, L.I. Zon, J. Kanki, and A.T. Look (2003). **Expression of Pu.1 In embryonic and adult transgenic zebrafish.** *Blood* 102(11): 574a. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: Pu.1 is a member of the ets family of transcription factors and plays an essential role in

the development of both myeloid and lymphoid cells. To examine zebrafish Pu.1 (zpu.1) expression in blood cell subpopulations during the embryonic, larval, and adult stages of zebrafish development, we generated transgenic zebrafish expressing green fluorescent protein (GFP) under the control of the zpu.1 promoter. GFP-positive, fluorescent myeloid precursors were observed migrating from the antero-lateral mesoderm in living microinjected embryos from 16 to 28 hours post-fertilization (hpf), in a cellular pattern similar to the expression of endogenous zpu.1 mRNA. Analysis of larval histologic sections from stable transgenic lines revealed GFP-expressing hematopoietic cells in the developing zebrafish kidney. Flow cytometric analysis of cells from adult whole kidney marrow documented discrete subpopulations of GFP-positive cells, which after cell sorting exhibited either myeloid or early lymphoid morphology. Thus, the zebrafish zpu.1 promoter fragment employed here is able to faithfully recapitulate expression in subsets of embryonic and adult hematopoietic cells. These lines will be useful to examine the movements of early myeloid cells in the developing zebrafish embryo, to test whether adult expression marks hematopoietic stem or progenitor cells through transplantation experiments, and to provide a visual means to follow defective myeloid populations resulting from forward genetic screens. Furthermore, the fragment described here is also able to drive zpu.1 expression in the myeloid cells of adult zebrafish, suggesting that it should provide a useful means to drive the expression of oncogenes in myeloid cells to develop zebrafish models of acute myeloid leukemia. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, blood and lymphatics, transport and circulation, acute myeloid leukemia, blood and lymphatic disease, blood cells, neoplastic disease, Pu.1 (zpu.1) expression.

Jagadeeswaran, P., V. Kulkarni, M. Carrillo, and S. Kim (2007). **Zebrafish: from hematology to hydrology.** *Journal of Thrombosis and Haemostasis* 5(Suppl 1): 300-4. ISSN: 1538-7933.

Abstract: Hemostasis is a defense mechanism that protects an organism from bleeding. Abnormal hemostasis results in bleeding disorders and thrombosis. Several factors are known to control hemostasis in mammals. Despite this progress, more factors remain to be identified. Classical genetic approaches have resolved physiological pathways. However, classical genetics could not be applied completely to hemostasis pathways a decade ago. We introduced zebrafish as a model system to study hemostasis and thrombosis using classical genetic methods. First, we established that zebrafish hemostasis is essentially similar to mammalian hemostasis. Secondly, we developed screening tools for isolating hemostatic mutants. These tools enabled us to identify hemostatic mutants, as well as providing a means to study hemostasis by knockdown methods. Continued characterization of the physiology of thrombus formation led to the novel finding of thrombocyte clustering. Recently, we have discovered that fish secrete proteases, which participate in hemostasis and may have direct relevance in humans. Future work to identify most of the players in thrombus formation is underway.

Descriptors: mutant zebrafish, hematology, hemostasis, mutagenesis, research model , thrombosis, isolated hemostatic mutants.

Jagadeeswaran, P., R. Paris, and P. Rao (2006). **Laser-induced thrombosis in zebrafish larvae: a novel genetic screening method for thrombosis.** *Methods in Molecular Medicine* 129: 187-95. ISSN: 1543-1894.

Abstract: Classical genetic approaches to study hemostasis and thrombosis have not been available until our recent introduction of the teleost, *Danio rerio* (the zebrafish), as an effective genetic model for in vivo coagulation assays. The genetic screen for this model is carried out using the genome saturation mutagenesis approach. The resulting mutants are screened for hemostatic or thrombotic defects. We developed a global physiological screening method for thrombosis by utilizing a laser to induce thrombosis in a specifically targeted area of the major artery and

vein. Using this assay, we have screened many fish for abnormal hemostasis, and have isolated a number of mutants with abnormal coagulation parameters. These mutants can be grown, bred, and further evaluated for the genetic etiology of their abnormal hemostatic pathways.
Descriptors: mutant zebrafish, blood coagulation disorders diagnosis, hemostasis physiology, research models, thrombosis, breeding, lasers, sepharose, genetics, in vivo coagulation assays.

Jin, H., J. Xu, and Z. Wen (2007). **Migratory path of definitive hematopoietic stem/progenitor cells during zebrafish development.** *Blood* 109(12): 5208-14. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: The development of vertebrate definitive hematopoiesis is featured by temporally and spatially dynamic distribution of hematopoietic stem/progenitor cells (HSPCs). It is proposed that the migration of definitive HSPCs, at least in part, accounts for this unique characteristic; however, compelling in vivo lineage evidence is still lacking. Here we present an in vivo analysis to delineate the migration route of definitive HSPCs in the early zebrafish embryo. Cell-marking analysis was able to first map definitive HSPCs to the ventral wall of dorsal aorta (DA). These cells were subsequently found to migrate to a previously unappreciated organ, posterior blood island (PBI), located between the caudal artery and caudal vein, and finally populate the kidney, the adult hematopoietic organ. These findings demonstrate that the PBI acts as an intermediate hematopoietic organ in a manner analogous to the mammalian fetal liver to sustain definitive hematopoiesis before adult kidney hematopoiesis occurs. Thus our study unambiguously documents the in vivo trafficking of definitive HSPCs among developmentally successive hematopoietic compartments and underscores the ontogenic conservation of definitive hematopoiesis between zebrafish and mammals.
Descriptors: zebrafish embryos, cell movement physiology, hematopoiesis physiology, hematopoietic stem cells physiology, aorta cytology, aorta embryology embryonic development, hematopoiesis genetics, kidney cytology and embryology.

Juarez, M.A., F. Su, M.J. Kiel, and S.E. Lyons (2004). **Persistence of gata1+erythroid progenitors in vivo in absence of zebrafish Sc1**. *Blood* 104(11, Part 1): 449A. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: The (SCL) gene encodes a bHLH transcription factor essential to the primitive and definitive hematopoietic systems in vertebrates. In the , studies of hematopoiesis have shown significant functional conservation with mammals. In order to assess the functions of scl in zebrafish primitive hematopoiesis, zebrafish embryos were analyzed after ablation of Set protein by morpholino-modified antisense RNA injection. Embryos injected with morpholinos targeting the scl translation initiation site or two independent splice donor sites lacked circulating blood and exhibited cardiac edema by 28 hours post fertilization. RNA in situ hybridization analysis with a panel of hematopoietic-specific genes showed that myeloid lineage development in the anterior lateral mesoderm was absent upstream of draculin and pit. I expression. In contrast, erythroid development in the posterior lateral mesoderm occurred withlittle or no functional Scl, as shown by expression of draculin, pu.1, and gata1 in this PLM region. Primitive erythroid cells failed to mature, as evidenced by the absence of circulating cells after 24 hpf. Only upon titration of scl morpholinos to near toxic levels was gata1 reduced or absent in embryos at 21 somites. These findings show that scl plays a vital role in development of all hematopoietic lineages in zebrafish. However, these data reveal differential effects of reduction or loss of Scl on hematopoietic development in the anterior lateral mesoderm versus the posterior lateral mesoderm. The results reveal that reduction or loss of Scl affects erythroid development in the posteriorlateral mesoderm or ICM at a progenitor stage of erythroid development, afterinitiation of gata1 and embryonic globin expression. These data suggest that early erythroid development in the zebrafish requires

little or no Scl, while maturation of erythroid progenitors cannot occur in the absence of Scl. Myeloid development in the anterior lateral mesoderm is dependent upon Set. This embryonic analysis in the zebrafish is the first to demonstrate a transient, in vivo population of in embryos with severely reduced or absent expression of Scl. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, development, stein cell leukemia, molecular genetics, SCL, blood and lymphatics, hematopoiesis, erythroid precursors, globin expression.

Kwan, T.T., R. Liang, C.M. Verfaillie, S.C. Ekker, L.C. Chan, S. Lin, and A.Y. Leung (2006). **Regulation of primitive hematopoiesis in zebrafish embryos by the death receptor gene.** *Experimental Hematology* 34(1): 27-34. ISSN: 0301 472X.
NAL Call Number: RB145.E97
Abstract: OBJECTIVE: We investigated the regulatory mechanism of primitive hematopoiesis in zebrafish (*Danio rerio*) embryos with particular reference to the role of a death receptor (zDR) gene, based on a morpholino (MO) knockdown approach. METHODS: MOs targeting the zDR and chordin (Chd) were injected into naturally spawned embryos at one- to four-cell stage. A random sequence (RS) MO was used as a control. Effects on hemoglobin formation (Hb), apoptosis, and lineage-specific gene expression were examined. Embryos injected with zDR, Chd, and RS-MOs were denoted zDR(mo), zChd(mo), and zRS(mo), respectively. Those co-injected with Chd+zDR-MOs and Chd+RS-MOs were abbreviated zChd+DR(mo) and zChd+RS(mo). RESULTS: zDR mRNA expression was restricted to the intermediate cell mass of wild-type (WT) and zChd(mo) embryos. At 48 hours postfertilization, zDR(mo) embryos showed increased Hb compared with WT or zRS(mo) embryos (2.36 x 10(-2) +/- 1.13 x 10(-3) vs 1.85 x 10(-2) +/- 5.60 x 10(-4) vs 1.79 x 10(-2) +/- 1.31 x 10(-3) U, $p < 0.05$). zChd+DR(mo) embryos also showed increased Hb compared with zChd(mo) or zChd+RS(mo) embryos (4.60 x 10(-2) +/- 2.79 x 10(-3) vs 3.17 x 10(-2) +/- 1.07 x 10(-3) vs 3.05 x 10(-2) +/- 1.25 x 10(-3) U, $p < 0.05$). zDR-MO reduced apoptosis, as shown by reduced terminal transferase-mediated dUTP nick end-labeling staining in zChd+DR(mo) compared with zChd+RS(mo) embryos and caspase-3 activity in zDR(mo) vs zRS(mo) (0.525 +/- 0.094 vs 0.953 +/- 0.113 U, $p < 0.05$), and zChd+DR(mo) vs zChd+RS(mo) embryos (0.247 +/- 0.121 vs 1.180 +/- 0.082, $p < 0.05$). zChd+DR(mo) embryos showed upregulation of erythroid-specific embryonic hemoglobin gene expression but not that of a myeloid-specific myeloperoxidase gene. CONCLUSION: Knockdown of zDR in zebrafish embryos decreased apoptosis and increased Hb, suggesting that zDR may regulate primitive hematopoiesis during development.
Descriptors: zebrafish embryology, hematopoiesis physiology, receptors, tumor necrosis factor administration and dosage, receptors, tumor necrosis factor genetics, metabolism, apoptosis drug effects, caspase 3, caspases drug effects and metabolism, disease models, developmental gene expression regulation drug effects, glycoproteins administration and dosage, hematopoiesis drug effects and genetics, hemoglobins biosynthesis and drug effects, intercellular signaling peptides and proteins administration and dosage, morpholines administration and dosage, receptors, tumor necrosis factor metabolism, .

Langenau, D.M., C. Jette, S. Berghmans, T. Palomero, J.P. Kanki, J.L. Kutok, and A.T. Look (2005). **Suppression of apoptosis by bcl-2 overexpression in lymphoid cells of transgenic zebrafish.** *Blood* 105(8): 3278-85. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: The zebrafish is an attractive vertebrate model for genetic studies of development, apoptosis, and cancer. Here we describe a transgenic zebrafish line in which T- and B-lymphoid cells express a fusion transgene that encodes the zebrafish bcl-2 protein fused to the enhanced green fluorescence protein (EGFP). Targeting EGFP-bcl-2 to the developing thymocytes of

transgenic fish resulted in a 2.5-fold increase in thymocyte numbers and a 1.8-fold increase in GFP-labeled B cells in the kidney marrow. Fluorescent microscopic analysis of living rag2-EGFP-bcl-2 transgenic fish showed that their thymocytes were resistant to irradiation- and dexamethasone-induced apoptosis, when compared with control rag2-GFP transgenic zebrafish. To test the ability of bcl-2 to block irradiation-induced apoptosis in malignant cells, we compared the responsiveness of Myc-induced leukemias with and without EGFP-bcl-2 expression in living transgenic zebrafish. T-cell leukemias induced by the rag2-EGFP-Myc transgene were ablated by irradiation, whereas leukemias in double transgenic fish expressing both Myc and EGFP-bcl-2 were resistant to irradiation-induced apoptotic cell death. The forward genetic capacity of the zebrafish model system and the ability to monitor GFP-positive thymocytes in vivo make this an ideal transgenic line for modifier screens designed to identify genetic mutations or small molecules that modify bcl-2-mediated antiapoptotic pathways.

Descriptors: zebrafish, apoptosis physiology, B lymphocytes physiology, proto oncogene proteins C Bcl 2 genetics, T lymphocytes physiology, amino acid sequence, genetically modified, apoptosis drug effects and radiation effects, B lymphocytes cytology, conserved sequence, dexamethasone pharmacology, gene expression physiology and radiation effects, glucocorticoids pharmacology, green fluorescent leukemia,T cell physiopathology, proto oncogene proteins C Bcl 2 metabolism and proteins C Myc physiology, recombinant fusion proteins genetics and metabolism, T lymphocytes cytology, thymus gland cytology.

Lee, D.W. and R.J. Arceci (2003). **Identification and analysis of the zebra fish homologue of human pasg, a snf-2-like chromatin remodeling regulator of dna methylation.** *Blood* 102(11): 202b-203b. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Epigenetic changes play key roles in directing normal embryogenesis as well as in the development of cancer through the regulation of transcription, replication and DNA repair. Chromatin remodeling factors are important components of multiprotein complexes that regulate these fundamental processes. PASG (Proliferation Associated SNF-2-like Gene) or lsh (lymphocyte specific helicase) are, respectively, the human and murine genes encoding a protein shown to contribute to global DNA methylation, cell proliferation and chromosome stability. An in-frame deletion of a conserved domain of PASG has been identified in human acute leukemias. The zebra fish (*Danio rerio*) has become an increasingly important genetic model to study development, hematopoiesis and cancer. In order to better define the ability of PASG in regulating epigenetic change during normal and neoplastic development, we identified and characterized the zebra fish homologue. The amino acid comparison of human PASG (hPASG) and murine PASG (lsh) shows >90% homology. The conserved domains that define this SWI/SNF protein family of chromatin remodelers are >98% conserved between human and murine PASG. While the overall amino acid composition of DDM1 (DNA demethylation), a closely related protein from the flowering plant Arabidopsis thaliana, differs substantially from PASG, its helicase domains are nearly 90-100% conserved. Based on these findings of evolutionarily conserved domains, we investigated whether there might be a zebra fish homologue of PASG. A BLAST search of hPASG amino acid sequence using the Sanger Institute WGS (Whole Genome Shotgun) and Zfin databases produced several possible matches. The BAC clone genomic sequences were translated in all six reading frames and putative amino acid sequences were aligned. Using the genomic structure of PASG for comparison, zebra fish genomic clones were analyzed for intron/exon boundaries. The resulting putative open reading frame (ORF) was then translated. The predicted protein is 853aa long (15aa larger than hPASG). The additional length is at 5' end of the molecule involving zebra fish exons 1 and 2. To verify these observations, the DNA sequence was used for the design of PCR primers. The primers mapped to the 5' ATG and the 3'TGA regions. Purified

RNA from 5 day post fertilization embryos and from adult kidney was reverse transcribed using oligo-dT and cDNAs amplified. The resulting PCR product was 2562 bp in length. Sequence analysis confirmed the existence of the zebra fish homologue of PASG. Overall amino acid homology with hPASG is 75% with the conserved domains having a homology that ranges from 80-100%. Zebra fish PASG has 23 exons, one more than hPASG; the additional exon is the first. Intron/exon boundaries are highly conserved, although the intron lengths are significantly different, resulting in a zebra fish PASG genomic length of approximately 15,400 bp and a hPASG gene length of approximately 56,300 bp. Expression and functional analyses reveal conserved characteristics between zebra fish and mammalian PASG. These findings should allow us to utilize the zebra fish to examine in greater genetic and mechanistic detail the role of PASG (lsh) in epigenetic change during development and cancer formation. Copyright©Thomson Reuters 2009

Descriptors: post fertilization zebrafish embryos, blood and lymphatics, immune system , molecular genetics, tumor biology, leukemia , role of PASG (lsh) in epigenetic change, blood and lymphatic disease, neoplastic disease, chromatin remodeling, cancer, adult kidney.

Leung, A.Y.H., S.C. Ekker, and C.M. Verfaillie (2003). **Differential gene expression of zebrafish chordin morphants with special reference to the mechanism of hematopoietic cell proliferation in developing embryos.** *Blood* 102(11): 328a-329a. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Introduction: We have phenocopied the zebrafish dino mutants using chordin morpholino (the Chordin morphants, Chdmo) and shown that the expanded ICM comprises hematopoietic cells that are proliferating under the influence of heightened BMP4 signaling. To investigate the mechanism of hematopoietic expansion, we used microarray analysis to identify genes differentially expressed in Chdmo and wild-type embryos. Methods and Materials: Anti-sense morpholino (1.5 ng) targeting at the 5' UTR of the zebrafish chordin gene was injected into the embryos at 1-4 cell stage. Total RNA was extracted from dechorionated Chdmo and uninjected embryos (in triplicates) at 24 hour-post-fertilization. cRNAs were linearly amplified and labeled with biotin-NTP. The biotin-labeled cRNA targets hybridized to oligo probes (65-mers) built on the chip and the signals of probe/target complex were detected by Streptavidin-Alex conjugates. The platform was an oligo-based microarray containing 16,399-zebrafish oligos derived from 16,288 zebrafish ESTs. The hybridization intensities were normalized and log2 transformed and zebrafish genes (or orthologs) that showed a significant difference (Students' unpaired t-test, $p<0.05$) between the Chdmo and WT embryos were analysed. Results: A total of 535 known genes (or orthologs) were differentially expressed between Chdmo and WT embryos. In addition to hematopoietic transcription factors (draculin, gata-1, scl, lmo-2, JunB, biklf, C/EBPs, ikaros), Chdmo expressed higher levels of mRNA for matrix metalloproteinase (members 9 and 2), cell junction proteins (claudin members a, b, f, 7 and h, junctional adhesion molecules, occludin), erythrocyte membrane protein (band 4.1 and 7.2), mitochondrial ribosomal proteins, proteases, chaperon proteins, secreted proteins (ARMET protein, thrombospondin 4, Factor XIIa, secreted protein acidic rich in cysteine and Osteoinductive factor precursor), apoptosis (death receptor, mDAM1, BAX, programmed cell death 10) and other transcription factors (homeobox protein VEGA, paraxis, hypoxia inducible gene1, terra, HoxA10, B10, D10, A13, B13, HNF-1alpha, DELTANp63alpha1, ATF-4, c-Myc). A number of genes were downregulated in Chdmo, including those encoding for Notch (delta-A, jagged1) and Wnt pathways (Wntl 1, Frizzled homolog, TCF3b), the POU genes (zp-50 POU gene, type III POU domain protein, zp-47 POU gene), the Sox genes (Sox31, Sox-11A, Sox 19, Sox-11B), the iroquios genes (ziro 5, 3, 1), muscle proteins (myosin light chain 2, Troponin,

parvalbumin, type I cytokeratin, myosin light polypeptide 3, Troponin T, myosin light chain 2, alpha tropomyosin, troponin C) as well as glycolytic enzymes (Aldolase A, glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate mutase, triosephosphate isomerase). Conclusion: While some of gene expression patterns in the Chdmo were compatible with what have been known about zebrafish hematopoiesis, the significance of may other genes that were differentially expressed remains unknown. Further studies are on-going to see if perturbation of these genes can recapitulate the ICM expansion phenotype in the chordin morphants. Copyright©Thomson Reuters 2009

Descriptors: zebrafish mutant embryos, wild type, Chordin morphants, Chdmo, blood and lymphatics, transport and circulation, cell proliferation, differential gene expression, zebrafish.

Leung, T., H. Chen, A.M. Stauffer, K.E. Giger, S. Sinha, E.J. Horstick, J.E. Humbert, C.A. Hansen, and J.D. Robishaw (2006). **Zebrafish G protein gamma2 is required for VEGF signaling during angiogenesis.** *Blood* 108(1): 160-6. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Vascular endothelial growth factor (VEGF) is a major mediator of pathologic angiogenesis, a process necessary for the formation of new blood vessels to support tumor growth. Historically, VEGF has been thought to signal via receptor tyrosine kinases, which are not typically considered to be G protein dependent. Here, we show that targeted knockdown of the G protein gng2 gene (Ggamma2) blocks the normal angiogenic process in developing zebrafish embryos. Moreover, loss of gng2 function inhibits the ability of VEGF to promote the angiogenic sprouting of blood vessels by attenuating VEGF induced phosphorylation of phospholipase C-gamma1 (PLCgamma1) and serine/threonine kinase (AKT). Collectively, these results demonstrate a novel interaction between Ggamma2- and VEGF-dependent pathways to regulate the angiogenic process in a whole-animal model. Blocking VEGF function using a humanized anti-VEGF antibody has emerged as a promising treatment for colorectal, non-small lung cell, and breast cancers. However, this treatment may cause considerable side effects. Our findings provide a new opportunity for cotargeting G protein- and VEGF-dependent pathways to synergistically block pathologic angiogenesis, which may lead to a safer and more efficacious therapeutic regimen to fight cancer.

Descriptors: zebrafish embryos, pathologic angiogenesis, GTP binding protein gamma subunits pharmacology, physiologic neovascularization drug effects, protein subunits pharmacology, signal transduction drug effects, vascular endothelialgrowth factor A drug effects, proteins pharmacology, GTP binding protein gamma subunits genetics, GTP binding protein gamma subunits, GTP binding proteins pharmacology, physiology and genetics, gene expression profiling, models, physiologic neovascularization physiology, phenotype, phospholipase C gamma metabolism, protein subunits genetics and physiology, proto oncogene proteins C AKT metabolism, reverse transcriptase PCR, signal transduction physiology, vascular endothelialgrowth factor A physiology and physiology.

Lewis, R.S., S.E. Stephenson, and A.C. Ward (2006). **Constitutive activation of zebrafish Stat5 expands hematopoietic cell populations in vivo.** *Experimental Hematology* 34(2): 179-87. ISSN: 0301 472X.

NAL Call Number: RB145.E97

Abstract: OBJECTIVE: Constitutive activation of Stat5 has been observed in a variety of malignancies, particularly myeloid leukemias. To directly investigate the in vivo consequences of Stat5 perturbation, we expressed constitutively active forms in zebrafish. METHODS: We generated mutants of the zebrafish stat5.1 protein (N646H, H298R/N714F, and N714F)

based on previously identified constitutively active mutants of murine Stat5a. The in vitro properties of these mutants were determined using phosphorylation-specific antibodies and luciferase reporter assays, and their in vivo effects were analyzed through microinjection of zebrafish embryos. RESULTS: Two of these stat5.1 mutants (N646H and H298R/N714F) showed increased tyrosine phosphorylation and transactivation activity compared to the wild-type protein. Expression of either mutant led to a range of hematological perturbations, which were more pronounced for the H298R/N714F mutant. Interestingly, expression of wild-type also produced generally similar phenotypes. Further analysis showed that expression of the H298R/N714F mutant led to increased numbers of early and late myeloid cells, erythrocytes, and B cells. Some nonhematopoietic developmental perturbations were also observed, but these were equally prominent with wild-type or mutant forms. CONCLUSION: These data implicate Stat5 activity as a direct critical regulator of hematological cell proliferation, suggesting a causal role for constitutively-active Stat5 in the etiology of hematological malignancies.

Descriptors: zebrafish wild type mutants, hematologic diseases genetics, hematopoietic stem cells metabolism, STAT5 transcription factor physiology, amino acid substitution, cell line, cell lineage physiology, cell proliferation drug effects, hematologic diseases pathology, hematopoietic stem cells cytology and drug effects, site directed mutagenesis mutation, phosphorylation, STAT5 transcription factor genetics and pharmacology, tyrosine drug effects and metabolism and pharmacology.

Lin, H.F., D. Traver, H. Zhu, K. Dooley, B.H. Paw , L.I. Zon, and R.I. Handin (2005). **Analysis of thrombocyte development in CD41-GFP transgenic zebrafish.** *Blood* 106(12): 3803-3810. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Thrombocytes are the nucleated equivalent of platelets in nonmammalian vertebrates such as the zebrafish, *Danio rerio*. We have cloned zebrafish CD41 cDNA (alpha(IIb), glycoprotein IIb [GPIIb]) and its promoter and have generated transgenic zebrafish lines with green fluorescent protein (GFP)-tagged thrombocytes. CD41 mRNA transcripts appeared 42 hours after fertilization (hpf) by reverse-transcriptase-polymerase chain reaction (RT-PCR) and at 48 hpf in circulating hematopoietic cells. Flow sorting of thrombocytes from the mesonephros of adult CD41-GFP zebrafish showed a GFP(high) subset, which had the morphologic appearance of mature thrombocytes, and a GFP(low) subset with an immature appearance, suggesting that they may be thrombocyte precursors. Confocal laser microscopy of embryos 40 and 48 hpf also showed a nonmobile population of GFP+ cells in a discrete area between the dorsal aorta and caudal vein. Production of circulating thrombocytes was inhibited by the injection of antisense morpholinos for the stem-cell transcription factor scl and c-mpl, the receptor for thrombopoietin. The nonmobile pool of GFP+ cells was abolished by scl knockdown and partially inhibited by c-mpl knockdown. These studies have shown that it is possible to identify thrombocytes, thrombocyte precursors, and, possibly, early hematopoietic stem cells in zebrafish embryos and track their proliferation and maturation.

Descriptors: zebrafish embryology, blood platelets cytology, platelet membrane glycoprotein IIb genetics, thrombopoiesis physiology, genetically modified, molecular cloning , flow cytometry, green fluorescent proteins, in situ hybridization, confocal microscopy , promoter regions genetics, reverse transcriptase PCR, amino acid sequence homology.

Liu, T.X., J. Rhodes, M. Deng, K. Hsu, H.S. Radomska, J.P. Kanki, D.G. Tenen, and A.T. Look (2007). **Dominant-interfering C/EBPalpha stimulates primitive erythropoiesis in zebrafish.** *Experimental Hematology* 35(2): 230-9. ISSN: 0301 472X.

NAL Call Number: RB145.E97

Abstract: OBJECTIVE: We investigated the role of CCAAT enhancer-binding protein-alpha (C/EBPalpha) during zebrafish embryonic blood development. METHODS: Whole-mount mRNA in situ hybridization was performed to determine the spatio-temporal expression pattern of zebrafish cebpa in developing hematopoietic progenitors. A deletion mutation of cebpa (zD420), which mimics the human dominant-negative mutations of C/EBPalpha, was transfected into CV1 cell line to evaluate its transcriptional activity in vitro and injected into zebrafish embryos at the one- to two-cell stage to examine its effects on primitive hematopoiesis during early zebrafish development. RESULTS: Zebrafish cebpa is expressed in the anterior and posterior lateral plate mesoderm at 12 hours postfertilization, along with scl, pu.1, and gata1 in developing hematopoietic progenitors. In vitro, the deletion mutation of cebpa (zD420) prevents expression of the full-length protein, allowing the expression of truncated isoforms from internal translational initiation sites. As in the human, the truncated zebrafish C/EBPalpha proteins did not activate the expression of known target granulocytic genes, and in fact suppressed transactivation that was induced in vitro by the full-length protein. Forced expression of the zD420 mRNA in zebrafish embryos led to an expansion of primitive erythropoiesis, without a discernible effect on granulopoiesis. CONCLUSION: Expression of the truncated isoforms of cebpa alters the developmental pattern of hematopoietic progenitor cells during embryogenesis.

Descriptors: zebrafish embryos, CCAAT enhancer binding protein alpha genetics and metabolism, erythropoiesis genetics, developmental gene expression regulation, dominant genes, base sequence, blood vessels embryology and metabolism, complementary DNA genetics, embryonic development genetics and physiology, gene deletion, hematopoietic stem cells cytology and metabolism, molecular sequence data, mutation, promoter regions genetics, promoter regions genetics physiology, messenger RNA, reverse transcriptase PCR methods, DNA sequence analysis, translocation, genetic, translocation, genetic physiology, heterologous transplantation, metabolism.

Liu, Y., L. Du, M. Osato, E.H. Teo, F. Qian, H. Jin, F. Zhen, J. Xu, L. Guo, H. Huang, J. Chen, R. Geisler, Y.J. Jiang, J. Peng, and Z. Wen (2007). **The zebrafish udu gene encodes a novel nuclear factor and is essential for primitive erythroid cell development.** *Blood* 110(1): 99-106. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Hematopoiesis is a complex process which gives rise to all blood lineages in the course of an organism's lifespan. However, the underlying molecular mechanism governing this process is not fully understood. Here we report the isolation and detailed study of a newly identified zebrafish ugly duckling (Udu) mutant allele, Udu(sq1). We show that loss-of-function mutation in the udu gene disrupts primitive erythroid cell proliferation and differentiation in a cell-autonomous manner, resulting in red blood cell (RBC) hypoplasia. Positional cloning reveals that the Udu gene encodes a novel factor that contains 2 paired amphipathic alpha-helix-like (PAH-L) repeats and a putative SANT-L (SW13, ADA2, N-Cor, and TFIIIB-like) domain. We further show that the Udu protein is predominantly localized in the nucleus and deletion of the putative SANT-L domain abolishes its function. Our study indicates that the Udu protein is very likely to function as a transcription modulator essential for the proliferation and differentiation of erythroid lineage.

Descriptors: zebrafish ugly duckling (Udu) mutant, erythroid progenitor cells cytology,

erythroid specific DNA binding proteins genetics, factors physiology, erythropoiesis, transcription factors physiology, cell differentiation and proliferation, erythrocytes, abnormal pathology, erythroid cells cytology, erythroid specific DNA binding proteins genetics, factors genetics, mutation.

Ma, A.C., R. Liang, and A.Y. Leung (2007). **The role of phospholipase C gamma 1 in primitive hematopoiesis during zebrafish development.** *Experimental Hematology* 35(3): 368-73. ISSN: 0301 472X.

NAL Call Number: RB145.E97

Abstract: OBJECTIVES: Phospholipase C (PLC) gamma 1 has been shown to mediate signal transduction of tyrosine kinases and affect function of hematopoietic cells. However, its role in hematopoiesis during embryonic development is currently unclear. In this study, we examined this issue using morpholino (MO) gene knockdown in zebrafish embryos METHODS: MO targeting at the exon-1-intron-1 junction of zebrafish PLC-gamma1 was injected into embryos at the one- to four-cell stage (referred herein zPLC-gamma1(MO) embryos). Primitive hematopoiesis was examined quantitatively by flow cytometry in Tg(gata1:GFP) embryos and by real-time quantitative polymerase chain reaction at 18 hours-post-fertilization (hpf), before the onset of circulation. The embryos were also treated with receptor inhibitors of vascular endothelial growth factor, fibroblast growth factor, and platelet-derived growth factor at 25, 1, and 30 micromol/L, respectively, from one cell until 48 hpf. RESULTS: Erythropoiesis was reduced in zPLC-gamma1(MO) embryos, as shown by the reduction in gata1(+) cells (wild-type: 4.32% +/- 0.10% vs zPLC-gamma1(MO): 2.38% +/- 0.11%, p = 0.021) and gata1 and alpha-embryonic hemoglobin expression [0.47 +/- 0.06-fold (p = 0.013) and 0.46 +/- 0.04-fold (p = 0.013)]. Expression of scl, lmo-2 (early hematopoietic progenitors), pu.1, and l-plastin (myelomonocytic lineages) as well as fli1 (vascular progenitors) were unaffected. Fli1(+) cells in Tg(fli1:GFP) embryos were also unaffected by zPLC-gamma1(MO). When embryos were incubated with receptor inhibitors of vascular endothelial growth factor (VEGFRTKI), fibroblast growth factor (SU5402), or platelet-derived growth factor (AG1296), only VEGFRTKI reduced erythropoiesis [VEGFRTKI: 2.10% +/- 0.07% (p = 0.021) vs SU5402: 4.08% +/- 0.12% (p = 0.248) vs AG1296: 4.12% +/- 0.14% (p = 0.149)]. CONCLUSION: PLC-gamma1 is involved in the regulation of primitive hematopoiesis in zebrafish embryos, which is distinct from its later effect on vascular formation.

Descriptors: zebrafish embryology, hematopoiesis physiology, phospholipase C gamma physiology, erythropoiesis drug effects and physiology, fibroblast growth factor 1 administration and dosage, flow cytometry methods, gene expression profiling, developmental gene expression regulation, gene targeting methods, green fluorescent hematopoiesis drug effects and genetics, antisense oligonucleotides administration and dosage, phospholipase C gamma drug effects and genetics, platelet derivedgrowth factor administration and dosage, reverse transcriptase PCR methods, vascular endothelialgrowth factor A administration and dosage, .

Ma, A.C., A.C. Ward, R. Liang, and A.Y. Leung (2007). **The role of jak2a in zebrafish hematopoiesis.** *Blood* 110(6): 1824-30. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Janus kinase 2 (Jak2) transduces signals from hematopoietic cytokines, and a gain-of-function mutation (Jak2(617V>F)) is associated with myeloproliferative diseases, particularly polycythemia vera. In this study, we examined the role of jak2a in zebrafish embryos in knock-down and overexpression studies using morpholinos (MOs) targeting the 5' untranslated region (UTR) (jak2a(UTR)-MO) and splice-site junction (jak2a(SS)-MO) of

jak2a, a Jak inhibitor AG490 and a constitutive-active form of jak2a (jak2a(ca)). At 18 and 24 hours after fertilization (hpf), jak2a is expressed predominantly in the intermediate cell mass (ICM; site of primitive hematopoiesis) of wild-type and chordin morphant embryos (characterized by expansion of ICM). Both jak2a MOs and AG490 reduced gata1(+) (erythroid) cells in Tg(gata1:GFP) embryos, signal transducer and activation of transcription 5 (stat5) phosphorylation, and gene expression associated with early progenitors (scl and lmo2) and erythroid (gata1, alphahe1 and betahe1) and myeloid (spi1 [early] and mpo [late]) lineages. The chordin morphant is associated with increased stat5 phosphorylation, and both jak2a MOs and treatment with AG490 significantly ameliorated ICM expansion and hematopoietic gene up-regulation in these embryos. Injection of plasmid encoding jak2a(ca) significantly increased erythropoiesis and expression of gata1, alphahe1 and betahe1, spi1, mpo, and l-plastin. In conclusion, zebrafish jak2a is involved in primitive hematopoiesis under normal and deregulated conditions.

Descriptors: wild-type and chordin morphant embryo zebrafish, developmental gene expression regulation, hematopoiesis, janus kinase 2 physiology, genetically modified, cell division and lineage, enzyme activation, erythroid progenitor cells metabolism, gene expression profiling, glycoproteins metabolism, intercellular signaling peptides and proteins metabolism, janus kinase 2 genetics, physiologic neovascularization, oligonucleotide array sequence analysis, phosphorylation, STAT5 transcription factor metabolism, signal transduction, proteins genetics.

MacDonald, B.A., M. Sund, M.A. Grant, K.L. Pfaff, K. Holthaus, L.I. Zon, and R. Kalluri (2006). **Zebrafish to humans: evolution of the alpha3-chain of type IV collagen and emergence of the autoimmune epitopes associated with Goodpasture syndrome.** *Blood* 107(5): 1908-15. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Goodpasture syndrome is an autoimmune vascular disease associated with kidney and lung failure, with pathogenic circulating autoantibodies targeted to a set of discontinuous epitope sequences within the noncollagenous domain-1 (NC1) of the alpha3 chain of type IV collagen (alpha3(IV)NC1), the Goodpasture autoantigen. We demonstrate that basement membrane extracted NC1 domain preparations from *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Danio rerio* do not bind Goodpasture autoantibodies, while *Xenopus laevis*, chicken, mouse and human alpha3(IV)NC1 domains bind autoantibodies. The alpha3(IV) chain is not present in *C. elegans* and *Drosophila melanogaster*, but is first detected in the *Danio rerio*. Interestingly, native *Danio rerio* alpha3(IV)NC1 does not bind Goodpasture autoantibodies. Next, we cloned, sequenced, and generated recombinant *Danio rerio* alpha3(IV)NC1 domain. In contrast to recombinant human alpha3(IV)NC1 domain, there was complete absence of autoantibody binding to recombinant *Danio rerio* alpha3(IV) NC1. Three-dimensional molecular modeling from existing x-ray coordinates of human NC1 domain suggest that evolutionary alteration of electrostatic charge and polarity due to the emergence of critical serine, aspartic acid, and lysine residues, accompanied by the loss of asparagine and glutamine, contributes to the emergence of the 2 major Goodpasture epitopes on the human alpha3(IV)NC1 domain, as it evolved from the *Danio rerio* over 450 million years.

Descriptors: zebrafish, anti glomerular basement membrane disease immunology, autoantibodies immunology, autoantigens immunology, collagen Type IV immunology, epitopes immunology, molecular evolution, anti glomerular basement membrane disease genetics, antibody specificity immunology, autoantigens genetics, collagen Type IV genetics, epitope

mapping methods, epitopes genetics, mice, amino acid sequence homology, species specificity, structural protein homology, zebrafish immunology.

McReynolds, L.J., M. Mullins, and T. Evans (2005). **Functional distinctions for smad1 and smad5 during hematopoiesis revealed by the zebrafish model system**. *Blood* 106(11, Part 1): 1005A. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Descriptors: zebrafish, development, molecular genetics, blood and lymphatics, hemolytic anemia, blood and lymphatic disease, chemically induced, flow cytometry, histology and cytology techniques, research model, hematopoiesis.

Mendenhall, E.M., C.E. Eckfeldt, S.C. Ekker, and C.M. Verfaillie (2004). **The role of sprouty family members in hematopiesis in zebrafish and mammals.** *Blood* 104(11, Part 1): 42A. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: In an effort to identify novel regulators of hematopoiesis, we identified a Sprouty family member, SPRY 1, significantly enriched (>1.5 fold, p-value <0.03)in human hematopoietic stem cells (HSC) compared to more committed hematopoietic progenitor cells (HPC) using Affymetrix-based global gene expression profiling (see Eckfeldt et al. abstract). Predominantly FGF, but also VEGF, and EGFsignaling induces expression of Sprouty family members (SPRY1-4). SPRY proteins form heterodimers with other Sprouty members along with homodimers. They and are intracellular antagonists and agonists of receptor tyrosine kinase signaling via the RAS/MAPK pathway. SPRY proteins are best characterized. as antagonists of the FGF, EGF, and VEGF pathways. As part of a high-throughput functional analysis of the genes identified by human gene array in zebrafish, we evaluated the role of the only identified zebrafish homologue for Sprouty, SPRY4, in zebrafish hematopoiesis. Using morpholino (MO) antisense technology we knocked-down expression of zebrafish SPRY4 during early zebrafish development in adouble transgenic GATA1:dsRed/Fli1:eGFP zebrafish line. The SPRY4 morpholino injected fish (SPRY4(MO)) showed an almost complete loss of blood with normal vasculature, which was confirmed by injection of a second unique morpholino designed against SPRY4. In situ hybridization of the hematopoietic markers SCL, GATA1 and Ikaros were all significantly reduced or absent at multipledevelopmental stages in the SPRY4(MO) embryo. Together with the normal development of the vasculature, this data indicates SPRY4 acts early in hematopoiesis and after the specification of the hemangioblast. In situ hybridization for SPRY4 demonstrated expression in the otic vesicle, tail bud and lateral plate mesoderm, the first site of hematopoietic stem cells. Coinjection of SPRY morpholino and low doses of VEGF morpbolino did not rescue the SPRY bloodphenotype, indicating that the blood phenotype is not likely through the VEGFpathway. Previous gain-of-function experiments injecting SPRY4 mRNA showed anexpansion of hematopoietic progenitors (Furthauer et al. Development 2001), aphenotype we confirmed with overexpression of SPRY4 cDNA.Experiments are ongoing to overexpress human SPRY I or zebrafish SPRY4 cDNAs in mammalian cells todetermine if the hematopoietic phenotype correlates with the zebrafish data. Additional genes differentially expressed in human HSC and HPC evaluated via the high throughput zebrafish screen will also be presented. This is the first description of functional validation of a gene from a human microarray in zebrafish, an essential step in obtaining meaningful in vivo data from global geneprofiling studies.
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Descriptors: transgenic zebrafish, blood and lymphatics, transport and circulation,

molecular genetics, cell biology, gene profiling, genetic techniques , morpholino antisense technology, hematopiesis , rAS, MApK pathway.

Onnebo, S.M., M.M. Condron, D.O. McPhee, G.J. Lieschke, and A.C. Ward (2005). **Hematopoietic perturbation in zebrafish expressing a tel-jak2a fusion.** *Experimental Hematology* 33(2): 182-8. ISSN: 0301 472X.
NAL Call Number: RB145.E97
Abstract: OBJECTIVE: Various TEL-JAK2 fusions have been identified in patients with lymphoblastic and myeloid leukemias that result in constitutive activation of the JAK2 kinase domain. Such fusions can mediate factor-independent growth of hematopoietic cell lines and induction of malignancy in mouse models. MATERIALS AND METHODS: To assess whether zebrafish could be utilized as a suitable model for the study of myeloid oncogenesis, we generated a zebrafish tel-jak2a fusion oncoprotein based on that seen in a case of chronic myeloid leukemia. This was transiently expressed in zebrafish embryos under the control of the spi1 promoter, which is strongly active in myeloid precursors. RESULTS: Visual, histological, and molecular analysis revealed disruption of normal embryonic hematopoiesis, including perturbation of the myeloid and erythroid lineages. CONCLUSION: These results indicate that the zebrafish tel-jak2a oncoprotein is functional, and suggest that this organism will be useful for the experimental study of myeloid malignancy.
Descriptors: zebrafish embryos, DNA binding proteins genetics, hematopoiesis physiology, leukemia, myeloid genetics, protein tyrosine kinases genetics, proto oncogene repressor acute disease, amino acid sequence, genetically modified, disease models, physiology, tel-jak2a oncoprotein, janus kinase 2, peptide fragments chemistry, proto oncogene proteins C ets, recombinant fusion proteins metabolism.

Onnebo, S.M., S.H. Yoong, and A.C. Ward (2004). **Harnessing zebrafish for the study of white blood cell development and its perturbation.** *Experimental Hematology* 32(9): 789-96. ISSN: 0301 472X.
NAL Call Number: RB145.E97
Abstract: Considerable progress has been made in understanding the molecular basis of normal white blood cell development and its perturbation in disease through the use of clinical studies and traditional animal and cell line models. Despite this, however, many questions are still being answered and white blood cell disorders, including leukemia and lymphoma, remain a significant health problem. The zebrafish *(Danio rerio*) has emerged as a powerful alternative vertebrate model for the study of development and disease. We review the recent application of zebrafish to the study of white blood cell development and its disruption, particularly leukemogenesis. Such studies have highlighted the overall conservation of these processes throughout vertebrates, and establish zebrafish as a useful experimental model. This organism is now poised to make an important contribution to our understanding of the underlying genetic control of white blood cell development and its disruption, as well as the identification of new therapeutic agents.
Descriptors: zebrafish disease model, leukocytes physiology, blood, leukemia etiology, white blood cell development, mutation, developoment disruption.

Paffett Lugassy, N., N. Hsia, P.G. Fraenkel, B. Paw, I. Leshinsky, B. Barut, N. Bahary, J. Caro, R. Handin, and L.I. Zon (2007). **Functional conservation of erythropoietin signaling in zebrafish.** *Blood* 110(7): 2718-26. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: Erythropoietin (Epo) and its cognate receptor (EpoR) are required for maintain-

ing adequate levels of circulating erythrocytes during embryogenesis and adulthood. Here, we report the functional characterization of the zebrafish epo and epor genes. The expression of epo and epor was evaluated by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) and whole-mount in situ hybridization, revealing marked parallels between zebrafish and mammalian gene expression patterns. Examination of the hypochromic mutant, weissherbst, and adult hypoxia-treated hearts indicate that zebrafish epo expression is induced by anemia and hypoxia. Overexpression of epo mRNA resulted in severe polycythemia, characterized by a striking increase in the number of cells expressing scl, c-myb, gata1, ikaros, epor, and betae1-globin, suggesting that both the erythroid progenitor and mature erythrocyte compartments respond to epo. Morpholino-mediated knockdown of the epor caused a slight decrease in primitive and complete block of definitive erythropoiesis. Abrogation of STAT5 blocked the erythropoietic expansion by epo mRNA, consistent with a requirement for STAT5 in epo signaling. Together, the characterization of zebrafish epo and epor demonstrates the conservation of an ancient program that ensures proper red blood cell numbers during normal homeostasis and under hypoxic conditions.

Descriptors: zebrafish embryos, erythropoietin metabolism, signal transduction, metabolism, amino acid sequence, anemia metabolism, anemia pathology, anoxia metabolism and pathology, conserved sequence, complementary DNA genetics, cytology, embryology, metabolism, erythroid cells cytology, erythropoiesis, erythropoietin chemistry, genetics, isolation and purification, developmental gene expression regulation, molecular sequence data, receptors, erythropoietin chemistry, genetics and metabolism, STAT5 transcription factor metabolism, sequence alignment.

Paffett Lugassy, N.N. and L.I. Zon (2005). **Analysis of hematopoietic development in the zebrafish.** *Methods in Molecular Medicine* 105: 171-98. ISSN: 1543-1894.

Abstract: The zebrafish (*Danio rerio*) has emerged as a powerful vertebrate genetic and developmental model that is particularly amenable to the study of hematopoiesis. The zebrafish embryo develops externally and its optical clarity allows the number and morphology of circulating blood cells to be visualized using a dissecting microscope. Both the morphology of the blood lineages and the expression of critical blood genes are highly conserved between zebrafish and mammals. The high fecundity and short generation time of zebrafish facilitate genetic analysis, and a number of large-scale mutagenesis screens have identified mutations in genes affecting blood development. The discovery of novel hematopoietic genes, as well as the cloning of zebrafish homologs of known hematopoietic genes, necessitates the use of efficacious and reliable methods for complete gene characterization. In this chapter, we illustrate frequently used techniques that are essential for evaluating hematopoiesis in the zebrafish, including whole-mount in situ hybridization, the detection of erythrocytes by o-dianisidine staining, and a description of the microinjection procedure, which has various applications, including overexpression of messenger ribonucleic acid, gene "knockdown" by antisense technology, and the creation of transgenic zebrafish. Also included is an explanation of the use of flow cytometry to separate hematopoietic lineages from the adult kidney and to isolate relatively pure populations of cell types from transgenic embryos based on the expression of fluorescent markers.

Descriptors: transgenic zebrafish, cell lineage physiology, developmental gene expression regulation physiology, gene transfer techniques, hematopoiesis physiology, mutagenesis genetics, genetically modified, cell lineage genetics, developmental gene expression regulation genetics, hematopoiesis genetics.

Patterson, L.J., M. Gering, C.E. Eckfeldt, A.R. Green, C.M. Verfaillie, S.C. Ekker, and R. Patient (2007). **The transcription factors Scl and Lmo2 act together during development of the hemangioblast in zebrafish.** *Blood* 109(6): 2389-98. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: The transcription factors Scl and Lmo2 are crucial for development of all blood. An important early requirement for Scl in endothelial development has also been revealed recently in zebrafish embryos, supporting previous findings in scl(-/-) embryoid bodies. Scl depletion culminates most notably in failure of dorsal aorta formation, potentially revealing a role in the formation of hemogenic endothelium. We now present evidence that the requirements for Lmo2 in zebrafish embryos are essentially the same as for Scl. The expression of important hematopoietic regulators is lost, reduced, or delayed, panendothelial gene expression is down-regulated, and aorta-specific marker expression is lost. The close similarity of the phenotypes for Scl and Lmo2 suggest that they perform these early functions in hemangioblast development within a multiprotein complex, as shown for erythropoiesis. Consistent with this, we find that scl morphants cannot be rescued by a non-Lmo2-binding form of Scl but can be rescued by non-DNA-binding forms, suggesting tethering to target genes through DNA-binding partners linked via Lmo2. Interestingly, unlike other hematopoietic regulators, the Scl/Lmo2 complex does not appear to autoregulate, as neither gene's expression is affected by depletion of the other. Thus, expression of these critical regulators is dependent on continued expression of upstream regulators, which may include cell-extrinsic signals.
Descriptors: zebrafish, basic helix loop helix transcription factors metabolism, DNA binding proteins genetics, proteins metabolism, hematopoiesis, metalloproteins metabolism, proto oncogene proteins metabolism, blood, metabolism, basic helix loop helix transcription factors genetics, cell line, DNA metabolism, DNA binding proteins genetics, proteins deficiency and genetics, cytology, embryology, metabolism, endothelial cells cytology and metabolism, erythroid cells cytology and metabolism, developmental gene expression regulation, hematopoietic stem cells cytology and metabolism, metalloproteins deficiency and genetics, mice, myeloid cells cytology and metabolism, phenotype, protein binding, proto oncogene proteins genetics.

Patterson, L.J., M. Gering, and R. Patient (2005). **Scl is required for dorsal aorta as well as blood formation in zebrafish embryos.** *Blood* 105(9): 3502-11. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: Blood and endothelial cells arise in close association in developing embryos, possibly from a shared precursor, the hemangioblast, or as hemogenic endothelium. The transcription factor, Scl/Tal1 (stem cell leukemia protein), is essential for hematopoiesis but thought to be required only for remodeling of endothelium in mouse embryos. By contrast, it has been implicated in hemangioblast formation in embryoid bodies. To resolve the role of scl in endothelial development, we knocked down its synthesis in zebrafish embryos where early precursors and later phenotypes can be more easily monitored. With respect to blood, the zebrafish morphants phenocopied the mouse knockout and positioned scl in the genetic hierarchy. Importantly, endothelial development was also clearly disrupted. Dorsal aorta formation was substantially compromised and gene expression in the posterior cardinal vein was abnormal. We conclude that scl is especially critical for the development of arteries where adult hematopoietic stem cells emerge, implicating scl in the formation of hemogenic endothelium.
Descriptors: zebrafish embryos, aorta growth and development, blood, DNA binding, proto oncogene, transcription factors physiology, aorta embryology, basic helix loop helix transcription factors, blood circulation genetics, DNA binding proteins genetics, proteins

deficiency vascular endothelium cytology and embryology, growth and development, developmental gene expression regulation, hematopoietic stem cells cytology, mice, physiologic neovascularization, proto oncogene proteins deficiency, transcription factors deficiency, proteins deficiency.

Paw, B.H., G.C. Shaw, J.J. Cope, K. Corson, C. Hersey, R. Wingert, G.E. Ackermann, D. Traver, N.S. Trede, B.A. Barut, and L.I. Zon (2004). **Frascati, a mitochondrial solute transporter, and its role in vertebrate erythropoiesis.** *Blood* 104(11, Part 1): 18A. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: Acquired and congenital defects in iron metabolism from either deficiency or excess are one of the most common human diseases. Here we present the characterization of the zebrafish frascati mutation, which results in a profound hypochromic anemia and a developmental arrest at the pro-erythroblast stage. Using apositional cloning strategy, we have identified the gene disrupted in frascati mutants as a novel member of the mitochondrial solute transporter family. Members of this family of solute carriers have related tripartite sequence and structure. They function in transporting various metabolites and substrates across the inner mitochondrial membrane. We have verified the identity of the gene in zebrafish by the following criteria: (a) tissue-restricted expression in erythroid progenitors, (b) identification of missense mutations from the five frascati alleles, (c) rescue of anemia by over-expression of frascati cRNA in mutant embryos, and (d) mimicry of anemia using inactivating antisense morpholinos in wildtype embryos. We have also identified the functional ortholog in the mouse which has an analogous tissue and developmental expression pattern. The frascati ortholog in the mouse is highly expressed in fetal liver and adultbone marrow and spleen. The routine frascaii transcript and protein are induced during terminal erythroid differentiation in MEL cells treated with either DMSO or HMBA. The over-expression of the mouse frascati cRNA in zebrafish frascati mutant embryos rescued their anemia with equal efficacy as the zebrafish clone. Given the identity of the gene and the requirement for iron in heme biosynthesis in the mitochondria of the developing erythron, we injected exogenous iron-dextran into frascati embryos. The embryos injected with iron-dextran were allowed to develop to 3 days post-fertilization, then stained for hemoglobinized cells with o-dianisidine and genotyped. Using this assay, the anemia caused by the frascati mutation could be partially rescued with exogenous iron supplementation. We therefore propose that the frascati gene functions as the essential transporter for iron importation into the mitochondria for heme biosynthesis and subsequent hemoglobin production in developing erythroid progenitors. Insight into the function of the frascati gene will be directly relevant to our understanding of human disorders of iron deficiency anemia and iron-overload sideroblastic anemia. Copyright©Thomson Reuters 2009
Descriptors: zebrafish frascati mutant, metabolism, molecular genetics, blood and lymphatics, transport and circulation, cell biology, hypochromic anemia, blood and lymphatic disease, erythropoiesis, metabolic disease, blood and lymphatic disease, iron metabolism defect, metabolic disease, positional cloning strategy, genetic techniques, developmental arrest.

Perkins, A.C., D. Gunaratnam, M.R. Gardiner, and K.C. Robinson (2005). **Klf12 is required for vessel organization in zebrafish embryos.** *Blood* 106(11, Part 1): 1029A. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: The process of forming a lumenised vessel from an angioblast cord is a crucial part of both vasculogenesis and angiogenesis. The Kruppel like factors (klfs) are a family of zinc finger transcription factors which play important roles in many aspects of differentia-

tion. Klf2 knockout mice die in utero from haemorrhaging due to arterial wall defects. Due to the similarity of vascular and haematopoietic systems to those of mammals, the zebrafish was chosen as a model in which to study the function of klf12, the only zebrafish representative of the repressor subfamily of mammalian klfs which includes klf3, klf8 and klf12. Klf12 is first detected by WISH at 12 somites in the lateral plate mesoderm (LPM) and continues in these cells as they from the ICM at 18-22hpf, the site of vasculogenesis and haematopoiesis. From 24hpf klf12 is expressed in short stripes extending from the ICM dorsally towards the notochord. This expression pattern is similar but not identical to that of fli1 and flk1, two vascular markers, Expression subsequently decreases and is absent by 30hpf.Targeted knockdown of klf12 by translantion-inhibiting and splicing morpholinos (MOs) produces a vascular defect. At 24hpf morphants display correct expression of primitive blood, kidney and vascular markers such as gata1, beta E3globin, biklf, pax2.1 and fli1. However, circulation is absent in 65% of embryos at 48 hpf and reduced in most, as seen by both brightfield microscopy and fluorescent microbead microangiography. Embryos also display a slower heartbeat, pericardial oedema and oedema over the yolk/duct of Cuvier, all likely to be secondary effects due to the circulatory defect. Injection of klf12 MOs into fli1-eGFP transgenic embryos reveals correct differentiation of endothelial cells, but disorganised angiogenesis.In summary, klf12 morphants display correct specification of angioblasts and differentiation of endothelial cells but a defect in tubulogenesis of endothelial cords. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, biochemistry, development, cardiovascular system, transport and circulation, hemorrhage, vascular disease, function of klf12, angiogenesis, vasculogenesis, edema, tubulogenesis, arterial wall defect.

Renshaw, S.A., C.A. Loynes, D.M. Trushell, S. Elworthy, P.W. Ingham, and M.K. Whyte (2006). **A transgenic zebrafish model of neutrophilic inflammation.** *Blood* 108(13): 3976-8. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: We have established an in vivo model for genetic analysis of the inflammatory response by generating a transgenic zebrafish line that expresses GFP under the neutrophil-specific myeloperoxidase promoter. We show that inflammation is induced after transection of the tail of zebrafish larvae and that this inflammation subsequently resolves over a similar time course to mammalian systems. Quantitative data can be generated from this model by counting of fluorescent cells or by digital image analysis. In addition, we show that the resolution of experimentally induced inflammation can be inhibited by the addition of a pan-caspase inhibitor, zVD.fmk, demonstrating that experimental manipulation of the resolution of inflammation is possible in this model.
Descriptors: zebrafish, neutrophils immunology, wounds and injuries immunology, zebrafish immunology, genetically modified, cell count methods, disease models, green fluorescent proteins, computer assisted image processing, inflammation genetics, immunology and pathology, neutrophils pathology, peroxidase genetics and immunology, promoter regions genetics, transgenes immunology, wounds and injuries genetics and pathology.

Rhodes, J., A. Amsterdam, K. Ho, J.P. Kanki, N. Hopkins, and A.T. Look (2005). **A role for fbxo5 in zebrafish myelopoiesis**. *Blood* 106(11, Part 1): 1005A-1006A. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: The zebrafish is a powerful model system for investigating embryonic vertebrate hematopoiesis that allows for the critical in vivo analysis of cell development and lineage determination. Conservation of the variety of blood cell types and key regulatory factors

between mammals and zebrafish suggests that the regulatory mechanisms directing lineage specification are similarly conserved. To identify novel genes necessary for normal myeloid cell development, we have analyzed a panel of zebrafish insertional mutants, provided by Nancy Hopkins at Massachusetts Institute of Technology (MIT), and identified mutants with deficient or abnormal distribution of myeloperoxidase (lope), a gene specifically expressed in zebrafish granulocytes. One of the mutants identified in this screen is disrupted in fbxo5, a gene not previously identified as having a role in hematopoiesis. Using whole mount RNA in situ analysis on mutant and morpholino-injected embryos (morphants) we have determined that decreased levels of fbxo5 result in severely diminished numbers of granulocytes, although erythropoiesis appears normal. Interestingly, fbxo5 morphants have normal levels of pu.1 and 1-plastin expression. Since we have previously shown that knock-down of pu.1 results in the loss of mpo-expressing cells, our data suggest that fbxo5 may function downstream or in a parallel pathway to pu.1 during granulopoiesis. To determine if the decreased number of granulocytes was due to programmed cell death, we examined the level of apoptosis in morphant versus control embryos. Whole mount acridine orange staining of apoptotic cells indicated that fbxo5 morphants had increased apoptosis, which can be mostly rescued by co-injection with p53 morpholino. However, the fbxo5/p53 double morphants continued to display a severe decrease in granulocytes, suggesting that the loss of granulocytes is not due to p53-mediated apoptosis. Fbxo5 has been examined previously in *Xenopus* oocytes, where the protein was found to regulate cell cycle progression by inhibiting APC. Interestingly, we have identified multiple mutants in genes that normally regulate the G2/M checkpoint and mitosis during the cell cycle all which display decreased numbers of granulocytes, suggesting a role for the regulation of the cell cycle in granulopoiesis. While further analysis is needed to determine where and how fbxo5 functions during myeloid cell development, as well as each of the genes disrupted in our additional cell cycle mutants, we have identified that fbxo5 is necessary specifically for the normal development of granulocytes. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, cell cycle, mutants, granulocytes, immune system, blood and lymphatics, circulation, molecular genetics , development, whole mount RNA in situ analysis, genetic techniques, whole mount acridine orange staining, histology and cytology techniques, apoptosis, mitosis, hematopoiesis, myelopoiesis.

Shaw, G.C., J.J. Cope, L. Li, K. Corson, C. Hersey, G.E. Ackermann, B. Gwynn, A.m.y.J. Lambert, R.A. Wingert, D. Traver, N.S. Trede, B.A. Barut, Y.i. Zhou, E. Minet, A. Donovan, A. Brownlie, R. Balzan, M.J. Weiss, L.L. Peters, J. Kaplan, L. Zon, and B.H. Paw (2006). **Mitoferrin is essential for erythroid iron assimilation**. *Nature* 440(7080): 96-100. ISSN: 0028-0836.

NAL Call Number: 472 N21

Descriptors: zebrafish model, anemia, biogenesis, biosynthesis, erythroblasts, erythrocytes, freshwater fish, hemoglobinss, heme, hemopoiesis, iron, metabolism, mitochondria, redox reactions, *Danio rerio*.

Sumanas, S., T. Jorniak, and S. Lin (2005). **Identification of novel vascular endothelial-specific genes by the microarray analysis of the zebrafish cloche mutants.** *Blood* 106(2): 534-41. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: The zebrafish cloche (clo) mutation affects the earliest known step in differentiation of blood and endothelial cells in vertebrates. We established clo/gata1-GFP transgenic line with erythroid-specific green fluorescent protein (GFP) expression, which allowed differ-

entiation of clo and wild-type siblings at the midsomitogenesis stages before morphologically visible phenotypes appeared. To discover novel genes potentially involved in hematopoietic and vascular development, we performed microarray analysis of more than 15,000 zebrafish genes or expressed sequence tags (ESTs) in clo mutant embryos. We isolated the full-length sequences and determined the expression patterns for 8 novel cDNAs that were significantly down-regulated in clo-/- embryos. Dual specificity phosphatase 5 (dusp5), cadherin 5 (cdh5; VE-cadherin), aquaporin 8 (aqp8), adrenomedullin receptor (admr), complement receptor C1qR-like (crl), scavenger receptor class F, member 1 (scarf1), and ETS1-like protein (etsrp) were specifically expressed in the vascular endothelial cells, while retinol binding protein 4 (rbp4) was expressed in the yolk syncytial layer and the hypochord. Further functional studies of these novel genes should help to elucidate critical early steps leading to the formation of vertebrate blood vessels.

Descriptors: zebrafish embryology, vascular endothelium embryology, mutation, amino acid sequence, genetically modified, antigens, cd, aquaporins genetics, cadherins genetics, expressed sequence tags, developmental gene expression regulation, green fluorescent in situ hybridization, ion channels genetics, membrane glycomolecular sequence data, oligonucleotide array sequence analysis, protein tyrosine phosphatase genetics, receptors, complement genetics, immunologic receptors genetics, receptors, peptide genetics, receptors, scavenger, recombinant retinol binding scavenger receptors, class F, amino acid sequence homology.

Thattaliyath, B., M. Cykowski, and P. Jagadeeswaran (2005). **Young thrombocytes initiate the formation of arterial thrombi in zebrafish.** *Blood* 106(1): 118-24. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: The zebrafish system is an excellent vertebrate genetic model to study hemostasis and thrombosis because saturation mutagenesis screens can identify novel genes that play a role in this vital physiologic pathway. To study hemostatic mutations, it is important to understand the physiology of zebrafish hemostasis and thrombosis. Previously, we identified zebrafish thrombocytes and have shown that they participate in arterial thrombus formation. Here, we recognized 2 populations of thrombocytes distinguishable by DiI-C18 (DiI) staining. DiI+ thrombocytes have a high density of adhesive receptors and are functionally more active than DiI- thrombocytes. We classified DiI+ thrombocytes as young and DiI- thrombocytes as mature thrombocytes. We found young and mature thrombocytes each formed independent clusters and that young thrombocytes clustered first. We have also shown that young thrombocytes initiate arterial thrombus formation. We propose that due to the increased adhesive receptor density on young thrombocytes, they adhere first to the subendothelial matrix, get activated rapidly, release agonists, and recruit more young thrombocytes, which further release more agonists. This increase in agonists activates the less active mature thrombocytes, drawing them to the growing thrombus. Since arterial thrombus formation is a fundamental hemostatic event, this mechanism may be conserved in mammals and may open new avenues for prevention of arterial thrombosis.

Descriptors: zebrafish thrombocytes, hemostatic mutations, blood platelets cytology and physiology, hemostasis physiology, thrombosis physiopathology, arteries, carbocyanines, cell aging, fluorescent dyes.

Traver, D. (2004). **Cellular dissection of zebrafish hematopoiesis.** *Methods in Cell Biology* 76: 127-49. ISSN: 0091-679X.

NAL Call Number: 442P92

Descriptors: zebrafish embryos, hematopoiesis physiology, genetically modified, cell lineage and separation methods, cytology, physiology, flow cytometry, fluorescent dyes metabolism,

hematopoietic stem cell transplantation methods, cytology and physiology, immunohistochemistry, kidney cytology and physiology, lectins metabolism, membrane glycoproteins immunology and metabolism, fluorescence microscopy, thymus gland and physiology.

Traver, D., A. Winzeler, H.M. Stern, E.A. Mayhall, D.M. Langenau, J.L. Kutok, A.T. Look, and L.I. Zon (2004). **Effects of lethal irradiation in zebrafish and rescue by hematopoietic cell transplantation.** *Blood* 104(5): 1298-305. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: The study of hematopoiesis has been greatly facilitated by transplantation of blood cell populations into recipient animals. Efficient engraftment of donor cells generally requires ablation of the host hematopoietic system. The zebrafish has recently emerged as a developmental and genetic system to study hematopoiesis. To enable the study of hematopoietic stem cell (HSC) biology, immune cell function, and leukemogenesis in zebrafish, we have developed hematopoietic cell transplantation (HCT) into adult recipient animals conditioned by gamma irradiation. Dose-response experiments showed that the minimum lethal dose (MLD) of 40 Gy led to the specific ablation of hematolymphoid cells and death by 14 days after irradiation. Sublethal irradiation doses of 20 Gy predominantly ablated lymphocytes and permitted transplantation of a lethal T-cell leukemia. Finally, transplantation of hematopoietic cells carrying transgenes yielding red fluorescent erythrocytes and green fluorescent leukocytes showed that HCT is sufficient to rescue the MLD, that recipient hematolymphoid tissues were repopulated by donor-derived cells, and that donor blood cell lineages can be independently visualized in living recipients. Together, these results establish transplantation assays to test for HSC function and oncogenic transformation in zebrafish.
Descriptors: zebrafish embryos, hematopoiesis radiation effects, hematopoietic stem cell transplantation mortality, lymphoid tissue radiation effects, genetically modified, gamma rays, green fluorescent proteins, kidney cytology, leukemia,T cell etiology and pathology, luminescent lymphoid tissue pathology, transplantation conditioning, whole body irradiation.

Ward, A.C., D.O. McPhee, M.M. Condron, S. Varma, S.H. Cody, S.M. Onnebo, B.H. Paw, L.I. Zon, and G.J. Lieschke (2003). **The zebrafish spi1 promoter drives myeloid-specific expression in stable transgenic fish.** *Blood* 102(9): 3238-40. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: The spi1 (pu.1) gene has recently been identified as a useful marker of early myeloid cells in zebrafish. To enhance the versatility of this organism as a model for studying myeloid development, the promoter of this gene has been isolated and characterized. Transient transgenesis revealed that a 5.3 kilobase promoter fragment immediately upstream of the spi1 coding sequence was sufficient to drive expression of enhanced green fluorescent protein (EGFP) in injected embryos in a manner that largely recapitulated the native spi1 gene expression pattern. This fragment was successfully used to produce a germ line transgenic line of zebrafish with EGFP-expressing myeloid cells. These TG(spi1:EGFP)pA301 transgenic zebrafish represent a valuable tool for further studies of myeloid development and its perturbation.
Descriptors: transgenic zebrafish embryo, EGFP-expressing myeloid cells metabolism, promoter regions genetics, proto oncogene trans activators genetics, genetically modified, complementary DNA, embryo, green fluorescent proteins, luminescent transgenes.

Weber, G.J., S.E. Choe, K.A. Dooley, N.N. Paffett Lugassy, Y. Zhou, and L.I. Zon (2005). **Mutant-specific gene programs in the zebrafish.** *Blood* 106(2): 521-30. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: Hematopoiesis involves the production of stem cells, followed by the orchestrated differentiation of the blood lineages. Genetic screens in zebrafish have identified mutants with defects that disrupt specific stages of hematopoiesis and vasculogenesis, including the cloche, spadetail (tbx16), moonshine (tif1g), bloodless, and vlad tepes (gata1) mutants. To better characterize the blood program, gene expression profiling was carried out in these mutants and in scl-morphants (scl(mo)). Distinct gene clusters were demarcated by stage-specific and mutant-specific gene regulation. These were found to correlate with the transcriptional program of hematopoietic progenitor cells, as well as of the erythroid, myeloid, and vascular lineages. Among these, several novel hematopoietic and vascular genes were detected, for instance, the erythroid transcription factors znfl2 and ncoa4. A specific regulation was found for myeloid genes, as they were more strongly expressed in vlt mutants compared with other erythroid mutants. A unique gene expression pattern of up-regulated isoprenoid synthesis genes was found in cloche and scl(mo), possibly in migrating cells. In conjunction with the high conservation of vertebrate hematopoiesis, the comparison of transcriptional profiles in zebrafish blood mutants represents a versatile and powerful tool to elucidate the genetic regulation of blood and blood vessel development.
Descriptors: zebrafish embryos, hematopoiesis genetics, mutation , blood vessels embryology, erythropoiesis genetics, gene expression profiling, developmental gene expression regulation, in situ hybridization, multigene family, myelopoiesis genetics, phenotype, genetic transcription.

Weber, G.J., K. Chiang, E.A. Mayhall, Y. Zhou, and L.I. Zon (2005). **Gene expression profiling of hematopoietic stem and progenitor cells in zebrafish**. *Blood* 106(11, Part 1): 1009A. ISSN: 0006 4971.
NAL Call Number: RB145.A1B57
Abstract: The differentiation of hematopoietic stem cells (HSCs) into distinct blood lineages is orchestrated by a tight regulation of critical genes during development. To describe the genetic program during the early steps of blood and blood vessel formation in greater detail, we analyzed the expression profiles of zebrafish cloche mutants (clo), in which blood and endothelial cells are completely abrogated, and of isolated hematopoietic and vascular progenitor cell populations from FAC-sorted transgenic lmo2-GFP zebrafish embryos. Consistent with a defect in hemangioblast differentiation, clo mutants at the 5 somite stage were characterized by a prominent downregulation of transcription factors, such as scl and lmo2. As a novel finding, clo mutants at this stage strongly upfegulated muscle-related genes, such as myosin, actin, or creatine kinase. FAC-sorted lmo2-GFP cells from 14 somite stage embryos expressed similar hematopoietic transcription factors including scl, lmo2, and gata1. In addition, sacs genes, tgfb,fos and jun related genes, and heat shock proteins were also strongly expressed. Comparison with the expression profile of human and mouse fetal liver HSCs revealed a similar regulation between fish and mammals: Expression of 102/ 168 genes from lmo2-GFP positive cells were also present in mammalian fetal HSCs, indicating an evolutionary highly conserved genetic program of blood formation. In sum, gene expression profiling of zebrafish mutants and of sorted progenitor cells from transgenic zebrafish represent a powerful and versatile tool to define the genetic regulation of vertebrate hematopoietic development. Copyright©Thomson Reuters 2009
Descriptors: zebrafish cloche mutants, development, biochemistry and molecular biophys-

ics, enzymology, gene expression profiling, genetic techniques, vertebrate hematopoietic development.

Wingert, R.A., B. Barut, H. Foott, P. Fraenkel, K. Dooley, P. Kingsley, J. Palis, H. Shubert, O. Chen, J. Kaplan, and L.I. Zon (2004). **The zebrafish hypochromic mutant shiraz encodes a novel mitochondrial glutaredoxin that establishes a link between heme and Fe/S production.** *Blood* 104(11, Part 1): 18A-19A. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Iron is required in the mitochondria both to produce heme, which is used for hemoglobin synthesis, and to make iron-sulfur (Fe/S) clusters, which confer electron transfer or catalytic functions to proteins. Cellular iron utilization and Fe/S cluster production are thought to occur independently, vet the processes are coordinated through currently uncharacterized pathways. The shiraz (sir) zebrafish mutant manifests a hypochromic, microcytic anemia. Positional cloning of sir discovered a deletion at the locus that included the zebrafish orthologue to glutaredoxin 5 (grx5), a gene required in yeast for Fe/S cluster assembly. We found that grx5 is highly expressed in the developing blood and fetal liver of both zebrafish and mouse embryos. Anti sense-mediated morpholino knockdown of grx5 prevented hemoglobin production, and overexpression of zebrafish, yeast, mouse, or human grx5 RNA in sir embryos completely rescued hemoglobin production. indicating that grx5 is the gene responsible for the sir phenotype. Expression of zebrafish grx5 was found to rescue Fe/S protein production in the yeast Delta grx5 strain, demonstrating that the role of grx5 in Fe/S cluster assembly is conserved among eukaryotes. The surprising finding that mutating a gene necessary for Fe/S cluster assembly caused a lack of hemoglobin synthesis suggested that we had discovered a connection between these pathways. In vertebrates, iron regulatory protein 1 (IRP1) acts as a sensor of intracellular iron levels and controls cellular iron homeostasis via posttranscriptional regulation of iron uptake, storage, and utilization genes. For instance, IRP1 binds to the 5' iron response element (IRE) in the aminolevulinate synthase 2 (ALAS2) mRNA, blocking translation when cellular iron is low. However, when cellular iron is replete, IRP1 binds a Fe/S cluster and its RNA-binding activity is abolished. We hypothesized that the loss of Fe/S cluster assembly in sirwould activate IRP1 and block ALAS2 synthesis, resulting in hypochromia. In support of this model, overexpression of ALAS2 RNA without the 5' IRE rescued sir hypochromia, while overexpression of ALAS2 including the IRE did not facilitate rescue. Furthermore, antisense morpholino knockdowns of IRP1 caused rescue of hemoglobin synthesis in sir embryos. The combination of these data indicate that hemoglobin production in the differentiating red cell is monitored through Fe-S cluster assembly as a mechanism to gauge iron levels and accordinglydirect heme synthesis. This finding illustrates a crucial role for the mitochondrial Fe/S cluster assembly machinery during hemoglobin production, and has broad implications for the role of such genes in human hypochromic anemias. Copyright©Thomson Reuters 2009

Descriptors: zebrafish shiraz mutant, blood and lymphatics, transport and circulation, molecular genetics, anemia hypochromic, blood and lymphatic disease, genetics, antisense morpholino knockdown, genetic techniques, phenotype.

Wingert, R.A., A. Brownlie, K. Dooley, P. Fraenkel, J. Axe, and L.I. Zon (2003). **Gene duplication in zebrafish provides a model for erythroid-specific disruption of the TfR1 gene.** *Blood* 102(11): 157a. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Transferrin receptor 1 (TfR1) is required for normal erythropoiesis and neurologic development, as the murine knockout exhibits both severe anemia and central nervous

system apoptosis. In this study we isolated the gene responsible for the zebrafish chianti (cia) mutant, which manifests a blood specific phenotype of hypochromic microcytic anemia after the onset of embryonic circulation. Synteny between the cia locus on linkage group 2 and human chromosome 3q29 suggested transferrin receptor 1 (TfR1) was a candidate gene. Hybridization techniques and degenerative oligonucleotide PCR were used to isolate the zebrafish orthologue of TfR1. In the process, we discovered two TfR1-like genes (a and b) and a single TfR2-like gene. Zebrafish TfR1a mapped to the cia locus and is specifically expressed in the developing blood by in situ hybridization; in contrast, zebrafish TfR1b mapped to an independent locus on linkage group 24 also syntenic to human 3q29, and was found to be ubiquitously expressed. An ancient duplication of the TfR1 gene in early teleosts is likely to have occurred. Sequence analysis found that three cia alleles represented missense mutations in TfR1a and one allele represented a splice site mutation. Injection of antisense TfR1a morpholinos into wildtype embryos caused hypochromia that could be rescued by concomitant overexpression of TfR1a RNA, illustrating that TfR1a function alone is required for hemoglobin synthesis in erythroid precursors. The hypochromia in cia embryos can be rescued by overexpression of either TfR1a or TfR1b, indicating that both receptors can function to deliver iron from the embryonic circulation. Furthermore, overexpression of mouse TfR1 or TfR1 can rescue the cia phenotype. This establishes that both mammalian TfR1 and TfR2 are biochemically sufficient to support hemoglobin production in vivo: In an effort to functionally demonstrate the requirement of TfR1a for iron delivery in da erythroid precursors, we provided either the 1-cell stage embryo or embryonic circulation at 2 days with iron. Injection of iron-dextran into the embryo at the 1-cell stage, but not iron-dextran injection into the circulation of 2 day old embryos, rescued the cia hypochromic phenotype. We hypothesize that the iron injected at the 1-cell stage uniformly distributes to the cytoplasm of the red cell precursors, bypassing the need for iron uptake via the TfR pathway, whereas excess iron in circulation cannot enter cia erythroid precursors. The zebrafish cia mutant thus provides an opportunity to examine the role of TfR1 in erythropoiesis in the absence of defects in other tissues, as exemplified by neural cell death in the mouse TfR1 knockout. Copyright©Thomson Reuters 2009

Descriptors: zebrafish chianti (cia) mutant, blood and lymphatics, transport and circulation, hypochromatic microcytic anemia, blood and lymphatic disease, neural cell death, phenotype.

Yabu, T., H. Tomimoto, Y. Taguchi, S. Yamaoka, Y. Igarashi, and T. Okazaki (2005). **Thalidomide-induced antiangiogenic action is mediated by ceramide through depletion of vegf receptors, and is antagonized by sphingosine-1-phosphate**. *Blood* 106(1): 118-134. ISSN: 0006 4971.

NAL Call Number: RB145.A1B57

Abstract: Thalidomide, which is clinically recognized as an efficient therapeutic agent for multiple myeloma, has been thought to exert antiangiogenic action through an unknown mechanism. We here show a novel mechanism of thalidomide-induced antiangiogenesis in zebrafish embryos. Thalidomide induces the defect of major blood vessels, which is demonstrated by their morphologic loss and confirmed by the depletion of vascular endothelial growth factor (VEGF) receptors such as neuropilin-1 and Flk-1. Transient increase of ceramide content through activation of neutral sphingomyelinase (nSMase) precedes thalidomide-induced vascular defect in the embryos. Synthetic cell permeable ceramide, N-acetylsphingosine (C2-ceramide) inhibits embryonic angiogenesis as well as thalidomide. The blockade of ceramide generation by antisense morpholino oligonucleotides for nSMase prevents thalidomide-Induced ceramide generation and vascular defect. In contrast to cer-

amide, sphingosine-1-phosphate (S1P) inhibits nSMase-dependent ceramide generation and restores thalidomide-induced embryonic vascular defect with an increase of expression of VEGF receptors. In human umbilical vein endothelial cells (HUVECs), thalidomide-induced inhibition of cell growth, generation of ceramide through nSMase, and depletion of VEGF receptors are restored to the control levels by pretreatment with S1P. These results suggest that thalidomide-induced antiangiogenic action is regulated by the balance between ceramide and S1P signal. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, thalidomide, pharmacology, tumor biology, blood and lymphatics, transport and circulation, multiple myeloma, neoplastic disease, immune system disease, blood and lymphatic disease, drug therapy, antiangiogenesis.

Yamasaki, K. and H. Nakayasu (2003). **Visualization of erythrocytes in the zebrafish brain.** *Zoological Science* 20(9): 1071-8. ISSN: 0289-0003.
NAL Call Number: 442P92
Abstract: We found that erythrocytes of zebrafish have cytoplasmic peroxidase activity. Blood in the zebrafish brain was visualized using a standard peroxidase staining method after formaldehyde fixation. The erythrocytes in the brain were heavily stained, but neurons and glias were not stained at all. This easy method enables the distribution of erythrocytes in the whole brain to be determined, and enables the actual number of erythrocytes in each area in the brain to be calculated. The paths of major, thick blood vessels in zebrafish brain are similar to those in higher vertebrates, however, the distribution of thin blood vessels is different. We also found that the erythrocytes were unevenly distributed in the brain. For example, the density of erythrocytes in the surface layer of the tectum was more than 30-fold higher than in the deeper granular layer. Very few erythrocytes were found in bundles of axons like cranial nerves and the medial longitudinal fascicle. In general, fewer erythrocytes were found in areas near the ventricle, whereas many more were found closer to the surface of the brain. The distribution of erythrocytes in the brains of sleeping, awake and actively moving fish were compared. In the brains of sleeping fish, most of the erythrocytes were present in large vessels. This was not observed in brains of awake or actively moving fish. We found that the blood supply to motor neurons in the ventral horn of the spinal cord increased during active movement compared to that in awake or sleeping fish.
Descriptors: zebrafish brains, awake and sleeping fish, brain anatomy and histology and blood supply, erythrocytes cytology, zebrafish blood, cell count, erythrocytes physiology, histological staining techniques, peroxidase metabolism, zebrafish anatomy and histology.

Yoder, M.C. (2005). **Hematopoietic stem cell development: an introduction**. *Experimental Hematology* 33(9): 953-954. ISSN: 0301 472X.
NAL Call Number: RB145.E97
Descriptors: zebrafish mdels, embryos, development, hematology, human medicine, medical sciences, embryonic stem cell models, hematopoietic studies.

Zhang, X.Y. and A.R. Rodaway (2007). **SCL-GFP transgenic zebrafish: in vivo imaging of blood and endothelial development and identification of the initial site of definitive hematopoiesis.** *Developmental Biology* 307(2): 179-94. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The bHLH transcription factor SCL plays a central role in the generation of hematopoietic cells in vertebrates. We modified a PAC containing the whole zebrafish scl locus, inserting GFP into the first coding exon of scl. In germline-transgenic zebrafish generated using this construct, GFP expression completely recapitulates the endogenous expression

of scl in blood, endothelium and CNS. We performed in vivo timelapse imaging of blood and endothelial precursor migration at the single-cell level and show that these cells migrate from the posterior lateral plate mesoderm to their site of differentiation in the intermediate cell mass. The anterior lateral plate domain of GFP expression gives rise to primitive macrophages and the blood vessels of the head. In later embryos, GFP expression identifies clusters of hematopoietic cells that develop between the dorsal aorta and posterior cardinal veins after primitive erythrocytes have entered circulation. Two treatments that block definitive hematopoiesis (treatment with dioxin (TCDD), and injection of an antisense morpholino oligonucleotide targeted to runx1) ablate these hematopoietic clusters. This indicates that these clusters represent the first site of definitive hematopoiesis in zebrafish. This site is anatomically homologous to the proposed source of hematopoietic stem cells in amniotes, the aorta-gonad-mesonephros (AGM) region. A second transgenic line, containing the promoter of scl driving GFP, lacks expression in the definitive clusters.

Descriptors: germline-transgenic zebrafish, basic helix loop helix transcription factors physiology, hematopoiesis physiology, proto oncogene, genetically modified, base sequence, basic helix loop helix transcription factors genetics, cell movement, DNA primers genetics, developmental gene expression regulation, green fluorescent hematopoiesis genetics, hematopoietic stem cells cytology, proto oncogene recombinant zebrafish blood, GFP expression.

Husbandry

Carvalho, A.P., L. Araujo, and M.M. Santos (2006). **Rearing zebrafish (*Danio rerio*) larvae without live food: evaluation of a commercial, a practical and a purified starter diet on larval performance**. *Aquaculture Research* 37(11): 1107-1111. ISSN: 1355 557X.

NAL Call Number: SH1.F8

Abstract: Owing to the increasing importance of zebrafish as a vertebrate model in many fields of research, efforts must be made to breed and maintain this species in laboratory. Zebrafish larvae are traditionally reared on cultured live paramecia during the first 9 days of exogenous feeding, followed by a combination of *Paramecia* and *Artemia nauplii* until day 21, making larval rearing expensive, labour intensive and unpredictable. Thus, a trial was conducted with zebrafish larvae in order to evaluate the suitability of artificial diets as an alternative to live food during the first 21 days of exogenous feeding. Five dietary treatments were tested: (1) *Artemia nauplii*; (2) a commercial; (3) a purified; (4) a practical diet, all delivered continuously; (5) the same practical diet delivered manually. The best overall larval performance was achieved in the group fed artemia nauplii (86% survival, 14.3 mm standard length, 46.1 mg wet weight). Compared with existing results obtained with the traditional live food schedule, our results suggest that paramecia might not be the most suitable first food for zebrafish, and that artemia nauplii could be used as the only live food. The present work demonstrates that zebrafish larvae can be reared without live food with a significant growth and a high survival, provided that an appropriate artificial diet is presented in a continuous way. Among the diets tested, the practical diet, if continuously delivered, led to the best performance assuring a mean standard length of 72% of that obtained with artemia and a similar survival rate. Moreover, the purified diet, supporting over 50% survival and an appreciable growth, could be useful in some toxicological studies in which a well-defined diet is needed. Copyright © 2008 CABI

Descriptors: zebrafish larvae, body length, artificial diets, fish feeding, growth rate, liveweight gain, survival, synthetic diets, *Artemia*, *Danio rerio*.

Cattin, P. and P. Crosier (2004). **A nursery that improves zebrafish fry survival.** *Methods in Cell Biology* 77: 593-8. ISSN: 0091-679X.

NAL Call Number: 442P92

Descriptors: raising zebrafish, animal husbandry methods, zebrafish growth and development.

Clay, H., J.M. Davis, D. Beery, A. Huttenlocher, S.E. Lyons, and L. Ramakrishnan (2007). **Dichotomous role of the macrophage in early *Mycobacterium marinum* infection of the zebrafish.** *Cell Host and Microbe* 2(1): 29-39. ISSN: 1931-3128.

Abstract: In tuberculosis, infecting mycobacteria are phagocytosed by macrophages, which then migrate into deeper tissue and recruit additional cells to form the granulomas that eventually contain infection. *Mycobacteria* are exquisitely adapted macrophage pathogens, and observations in the mouse model of tuberculosis have suggested that mycobacterial growth is not inhibited in macrophages until adaptive immunity is induced. Using the optically transparent and genetically tractable zebrafish embryo-*Mycobacterium marinum* model of tuberculosis, we have directly examined early infection in the presence and absence of macrophages. The absence of macrophages led rapidly to higher bacterial burdens, suggesting that macrophages control infection early and are not an optimal growth niche. However, we show that macrophages play a critical role in tissue dissemination of mycobacteria. We propose that residence within macrophages represents an evolutionary trade-off for pathogenic mycobacteria that slows their early growth but provides a mechanism for tissue dissemination.

Descriptors: zebrafish embryology, fish diseases microbiology, macrophages microbiology,

Mycobacterium infections, atypical microbiology, *Mycobacterium marinum*, tuberculosis, zebrafish microbiology, microbiology.

Harper, C. (2004). **Zebrafish aquaculture.** *Aquaculture Magazine* 30(6): 38-41. ISSN: 0199 1388.
NAL Call Number: SH1.C65
Descriptors: zebrafish, fish culture, *BrachyDanio rerio*, fish diseases, raising zebrafish.

Harris, M. (2006). **Using my ARMADA Research Experience to Enhance Teaching**. *American Geophysical Union Fall Meeting,December 11, 2006-December 15, 2006, San Francisco* , American Geophysical Union: 2000 Florida Ave., N.W. Washington DC 20009 USA,
Descriptors: zebrafish, aquatic reptiles, CTD observations, education, fish culture, food webs, freshwater aquaculture, greenhouse effect, marine mammals, Salt lakes, cetacea, *Danio rerio*, penaeidae.

Hong, J.R., L.J. Huang, and J.L. Wu (2005). **Aquatic birnavirus induces apoptosis through activated caspase-8 and -3 in a zebrafish cell line**. *Journal of Fish Diseases* 28(3): 133-140. ISSN: 0140 7775.
NAL Call Number: SH171.A1J68
Abstract: In this study, the possible influence of temperature on infectious pancreatic necrosis virus (IPNV)-induced apoptosis in a zebrafish liver epithelium (ZLE) cell line was investigated. At a lower temperature (18 degrees C), there was expression of viral proteins VP2 and VP3 at 4 h post-infection (p.i.). At this time no expression was found in the high temperature group at 28 degrees C. The cell survival ratio was 52 and 18% at 24 and 48 h p.i., respectively, during IPNV infection at 18 degrees C. In addition, we assayed for apoptosis in IPNV-infected cells with terminal deoxynucleotidyl transferase (TdT)-mediated end labelling (TUNEL) of DNA at different dosages of virus. We found a ratio of apoptotic cells of 8 and 25% at 12 and 18 h p.i., respectively, in the multiplicity of infection (MOI) 1 group. The MOI 10 group had 20 and 45% apoptotic cells at 12 and 18 h, respectively. Furthermore, at 18 degrees C IPNV activated the caspase-8 and 3 from 1.5 to 2 times at 12 and 18 h p.i., respectively. Taken together, these findings suggest that successful virus replication occurs at the low temperature (18 degrees C) compared with the non-permissive temperature of 28 degrees C. Thus, IPNV replication is capable of activating caspase-8 and -3 and inducing host apoptosis. Copyright © 2008 CABI
Descriptors: zebrafish, apoptosis, cell culture, hepatic cell lines, epithelium, in vitro, liver, pathogenesis, signal transduction, survival, temperature, viral proteins and replication, birnavirus, *Danio rerio.*

Kent, M.L. and J.W. Fournie (2007). **Parasites of fishes**. D.G. Baker *Flynn' s Parasites of Laboratory Animals,* 2 edition, Blackwell Publishing: Oxford, UK, p. 69-116. ISBN: 9780813812021.
NAL Call Number: SF996.5.F59
Abstract: This chapter describes the parasites of importance to fishes maintained and used in laboratory settings. The focus will be only on those parasites that pose a serious threat to or are common in fishes held in these confined environments. The parasites emphasized are those capable of spreading, proliferating and causing significant disease in research facilities. Although the use of aquatic animal models has increased significantly in recent year, the coverage in this chapter is limited to the following fish species: zebrafish *Danio rerio*, goldfish *Carassius auratus*, rainbow trout *Oncorhynchus mykiss*, stickleback *Gasterosteus*, mummichog *Fundulus heteroclitus* , gulf killifish *Fundulus grandis*, sheepshead minnow *Cyprinodon variegatus*, fathead minnow *Pimephales promelas* and swordtails and platys *Xiphophorus* spp. The text is organized by parasite taxa rather than hosts, because the most important parasites show very broad host specificity.

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Descriptors: zebrafish and other fish species, animal parasitic nematodes, ectoparasites, ectoparasitoses, helminthoses, laboratory animals, nematode infections, parasites, parasitoses, protozoal infections, Apicomplexa, arthropods, ciliophora, dinoflagellida, fishes, microspora, Myxozoa, Nematoda, platyhelminthes, protozoa.

Lawrence, C. (2007). **The husbandry of zebrafish *(Danio rerio*): a review**. *Aquaculture* 269(1/4): 1-20. ISSN: 0044 8486.
NAL Call Number: SH1.A6
Abstract: The zebrafish (*Danio rerio*) has recently emerged as a pre-eminent vertebrate biomedical research model. The same favorable characteristics that have contributed to its popularity as a model of human disease and development; i.e. high fecundity, small size, rapid generation time, optical transparency during early embryogenesis, have also long endeared it to investigators in numerous other disciplines, including animal behavior, fish physiology, and aquatic toxicology. Despite this, the scientific rigour of zebrafish husbandry techniques is poorly developed. While there is a considerable body of literature on zebrafish that has both direct and indirect relevance to their husbandry, this information is from disparate sources, and little of it is has been applied to developing standard protocols. This review is an attempt to integrate the available scientific information related to zebrafish biology and culture into an overview of the field that can be used to improve the efficiency with which this important model animal is used in research. The review also highlights those areas in which further studies are needed. Copyright © 2008 CABI
Descriptors: zebrafish, behavior, animal breeding, breeding methods, breeding programs, diet, dissolved oxygen, fish culture, fish feeding, geographical distribution, growth rate, habitats, larvae, lifespan, nitrogen, nutrient requirements, pH, reproduction, reviews, salinity, spawning, stocking density, stocking rate, survival, wastes, water hardness, quality and temperature, *Danio rerio.*

Lefebvre, K.A., V.L. Trainer, and N.L. Scholz (2004). **Effects of a common marine algal toxin on early development and behavioral performance in fish**. *2003 Georgia basin/Puget sound resarch conference,March 3, 1931-April 3, 2003, Vancouver, BC (Canada),* Series T.W. Droscher, and D.A. Fraser (Editors), Puget Sound Action Team:
Descriptors: zebrafish, abnormalities, algal blooms, behavior, bioassays , biological poisons, embryonic development, eyes, fish eggs, fish larvae, ichthyoplankton , larval development, neurotoxins, phytoplankton, toxicity tests, *Danio rerio*, saxitoxin.

Lin, J.H.Y., C.C. Yu, C.C. Lin and H.L. Yang (2005). **An oral delivery system for recombinant subunit vaccine to fish**. P.J. Midtlyng *Progress in Fish Vaccinology 3rd International Symposium on Fish Vaccinology,* Vol. 121, S Karger AG: Basel, Switzerland, p. 175-180. ISBN: 3805579365.
NAL Call Number: QR180.3.D4 v.121
Abstract: Oral vaccination is considered as the most desirable method for immunizing fish because it is non-stressful, user-friendly and is capable of easy administration to large numbers of fish. However, many publications have indicated that the current oral vaccines still lack the desired efficacy. Here we reported on an oral vaccine method to deliver recombinant subunit vaccine based on the food chain of fish, in that live *Artemia nauplii* are encapsulated with recombinant bacteria containing the antigen. The feasibility and efficacy of this method for delivering protein antigen was tested in a zebrafish model, where immunisation took place through feeding Artemia encapsulated with recombinant *E. coli*. By this oral vaccine method the antigen could be delivered to the hindgut as confirmed by immuno-histochemistry, and its efficacy was demonstrated by the ability to protect vaccinated fish significantly from a direct injection of a native *Pseudomonas exotoxin* and bacterial pathogen. Combined with the recombinant technology for

producing desired protein antigens, this oral vaccine delivery method provides the capacity to deliver a variety of different antigens, including the option for delivery of multivalent vaccines by the oral route. Copyright © 2008 CABI

Descriptors: zebrafish, animal models, antigens, drug delivery systems, fish feeding, food chains, immunization, methodology, oral vaccination, potency, recombinant antigens, recombinant vaccines, techniques, vaccines, Artemia, *Danio rerio*, *Escherichia coli*, fishes.

Markovich, M.L., N.V. Rizzuto, and P.B. Brown (2007). **Diet affects spawning in zebrafish.** *Zebrafish* 4(1): 69-74. ISSN: 1545-8547.

Abstract: Seven-month-old zebrafish (*Danio rerio*) were fed four different diets to test the hypothesis that diet affects spawning success and resulting characteristics of eggs and offspring. The diets were: the recommended feeding regime for zebrafish (a mixture of *Artemia*, flake feed, and liver paste); *Artemia*; a flake feed; and a commercially available trout diet. The number of eggs laid and average egg diameter were significantly different as functions of male, female, and individual matings. Fish fed the flake diet produced significantly fewer eggs (mean, 116) than fish fed all other diets (means, 166-187). However, the percent hatch of eggs from fish fed the flake diet (62.5%) was significantly higher than from fish fed the trout diet (19.5%). The percentages of hatched eggs from fish fed the control diet (36.2%) or *Artemia* (35.6%) were not significantly different from each other or from fish fed the other two diets. Wet weight and diameter of eggs were not significantly affected by diet. Larval length was significantly higher from parents fed the flake diet (14.5 mm) compared to larvae from parents fed *Artemia* (13.7 mm). Length of larvae from fish fed the control or trout diets was intermediate and not significantly different from fish fed the flake diet or Artemia. Larval weight was not significantly affected by dietary treatment, but offspring from fish fed the flake diet were heavier than larvae from adults fed any of the other diets. Feeding adult zebrafish the flake diet alone resulted in more viable offspring and larger larvae and is a simpler feeding regime than the current recommendation. The authors recommend feeding adult zebrafish flake diets to satiation three times daily for maximum production of viable offspring.

Descriptors: zebrafish, diet, breeding, *Artemia* , flake diets, egg diameter, larval length, effects on spawning.

Matthews, J.L. (2004). **Common diseases of laboratory zebrafish.** *Methods in Cell Biology* 77: 617-43. ISSN: 0091-679X.

NAL Call Number: 442P92

Descriptors: zebrafish, fish diseases etiology, fish diseases diagnosis, fish diseases microbiology, neoplasms genetics, neoplasms veterinary, care and procedures, water chemistry, zoonoses transmission.

McClure, M.M., P.B. McIntyre, and A.R. McCune (2006). **Notes on the natural diet and habitat of eight danionin fishes, including the zebrafish *Danio rerio*.** *Journal of Fish Biology* 69(2): 553-570. ISSN: 0022-1112.

NAL Call Number: QL614.J68

Descriptors: zebrafish, aquatic insects, detritus, diets, environmental conditions, food organisms, freshwater fish, habitat selection, light penetration, *Danio rerio.*

Moyer, T.R. and D.W. Hunnicutt (2007). **Susceptibility of zebra fish *Danio rerio* to infection by *Flavobacterium columnare* and *F. johnsoniae.*** *Diseases of Aquatic Organisms* 76(1): 39-44. ISSN: 0177 5103.

Abstract: *Flavobacterium columnare* is a serious pathogen in a wide range of fish species. *F.*

johnsoniae is an opportunistic pathogen of certain fish. Both are gliding bacteria. These species were tested for their ability to infect the zebra fish *Danio rerio*. Both injection and bath infection methods were tested. The results indicate that *F. johnsoniae* is not an effective pathogen in *D. rerio*, but that *F. columnare* is an effective pathogen. *F. johnsoniae* did not cause increased death rates following bath infection, but did cause increased death rates following injection, with an LD50 (mean lethal dose) of approximately 3 x 10(10) cfu (colony-forming units). Non-motile mutants of *F. johnsoniae* produced a similar LD50. *F. columnare* caused increased death rates following both injection and bath infections. There was considerable strain variation in LD50, with the most lethal strain tested producing an LD50 of 3.2 x 10(6) cfu injected and 1.1 x 10(6) cfu ml(-1) in bath experiments, including skin damage. The LD50 of F. columnare in zebra fish without skin damage was > 1 x 10(8), indicating an important effect of skin damage.
Descriptors: zebrafish, fish diseases microbiology, Flavobacteriaceae infections veterinary, *Flavobacterium* pathogenicity, zebrafish microbiology, disease susceptibility, fish diseases transmission, flavobacteriaceae infections microbiology, transmission, growth and development, lethal dose 50, ribosomal RNA 16s genetics, survival analysis.

Olascoaga, T.J. and J. Luna Figueroa (2005). **Aprovechamiento de alimento vivo *Culex quinquefasciatus* en la dieta del pez cebra *Brachidanio rerio* (Pisces: Cyprinidae) con enfasis en la reproduccion [Use of living food *Culex quinquefasciatus* in diet of zebra fish *brachydanio rerio* (Pisces: Cyprinidae) with emphasis in reproduction]**. *AquaTIC*(22): 20-25. ISSN: 1578 4541.
Abstract: In this study the use of live *C. quinquefasciatus* in feeding zebra fish *B. rerio* was evaluated to find out its effect on the reproductive performance of the fish. The fishes which were fed with a diet that contains *C. quinquefasciatus* was then compared with fishes fed with a commercial feed (control) in terms of reproductive performance and results showed that the fish which were fed with the live feed *(C. quinquefasciatus*) showed better performance as compared with the control group. The following data were obtained as proof of improved reproductive performance: frequency of spawning (57.54%), fish eggs production (55.06%), and fingerlings survival (49%). Copyright © 2008 CABI
Descriptors: zebrafish, live food, diets, fish eggs, fish feeding, fish production, fry, reproduction, reproductive performance, spawning, survival, *culex quinquefasciatus*, *Danio rerio.*
Language of Text: Spanish.

Onal, U. and C. Langdon (2004). **Lipid spray beads for delivery of riboflavin to first-feeding fish larvae**. *Aquaculture* 233(1/4): 477-493. ISSN: 0044 8486.
NAL Call Number: SH1.A6
Abstract: High nutrient loss due to leakage from microparticulate diets is an important problem in the development of artificial diets for marine fish larvae. Lipid spray beads (LSB) are promising as they can be used to deliver low-molecular weight, water-soluble nutrients. Here, we describe a simple method for the production of LSB composed of methyl palmitate (MP), a low melting point wax, for delivering riboflavin to first-feeding freshwater fish larvae of zebra fish, *Brachydanio rerio*, and glowlight tetra, *Hemigrammus erythrozonus*. Characteristics of LSB, such as inclusion, retention and delivery efficiencies as well as overall leaching patterns over time, were used to compare the performances of LSB prepared with different materials. The mean inclusion efficiency (IE) of riboflavin within LSB was 96.70% and it was improved by addition of emulsifiers to the lipid matrix. Addition of the solid (at room temperature) emulsifier, sorbitan monopalmitate (SMP) resulted in higher retention efficiencies compared to the additions of liquid emulsifier sorbitan sesquioleate (SSO). Additions of SMP were found to have a positive effect on retention and delivery efficiencies of LSB at concentrations of 10% and 25% (w/w) of lipid. LSB composed

of a mixture of MP+10% SMP+10% ethyl cellulose (EC, w/w) were shown to retain riboflavin with the highest retention efficiencies over a period of 6 h of aqueous suspension. Use of MP facilitated digestive breakdown of LSB by larvae of zebrafish and glowlight tetra as indicated by release of free riboflavin from ingested LSB. There is a need to balance the wall stability of LSB with the digestion requirements of target species. The results suggest that it is possible to formulate a carrier lipid matrix that both retains riboflavin and is digested by fish larvae. Besides riboflavin delivery, LSB may also be useful as a carrier for other water-soluble nutrients, such as dietary amino acids and peptides. Copyright © 2008 CABI
Descriptors: zebrafish larvae, diets, digestion, emulsifiers, fish feeding, larvae, leaching, lipids, methodology, nutrition physiology, palmitates, riboflavin, sprays, *Danio rerio.*

Pilati, A. and M.J. Vanni (2007). **Ontogeny, diet shifts, and nutrient stoichiometry in fish**. *Oikos* 116(10): 1663-1674. ISSN: 0030-1299.
Descriptors: zebrafish, detritus, diets, excretion, life cycle, molars, nutrients, ontogeny, osteogenesis, *Danio rerio*, *Dorosoma cepedianum.*

Poling, K.R., E. Jaworski, K.R. Fantetti, and D.M. Higgs (2005). **Do laboratory rearing conditions affect auditory and mechanosensory development of zebrafish (*Danio rerio*)?** *Journal of the Acoustical Society of America* 117(4): 2467. ISSN: 0001-4966.
Descriptors: zebrafish, laboratory environment effects, acoustic data, anthropogenic factors, aquarium culture, audition, auditory organs, body size, environmental impact, fish culture, larval development, sound sources, underwater noise, *Danio rerio*.

Rogers, G. (2005). **Total gas saturation considerations for recirculating aquatic systems**. *International Journal of Recirculating Aquaculture* 6 ISSN: 1547-917X.
Descriptors: zebrafish, aquaculture techniques, dissolved gases, fish culture, freshwater fish, recirculating systems, supersaturation, water quality, *Danio rerio.*

Russo, R.E. and D. Dillehay (2005). **Use of broad-spectrum antimicrobials in the eradication of unknown aquatic pathogens in a zebrafish larval rearing system**. *Contemporary Topics in Laboratory Animal Science* 44(2): 49-51. ISSN: 1060 0558.
NAL Call Number: SF405.5.A23
Abstract: A zebrafish larval rearing system experienced a surge in mortality rates soon after the introduction of new stocks. A comprehensive water analysis of pH, nitrites, nitrates, ammonia, chlorine, carbonate hardness, general hardness, and conductivity identified no anomalies. Observations via light microscopy of affected fry revealed consistent signs of impaired mobility, blood clotting, and eventual heart haemorrhage resulting to the death of 90 to 100% of the fry by the age of 2 weeks. Collection of sufficient tissue samples for a histological investigation proved problematic due to the fry's diminutive size. Because a causal agent could not be isolated satisfactorily, the use of a broad-spectrum antibiotic was deemed necessary. After considering many broad-spectrum antibiotics for treatment, we implemented a two-tiered approach for the treatment. The rearing system was treated with a nitrofurazone derivative, whereas the adult populations were treated using multi-antibiotic food pellets. The rearing system was treated for 3 weeks, and the adult population was treated for 2 weeks. After the completion of the antibiotic treatments, the biological filters of all of the medicated systems were seeded with nitrifying bacterial cultures. Upon the maturation of the rearing systems' biological filters, mortality rates returned to pre-outbreak levels. There have been no re-occurrences of the fish mortality since the completion of treatment. This epidemic provided some valuable lessons, lessons that if followed, will ensure

faster response to unknown pathogens in the future. Copyright © 2008 CABI
Descriptors: zebrafish larvae, antibiotics, pathogens, aetiology, antiinfective agents, control programs, disease control, disease prevention, fish diseases, nitrofural, treatment, *Danio rerio.*

Spence, R., M.K. Fatema, S. Ellis, Z.F. Ahmed, and C. Smith (2007). **Diet, growth and recruitment of wild zebrafish in Bangladesh**. *Journal of Fish Biology* 71(1): 304-309. ISSN: 0022-1112.
NAL Call Number: QL614.J68
Descriptors: wild zebrafish, age, aquatic insects, diets, freshwater fish, growth rate, monsoons, population dynamics, recruitment, reproduction, zooplankton, *Danio rerio.*

Spence, R. and C. Smith (2005). **Male territoriality mediates density and sexratio effects on oviposition in the zebrafish, *Danio rerio*.** *Animal Behaviour* 69(6): 1317-1323. ISSN: 0003 3472.
NAL Call Number: 410 B77
Descriptors: zebrafish, *Danio rerio*, egg laying, oviposition, density and sex ratio effects related to male territoriality, aggressive behavior, male territoriality, population density, population sex ratio, intraspecific competition.

Stewart, K.L., P. Skibins, and D.A. Bang (2004). **Brine shrimp dispenser**. *Contemporary Topics in Laboratory Animal Science* 43(4): 33-34. ISSN: 1060 0558.
NAL Call Number: SF405.5.A23
Descriptors: zebrafish, fish feeding, laboratory husbandry, brine shrimp dispensers, feeding equipment, *Danio rerio.*

Trevarrow, B. (2004). **Zebrafish facilities for small and large laboratories.** *Methods in Cell Biology* 77: 565-91. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, laboratory culture, adaptable to large and small stuations, models.

Whipps, C.M. and M.L. Kent (2006). **Polymerase chain reaction detection of *Pseudoloma neurophilia*, a common microsporidian of zebrafish (*Danio rerio*) reared in research laboratories**. *Journal of the American Association for Laboratory Animal Science* 45(1): 36-39. ISSN: 1060 0558.
NAL Call Number: SF405.3 .A23
Abstract: One of the most prevalent pathogens found in zebrafish (*Danio rerio*) research facilities is the microsporidian parasite *Pseudoloma neurophilia*. Infections occur primarily in the spinal cord and are associated with emaciation and scoliotic changes. It is unclear why *P. neurophilia* is so widespread among research colonies, although transfer of infected animals and eggs between laboratories is a likely contributor. In addition to preventing the spread of this pathogen among facilities, it is desirable to have parasite-free fish for use in experiments. Therefore we have developed a polymerase chain reaction (PCR)-based diagnostic test for *P. neurophilia*. Compared with conventional diagnostic methods, PCR diagnosis is rapid, allows for screening of large numbers of fish, and can be applied to eggs, water filtrates, biofilms, and other samples. Using PCR primers specific to the small subunit ribosomal DNA of *P. neurophilia*, the test was consistently capable of detecting 10 spores per reaction and often as few as 0.1 spore per reaction, and it did not cross-react with other selected microsporidian species from fish. We recommend this PCR diagnostic assay for use by the research community to determine the presence (or absence) of P. neurophilia in colonies and for screening fish shipped between facilities, especially when parasite-free fish are required for experiments. Furthermore, we currently are using this PCR method to investigate the potential role of vertical transmission in the spread of *P. neurophilia*. Copyright © 2008 CABI

Descriptors: zebrafish, diagnosis, diagnostic techniques, *Pseudoloma neurophilia*, laboratory animals, PCR, ribosomal DNA, *Danio rerio* , *microspora*.

Wright, D. and J. Krause (2006). **Repeated measures of shoaling tendency in zebrafish (*Danio rerio*) and other small teleost fishes.** *Nature Protocols* 1(4): 1828-31. ISSN: 1754-2189.
Abstract: This protocol details a method for constructing and using both a holding tank and a test tank to assess the shoaling tendency of zebrafish and other teleosts. The test tank consists of a central compartment with two side compartments, one of which contains a shoal of stimulus fish. The focal fish is released and the amount of time spent associating with the stimulus shoal is recorded over a 10-min period. A holding tank enables individual fish to be accurately retested with a minimum of stress. Testing takes around 15 min per fish.
Descriptors: zebrafish, shoaling behavior, behavioral research instrumentation, equipment design, holding and test tank design, compartments.

Immunology

Arenzana, F.J., R. Arevalo, R. Sanchez Gonzalez, D. Clemente, J. Aijon, and A. Porteros (2006). **Tyrosine hydroxylase immunoreactivity in the developing visual pathway of the zebrafish.** *Anatomy and Embryology* 211(4): 323-34. ISSN: 0340 2061.

NAL Call Number: QL951

Abstract: We analyzed the distribution of tyrosine hydroxylase immunoreactivity in the central nervous zones involved in the processing of visual information during zebrafish ontogeny, employing a segmental approach. In the retina, we observed immunolabeled cells in the inner nuclear layer after hatching. From the juvenile stages onwards, some of these cells presented two immunolabeled processes towards the inner and outer plexiform layers of the retina, which are identified as interplexiform cells. In the adult zebrafish retina, we have identified two cellular types displaying immunoreactivity for tyrosine hydroxylase: interplexiform and amacrine cells. In the optic tectum, derived from the mesencephalon, no immunolabeled neurons were observed in any of the stages analyzed. The periventricular gray zone and the superficial white zone display immunostained neuropile from the end of fry life onwards. At the 30-day postfertilization, the tyrosine hydroxylase immunoreactive neuropile in the optic tectum presents two bands located within the retinorecipient strata and deeper strata, respectively. All diencephalic regions, which receive direct retinal inputs, show immunolabeled cells in the preoptic area, in the pretectum, and in the ventral thalamus from embryonic stages onwards. During the fry development, the immunolabeled neurons can be observed in the periventricular pretectum from 15-days postfertilization and in both the ventrolateral thalamic nucleus and suprachiasmatic nucleus from 30-days postfertilization. The transient expression of tyrosine hydroxylase is observed in fibers of the optic tract during fry and juvenile development. The existence of immunolabeled neuropile in the zebrafish retinorecipient strata could be related to the turnover of retinotectal projections.

Descriptors: juvenile zebrafish, tyrosine 3 monooxygenase metabolism, visual pathways enzymology, diencephalon cytology and enzymology, immunohistochemistry, nerve fibers enzymology, retina cytology and enzymology, superior colliculus cytology and enzymology, visual pathways cytology.

Bei JinXin, H. Suetake, K. Araki, K. Kikuchi, Y. Yoshiura, Lin HaoRan, and Y. Suzuki (2006). **Two interleukin (IL)-15 homologues in fish from two distinct origins**. *Molecular Immunology* 43(7): 860-869. ISSN: 0161 5890.

NAL Call Number: 448.3Im62

Abstract: Here, we report two distinct genes in teleosts that are homologous to interleukin (IL)-15. The two genes, isolated from fugu (*Takifugu rubripes*), resemble to mammalian IL-15 but differ from IL-2 and IL-21 in their amino acid sequences, the possessing of an extraordinary long signal peptide and more widespread tissue localization. In addition, multiple out-of-frame AUG codons, the negative translational regulators of mammalian IL-15 genes were also detected in the 5'-UTR of the two genes. Fugu IL-15 homologues also contain four conserved cysteines allowing the formation of two disulfide bridges along with four predicted alpha -helices. Genomic analysis showed that one of the fugu IL-15 homologues possessed six coding exons and exhibited a similar exon-intron organization and synteny structure to that of mammalian and chicken IL-15 genes. Conversely, the other fugu IL-15 homologue possesses four exons and exhibits a different synteny structure with that of IL-15, suggesting that the two genes were derived from two different origins. Moreover, the two genes also differ from each other in tissue localizations and in their expression in response to mitogens. The existence of these two IL-15 homologues in teleosts was further supported by their characterization in zebrafish *Danio rerio*, and the green-spotted pufferfish *Tetraodon nigroviridis*. The discovery of two distinct IL-15 homologues in fish will assist

investigations into the evolution of these genes and their relative contribution to the fish immune system. Copyright © 2008 CABI

Descriptors: zebrafish, cytokines, exons, genes, genomes, interleukins, mitogens, nucleotide sequences, *Danio rerio*, fishes, *Tetraodon nigroviridis*, *Tetraodontidae*.

Bowden, T.J., P. Cook, and J.H.W.M. Rombout (2005). **Development and function of the thymus in teleosts**. *Fish and Shellfish Immunology* 19(5): 413-427. ISSN: 1050 4648.

NAL Call Number: QL638.97.F55

Abstract: The thymus plays a pivotal role in the development of the adaptive immune system, an important factor that separates higher vertebrates from the rest of the animal phyla. The development of functional T-cells from thymocytes is a crucial step in the development of a functional vertebrate immune system and whilst recent advances in molecular and developmental biology have advanced our understanding of T-cell development, they have also provided potential model species across the vertebrate phyla including the zebrafish (*Danio rerio*). However, this species is one of more than 20,000 species of fish that could assist in elucidating the development of the vertebrate thymus and, consequently, the evolution of the vertebrate immune response. In this paper we review the knowledge of the teleost thymus through the organogenesis and development studies in teleosts together with advances in molecular and functional approaches. Where necessary we will combine this knowledge with that obtained in higher vertebrates. Copyright © 2008 CABI

Descriptors: zebrafish, teleost anatomy, biological development, morphology, ontogeny, organs, reviews, thymus gland, fishes, T-cell development.

Notes: A Review.

Bruecker, M., Z. Sun, R. Titcombe, and I. Lin (2006). **Cilia and gap junctions in left-right development**. *Federation of American Societies for Experimental Biology Journal* 20(4, Part 1): A437. ISSN: 0892 6638.

NAL Call Number: QH301.F3

Abstract: In mouse, two types of monocilia found on cells comprising the node establish LR asymmetry. Motile node monocilia generate leftward flow (nodal flow) of the perinodal extraembryonic fluid. Immotile cilia containing polycystin-2 are mechanosensors, responding to nodal flow by increasing intracellular calcium in cells at the left of the node.-We provide evidence for the nature of the midline barrier that limits the calcium signal to the left side of the node. E7.5 mouse embryos were cultured for 20 hrs in still culture with the gap junction blocker heptanol (3.5mM). Normal left-sided expression of Pitx2 was observed in 75%(n=36) control embryos and only 8%(n=12) embryos cultured in 3.5mM heptanol. Gap junctions at the node were visualized in fixed e7.75 embryos with antiCx43 antibodies. Abundant Cx43 containing gap junctions were at the margins and lateral to the node, and very few Cx43 containing gap junctions were within the node itself, or at the midline. Zebrafish embryos at the Kuppfer's vesicle (KV) stage (8-10somites) were examined for the distribution of Gap junctions. Reflecting the distribution of gap junctions at the mouse node, there were Cx43 containing gap junctions around, but not within KV. Overexpression of rat Cx43 in zebrafish embryos resulted in bilateral expression of the normally left-sided marker southpaw. In summary, we show that gap junctions are required for normal LR development at a timepoint corresponding to cilia-generated nodal flow in mouse, and that the expression pattern of Cx43 at this timepoint is conserved in zebrafish. Absence of Cx43 in the node and KV suggests that absence of gap junctions at the node functions as the midline barrier with respect to asymmetric perinodal calcium signals. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, mouse embryos, molecular biophysics, immune system, diges-

tive system, ingestion , gap junction, left-right development, node function, cilia junction, species comparison.

Chang, M.X. and P. Nie (2007). **Intelectin gene from the grass carp *Ctenopharyngodon idella*: cDNA cloning, tissue expression, and immunohistochemical localization**. *Fish and Shellfish Immunology* 23(1): 128-140. ISSN: 1050 4648.
NAL Call Number: QL638.97.F55
Abstract: The cDNA encoding grass carp intelectin was isolated from a head kidney cDNA library, and termed gcIntL. The deduced amino acid sequence of gcIntL consists of 318 amino acids, and about 55% identical and 74% similar to human intelectin, which is a new type of lectin recognizing galactofuranose, and plays a role in the recognition of bacteria-specific components in animal hosts. The gcIntL gene consists of seven exons and six introns, spacing over approximately 3 kb of genomic sequence. Phylogenetic analysis clearly demonstrated that the gcIntL formed a clade with *Danio rerio* intelectin and 35 kDa serum lectin. By real-time quantitative RT-PCR analysis, gcIntL transcripts were significantly induced in head kidney, trunk kidney, spleen, and intestine from LPS-stimulated fish. RT-PCR and Western blotting analysis demonstrated that the mRNA and protein of gcIntL gene have the same expression pattern, and both were detected in brain, gill, intestine, head kidney, trunk kidney, spleen, and heart. Furthermore, gcIntL protein could be detected in gill, intestine, trunk kidney, head kidney, spleen, heart, and brain including medulla oblongata and optic lobe, as determined by immunohistochemistry. This is the first report of intelectin expression pattern in fish, and of recombinant gcIntL and polyclonal antibody against gcIntL. Copyright © 2008 CABI
Descriptors: zebrafish, complementary DNA, DNA cloning, exons, gene expression, genes, genomes, immunohistochemistry, introns, lectins, messenger RNA, nucleotide sequences, phylogeny, *Ctenopharyngodon idella.*

Chang, M.X., P. Nie, and L.L. Wei (2007). **Short and long peptidoglycan recognition proteins (PGRPs) in zebrafish, with findings of multiple PGRP homologs in teleost fish.** *Molecular Immunology* 44(11): 3005-23. ISSN: 0161-5890.
NAL Call Number: 448.3Im62
Abstract: Peptidoglycan recognition protein (PGRP) specifically binds to peptidoglycan and is considered to be one of the pattern recognition proteins in the innate immunity of insect and mammals. Using a database mining approach and RT-PCR, multiple peptidoglycan recognition protein (PGRP) like genes have been discovered in fish including zebrafish *Danio rerio*, Japanese pufferfish *TakiFugu rubripes* and spotted green pufferfish *Tetraodon nigroviridis*. They share the common features of those PGRPs in arthropod and mammals, by containing a conserved PGRP domain. Based on the predicted structures, the identified zebrafish PGRP homologs resemble short and long PGRP members in arthropod and mammals. The identified PGRP genes in *T. nigroviridis* and *TakiFugu rubripes* resemble the long PGRPs, and the short PGRP genes have not been found in *T. nigroviridis* and *TakiFugu rubripes* databases. Computer modelling of these molecules revealed the presence of three alpha-helices and five or six beta-strands in all fish PGRPs reported in the present study. The long PGRP in teleost fish have multiple alternatively spliced forms, and some of the identified spliced variants, e.g., tnPGRP-L3 and tnPGRP-L4 (tn: *Tetraodon nigroviridis)*, exhibited no characters present in the PGRP homologs domain. The coding regions of zfPGRP6 (zf: zebrafish), zfPGRP2-A, zfPGRP2-B and zfPGRP-L contain five exons and four introns; however, the other PGRP-like genes including zfPGRPSC1a, zfPGRPSC2, tnPGRP-L1-, tnPGRP-L2 and frPGRP-L (fr: *Takifugu rubripes*) contain four exons and three introns. In zebrafish, long and short PGRP genes identified are located in different chromosomes, and an unknown locus containing another long PGRP-like gene has also been found in zebrafish,

demonstrating that multiple PGRP loci may be present in fish. In zebrafish, the constitutive expressions of zfPGRP-L, zfPGRP-6 and zfPGRP-SC during ontogeny from unfertilized eggs to larvae, in different organs of adult, and the inductive expression following stimulation by *Flavobacterium columnare*, were detected by real-time PCR, but the levels and patterns varied for different PGRP genes, implying that different short and long PGRPs may play different roles in innate immune response.
Descriptors: zebrafish, fish species, carrier proteins chemistry, genetics and immunology, molecular models, amino acid and base sequence, genetic databases, natural immunity, molecular sequence data, molecular structure, phylogeny, secondary protein structure, tertiary protein structure, sequence alignment, sequence homology, species specificity, *Takifugu*, *Tetraodontiformes*.

Chen, J.S.C., T.Y. Wang, T.D. Tzeng, C.Y. Wang, and D. Wang (2008). **Evidence for positive selection in the TLR9 gene of teleosts**. *Fish & Shellfish Immunology* 24(2): 234-242. ISSN: 1050-4648.
NAL Call Number: QL638.97.F55
Descriptors: zebrafish, adaptations, evolution, models, nucleotide sequence, oligonucleotides, positive selection, TLR9 protein, toll-like receptors, *Danio rerio*, *Paralichthys olivaceus*, *pleuronectiformes*, *Takifugu rubripes*, *Teleostei.*

Danilova, N. (2005). **Analysis of recombination signal sequences in zebrafish.** *Molecular Immunology* 42(10): 1243-9. ISSN: 0161-5890.
NAL Call Number: 448.3Im62
Abstract: Recombination signal sequences (RSS) from immunoglobulin and TCRalpha genes of zebrafish were analyzed in comparison with RSS from human and species-specific features were revealed. In contrast to human RSS, in zebrafish RSS from both V(H) and TCRalpha genes the last nonamer position is not conserved. On the contrary, the fourth nonamer position, which is not conserved in human or mouse is conserved in zebrafish. The 12 bp spacers from human and zebrafish RSS contain 9 bp motif resembling nonamer sequence. Spacers in zebrafish 23 bp RSS from both immunoglobulins and TCRalpha contain 7 bp motif also resembling nonamer sequence while corresponding human sequences do not contain analogous motif. RSS are recognized by RAG1 protein, which also has specific features in teleost suggesting co-evolution of RAG1 with corresponding RSS.
Descriptors: zebrafish, genes, immunoglobulin, T cell receptors antigen genetics, genetic recombination immunology, amino acid and base sequence, conserved sequence, DNA, intergenic chemistry and genetics, molecular evolution, homeodomain proteins chemistry and genetics, immunoglobulin variable region genetics, molecular sequence data, amino acid sequence homology, species specificity.

Danilova, N., J. Bussmann, K. Jekosch, and L.A. Steiner (2005). **The immunoglobulin heavy-chain locus in zebrafish: identification and expression of a previously unknown isotype, immunoglobulin Z.** *Nature Immunology* 6(3): 295-302. ISSN: 1529-2908.
NAL Call Number: QR180
Abstract: The only immunoglobulin heavy-chain classes known so far in teleosts have been mu and delta. We identify here a previously unknown class, immunoglobulin zeta, expressed in zebrafish and other teleosts. In the zebrafish heavy-chain locus, variable (V) gene segments lie upstream of two tandem diversity, joining and constant (DJC) clusters, resembling the mouse T cell receptor alpha (Tcra) and delta (Tcrd) locus. V genes rearrange to (DJC)(zeta) or to (DJC)(mu) without evidence of switch rearrangement. The zebrafish immunoglobulin zeta gene (ighz) and mouse Tcrd, which are proximal to the V gene array, are expressed earlier in development. In adults, ighz was expressed only in kidney and thymus, which are primary lymphoid organs

in teleosts. This additional class adds complexity to the immunoglobulin repertoire and raises questions concerning the evolution of immunoglobulins and the regulation of the differential expression of ighz and ighm.

Descriptors: zebrafish, genes, immunoglobulin, immunoglobulin heavy chains genetics, zebrafish immunology, base sequence, fishes genetics, gene rearrangement, immunoglobulin heavy chains metabolism isotypes and genetics, immunoglobulin isotypes metabolism, immunoglobulin delta chains genetics, immunoglobulin mu chains genetics, molecular sequence data, phylogeny.

Danilova, N., V.S. Hohman, F. Sacher, T. Ota, C.E. Willett, and L.A. Steiner (2004). **T cells and the thymus in developing zebrafish.** *Developmental and Comparative Immunology* 28(7-8): 755-67. ISSN: 0145 305X.

NAL Call Number: QR180.D4

Abstract: The expression of genes encoding T cell receptor (TCR) alpha was used to follow the development of the thymus and to analyze the distribution of T cells in zebrafish. In the thymus, expression was first detected, by in situ hybridization, at four days post fertilization. In RNA extracted from whole fish, TCRalpha transcripts were also detected at four days and reached adult levels at three weeks. At six weeks, TCRalpha was expressed throughout the thymus, whereas rag1 expression was localized to the peripheral regions. Expression of TCRalpha outside the thymus was detected at nine days. In adult peripheral organs, the greatest expression was in the pronephros, mesonephros and intestine; expression in the spleen became greater as fish age. Three new, relatively highly expressed, TCR Valpha families were identified.

Descriptors: zebrafish embryos, intestines metabolism, spleen metabolism, T lymphocytes metabolism, thymus gland metabolism, amino acid sequence, developmental gene expression regulation genetics, homeodomain proteins metabolism, in situ hybridization, intestines embryology, molecular sequence data, T cell receptors antigen metabolism, spleen embryology, thymus gland embryology.

Diamant, A., S. Ram, and I. Paperna (2006). **Experimental transmission of *Enteromyxum leei* to freshwater fish**. *Diseases of Aquatic Organisms* 72(2): 171-178. ISSN: 0177 5103.

Abstract: The myxosporean *Enteromyxum leei* is known to infect a wide range of marine fish hosts. The objective of the present study was to determine whether freshwater fish species are also receptive hosts to this parasite. Seventeen species of freshwater fish were experimentally fed *E. leei*-infected gut tissue from donor gilthead sea bream *Sparus aurata* obtained from a commercial sea bream cage farm. Four of the tested species, tiger barb *Puntius tetrazona*, zebra danio *Danio rerio*, oscar *Astronotus ocellatus* and *Mozambique tilapia Oreochromis mossambicus*, were found to be susceptible with prevalences ranging from 53 to 90%. The course of infection and pathology was limited to the gut mucosa epithelium and was similar to that observed in marine hosts. Little is known of the differences in physiological conditions encountered by a parasite in the alimentary tract of freshwater vs. marine teleost hosts, but we assume that a similar osmotic environment is maintained in both. Parasite infectivity may be influenced by differences in the presence or absence of a true stomach, acidic gastric pH and digestive enzyme activity both in the stomach and intestine. Variability in susceptibility among species may also stem from differences in innate immunity. Dimensions of spores produced in the donor sea bream and recipient freshwater species are variable in size, as previously observed in other captive marine host species.
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Descriptors: zebrafish, disease transmission, experimental infection, freshwater fishes, immunity, stomach parasite, susceptibility, *Danio rerio*, fishes, *Myxozoa*, *Oreochromis mossambicus*, *Pagrus aurata*.

Dong, C.W., Y.B. Zhang, A.J. Lu, R. Zhu, F.T. Zhang, Q.Y. Zhang, and J.F. Gui (2007). **Molecular characterisation and inductive expression of a fish protein arginine methyltransferase 1 gene in response to virus infection**. *Fish & Shellfish Immunology* 22(4): 380-393. ISSN: 1050-4648.
NAL Call Number: QL638.97.F55
Descriptors: zebrafish embryos, DNA, defence mechanisms, fish diseases, freshwater fish, diseases, gene expression, homology, immune response, immunity, immunofluorescence, infection, interferon, intestine, kidney, marine fish, nuclei, signal transduction, spleen, transcription, protein arginine methyltransferase, *Danio rerio*, *Paralichthys olivaceus*, *Pleuronectiformes*, Rhabdovirus, *Scophthalmus maximus*, flatfishes, community, Japanese flounder, olive flounder, turbot.

Ferguson, J.A., V. Watral, A.R. Schwindt, and M. Kent (2007). **Spores of two fish microsporidia (*Pseudoloma neurophilia* and *Glugea anomala*) are highly resistant to chlorine**. *Diseases of Aquatic Organisms* 76(3): 205-214. ISSN: 0177-5103.
Descriptors: zebrafish, chlorine, disease control, disease transmission, disinfection, eggs, fish diseases, fish eggs, freshwater fish, infection, parasites, pathogens, progeny, spores, stains, microsporidia, pH effects, *Danio rerio*, *Encephalitozoon cuniculi*, glugea, microsporidia, *Pseudoloma neurophilia*.

Garner, J.N., B. Joshi, and R. Jagus (2003). **Characterization of rainbow trout and zebrafish eukaryotic initiation factor 2alpha and its response to endoplasmic reticulum stress and IPNV infection.** *Developmental and Comparative Immunology* 27(3): 217-31. ISSN: 0145 305X.
NAL Call Number: QR180.D4
Abstract: The cDNAs of rainbow trout and zebrafish eIF2alpha have been isolated and found to encode proteins of similar molecular weight and isoelectric point to the alpha-subunit of the human translational initiation factor, eIF2. The rainbow trout (36.0kDa) and zebrafish (36.2kDa) eIF2alphas share 93 and 91% identity to the human protein, respectively, and are recognized by antibodies raised to the human form. In mammals, the phosphorylation of the alpha-subunit of eIF2 plays a key role in the regulation of protein synthesis in response to a range of cellular stresses. Regions corresponding to the human phosphorylation and kinase-docking sites are identical in the proteins of both fish species, as are residues that interact with the eIF2 recycling factor, eIF2B. Moreover, both recombinant rainbow trout and zebrafish eIF2alphas can be phosphorylated in vitro by the mammalian heme-sensitive eIF2alpha-kinase, HRI/HCR, as well as the interferon-inducible, dsRNA sensitive kinase, PKR. Phosphorylation of rainbow trout and zebrafish eIF2alpha can also occur in vivo. RTG-2 and ZFL cells subjected to endoplasmic reticulum (ER) stress by treatment with the Ca(2+)-ionophore A23187 showed increased levels of eIF2alpha phosphorylation, suggesting similarity between the ER stress response in fish and other higher eukaryotes. Furthermore, RTG-2 cells responded to treatment with poly(I).poly(C) or to infection by infectious pancreatic necrosis virus, IPNV, by increasing eIF2alpha phosphorylation. These data imply that RTG-2 cells express the interferon-induced eIF2alpha-kinase, PKR and suggests that the interferon/eIF2alpha/PKR response to virus infection may be a conserved vertebrate characteristic. Overall these data are consistent with the premise that fish are able to regulate protein synthesis in response to cellular stresses through phosphorylation of eIF2alpha.
Descriptors: zebrafish, endoplasmic reticulum metabolism, eukaryotic initiation factor 2 metabolism, infectious pancreatic necrosis virus pathogenicity, *Oncorhynchus mykiss* immunology and virology, zebrafish immunology and virology, amino acid sequence, calcimycin pharmacology, cell line, molecular cloning, eukaryotic initiation factor 2 chemistry and genetics, molecular sequence data, phosphorylation, poly I and C pharmacology, stress metabolism, eIF 2 kinase metabolism.

Gunimaladevi, I., Ram Savan, K. Sato, R. Yamaguchi, and M. Sakai (2007). **Characterization of an interleukin-15 like (IL-15L) gene from zebrafish (*Danio rerio*)**. *Fish and Shellfish Immunology* 22(4): 351-362. ISSN: 1050 4648.

NAL Call Number: QL638.97.F55

Abstract: In fish, interleukin (IL)-2, IL-21 and IL-15 genes have recently been identified by in-silico cloning. Fish IL-15 gene is similar to counterparts from mammals and other vertebrates. A zebrafish genomic database-search initiated to find IL-2 and IL-21 genes from zebrafish (*Danio rerio*) led to the identification of an IL-15 like gene (IL-15L). This gene was cloned by prediction and the transcripts were subsequently cloned by PCR. The predicted translation yielded a 162 amino acid protein with a 42 amino acid-long signal peptide. This protein shared identities of 28.4% to 31.5% with other mammalian and vertebrate IL-2, IL-15 and IL-21 genes. The gene occupies 7.7 kb of the genomic DNA and the coding region spans into four exons and is interrupted by three introns, which is similar to the genomic structure of IL-2 gene. The chromosomal synteny and phylogenetic analyses support our view that this IL-15L gene is specific to teleosts. Furthermore, two alternatively spliced forms have been identified with differential exon usage translating for proteins of 108 and 120 amino acids in length. The analysis of the alternative splicing suggests it may play an important role in regulating the function of this novel gene. Analyses by RT-PCR and in situ hybridization show gene expression in lymphoid tissues like intestine, gills, spleen, pancreas and kidney suggestive of a role in immunity of fish. Copyright © 2008 CABI

Descriptors: zebrafish, characterization, DNA cloning, gene expression, genes, genomes, gills, immunogenetics, interleukins, intestines, pancreas, PCR, spleen, *Danio rerio*, fishes.

Gunimaladevi, I., R. Savan, and M. Sakai (2006). **Identification, cloning and characterization of interleukin-17 and its family from zebrafish.** *Fish and Shellfish Immunology* 21(4): 393-403. ISSN: 1050 4648.

NAL Call Number: QL638.97.F55

Abstract: Cytokines are one of the major signaling molecules involved in immunity. Many of these cytokines have been isolated in vertebrates and found to play a significant role in host defense mechanism. Interleukin-17 (IL-17) family of genes are known to have pro-inflammatory actions and associated with specific disease conditions, these genes are conserved across vertebrate evolution. In this study, computational screening for the zebrafish (*Danio rerio*) genome resulted in identification of five contigs harboring IL-17 genes. Zebrafish cDNA encoding five IL-17 genes exhibited percentage identities of 19.3%-61.9% with that of human homologs. The molecules show conservation of cysteines, important for disulphide bonds for IL-17 molecules. The structural composition of these genes shows two introns and three exons except for IL-17D gene that has only one intron and two exons. Phylogenetic analysis using maximum parsimony algorithm showed that zebrafish IL-17 genes clustered well with other IL-17 homologs further proving the structural similarity with IL-17 genes from other organisms. Expression analysis by RT-PCR revealed expression of IL-17 genes in normal and stimulated tissues of kidney, spleen, gills and intestine. The expression of IL-17 in un-stimulated tissues indicates that these genes may play important roles in normal conditions as well.

Descriptors: zebrafish genome screening, fish interleukin 17 genetics, DNA primers chemistry, fish proteins biosynthesis, chemistry and drug effects, gene order, interleukin 17 biosynthesis and chemistry, lipopolysaccharides pharmacology, molecular sequence data, phylogeny, PCR methods, sequence alignment veterinary, amino acid sequence homology and immunology.

Harriff, M.J., L.E. Bermudez, and M.L. Kent (2007). **Experimental exposure of zebrafish, *Danio rerio* (Hamilton), to *Mycobacterium marinum* and *Mycobacterium peregrinum* reveals the gastrointestinal tract as the primary route of infection: a potential model for environmental mycobacterial infection.** *Journal of Fish Diseases* 30(10): 587-600. ISSN: 0140-7775.
NAL Call Number: SH171.A1J68
Abstract: The natural route by which fish become infected with mycobacteria is unknown. *Danio rerio* (Hamilton) were exposed by bath immersion and intubation to *Mycobacterium marinum* and *Mycobacterium peregrinum* isolates obtained from diseased zebrafish. Exposed fish were collected over the course of 8 weeks and examined for the presence of mycobacteriosis. Mycobacteria were consistently cultured from the intestines, and often from the livers and spleens of fish exposed by both methods. Mycobacteria were not observed in the gills. Histological analysis revealed that fish infected with *M. marinum* often developed granulomas accompanied by clinical signs of mycobacteriosis, while infection with *M. peregrinum* infrequently led to clinical signs of disease. Passage of the bacteria through environmental amoebae (*Acanthamoeba castellani*) was associated with increased growth of *M. peregrinum* over the course of 8 weeks, when compared to infection with the bacteria not passed through amoebae. The results provide evidence that zebrafish acquire mycobacteria primarily through the intestinal tract, resulting in mycobacterial dissemination.
Descriptors: zebrafish mycobacterial infection, fish diseases microbiology, intestines microbiology, *Mycobacterium* pathogenicity and infections, veterinary care, *Acanthamoeba castellanii* microbiology, colony count, microbial, disease models, fish diseases pathology, liver microbiology, *Mycobacterium* infections microbiology and pathology, *Mycobacterium marinum* pathogenicity, spleen microbiology, time factors, virulence, water microbiology.

Hermann, A.C. and C.H. Kim (2005). **Effects of arsenic on zebrafish innate immune system.** *Marine Biotechnology* 7(5): 494-505. ISSN: 1436-2228.
NAL Call Number: TP248.27.M37M37
Abstract: The innate immune response, the first line of defense against invading pathogens, can be perturbed by environmental toxicants such as arsenic. This study reports the effects of arsenic on innate immunity of zebrafish. Respiratory burst activity, messenger RNA expression of tumor necrosis factor alpha (TNF-alpha), a primer of the respiratory burst response, and mRNA expression of the antiviral cytokines interferon (IFN) and MX, : before and after viral infection, were examined in arsenic-exposed zebrafish larvae. Respiratory burst activity and TNF-alpha expression were decreased upon arsenic exposure, indicating inhibition of TNF-alpha priming of the respiratory burst response. Arsenic enhanced IFN expression slightly over time, but reduced MX : expression. In zebrafish infected with snakehead rhabdovirus, arsenic decreased induction and altered the kinetics of IFN and MX : upon infection. Differences in IFN and MX : expression in arsenic-exposed larvae point toward an interruption of the Janus kinase-signal transducer and activator of transcription (JAK/STAT) pathway.
Descriptors: zebrafish immunology, arsenic toxicity, natural immunity drug effects, respiratory burst drug effects, metabolism, arsenic antagonists and inhibitors, DNA primers and virus infections metabolism, fish diseases immunology, gene expression and immunocompetence drug effects, interferons biosynthesis, interferons genetics, larva metabolism, novirhabdovirus immunology, PCR methods, messenger RNA biosynthesis and drug effects , rhabdoviridae infections immunology and veterinary diagnosis, tumor necrosis factor alpha biosynthesis and genetics.

Hermann, A.C., P.J. Millard, S.L. Blake, and C.H. Kim (2004). **Development of a respiratory burst assay using zebrafish kidneys and embryos**. *Journal of Immunological Methods* 292(1/2): 119-129. ISSN: 0022 1759.
NAL Call Number: QR180.J6

Abstract: The innate immune response constitutes the first line of defence against invading pathogens and consists of a variety of immune defence mechanisms including the respiratory burst of phagocytes. Respiratory burst can be used as a reliable measure of the immune response of a host, and numerous assays have been developed to measure this response in a variety of mammal and fish species. Phagocytes, like granulocytes and macrophages, that are derived from different tissues, or grown in cell culture, have been employed in a range of assay formats employing a variety of detection methods. The small size of the zebrafish has prevented the large-scale extraction of these cells for respiratory burst assays in the zebrafish. In this work, we describe a respiratory burst assay developed for the zebrafish using intact kidneys and embryos as sources of phagocytes. Phorbol myristate acetate (PMA)-inducible reactive oxygen species (ROS) were detected following the oxidation of a non-fluorescent dye 2',7'-dihydrodichlorofluorescein diacetate (H2DCFDA) to dichlorofluorescein (DCF), a fluorescent product. Embryos from 1 day post-fertilization until 5 days post-fertilization (dpf) were employed in this assay. Abrogation of H2DCFDA oxidation by the protein kinase C (PKC) inhibitor bisindolylmaleimide I (BisI) indicated a reduction in the respiratory burst. Fluorescence from the PMA-induced respiratory burst in kidneys and embryos was significantly elevated above DMSO-treated controls, while preincubation with BisI inhibited the increase in fluorescence. Colocalization of cell-associated chloromethyl-dihydrodichlorofluorescein diacetate (CM-H2DCFDA) with the phagocyte-selective dye neutral red is consistent with the observation that macrophages and granulocytes are the ROS-producing cells in the zebrafish. Copyright © 2008 CABI
Descriptors: zebrafish embryo, assays, embryos, free radicals, granulocytes, immune response, kidneys, macrophages, oxidation, oxygen, phagocytes, *Danio rerio.*

Hirono, I., H. Kondo, T. Koyama, N.R. Arma, J.Y. Hwang, R. Nozaki, N. Midorikawa, and T. Aoki (2007). **Characterization of Japanese flounder (*Paralichthys olivaceus*) NK-lysin, an antimicrobial peptide**. *Fish & Shellfish Immunology* 22(5): 567-575. ISSN: 1050-4648.
NAL Call Number: QL638.97.F55
Descriptors: zebrafish, amino acid sequence, antimicrobial activity and peptides, brain, cardiac muscle, cysteine, DNA, disease resistance, exons, freshwater fish, gene expression, gills, intestine, introns, kidney, leukocytes, lipopolysaccharides, liver, marine fish, muscles, open reading frames, ovaries, peptides, peripheral blood, protein folding, skin, spleen, Stomach, *Danio rerio*, *Escherichia coli*, *Klebsiella pneumoniae*, *Paralichthys olivaceus*, *Photobacterium damselae*, *Pleuronectiformes*, *Pseudomonas aeruginosa.*

Hogan, B.M., J.A. Danks, J.E. Layton, N.E. Hall, J.K. Heath, and G.J. Lieschke (2006). **Localization of parathyroid hormone in developing zebrafish embryos.** *Journal of Experimental Zoology* 305A(2): 133. ISSN: 1548-8969.
NAL Call Number: QL1.J854
Descriptors: zebrafish embryos, freshwater ecology, environmental sciences, development, molecular genetics, endocrine system, chemical coordination and homeostasis, immunohistochemistry, histology and cytology techniques, immunologic techniques.

Hutson, L.D., D.S. Campbell, and C.B. Chien (2004). **Analyzing axon guidance in the zebrafish retinotectal system.** *Methods in Cell Biology* 76: 13-35. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, axons physiology, retina embryology, genetically modified, antibodies immunology, axons immunology, eye transplantation, fluorescent dyes chemistry, green fluorescent proteins chemistry and genetics, luminescent proteins chemistry and genetics, mutation,

organ transplantation methods, retina physiology, retinal ganglion cells physiology, staining and labeling methods, superior colliculus physiology.

Igawa, D., M. Sakai, and R. Savan (2006). **An unexpected discovery of two interferon gamma-like genes along with interleukin (IL)-22 and -26 from teleost: IL-22 and -26 genes have been described for the first time outside mammals**. *Molecular Immunology* 43(7): 999-1009. ISSN: 0161 5890.
NAL Call Number: 448.3Im62
Abstract: By comparative genomics based on chromosome synteny between human and fish, IL-22, IL-26 and two IFN- gamma -like genes have been isolated in fish. Interleukin-22 and -26 have been identified for the first time outside of mammals and their organization and synteny demonstrates that this cluster of cytokines is well conserved during evolution. The cloned zebrafish IL-22 and -26 homologs are 161 and 169-aa long, respectively. Both the genes harbor a well-conserved IL-10-family signature in their respective C-terminus. The coding regions of these genes are spread in five exons and at the same position as in human homologs. Furthermore, surprisingly two IFN- gamma -like genes are present in tandem, 7.0 kb apart from each other, along this locus. IFN- gamma 1 and 2 genes are composed of 170- and 185-aa and share a low amino acid identity of 17.0%. The genomic structures of the isolated fish IFN- gamma genes were similar to its mammalian homologs, sharing a four exon and three phase 0 introns. The presence two IFN- gamma genes in both zebra and pufferfishes demonstrate that the duplication of IFN-gamma might have occurred prior to the teleostean split. The structural homology is further validated by phylogenetic analysis showing that the isolated cytokine genes are homologous to mammalian counterparts. Expression analysis of IL-22, IL-26 and two IFN- gamma genes suggests an active role of these genes in immune responses in fish. Copyright © 2008 CABI
Descriptors: cloned zebrafish, pufferfish, amino acid sequences, cytokines, exons, genes, interferon, interleukins, introns, nucleotide sequences, phylogenetics, *Danio rerio*, Tetraodontidae.

Imai, E., J. Ishikawa, T. Moritomo, and M. Tomana (2005). **Characterisation of T cell antigen receptor alpha chain isotypes in the common carp**. *Fish & Shellfish Immunology* 19(3): 205-216. ISSN: 1050-4648.
NAL Call Number: QL638.97.F55
Descriptors: zebrafish, amino acid sequence, antigens, DNA, defence mechanisms, freshwater fish, genetic diversity, immunology, nucleotide sequence, phylogeny, ploidy, PCR, receptors, V(d)J recombination, double prime T-cell receptor, cDNA, cyprinus, *Cyprinus carpio*, *Danio rerio*, common carp, European carp, T cell antigen receptor alpha chain.

Ishikawa, J., E. Imai, T. Moritomo, M. Nakao, T. Yano, and M. Tomana (2004). **Characterisation of a fourth immunoglobulin light chain isotype in the common carp**. *Fish & Shellfish Immunology* 16(3): 369-379. ISSN: 1050-4648.
NAL Call Number: QL638.97.F55
Descriptors: zebrafish, cloning, cultured organisms, cysteine, DNA, disease resistance, fish culture and physiology, freshwater aquaculture and fish, globulins, immunoglobulins, immunology, molecular structure, phylogenetics, PCR, recombination, reverse transcription, *Cyprinus carpio*, *Danio rerio*, *Oncorhynchus mykiss*.

Jault, C., L. Pichon, and J. Chluba (2004). **Toll-like receptor gene family and TIR-domain adapters in *Danio rerio.*** *Molecular Immunology* 40(11): 759-71. ISSN: 0161-5890.
NAL Call Number: 448.3Im62
Abstract: The toll-like family of receptors (TLR) is an ancient pattern recognition receptor

family, conserved from insects to mammals. We have identified in zebrafish (*Danio rerio*) 19 putative TLR variants, the orthologs of mammalian TLR2-5, 7-9, a fish specific receptor type group and three putative splice variants. One receptor is very close to mammalian TLR1, 6 and 10 and seems to be their common ancestor. However, in contrast to the pufferfish, *Fugu rubripes*, we found two receptors homologous to TLR4, showing that lack of TLR4 is not general for fish. In addition, we identified two members close to mammalian TLR8 and five members close to FuguTLR21 and goldfish TLR, a TLR group which now has only been found in fish. By RT-PCR we showed that all TLR are widely expressed in adult tissues, but also at different stages of development. All these TLRs contain very conserved toll/interleukin-1 receptor (TIR) domains able to interact with TIR-domain of adapter molecules. We demonstrate here that TIR-domain containing adapters MyD88 and SARM are present in zebrafish, showing that TLR adapter molecules are highly conserved in evolution.

Descriptors: zebrafish TLR mutants , membrane glycomultigene family, cell surface receptors genetics, amino acid sequence, molecular evolution, membrane glyco, molecular sequence data, phylogeny, tertiary protein structure, cell surface receptors physiology, sequence alignment, protein sequence analysis, sequence homology, signal transduction genetics and physiology, toll like receptor 1, 2, 4, 8, toll like receptors.

Jin HongJian, Shao JianZhong, and Xiang LiXin (2007). **Identification and characterization of suppressor of cytokine signalling 3 (SOCS-3) homologues in teleost fish**. *Molecular Immunology* 44(5): 1042-1051. ISSN: 0161 5890.

NAL Call Number: 448.3Im62

Abstract: The suppressor of cytokine signalling 3 (SOCS-3) is a member of a newly discovered protein family, which have been shown to regulate the responses of many immune cytokines, such as interferon (IFN), interleukin-2 (IL-2) and IL-6, etc., by inhibiting Janus kinase (JAK)-signal transducers and activators of transcription (STAT) signalling in a negative auto-regulatory manner. Although SOCS-3 was well characterized in several mammal species, there was still no report in fish. In present study, we initially identified and characterized the SOCS-3 genes from three fishes, the *Tetraodon nigroviridis*, the *Danio rerio* and the *Fugu rubripes*. The results showed that Tetraodon SOCS-3 gene located within a 2666 bp genomic fragment of chromosome 3, transcribed into a 1445 bp mRNA including 273 bp 5' UTR (untranslated region), 606 bp ORF (open reading frame) and 566 bp 3' UTR. Tetraodon SOCS-3 with 201aa (amino acid) has a calculated molecular mass of 22.76 kDa and a theoretical pI of 8.99. Danio SOCS-3 gene located within a 3617 bp genomic fragment of chromosome 3, transcribed into a 1927 bp mRNA including 178 bp 5' UTR, 624 bp ORF and 1125 bp 3' UTR. Danio SOCS-3 with 207aa has a calculated molecular mass of 23.68 kDa and a theoretical pI of 9.19. Fugu SOCS-3 gene located within a 2842 bp genomic fragment of Scaffold_1118, transcribed into a 1528 bp mRNA including 209 bp 5' UTR, 606 bp ORF and 713 bp 3' UTR. Fugu SOCS-3 with 201aa has a calculated molecular mass of 22.76 kDa and a theoretical pI of 8.18. The fish SOCS-3-encoding genes with the same organization as the mammalians consist of two exons and a single intron that lies in the 5' UTR of the transcript. The deduced amino acid sequences of the fish SOCS-3s showed: 60.7-61.7% sequence identity to mammalian SOCS-3s; 62.3-63.2% sequence identity to bird SOCS-3s; and 55.3-57.8% sequence identity to amphibian SOCS-3s. Phylogenetic analysis separates the fish SOCS-3s into an exclusive group. Expression study of Tetraodon SOCS-3 mRNA in ten selected tissues showed that it was constitutively expressed and induced by lipopolysaccharide (LPS) strikingly. These results indicated that SOCS-3s in fish may be involved in inflammatory responses. This is the first report of cloning and characterization of SOCS-3 cDNAs and genes in fish. Copyright © 2008 CABI

Descriptors: zebrafish, chromosomes, cytokines, DNA cloning, exons, gene expression, genes,

genome analysis, inflammation, interferon, interleukin 2 and 6, introns, lipopolysaccharides, messenger RNA, nucleotide sequences, open reading frames, phylogeny, proteins, signal transduction, transcription, *Danio rerio*, Tetraodontidae, *Tetraodon nigroviridis*, *Fugu rubripes*.

Kent, M. and J. Bishop Stewart (2003). **Transmission and tissue distribution of *Pseudoloma neurophilia* (Microsporidia) of zebrafish, *Danio rerio* (Hamilton).** *Journal of Fish Diseases* 26(7): 423-426. ISSN: 0140 7775.
NAL Call Number: SH171.A1J68
Descriptors: zebrafish, *Danio rerio*, protozoan parasites, *Pseudoloma neurophilia*, infection, transmission and tissue distribution, transmission of parasites.

Lam, S.H., H.L. Chua, Z. Gong, T.J. Lam, and Y.M. Sin (2004). **Development and maturation of the immune system in zebrafish, *Danio rerio*: a gene expression profiling, in situ hybridization and immunological study.** *Developmental and Comparative Immunology* 28(1): 9-28. ISSN: 0145 305X.
NAL Call Number: QR180.D4
Abstract: The development and maturation of the immune system in zebrafish was investigated using immune-related gene expression profiling by quantitative real-time polymerase chain reaction, in situ hybridization (ISH), immunoglobulin (Ig) detection by immuno-affinity purification and Western blotting as well as immersion immunization experiments. Ikaros expression was first detected at 1 day post-fertilization (dpf) and thereafter increased gradually to more than two-fold between 28 and 42dpf before decreasing to less than the initial 1dpf expression level in adult fish (aged 105dpf). Recombination activating gene-1 (Rag-1) expression levels increased rapidly (by 10-fold) between 3 and 17dpf, reaching a maximum between 21 and 28dpf before decreasing gradually. However, in adult fish aged 105dpf, the expression level of Rag-1 had dropped markedly, and was equivalent to the expression level at 3dpf. T-cell receptor alpha constant region and immunoglobulin light chain constant region (IgLC) isotype-1, 2 and 3 mRNAs were detected at low levels by 3dpf and their expression levels increased steadily to the adult range between 4 and 6 weeks post-fertilization (wpf). Using tissue-section ISH, Rag-1 expression was detected in head kidney by 2wpf while IgLC-1, 2 and 3 were detected in the head kidney and the thymus by 3wpf onwards. Secreted Ig was only detectable using immuno-affinity purification and Western blotting by 4wpf. Humoral response to T-independent antigen (formalin-killed Aeromonas hydrophila) and T-dependent antigen (human gamma globulin) was observed in zebrafish immunized at 4 and 6wpf, respectively, indicating that immunocompetence was achieved. The findings reveal that the zebrafish immune system is morphologically and functionally mature by 4-6wpf.
Descriptors: zebrafish embryology, messenger RNA metabolism, zebrafish immunology, antibody formation, Western blotting, cell differentiation, electrophoresis, agar gel, gene expression profiling , homeodomain proteins analysis and metabolism, immunoglobulins analysis and immunology, in situ hybridization, kidney cytology, PCR, messenger RNA analysis, reverse transcriptase PCR, thymus gland cytology.

Lam, S.H., Y.M. Sin, Z. Gong, and T.J. Lam (2005). **Effects of thyroid hormone on the development of immune system in zebrafish.** *General and Comparative Endocrinology* 142(3): 325-35. ISSN: 0016-6480.
NAL Call Number: 444.8G28
Abstract: Effects of thyroxine (T4) and methimazole (MMI) on the development of the zebrafish immune system were investigated using continuous immersion treatment experiments. The effects of the treatments on thymus development were determined using computer-aided thymus morphometric analyses on in situ hybridization serial sections of the thymus while the

effects on immune-related gene expression levels were monitored using quantitative real-time PCR. The findings indicate that thymus development and thymopoiesis, as indicated by thymus size, thymus Rag-1-positive region, and TCRAC expression level, were affected by T4 and MMI-treatments. With the exception of Ikaros, MMI-treated fish has lower immune-related gene expression levels, although it is not certain whether the effect resulted indirectly from the concomitant growth-retardation and/or directly from an effect on lymphopoiesis itself. The findings were comparable with those in mammalian system, thus providing the first evidence that the thyroid relationship with thymus development and lymphopoiesis is likely to be conserved from fish to higher vertebrates. It suggests the possibility of using zebrafish as a model system to investigate the molecular mechanisms involved in thyroid hormone-dependent disorders in the immune system.

Descriptors: zebrafish embryos, antithyroid agents pharmacology, methimazole pharmacology, thymus gland immunology, thyroxine pharmacology, zebrafish immunology, homeodomain proteins biosynthesis, genetics and immunology, in situ hybridization, messenger RNA biosynthesis and genetics, reverse transcriptase PCR, thymus gland drug effects.

Lee, E., B.S. Chang, G.H. Mun, Y.H. Chung, J. Kim, and D.H. Shin (2005). **An ultramicroscopic study on the distribution of Muller cell processes in the outer retinal layers of the zebrafish.** *Annals of Anatomy* 187(1): 43-50. ISSN: 0940 9602.

NAL Call Number: QL801

Abstract: The zebrafish, *Brachydanio rerio*, is a good model for studying the development of various organs. We have assayed the distribution pattern of Muller cell processes in zebrafish retinas by electron microscopy. In the outer nuclear (ON) layer, multiple layers of Muller cell processes were present along both sides of the large pyramidal endings of the synaptic terminals. We found that the inner segments (ISs) of the zebrafish photoreceptors (PRs), including the cones, double cones and rods, were arranged in different planes, and that the Muller cell processes formed multilayered sheaths around virtually all PR compartments except their outer segments. Thus, Muller cell processes beyond the outer limiting membrane (OLM) are more easily observed in zebrafish retina than in the retinas of other species. To our knowledge, this is the first study to show the exact ultrastructural distribution of Muller cell processes around the OLM and the PR layer in zebrafish retina.

Descriptors: zebrafish embryos, retina development and cytology, anatomy and histology, Muller cell, immunohistochemistry, electron microscopy, retina ultrastructure, vimentin analysis, photoreceptors, cone and rod arrangement.

Lennard, M.L., M.R. Wilson, N.W. Miller, L.W. Clem, G.W. Warr, and J. Hikima (2006). **Oct2 transcription factors in fish - a comparative genomic analysis**. *Fish and Shellfish Immunology* 20(2): 227-238. ISSN: 1050 4648.

NAL Call Number: QL638.97.F55

Abstract: The Oct2 transcription factor is important in driving expression of the IgH locus of the channel catfish, *Ictalurus punctatus*. Two isoforms, catfish Oct2 alpha and Oct2 beta , have been characterized at the level of expression and function, but little is known of the structure of the Oct2 gene in catfish. To gain insight into the diversity of Oct2 gene structure and expression in the teleost fish, a comparative genomic analysis of Oct2 was undertaken in the pufferfish (*Fugu rubripes*) and the zebrafish (*Danio rerio*). The orthologues of zebrafish and Fugu Oct2 were identified, and share with catfish Oct2 the expression of a limited number (two in zebrafish, three in Fugu) of isotypes produced by alternative pathways of RNA processing. The alternatively spliced variants of catfish Oct2 showed a different pattern of exon use from those of Fugu and zebrafish. The analysis also identified a novel homologue of Oct2 in both zebrafish and Fugu. This homo-

logue, termed Oct2x, shares similarities to both Oct1 and Oct2. A phylogenetic analysis of the relationships of Oct2x gave an unexpected result, with Oct2x occupying a position basal to the Oct gene families of both vertebrates and *Drosophila*. Copyright © 2008 CABI
Descriptors: zebrafish, exons, gene expression, genes, genomes, loci, phylogenetics, phylogeny, transcription factors, *Danio rerio*, fishes, *Ictalurus punctatus*, channel catfish, pufferfish.
Notes: Special issue:Reviews in Fish Immunology.

Li, X., S. Wang, J. Qi, S.F. Echtenkamp, R. Chatterjee, M. Wang, G.J. Boons, R. Dziarski, and D. Gupta (2007). **Zebrafish peptidoglycan recognition proteins are bactericidal amidases essential for defense against bacterial infections.** *Immunity* 27(3): 518-29. ISSN: 1074-7613.
Abstract: Peptidoglycan recognition proteins (PGRPs) are structurally conserved through evolution, but their functions in innate immunity are different in invertebrates and vertebrates. We asked what the functions of PGRPs in fish are and whether they are indispensable for defense against infection because fish are the first vertebrates that developed adaptive immunity, but they still rely solely on innate immunity during early development of embryos. We identified and cloned three zebrafish PGRPs and showed that they are highly expressed in eggs, developing embryos, and adult tissues that contact external environment. Zebrafish PGRPs have both peptidoglycan-lytic amidase activity and broad-spectrum bactericidal activity, which is a unique feature. Furthermore, we demonstrated that in the developing zebrafish embryo, one of these PGRPs is essential for defense and survival during bacterial infections. These data demonstrate an absolute requirement for innate immunity in defense against infections in fish embryos and for a PGRP protein for survival in vertebrates.
Descriptors: zebrafish, eggs, embryos, adult tissues, PGRP clones, bacterial infections enzymology and immunology, carrier proteins immunology, amidohydrolases genetics and immunology, amino acid sequence , Northern blotting, Western blotting, carrier molecular cloning, immunology, immunohistochemistry, in situ hybridization, molecular sequence data, amino acid sequence homology.

Li, Z. and Y. Chang (2007). **V(D)J recombination in zebrafish: Normal joining products with accumulation of unresolved coding ends and deleted signal ends.** *Molecular Immunology* 44(7): 1793-802. ISSN: 0161-5890.
NAL Call Number: 448.3Im62
Abstract: V(D)J recombination proceeds from a site-specific cleavage to an imprecise end joining, via generation and resolution of recombination ends. Although rearranged antigen receptor genes isolated from zebrafish *(Danio rerio*) resemble those made in mammals, differences may arise during evolution from lower to higher vertebrates, in regard to efficiency, fidelity and regulation of this recombination. To elucidate the V(D)J recombination reaction in zebrafish, we characterized recombination ends transiently produced by zebrafish lymphocytes, as well as joining products. Similar to their mammalian counterpart, zebrafish lymphocytes make perfect signal joints and normal coding joints, indicating their competent end resolution machinery. However, recombination ends recovered from the same zebrafish lymphoid tissues exhibit some features that are not readily seen in normal mammalian counterpart: deleted signal ends and accumulation of opened coding ends. These results indicate that the recombination reaction in zebrafish lymphocytes is inefficient and less stringently regulated, which may result from unstable post-cleavage complexes, and/or slow transition from cleavage to resolution. Our data suggests that the V(D)J recombination machinery may have undergone evolution selection to become more efficient in higher jawed vertebrates.
Descriptors: zebrafish lymphocytes, B lymphocyte gene rearrangement heavy chain genetics

and light chain genetics, base sequence, molecular sequence data, genetic recombination, V(D)J recombinases metabolism, zebrafish immunology.

Lin Bin, Chen ShangWu, Cao Zhen, Lin YiQun, Mo DunZhou, Zhang HaiBo, Gu Juda, Dong MeiLing, Liu ZeHuan, and Xu AnLong (2007). **Acute phase response in zebrafish upon Aeromonas salmonicida and Staphylococcus aureus infection: striking similarities and obvious differences with mammals**. *Molecular Immunology* 44(4): 295-301. ISSN: 0161 5890.
NAL Call Number: 448.3Im62
Abstract: Zebrafish has emerged as a valuable model for immunological studies. However, little is known about the overall picture of its immune response to infectious pathogens. Here we present the first systematic study of its immune response to *Aeromonas salmonicida* and *Staphylococcus aureus*, a Gram-negative and a Gram-positive bacteria, respectively. Genes induced upon infection were identified with suppression subtractive hybridization, with many of them encoding acute phase proteins (APPs). When compared with mammals, striking similarities and obvious differences have been observed. Both similar APPs (SAA, hepcidin and haptoglobin, etc.) and a similar system for the induction of APPs (which involves the TLRs, pro-inflammatory cytokines and C/EBPs) were identified, implying evolutionary conserved mechanisms among fish and mammals. Some novel APPs were also discovered, suggesting different immune strategies adopted by fish species. Among which, LECT2 was induced by up to 1000-fold upon infection, shedding new lights on the function of this gene. Our results constitute the first demonstration of a similar while different immune response in zebrafish and open new avenues for the investigation of evolutionary conserved and fish specific mechanisms of innate immunity. Copyright © 2008 CABI
Descriptors: zebrafish, acute phase proteins, animal models, comparison with mammalian responses, genes, immune response, immunity, *Aeromonas salmonicida*, *Danio rerio*, fishes, *Staphylococcus aureus.*

Lopez Castejon, G., M.T. Young, J. Meseguer, A. Surprenant, and V. Mulero (2007). **Characterization of ATP-gated P2X7 receptors in fish provides new insights into the mechanism of release of the leaderless cytokine interleukin-1 beta**. *Molecular Immunology* 44(6): 1286-1299. ISSN: 0161 5890.
NAL Call Number: 448.3Im62
Abstract: Mammalian interleukin-1 beta (IL-1 beta) is produced as a biologically inactive precursor molecule, which is proteolytically cleaved to an active form by IL-1 beta -converting enzyme (ICE) after the activation of P2X7 receptor by extracellular ATP. The mechanism of IL-1 beta release in non-mammalian vertebrates is largely unknown, although most of the IL-1 beta gene sequences lack a conserved ICE recognition site. Here we have cloned the P2X7 receptor from the bony fish seabream and compared agonist and antagonist profiles at this and other non-mammalian P2X7 receptors expressed in HEK cells, as well in seabream SAF-1 cells expressing endogenous P2X7 receptors. We used this information to further investigate the mechanisms of IL-1 beta release induced by mammalian and fish P2X7 receptors. Despite phosphatidylserine externalization and cell permeabilization in seabream leukocytes after the addition of high BzATP concentrations, IL-1 beta remained unprocessed within the cell. However, activation of rat P2X7 receptors ectopically expressed in HEK293 together with human ICE led to the specific secretion of unprocessed seabream IL-1 beta . In contrast, neither seabream nor zebrafish P2X7 receptors induced the secretion of mammalian or fish IL-1 beta when expressed in HEK293, while a chimeric receptor harboring the ATP-binding domain of seabream P2X7 and the intracellular region of its rat counterpart did so. These findings indicate that P2X7 receptor-mediated activation of ICE and release of IL-1 beta result from different downstream signaling pathways and suggest that although the mechanisms involved in IL-1 beta secretion are conserved throughout

evolution, distinct inflammatory signals have been selected for the secretion of this cytokine in different vertebrates. Copyright © 2008 CABI

Descriptors: zebrafish, ATP, biochemical receptors, cytokines, DNA cloning, immune system, immunity, interleukin 1, leukocytes, nucleotide sequences, *Danio rerio*, sea bream.

Lu, M.W., Y.M. Chao, T.C. Guo, N. Santi, O. Evensen, S.K. Kasani, J.R. Hong, and J.L. Wu (2008). **The interferon response is involved in nervous necrosis virus acute and persistent infection in zebrafish infection model.** *Molecular Immunology* 45(4): 1146-52. ISSN: 0161-5890.

NAL Call Number: 448.3Im62

Abstract: Betanodavirus, a small positive-sense bipartite RNA virus notoriously affecting marine aquaculture worldwide has been extensively studied in vitro. However, impending studies in elucidating virus-host interactions have been limiting due to the lack of appropriate animal disease models. Therefore, in this study, we have attempted to successfully establish NNV infection in zebrafish (*Danio rerio*) showing typical NNV symptoms and which could potentially serve as an in vivo model for studying virus pathogenesis. Zebrafish being already a powerful research tool in developmental biology and having its genome completely sequenced by the end of 2007 would expedite NNV research. We have observed viral titers peaked at 3 days post-infection and histological study showing lesions in brain tissues similar to natural host infection. Further, we used this infection model to study the acute and persistence infection during NNV infection. Interestingly, RT-PCR and immunoblotting assays revealed that the acute infection in larvae and juveniles is largely due to inactive interferon response as opposed to activated innate immune response during persistent infection in adult stage. This study is the first to demonstrate NNV infection of zebrafish, which could serve as a potential animal model to study virus pathogenesis and neuron degeneration research.

Descriptors: zebrafish viral in vivo disease model, interferon, response, nervous necrosis, pathogenic virus, acute, persistent, infection, NNV infection.

Lucini, C., L. Maruccio, S. Tafuri, N. Staiano, and L. Castaldo (2004). **Artemin-like immunoreactivity in the zebrafish, *Danio rerio*.** *Anatomy and Embryology* 208(5): 403-10. ISSN: 0340 2061.

NAL Call Number: QL951

Descriptors: zebrafish artemin distribution, nerve growth factors metabolism, nervous system metabolism, digestive system cytology and metabolism, endocrine system cytology and metabolism, glial cell line derived neurotrophic factor, gonads cytology, gonads metabolism, immune system cytology and metabolism, immunohistochemistry, nervous system cytology, neuroglia cytology, neuroglia metabolism, neurons cytology and metabolism, organ specificity, respiratory system cytology and metabolism, zebrafish anatomy and histology.

MacKay, A.B., A.A. Mhanni, R.A. McGowan, and P.H. Krone (2007). **Immunological detection of changes in genomic DNA methylation during early zebrafish development.** *Genome* 50(8): 778-85. ISSN: 0831-2796.

NAL Call Number: QH431.G452

Abstract: DNA methylation reprogramming, the erasure of DNA methylation patterns shortly after fertilization and their reestablishment during subsequent early development, is essential for proper mammalian embryogenesis. In contrast, the importance of this process in the development of non-mammalian vertebrates such as fish is less clear. Indeed, whether or not any widespread changes in DNA methylation occur at all during cleavage and blastula stages of fish in a fashion similar to that shown in mammals has remained controversial. Here we have addressed this issue by applying the techniques of Southwestern immunoblotting and immunohistochemistry with an anti-5-methylcytosine antibody to the examination of DNA methylation in early zebrafish

embryos. These techniques have recently been utilized to demonstrate that development-specific changes in genomic DNA methylation also occur in *Drosophila melanogaster* and *Dictyostelium discoideum*, both organisms for which DNA methylation was previously not thought to occur. Our data demonstrate that genome-wide changes in DNA methylation occur during early zebrafish development. Although zebrafish sperm DNA is strongly methylated, the zebrafish genome is not detectably methylated through cleavage and early blastula stages but is heavily remethylated in blastula and early gastrula stages.
Descriptors: zebrafish embryos, developmental changes, DNA, methylation, embryogenesis.

Mathias, J.R., B.J. Perrin, T.X. Liu, J. Kanki, A.T. Look, and A. Huttenlocher (2006). **Resolution of inflammation by retrograde chemotaxis of neutrophils in transgenic zebrafish.** *Journal of Leukocyte Biology* 80(6): 1281-88. ISSN: 0741-5400.
NAL Call Number: QP185.R4
Abstract: Neutrophil chemotaxis to sites of inflammation is a critical process during normal immune responses to tissue injury and infection and pathological immune responses leading to chronic inflammation. Although progress has been made in understanding the mechanisms that promote neutrophil recruitment to inflamed tissue, the mechanisms that regulate the resolution phase of the inflammatory response have remained relatively elusive. To define the mechanisms that regulate neutrophil-mediated inflammation in vivo, we have developed a novel transgenic zebrafish in which the neutrophils express GFP under control of the myeloperoxidase promoter (zMPO:GFP). Tissue injury induces a robust, inflammatory response, which is characterized by the rapid chemotaxis of neutrophils to the wound site. In vivo time-lapse imaging shows that neutrophils subsequently display directed retrograde chemotaxis back toward the vasculature. These findings implicate retrograde chemotaxis as a novel mechanism that regulates the resolution phase of the inflammatory response. The zMPO:GFP zebrafish provides unique insight into the mechanisms of neutrophil-mediated inflammation and thereby offers opportunities to identify new regulators of the inflammatory response in vivo.
Descriptors: transgenic zebrafish, neutrophils express GFP, chemotaxis immunology, neutrophil infiltration immunology, neutrophils immunology, wounds and injuries immunology, chemotaxis genetics, genetic markers immunology, green fluorescent proteins genetics and immunology, inflammation genetics, immunology and pathology, fluorescence microscopy methods, video microscopy methods, neutrophil infiltration genetics, neutrophils pathology, peroxidase genetics and immunology, promoter regions genetics and immunology, wounds and injuries genetics and pathology, .

Meijer, A.H., S.F. Gabby Krens, I.A. Medina Rodriguez, S. He, W. Bitter, B. Ewa Snaar Jagalska , and H.P. Spaink (2004). **Expression analysis of the Toll-like receptor and TIR domain adaptor families of zebrafish.** *Molecular Immunology* 40(11): 773-83. ISSN: 0161-5890.
NAL Call Number: 448.3Im62
Abstract: The zebrafish genomic sequence database was analysed for the presence of genes encoding members of the Toll-like receptors (TLR) and interleukin receptors (IL-R) and associated adaptor proteins containing a TIR domain. The resulting predictions show the presence of one or more counterparts for the human TLR1, TLR2, TLR3, TLR4, TLR5, TLR7, TLR8, TLR9, IL-1R and IL-18R genes and one copy of the adaptor genes MyD88, MAL, TRIF and SARM. In contrast to data for the pufferfish *Fugu rubripes*, zebrafish has two genes that are highly similar to human TLR4. In addition, one fish-specific TLR group can be distinguished that is closely related to the *Drosophila melanogaster* Toll-9 gene. The sequence of cloned cDNAs for TLR4, TLR2 and MyD88 show the same intron-exon organi-

sation as in the human counterparts. Expression analysis using reverse transcriptase-PCR (RT-PCR) shows that 17 of the predicted zebrafish TLR genes and all the genes encoding adaptor proteins are expressed in the adult stage. A subset of the TLR genes are expressed at higher levels in fish infected with the pathogen *Mycobacterium marinum*. The induced genes include the homologues of the human TLR1 and TLR2 genes, whose functions are associated with mycobacterial infections, underscoring the suitability of zebrafish as a model for analysis of the vertebrate innate immune system.

Descriptors: zebrafish, vertebrate innate immunity model, membrane glycomultigene family, cell surface receptors genetics, gene expression profiling, interleukin 18 receptor alpha subunit, membrane glycoproteins biosynthesis, phylogeny , tertiary protein structure, cell surface receptors biosynthesis, interleukin receptor genetics, interleukin 1 receptor genetics, interleukin 18 receptor, reverse transcriptase PCR, sequence alignment , DNA sequence analysis, protein sequence analysis, sequence homology, toll like receptor 1, 2, 3, 4, 5, 7, 8.

Meijer, A.H., F.J. Verbeek, E. Salas Vidal, M. Corredor Adamez, J. Bussman, A.M. van der Sar, G.W. Otto, R. Geisler, and H.P. Spaink (2005). **Transcriptome profiling of adult zebrafish at the late stage of chronic tuberculosis due to *Mycobacterium marinum* infection.** *Molecular Immunology* 42(10): 1185-203. ISSN: 0161-5890.

NAL Call Number: 448.3Im62

Abstract: The *Mycobacterium marinum*-zebrafish infection model was used in this study for analysis of a host transcriptome response to mycobacterium infection at the organismal level. RNA isolated from adult zebrafish that showed typical signs of fish tuberculosis due to a chronic progressive infection with *M. marinum* was compared with RNA from healthy fish in microarray analyses. Spotted oligonucleotide sets (designed by Sigma-Compugen and MWG) and Affymetrix GeneChips were used, in total comprising 45,465 zebrafish transcript annotations. Based on a detailed comparative analysis and quantitative reverse transcriptase-PCR analysis, we present a validated reference set of 159 genes whose regulation is strongly affected by mycobacterial infection in the three types of microarrays analyzed. Furthermore, we analyzed the separate datasets of the microarrays with special emphasis on the expression profiles of immune-related genes. Upregulated genes include many known components of the inflammatory response and several genes that have previously been implicated in the response to mycobacterial infections in cell cultures of other organisms. Different marker genes of the myeloid lineage that have been characterized in zebrafish also showed increased expression. Furthermore, the zebrafish homologs of many signal transduction genes with relationship to the immune response were induced by *M. marinum* infection. Future functional analysis of these genes may contribute to understanding the mechanisms of mycobacterial pathogenesis. Since a large group of genes linked to immune responses did not show altered expression in the infected animals, these results suggest specific responses in mycobacterium-induced disease.

Descriptors: zebrafish tuberculosis infection, gene expression profiling, *Mycobacterium* infections, atypical microbiology, *Mycobacterium marinum* pathogenicity and genetics, genetic transcription, tuberculosis microbiology, biological markers, chronic disease, disease models, gene expression, inflammation genetics, microarray analysis, *Mycobacterium* infections, atypical genetics, RNA analysis, isolation and purification, reverse transcriptase PCR, signal transduction genetics, time factors, tuberculosis genetics and pathology, up regulation genetics, zebrafish microbiology.

Miller, J.D. and M.N. Neely (2005). **Large-scale screen highlights the importance of capsule for virulence in the zoonotic pathogen *Streptococcus iniae***. *Infection and Immunity* 73(2): 921-934. ISSN: 0019 9567.

NAL Call Number: QR1.I57

Abstract: Zoonotic pathogens have the unique ability to cross the species barrier, causing disease in both humans and specific animal hosts. *Streptococcus iniae* is a zoonotic pathogen of both fish and humans, and the clinical presentations of S. iniae infections in fish and humans are very similar to those caused by various human-specific streptococcal pathogens. Virulence mechanisms required for infection by this pathogen of either host have yet to be determined. Using the previously reported zebrafish infectious disease model, we performed a large-scale screening to determine genes required for systemic infection. Screening 1128 signature-tagged transposon mutants through the zebrafish model allowed identification of 41 potential mutants that were unable to survive within the host environment. Greater than 50% of the mutants that could be identified through homology searches were highly homologous to genes found in other human-specific streptococcal pathogens, while 32% were found to have no homology to any sequences found in the databases, suggesting as yet unknown gram-positive bacterial virulence factors. A large percentage of the insertions were found to be located in several putative capsule synthesis genes, an important virulence component for other systemic pathogens. Density gradient assays demonstrated that several of these putative capsule mutants have dissimilar buoyant densities, suggesting different levels of capsule synthesis. Putative capsule mutants were also less resistant to phagocytosis in whole-blood assays than wild-type *S. iniae*. Our initial large-scale characterization of *S. iniae* virulence highlights the importance of the capsule for successful infection. Copyright © 2008 CABI

Descriptors: zebrafish, cross infection, disease models, genes, immune response, laboratory animals, mutants, pathogenesis, phagocytosis, virulence, zoonoses, *Danio rerio*, *Streptococcus.*

Mulero, I., A. Garcia Ayala, J. Meseguer, and V. Mulero (2007). **Maternal transfer of immunity and ontogeny of autologous immunocompetence of fish: a minireview**. *Aquaculture* 268(1/4): 244-250. ISSN: 0044 8486.

NAL Call Number: SH1.A6

Abstract: The effectiveness of immunostimulation and vaccination of cultured fish heavily depends on the degree of maturity of their immune system. In this review, we summarise the recent data on the maternal transfer of humoral innate and adaptive immune parameters of fish larvae that seems to be responsible for the protection of embryos and larvae in the early stages of their development. We also look at the onset of fish immunocompetence in relation to the appearance of the different cellular components of both innate (macrophages and granulocytes) and adaptive (T and B lymphocytes) immunity. In addition, the advantages of using the powerful zebrafish model to study the development of teleost immunity are mentioned. Finally, all these data are taken into account before proposing future directions that are likely to shed light on the ontogeny of immunity in teleosts and to help in the development of appropriate vaccination regimens for either broodstock or larvae. Copyright © 2008 CABI

Descriptors: zebrafish, animal models, B lymphocytes, biological development, granulocytes, humoral immunity, immune competence, immune response, immunity, macrophages, maternal immunity, morphogenesis, ontogeny, T lymphocytes, vaccine development, *Danio rerio*, fishes.

Notes: A review.

Murayama, E., K. Kissa, A. Zapata, E. Mordelet, V. Briolat, H.F. Lin, R.I. Handin, and P. Herbomel (2006). **Tracing hematopoietic precursor migration to successive hematopoietic organs during zebrafish development.** *Immunity* 25(6): 963-75. ISSN: 1074-7613.

Abstract: Although the ontogeny of hematopoietic stem cells (HSCs) in vertebrates has been studied intensely, a lineage relationship between the HSCs found in the developmentally successive hematopoietic organs remains to be shown. By using an in situ photoactivatable cell tracer in the transparent zebrafish embryo, we demonstrated that definitive blood precursors appeared between the dorsal aorta and axial vein, validating the homology of this tissue with the AGM (aorta-gonad-mesonephros) of amniotes. These cells first migrated through the blood to a previously undescribed caudal hematopoietic tissue (CHT), where they differentiated, expanded, and further migrated to seed the definitive hematopoietic organs, the thymus and kidney. Immigrants on the way to the thymus expressed c-myb and ikaros but not rag1; they were probably no longer HSCs, however, because they lacked scl and runx1 expression, unlike immigrants to the kidney. The CHT thus has a hematopoietic function similar to that of the mammalian fetal liver.

Descriptors: zebrafish embryos, chemotaxis, leukocyte immunology, hematopoiesis, extramedullary, hematopoietic stem cells cytology, hematopoietic system embryology, tail embryology, zebrafish immunology, cell differentiation immunology, cell lineage in situ hybridization, kidney cytology and embryology, electron transmission microscopy, tail blood supply, thymus gland cytology and embryology.

Nilsen, B.M., K. Berg, J.K. Eidem, S.I. Kristiansen, F. Brion, J.M. Porcher, and A. Goksoeyr (2004). **Development of quantitative vitellogenin-ELISAs for fish test species used in endocrine disruptor screening**. *Analytical and Bioanalytical Chemistry* 378(3): 621-633. ISSN: 1618-2642.

NAL Call Number: QD71.F7

Descriptors: zebrafish, analytical techniques, antibodies, bioassays, biomarkers, ELISA, endocrinology, enzyme-linked immunosorbent assay, freshwater and marine fish, immunoassays, monoclonal antibodies, pollution detection, screening, sex hormones, vitellogenesis, vitellogenin, endocrine disruptors, *Cyprinus carpio* , *Danio rerio*, *Oryzias latipes*, *Pimephales promelas*, test species, materials and methods, analytical procedures.

Novak, A.E. and A.B. Ribera (2003). **Immunocytochemistry as a tool for zebrafish developmental neurobiology.** *Methods in Cell Science* 25(1-2): 79-83. ISSN: 1381-5741.

NAL Call Number: QH573.T52

Abstract: Two methods are presented here that allow clear visualization of antibody localization in zebrafish whole mount preparations, both for immunocytochemistry (ICC) alone and in combination with in situ hybridization (ISH). The first protocol describes ICC performed using a modified permeabilization technique and the chromogen AEC (3-Amino-9-ethylcarbazole). The second protocol describes the co-localization of transcriptional and translational products using a combined ISH/ICC protocol. A fluorescing chromogen (Fast Red, FR) is used to detect mRNA transcripts by ISH, and is combined with ICC that uses a secondary antibody conjugated to a different fluorescent molecule (Alexa 488). These procedures allow the identification of gene expression patterns in cell types identifiable with known antibodies.

Descriptors: zebrafish methods, immunohistochemistry methods, whole mount preparation, neurons metabolism, spinal cord metabolism, fluorescent dyes chemistry, immunoenzyme techniques, in situ hybridization, fluorescence, antibody localization.

O' Toole, R., J.v. Hofsten, R. Rosqvist, P.E. Olsson, and H. Wolf Watz (2004). **Visualisation of zebrafish infection by GFP-labelled *Vibrio anguillarum***. *Microbial Pathogenesis* 37(1): 41-46. ISSN: 0882 4010.

NAL Call Number: QR175.M53

Abstract: *Vibrio anguillarum* is an invasive pathogen of fish causing a septicaemia called vibriosis. In this work, transparent zebrafish were immersed in water containing green fluorescent protein labelled V. anguillarum. The infection was visualised at the whole fish and single bacterium levels using microscopy. The gastrointestinal tract was the first site where the pathogen was detected. This enteric localisation occurred independently of the flagellum or motility. On the other hand, chemotactic motility was essential for association of the pathogen with the fish surface. In conclusion, the zebrafish infection model provides evidence that the intestine and skin represent sites of infection by *V. anguillarum* and suggests a host site where chemotaxis may function in virulence. Copyright © 2008 CABI

Descriptors: zebrafish, infection model, chemotaxis, digestive tract, experimental infections, intestines, localization, microscopy, motility, proteins, septicemia, skin, techniques, *Danio rerio*, *Listonella anguillarum*, *Vibrio anguillarum*.

Palic, D., C.B. Andreasen, J. Ostojic, R.M. Tell, and J.A. Roth (2007). **Zebrafish (*Danio rerio*) whole kidney assays to measure neutrophil extracellular trap release and degranulation of primary granules.** *Journal of Immunological Methods* 319(1-2): 87-97. ISSN: 0022-1759.

NAL Call Number: QR180.J6

Abstract: The zebrafish (*Danio rerio*) is an excellent model system for studies in developmental biology, genetics, and toxicology, and is increasingly gaining importance in the field of immunology. The use of whole zebrafish kidneys as source of neutrophils for degranulation assays and detection of neutrophil extracellular traps is described for the first time. Neutrophils from zebrafish kidneys released neutrophil extracellular traps (NETs) and myeloperoxidase (MPO) upon stimulation with calcium ionophore, phorbol myristate acetate, and beta-glucan. Immunocytochemical study of zebrafish kidney cells revealed that NETs are made of DNA fibers associated with neutrophil granular proteins, but not with cytoskeleton. Rapid, direct MPO and extracellular DNA detection assays were developed to quantify NET release and degranulation of neutrophil primary granules from whole zebrafish kidneys. The assays were used to measure the effects of acute crowding and handling stress on neutrophils, and to examine the potential for use of zebrafish whole kidney assays in evaluation of neutrophil function under different conditions in vivo. The whole kidney NET release and degranulation assays are quantitative, can rapidly measure a large number of samples, and are capable of detecting inhibition of neutrophil activity in stressed fish, overcoming the limitations that prevented use of zebrafish in the investigations of cellular innate immune function. The assays can be used as a new research model to study effects of stress, immunomodulators, toxicants, and diseases on fish neutrophil biology.

Descriptors: zebrafish, cell degranulation immunology, cytoplasmic granules immunology, kidney immunology, neutrophil activation immunology, neutrophils immunology, neutrophils secretion, cytoplasmic granules secretion, extracellular space immunology and metabolism, immunohistochemistry, kidney cytology, kidney secretion.

Parmentier, C., J. Taxi, R. Balment, G. Nicolas, and A. Calas (2006). **Caudal neurosecretory system of the zebrafish: ultrastructural organization and immunocytochemical detection of urotensins.** *Cell and Tissue Research* 325(1): 111-24. ISSN: 0302 766X.

NAL Call Number: QH581.A1Z4

Abstract: The caudal neurosecretory system is described here for the first time in the

zebrafish, one of the most important models used to study biological processes. Light- and electron-microscopical approaches have been employed to describe the structural organization of Dahlgren cells and the urophysis, together with the immunohistochemical localization of urotensin I and II (UI and UII) peptides. Two latero-ventral bands of neuronal perikarya in the caudal spinal cord project axons to the urophysis. The largest secretory neurons (approximately 20 microm) are located rostrally. UII-immunoreactive perikarya are much more numerous than those immunoreactive for UI. A few neurons are immunopositive for both peptides. Axons contain 75-nm to 180-nm dense-core vesicles comprising two populations distributed in two axonal types (A and B). Large dense vesicles predominate in type A axons and smaller ones in type B. Immunogold double-labelling has revealed that some fibres contain both UI and UII, sometimes even within the same neurosecretory granule. UII is apparently the major peptide present and predominates in type A axons, with UI predominating in type B. A surprising finding, not previously reported in other fish, is the presence of dense-core vesicles, similar to those in neurons, in astrocytes including their end-feet around capillaries. Secretory type vesicles are also evident in ependymocytes and cerebrospinal-fluid-contacting neurons in the terminal spinal cord. Thus, in addition to the urophysis, this region may possess further secretory systems whose products and associated targets remain to be established. These results provide the basis for further experimental, genetic and developmental studies of the urophysial system in the zebrafish.
Descriptors: zebrafish, neurosecretory systems metabolism and ultrastructure, urotensins metabolism, immunohistochemistry, spinal cord metabolism , and ultrastructure, anatomy and histology.

Phelan, P.E., M.T. Mellon, and C.H. Kim (2005). **Functional characterization of full-length TLR3, IRAK-4, and TRAF6 in zebrafish (*Danio rerio*)**. *Molecular Immunology* 42(9): 1057-1071 . ISSN: 0161 5890.
NAL Call Number: 448.3Im62
Abstract: Recently, the zebrafish, *Danio rerio*, has been recognized as a useful model for infectious disease and immunity. The Toll-like receptor (TLR) family is an evolutionarily conserved component of the innate immune system that responds to specific pathogen-associated molecular patterns (PAMPs) during an infection. This study reports the identification and characterization of a full-length orthologue of mammalian TLR3, and the key TLR pathway signaling molecules IRAK-4 and TRAF6 in the zebrafish. Sequence analysis of zebrafish TLR3 (zfTLR3), IRAK-4 (zfIRAK-4), and TRAF6 (zfTRAF6) revealed conserved domains shared with insect and mammalian genes. Quantitative real-time PCR showed that all three genes are expressed in a variety of adult tissues and during embryonic development. In in situ hybridization, we showed that zfTLR3, zfIRAK-4, and zfTRAF6 are present in distinct regions of the developing brain at 22 hpf and that zfTRAF6 was observed in the developing medial neural tube. Overexpression of zfIRAK-4, zfTRAF6, or a mutant zfTLR3 construct was able to stimulate NF- kappa B activation in ZFL cells as measured by a cotransfected NF- kappa B-luciferase reporter plasmid. Messenger RNA expression profiles of each gene in zebrafish embryos and adults were examined by quantitative real-time PCR following infection with snakehead rhabdovirus (SHRV) or *Edwardsiella tarda*. Following exposure to SHRV, only zfTLR3 and zfTRAF6 mRNA transcripts were upregulated. Interestingly, exposure of fish to E. tarda resulted in an unexpected increase in mRNA expression of zfTLR3, as well as the anticipated upregulation of zfIRAK-4 and zfTRAF6 mRNA transcripts. These results demonstrate that zebrafish possess conserved TLR-signaling pathways, further emphasizing the utility of the zebrafish as a model for vertebrate immunology. Copyright © 2008 CABI

Descriptors: zebrafish embryo, TLR family, brain tissues, vertebrate immunology model, gene expression, genes, immunology, nucleotide sequences, *Danio rerio.*

Pressley, M.E., P.E.I. Phelan, P.E. Witten, M.T. Mellon, and C.H. Kim (2005). **Pathogenesis and inflammatory response to *Edwardsiella tarda* infection in the zebrafish**. *Developmental and Comparative Immunology* 29(6): 501-513. ISSN: 0145 305X.
NAL Call Number: QR180.D4
Abstract: The zebrafish (*Danio rerio*) is a widely used model for developmental biology, neurobiology, toxicology, and genetic disease. Recently, the zebrafish has been recognized as a valuable model for infectious disease and immunity. In this study the pathogenesis and inflammatory cytokine response of zebrafish to experimental *Edwardsiella tarda* infection was characterized. In challenge experiments, zebrafish embryos were susceptible to infection by immersion. Adult fish were susceptible to challenge by intraperitoneal (ip) injection but not static immersion unless the epithelial layer was perturbed by scraping prior to exposure. To determine if E. tarda infection induces a typical acute inflammatory response, mRNA expression levels of interleukin-1 beta (IL-1 beta) and tumor necrosis factor- alpha (TNF alpha) were assessed by quantitative real-time PCR. The expression levels of IL-1 beta and TNF alpha were significantly upregulated in infected zebrafish embryos and adults. The methods developed in this study will be particularly valuable for targeted gene disruption studies of host immune components and in zebrafish genetic screens. Copyright © 2008 CABI
Descriptors: zebrafish embryos and adults, cytokines, experimental infection, immune response, inflammation, interleukins, pathogenesis, tumor necrosis factor, *Danio rerio*, *Edwardsiella tarda*, fish, pathogen.

Prouty, M.G., N.E. Correa, L.P. Barker, P. Jagadeeswaran, and K.E. Klose (2003). **Zebrafish -*Mycobacterium marinum* model for mycobacterial pathogenesis.** *Federation of European Microbiological Societies Microbiology Letters* 225(2): 177-82. ISSN: 0378 1097.
NAL Call Number: QR1.F44
Abstract: We report here the development of a pathogenesis model utilizing *Mycobacterium marinum* infection of zebrafish (*Danio rerio*) for the study of mycobacterial disease. The zebrafish model mimics certain aspects of human tuberculosis, such as the formation of granuloma-like lesions and the ability to establish either an acute or a chronic infection based upon inoculum. This model allows the genetics of mycobacterial disease to be studied in both pathogen and host.
Descriptors: zebrafish, disease models, *Mycobacterium* infections, atypical microbiology and atypical pathology, *Mycobacterium marinum* pathogenicity, zebrafish microbiology, colony count, microbial, *Mycobacterium* infections, atypical genetics, *Mycobacterium marinum* growth and development, virulence, .

Radaelli, G., A. Rowlerson, F. Mascarello, M. Patruno, and B. Funkenstein (2003). **Myostatin precursor is present in several tissues in teleost fish: a comparative immunolocalization study**. *Cell and Tissue Research* 311(2): 239-250. ISSN: 0302-766X.
NAL Call Number: QH581.A1Z4
Descriptors: zebrafish, gene expression, histochemistry, larval development, muscles, *Danio rerio*, *Solea solea*, *Sparus aurata*, gilthead seabream.

Reis, M.I.R., A.d. Vale, C. Pinto, D.S. Nascimento, C. Costa Ramos, D.S.P. Silva, M.T. Silva, and N.M.S.d. Santos (2007). **First molecular cloning and characterisation of caspase-9 gene in fish and its involvement in a gram negative septicemia**. *Molecular Immunology* 44(7):

1754-1764. ISSN: 0161 5890.
NAL Call Number: 448.3Im62
Abstract: Caspase-9 is an initiator caspase in the apoptotic process whose function is to activate effector caspases that are downstream in the mitochondrial pathway of apoptosis. This work reports for the first time the complete sequencing and characterisation of caspase-9 in fish. A 1924 bp cDNA of sea bass caspase-9 was obtained, consisting of 1308 bp open reading frame coding for 435 amino acids, 199 bp of the 5'-UTR and 417 bp of the 3'-UTR including a canonical polyadenilation signal 10 nucleotides upstream the polyadenilation tail. The sequence retains the pentapeptide active-site motif (QACGG) and the putative cleavage sites at Asp121, Asp325 and Asp343. The sequence of sea bass caspase-9 exhibits a very close homology to the sequences of caspase-9 from other vertebrates, particularly with the putative caspases-9 of *Danio rerio* and *Tetraodon nigroviridis* (77.5 and 75.4% similarity, respectively), justifying the fact that the phylogenetic analysis groups these species together with sea bass. The sea bass caspase-9 gene exists as a single copy gene and is organised in 9 introns and 10 exons. The sea bass caspase-9 showed a basal expression in all the organs analysed, although weaker in spleen. The expression of sea bass caspase-9 in the head kidney of sea bass infected with the *Photobacterium damselae* ssp. piscicida (Phdp) strain PP3, showed increased expression from 0 to 12 h returning to control levels at 24 h. Caspase-9 activity was detected in Phdp infected sea bass head kidney from 18 to 48 h post-infection, when the fish were with advanced septicaemia. Copyright © 2008 CABI
Descriptors: zebrafish, amino acid sequences, bacterial diseases, complementary DNA, defence mechanisms, DNA sequencing, enzyme activity, experimental infection, gene expression, genes, gram negative bacteria, kidneys, nucleotide sequences, phylogenetics, septicemia, *Dicentrarchus labrax*, fishes, *Photobacterium damselae subsp piscicid*a, sea bass.

Rojo, I., O.M. de Ilarduya, A. Estonba, and M.A. Pardo (2007). **Innate immune gene expression in individual zebrafish after *Listonella anguillarum* inoculation.** *Fish and Shellfish Immunology* 23(6): 1285-93. ISSN: 1050 4648.
NAL Call Number: QL638.97.F55
Abstract: Zebrafish were intraperitoneally injected with 10(6)CFU (LD(50)) *Listonella anguillarum*. Three inoculated and control fish were collected at 1, 2, 4, 6 and 22h post infection (hpi) and the expression of genes related to the immune response (il1b, cebpb, tfa, mpx, tnfa, nitr9, tlr22, hsc70, cp, mrlp1, c3b and lyz) in each fish was monitored by means of real-time RT-PCR. A similar experiment was performed considering an intermediate time point at 15hpi. Different relative levels of expression were found among genes. Also, wide interindividual variation in gene expression for most genes was detected among fish, inoculated or not. A steady increase of expression starting from the initial stages of the interaction was found for interleukin-1beta. An initial increase in levels of gene expression was found for the genes coding for the CCAAT/Enhancer Binding Protein subunit beta and the Novel Immune-Type Receptor 9, although their levels decreased later on and were indistinguishable from the controls at 22hpi. Finally, some genes (Transferrin, Myeloid-specific Peroxidase and Tumour Necrosis Factor alpha) were upregulated at 22hpi. Taken together, our results show an induction in gene expression of genes involved in the inflammatory and immune response upon *L. anguillarum* infection but also reveal the existence of a wide variation in the levels of expression of the studied genes in the zebrafish population.
Descriptors: zebrafish inflammatory and immune response, immune gene, expression, *Listonella anguillarum*, experimental infection, intraperitoneal inoculation.

Rombout, J.H.W.M., H.B.T. Huttenhuis, S. Picchietti, and G. Scapigliati (2005). **Phylogeny and ontogeny of fish leucocytes**. *Fish and Shellfish Immunology* 19(5): 441-455. ISSN: 1050 4648.

NAL Call Number: QL638.97.F55

Abstract: In contrast to higher vertebrates, most fish species hatch at the embryonic stage of life. Consequently, they have to defend against a variety of micro-organisms living in their aquatic environment. This paper is focussed on the development of leucocytes functioning within this early innate system and later on in the acquired immune system (B and T cells). Most of the data are derived from cyprinid fish (zebrafish, carp), which are excellent models to study early ontogeny. Attention is also paid to the phylogeny of leucocytes, with special attention to early chordates. It is clear that young fish use innate mechanisms during the first weeks/months of their development. In zebrafish, a variety of hematopoietic genes have been sequenced which allow a detailed picture of the development of the distinct leucocytes and their precursors. In cyprinids and sea bass, the thymus is the first lymphoid organ and T cells appear to be selected there much earlier than the first detection of T cell-dependent antibody responses. The first B cells are most probably generated in head kidney. Although T cells are selected earlier than B cells, T cell independent responses occur earlier than the T cell-dependent responses. The very early (pre-thymic) appearance of T-like cells in gut of sea bass and carp suggests an extra-thymic origin of these cells. However, B cells populate the GALT much later than spleen or kidney, indicating a rather late appearance of mucosal immunity. The first plasma cells are found long after the intake of food in cyprinids, but in many marine fish they appear around the first food intake. In general, acquired immunity is not correlated to food intake. Copyright © 2008 CABI

Descriptors: zebrafish leukocyte functioning, immune system, immunity, leukocytes, ontogeny, phylogeny, reviews, T and B lymphocytes, fish, plasma cells, food intake.

Sanders, G.E., W.N. Batts, and J.R. Winton (2003). **Susceptibility of zebrafish (*Danio rerio*) to a model pathogen, spring viremia of carp virus.** *Comparative Medicine* 53(5): 514-21. ISSN: 1532 0820.

NAL Call Number: SF77.C65

Abstract: To improve our understanding of the genetic basis of fish disease, we developed a pathogen model, using zebrafish (*Danio rerio*) and spring virema of carp virus (SVCV). Replicate groups of 10 fish were acclimated to 20 or 24 degrees C, then were exposed to SVCV concentrations of 10(3) to 10(5) plaque-forming units per milliliter (PFU/ml) of water and observed daily. In a second trial, fish were acclimated to 15 degrees C, and replicate groups of 10 fish were exposed to SVCV at a concentration of 10(5) PFU/ml; however, the temperature was raised 1 degrees C/wk. Moribund fish were collected for histologic examination, and dead fish were assayed for virus by use of cell culture and reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Mortality exceeded 50% in fish exposed to 10(5) PFU of SVCV/ml at the lower temperatures. Clinical signs of disease became evident seven days after viral exposure and were observed most consistently in fish of the 10(5) PFU/ml groups. Affected zebrafish were anorectic and listless, with epidermal petechial hemorrhages followed by death. Use of plaque assays and RT-PCR analysis confirmed presence of SVCV at titers > or = 10(4) PFU/g of tissue. Histologic lesions included multifocal brachial necrosis and melanomacrophage proliferation in gills, liver, and kidneys. These results indicate that zebrafish are susceptible to infection by SVCV under conditions that mimic a natural route of exposure.

Descriptors: zebrafish, carps, disease susceptibility veterinary, fish SVCV viral diseases, virology, novirhabdovirus pathogenicity and infections, cell culture techniques veterinary, viral

DNA analysis, disease susceptibility immunology, dose response relationship, immunologic, fish diseases immunology and pathology, hemorrhage pathology, novirhabdovirus immunology, isolation and purification, reverse transcriptase PCR veterinary, rhabdoviridae infections immunology and mortality and transmission, skin pathology, survival rate, viremia immunology and pathology, viremia veterinary.

Seok, S.H., C. Koo Hye, A. Kasuga, Kim Yeun, G. Lee Eun, Y. Lee Hye, H. Park Jong, W. Baek Min, Y. Lee Hui, J. Kim Dong, H. Lee Byeung, S. Lee Yong, N. Cho Sang, and H. Park Jae (2006). **Use of pcr-restriction fragment length polymorphism for the identification of zoonotic mycobacteriosis in zebrafish caused by *Mycobacterium abscessus* and *Mycobacterium chelonae***. *Veterinary Microbiology* 114(3/4): 292-297. ISSN: 0378 1135.
NAL Call Number: SF601.V44
Abstract: Skin ulcers, scoliosis, and dropsy-like scale edema were observed in laboratory-maintained zebrafish. Affected fish had multifocal granulomas not only in internal organs such as the liver, intestine, genital organs, kidney, muscle, and spleen but also in the fin, epithelium, gills, and sclera of the eyes. Large numbers of acid-fast-rod-shaped bacteria were observed within the necrotic centers of well-demarcated, multifocal granulomas with Gram's stain and Ziehl-Neelson's stain. The size of the *Mycobacterium* spp. was 1-2 micro m x 2-3 micro m with a double-layered cell wall, based upon electron-microscopical features. Definitive diagnosis of these outbreaks was obtained by culture on selective media followed by PCR-restriction fragment length polymorphism analysis (PRA) of the rpoB gene for species identification. The amplified 360-bp products of the rpoB gene of mycobacteria isolated from zebrafish were digested with MspI restriction enzyme, which revealed unique band patterns matching those of *Mycobacterium abscessus* and *Mycobacterium chelonae* which are responsible for skin and soft tissue infection caused by rapidly growing mycobacteria in humans. This is the first documentation of the precise identification of zoonotic non-tuberculous mycobacteria isolated from laboratory-maintained zebrafish by the PRA of the rpoB gene; this study thus provides a great deal of useful epidemiological information and reduces the likelihood that epizootics will occur. Copyright © 2008 CABI
Descriptors: zebrafish, diagnosis, diagnostic techniques, granuloma, necrosis, PCR, skin diseases, zoonoses, *Danio rerio*, *Mycobacterium chelonae*.

Silva, A.B., D. Aw, and D.B. Palmer (2006). **Evolutionary conservation of neuropeptide expression in the thymus of different species**. *Immunology* 118(1): 131-140. ISSN: 0019 2805.
NAL Call Number: 448.3IM6
Abstract: Evidence suggests that the immune and neuroendocrine systems cross talk by sharing ligands and receptors. Hormones and neuropeptides produced by the neuroendocrine system often modulate the function of lymphoid organs and immune cells. We have previously reported the intrathymic expression of somatostatin (SOM) in the mouse and that several neuropeptides, most notably calcitonin-gene-related peptide (CGRP), neuropeptide Y (NPY), SOM and substance P (SP), can modulate thymocyte development. However, little is known about the intrathymic expression of these neuropeptides either in the mouse or in other species. Moreover, a comparative analysis of the expression of these molecules would highlight the evolutionary importance of intrathymic neuroendocrine interactions in T-cell development. We have studied the expression of different neuropeptides in the thymus of zebrafish, Xenopus, avians, rodent, porcine, equine and human by immunohistochemistry and reverse transcription-polymerase chain reaction. We found that CGRP, NPY, SOM, SP and vasointestinal polypeptide (VIP) are expressed in the thymus of all species investigated. The thymic location of many of these neuropeptides was conserved and appears to be within

the stromal compartments. Interestingly, in the avian thymus the expression of CGRP, SOM and SP appears to change depending on the age of the tissue. These findings suggest that neuropeptides may play an important role in T-cell development and provide further evidence of cross talk between the immune and neuroendocrine systems. Copyright © 2008 CABI

Descriptors: zebrafish, endocrine system, evolution, gene expression, immune system, immunity, immunohistochemistry, neuropeptides, PCR, poultry, thymus gland, tissue distribution, *Danio rerio*, fowls, horses, man, mice, pigs, rats, *Xenopus*.

Swaim, L.E., L.E. Connolly, H.E. Volkman, O. Humbert, D.E. Born, and L. Ramakrishnan (2006). ***Mycobacterium marinum* infection of adult zebrafish causes caseating granulomatous tuberculosis and is moderated by adaptive immunity**. *Infection and Immunity* 74(11): 6108-6117. ISSN: 0019 9567.

NAL Call Number: QR1.I57

Abstract: The zebrafish, a genetically tractable model vertebrate, is naturally susceptible to tuberculosis caused by *Mycobacterium marinum*, a close genetic relative of the causative agent of human tuberculosis, *Mycobacterium* tuberculosis. We previously developed a zebrafish embryo-*M. marinum* infection model to study host-pathogen interactions in the context of innate immunity. Here, we have constructed a flowthrough fish facility for the large-scale longitudinal study of *M. marinum*-induced tuberculosis in adult zebrafish where both innate and adaptive immunity are operant. We find that zebrafish are exquisitely susceptible to *M. marinum* strain M. Intraperitoneal injection of five organisms produces persistent granulomatous tuberculosis, while the injection of ~9,000 organisms leads to acute, fulminant disease. Bacterial burden, extent of disease, pathology, and host mortality progress in a time- and dose-dependent fashion. Zebrafish tuberculous granulomas undergo caseous necrosis, similar to human tuberculous granulomas. In contrast to mammalian tuberculous granulomas, zebrafish lesions contain few lymphocytes, calling into question the role of adaptive immunity in fish tuberculosis. However, like rag1 mutant mice infected with *M. tuberculosis*, we find that rag1 mutant zebrafish are hypersusceptible to *M. marinum* infection, demonstrating that the control of fish tuberculosis is dependent on adaptive immunity. We confirm the previous finding that *M. marinum* Delta RD1 mutants are attenuated in adult zebrafish and extend this finding to show that Delta RD1 predominantly produces nonnecrotizing, loose macrophage aggregates. This observation suggests that the macrophage aggregation defect associated with Delta RD1 attenuation in zebrafish embryos is ongoing during adult infection. Copyright © 2008 CABI

Descriptors: zebrafish embryos, experimental infections, granuloma, immunity, lymphocytes, macrophages, mortality, necrosis, pathology, susceptibility, tuberculosis, fishes, *Mycobacterium marinum.*

Takizawa, F., K. Araki, K. Ito, T. Moritomo, and T. Nakanishi (2007). **Expression analysis of two Eomesodermin homologues in zebrafish lymphoid tissues and cells.** *Molecular Immunology* 44(9): 2324-31. ISSN: 0161-5890.

NAL Call Number: 448.3Im62

Abstract: Eomesodermin (Eomes) is a T-box transcription factor that is involved in mesoderm formation in most vertebrates. Eomes is also expressed in CD8+ T cells and NK cells. No information is available on the role of Eomes in the immune system of lower vertebrates to date, although developmental studies on Eomes (Eomes1) have been performed in zebrafish. Here we report the identification of a second Eomes (Eomes2) in zebrafish and compare expression of the two Eomes genes in the immune system. Zebrafish Eomes1 and

Eomes2, composed of 661 and 534 amino acids, respectively, share 49.3% amino acid identity in their coding regions and 88.7% amino acid identity in their T-box regions. Conserved synteny between regions of the human and zebrafish genomes, gene organization and phylogenetic analysis all indicate that the zebrafish Eomes2 gene is a homologue of mammalian Eomes, as previously found for zebrafish Eomes1. Eomes1 mRNA was found to be expressed in the gonad, body kidney, spleen and gill, while Eomes2 mRNA was not detected in any of these tissues. However, strong expression of both Eomes mRNAs was detected in the leukocytes from the spleen, followed by those from body kidney and peripheral blood, with expression of Eomes1 always stronger than that of Eomes2. RT-PCR analysis of body kidney cells sorted by FACS revealed that Eomes1 was expressed strongly in lymphocytes, weakly in blast cells, and was not expressed in granulocytes, while Eomes2 was expressed weakly in lymphocytes. These results suggest that both Eomes genes are involved in the zebrafish immune response, particularly in lymphocyte function as has been found in mammals.
Descriptors: zebrafish Eomes genes, immune response, gene expression profiling, leukocytes metabolism, lymphoid tissue metabolism, amino acid sequence homology, T-box domain proteins genetics, amino acid and base sequence, complementary DNA genetics, flow cytometry, gene expression regulation, genome genetics, molecular sequence data, phylogeny, messenger RNA genetics and metabolism, sequence alignment, DNA sequence analysis, T-box domain proteins chemistry, proteins chemistry.

Trede, N.S., D.M. Langenau, D. Traver, A.T. Look, and L.I. Zon (2004). **The use of zebrafish to understand immunity.** *Immunity* 20(4): 367-79. ISSN: 1074-7613.
Abstract: For decades immunologists have relied heavily on the mouse model for their experimental designs. With the realization of the important role innate immunity plays in orchestrating immune responses, invertebrates such as worms and flies have been added to the repertoire. Here, we discuss the advent of the zebrafish as a powerful vertebrate model organism that promises to positively impact immunologic research.
Descriptors: zebrafish, natural immunity, zebrafish immunology, cell lineage immunology, vertebrate immunity models, preclinical drug evaluation, gene expression, mice.

Van der Sar, A.M., O.W. Stockhammer, C. van der Laan, H.P. Spaink, W. Bitter, and A.H. Meijer (2006). **MyD88 innate immune function in a zebrafish embryo infection model.** *Infection and Immunity* 74(4): 2436-41. ISSN: 0019-9567.
NAL Call Number: QR1.I57
Abstract: Innate immunity signaling mechanisms during vertebrate embryogenesis are largely unknown. To study Toll-like receptor (TLR) signaling function in the zebrafish embryo model, we designed an experimental setup for antisense morpholino knockdown under conditions of bacterial infection. Clearance of *Salmonella enterica* serovar Typhimurium Ra bacteria was significantly impaired after knockdown of myeloid differentiation factor 88 (MyD88), a common adaptor protein in TLR and interleukin-1 receptor signaling. Thereby, we demonstrate for the first time that the innate immune response of the developing embryo involves MyD88-dependent signaling, which further establishes the zebrafish embryo as a model for the study of vertebrate innate immunity.
Descriptors: zebrafishembryos, signal transducing adaptor, bacterial outer membrane proteins biosynthesis, natural immunity genetics, signal transduction immunology, signal transducing adaptor bacterial outer membrane proteins genetics and metabolism, disease models, molecular sequence data, myeloid differentiation factor 88, antisense oligonucleotides metabolism, reverse transcriptase PCR, salmonella infections genetics and

immunology, *salmonella typhimurium* genetics and immunology, signal transduction genetics, toll like receptors genetics, toll like receptors physiology.

Volkman, H.E., H. Clay, D. Beery, J.C.W. Chang, D.R. Sherman, and L. Ramakrishnan (2004). **Tuberculous granuloma formation is enhanced by a mycobacterium virulence determinant**. *Public Library of Science: Biology* 2(11): 1946-1956. ISSN: 1544 9173.
NAL Call Number: QH301.P56
Abstract: Granulomas are organized host immune structures composed of tightly interposed macrophages and other cells that form in response to a variety of persistent stimuli, both infectious and noninfectious. The tuberculous granuloma is essential for host containment of mycobacterial infection, although it does not always eradicate it. Therefore, it is considered a host-beneficial, if incompletely efficacious, immune response. The *Mycobacterium* RD1 locus encodes a specialized secretion system that promotes mycobacterial virulence by an unknown mechanism. Using transparent zebrafish embryos to monitor the infection process in real time, we found that RD1-deficient bacteria fail to elicit efficient granuloma formation despite their ability to grow inside of infected macrophages. We showed that macrophages infected with virulent mycobacteria produce an RD1-dependent signal that directs macrophages to aggregate into granulomas. This Mycobacterium-induced macrophage aggregation in turn is tightly linked to intercellular bacterial dissemination and increased bacterial numbers. Thus, mycobacteria co-opt host granulomas for their virulence. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, immune system, chemical coordination, homeostasis, infection, RD1-deficient bacteria, mycobacterial infection, bacterial pathogen immunology, tuberculous granuloma formation, virulence.

Watzke, J., K. Schirmer, and S. Scholz (2007). **Bacterial lipopolysaccharides induce genes involved in the innate immune response in embryos of the zebrafish (*Danio rerio*)**. *Fish and Shellfish Immunology* 23(4): 901-905. ISSN: 1050 4648.
NAL Call Number: QL638.97.F55
Abstract: The innate immune response in fish represents an early, rapid defence against pathogens. Environmental contaminants could disturb this defence and negatively influence the ability to protect against infection. However, analysis of immune-modulation has not yet been included in testing strategies for environmental risk assessment of chemicals. In order to establish an efficient, small scale test system, the ability to induce the innate immune response by bacterial lipopolysaccharides in zebrafish embryos was investigated. The level of expression of various genes involved in inflammation was used as the endpoint. We could show that immersion of embryos in LPS induced the gene expression of two key pro-inflammatory cytokines, tumor necrosis factor alpha and interleukin 1 beta in 32 h old zebrafish embryos. The gene induction required the removal of the chorion prior to lipopolysaccharide exposure. Copyright © 2008 CABI
Descriptors: zebrafishembryos, cytokines, embryos, gene expression, genes, immune response, inflammation, interleukin 1, lipopolysaccharides, tumor necrosis factor, *Danio rerio*.

Whipps, C.M., S.T. Dougan, and M.L. Kent (2007). ***Mycobacterium haemophilum* infections of zebrafish (*Danio rerio*) in research facilities.** *Federation of European Microbiological Societies Microbiology Letters* 270(1): 21-26. ISSN: 0378 1097.
NAL Call Number: QR1.F44
Abstract: In May 2005, a disease outbreak was investigated at a zebrafish (*Danio rerio*)

research facility experiencing severe losses. *Mycobacterium haemophilum* was isolated from these fish and the disease was subsequently recreated in experimentally infected zebrafish. Fish exhibited signs characteristic of mycobacteriosis, including granuloma formation and severe, diffuse, chronic inflammation. Bacteria were observed in multiple tissues, including the central nervous system. Biofilm samples from the outbreak facility were PCR positive for *M. haemophilum*, suggesting biofilms might act as a reservoir for infection. Zebrafish appear to be particularly vulnerable to *M. haemophilum*, and measures such as quarantine and treatment of incoming water should be implemented to minimize the likelihood of introduction of this bacterium to zebrafish research facilities. Zebrafish are already a well-established laboratory animal model for genetics, toxicology and disease, their susceptibility to *M. haemophilum* may make them useful for the study of this bacterium in the future.
Descriptors: zebrafish disease model, *Mycobacterium haemophilum*, *Danio rerio*, zoonoses, heat-shock-protein-65, RNA-polymerase-subunit-beta, internet resource.

Wu, H.C., C.S. Chiu, J.L. Wu, H.Y. Gong, M.C. Chen, M.W. Lu, and J.R. Hong (2008). **Zebrafish anti-apoptotic protein zfBcl-x sub(L) can block betanodavirus protein alpha -induced mitochondria-mediated secondary necrosis cell death**. *Fish & Shellfish Immunology* 24(4): 436-449. ISSN: 1050-4648.
NAL Call Number: QL638.97.F55
Descriptors: zebrafish, anti-apoptotic protein, zfBcl-x sub(L), betanodavirus, protein, alpha -induced, mitochondria-mediated, necrosis, cell death.

Yoder, J.A., R.T. Litman, M.G. Mueller, S. Desai, K.P. Dobrinski, J.S. Montgomery, M.P. Buzzeo, T. Ota, C.T. Amemiya, N.S. Trede, S. Wei, J.Y. Djeu, S. Humphray, K. Jekosch, J.A. Hernandez Prada, D.A. Ostrov, and G.W. Litman (2004). **Resolution of the novel immune-type receptor gene cluster in zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 101(44): 15706-11. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: The novel immune-type receptor (NITR) genes encode a unique multigene family of leukocyte regulatory receptors, which possess an extracellular Ig variable (V) domain and may function in innate immunity. Artificial chromosomes that encode zebrafish NITRs have been assembled into a contig spanning approximately 350 kb. Resolution of the complete NITR gene cluster has led to the identification of eight previously undescribed families of NITRs and has revealed the presence of C-type lectins within the locus. A maximum haplotype of 36 NITR genes (138 gene sequences in total) can be grouped into 12 distinct families, including inhibitory and activating receptors. An extreme level of interindividual heterozygosity is reflected in allelic polymorphisms, haplotype variation, and family-specific isoform complexity. In addition, the exceptional diversity of NITR sequences among species suggests divergent evolution of this multigene family with a birth-and-death process of member genes. High-confidence modeling of Nitr V-domain structures reveals a significant shift in the spatial orientation of the Ig fold, in the region of highest interfamily variation, compared with Ig V domains. These studies resolve a complete immune gene cluster in zebrafish and indicate that the NITRs represent the most complex family of activating/inhibitory surface receptors thus far described.
Descriptors: zebrafish, immunologic receptors genetics, zebrafish immunology, alleles, alternative splicing, amino acid sequence, chromosome mapping, complementary DNA genetics, molecular evolution, C type lectins chemistry and genetics, molecular models, molecular sequence data, multigene family, phylogeny, tertiary protein structure, immunologic receptors chemistry, variation genetics, proteins chemistry, proteins immunology.

Zapata, A., B. Diez, T. Cejalvo, C. Gutierrez de Frias, and A. Cortes (2006). **Ontogeny of the immune system of fish**. *Fish and Shellfish Immunology* 20(2): 126-136. ISSN: 1050 4648.
NAL Call Number: QL638.97.F55
Abstract: Information on the ontogeny of the fish immune system is largely restricted to a few species of teleosts (e.g., rainbow trout, catfish, zebrafish, sea bass) and has previously focused on morphological features. However, basic questions including the identification of the first lympho-hematopoietic sites, the origin of T- and B-lymphocytes and the acquisition of full immunological capacities remain to be resolved. We review these three main topics with special emphasis on recent results obtained from the zebrafish, a new experimental model particularly suitable for study of the ontogeny of the immune system because of its rapid development and easy manipulation. This species also provides an easy way of creating mutations that can be detected by various types of screens. In some teleosts (i.e., angelfish) the first blood cells are formed in the yolk sac. In others, such as zebrafish, the first hematopoietic site is an intraembryonic locus, the intermediate cell mass (ICM), whereas in both killifish and rainbow trout the first blood cells appear for a short time in the yolk sac but later the ICM becomes the main hematopoietic area. Erythrocytes and macrophages are the first blood cells to be identified in zebrafish embryos. They occur in the ICM, the duct of Cuvier and the peripheral circulation. Between 24 and 30 hour post-fertilization (hpf) at a temperature of 28 degrees C a few myeloblasts and myelocytes appear between the yolk sac and the body walls, and the ventral region of the tail of 1-2 day-old zebrafish also contains developing blood cells. The thymus, kidney and spleen are the major lymphoid organs of teleosts. The thymus is the first organ to become lymphoid, although earlier the kidney can contain hematopoietic precursors but not lymphocytes. In freshwater, but not in marine, teleosts the spleen is the last organ to acquire that condition. We and other authors have demonstrated an early expression of Rag-1 in the zebrafish thymus that correlates well with the morphological identification of lymphoid cells. On the other hand, the origins and time of appearance of B lymphocytes in teleosts are a matter of discussion and recent results are summarized here. The functioning rather than the mere morphological evidence of lymphocytes determines when the full immunocompetence in fish is attained. Information on the histogenesis of fish lymphoid organs can also be obtained by analysing zebrafish mutants with defects in the development of immune progenitors and/or in the maturation of non-lymphoid stromal elements of the lymphoid organs. The main characteristics of some of these mutants will also be described. Copyright © 2008 CABI
Descriptors: zebrafish ontogeny of the immune system, B lymphocytes, biological development, cell mediated immunity, erythrocytes, hematopoiesis, humoral immunity, immune competence, immune system, lymphatic system, macrophages, ontogeny, T lymphocytes, thymus gland, yolk sac, *Danio rerio*, fish.
Notes: Special issue:Reviews in Fish Immunology.

Zhong, H., Z. Li, S. Lin, and Y. Chang (2007). **Initiation of V(D)J recombination in zebrafish (*Danio rerio*) ovaries.** *Molecular Immunology* 44(7): 1784-92. ISSN: 0161-5890.
NAL Call Number: 448.3Im62
Abstract: The assembly of mammalian antigen receptor genes is a lymphoid-specific process. However, rearranged immunoglobulin genes can also be recovered from non-lymphoid tissues of cartilaginous fish. This event, known as germline rearrangement, has been speculated to arise from recombination-activating gene (RAG)-mediated recombination in germ cells. In this report, we demonstrate that zebrafish (*Danio rerio*) oocytes expressing high levels of RAG-RNA can readily initiate recombination cleavage at immunoglobulin gene loci, providing direct evidence for an ongoing process of attempted germline rearrangement in

zebrafish ovaries. This attempted rearrangement is largely unproductive, yielding no accumulation of germline-joined immunoglobulin genes in zebrafish, which is consistent with their general absence in this species. Our data, therefore, substantiate the speculation that RAG might have been derived from a transposase, invading germ cells of ancient species, and later become a dedicated recombinase only expressed in developing lymphocytes.
Descriptors: zebrafish, DNA binding proteins genetics, gene expression, genes, immunoglobulin genetics, homeodomain ovary immunology, zebrafish immunology, base sequence, gene rearrangement, molecular sequence data, ovary chemistry, messenger RNA analysis, messenger RNA metabolism, genetic recombination.

Zou Jun, C. Mercier, A. Koussounadis, and C. Secombes (2007). **Discovery of multiple beta-defensin like homologues in teleost fish**. *Molecular Immunology* 44(4): 638-647. ISSN: 0161 5890.
NAL Call Number: 448.3Im62
Abstract: Using a database mining approach, multiple defensin like genes have been discovered for the first time in fish, in species including zebrafish *Danio rerio* and the pufferfish, *Takifugu rubripes* and *Tetraodon nigroviridis*. They share the common features of vertebrate defensins, including small size, net cationic charge, and six conserved cysteines in the mature region. Based on their cysteine arrangement, the identified fish defensin like peptides resemble beta-defensin family members in birds and mammals. Computing modelling detected three beta-strands in all three zebrafish defensins and an extra N-terminal alpha-helix in one of the peptides. The coding regions of the fish genes contain three exons and two introns, the same as avian defensin genes. In zebrafish and tetraodon, two defensin genes identified are located in the same chromosome. An additional locus containing a third defensin gene has also been found in a different chromosome in zebrafish, demonstrating that multiple defensin loci may be present in fish. Comparative studies suggest that beta -defensins may represent the primitive form of the defensin family, which expanded during evolution by gene or genome duplication. In healthy zebrafish, constitutive expression of defensins was detected by RT-PCR in gill, gonad, gut, kidney, muscle, skin and spleen but the levels and patterns varied for individual defensin genes. Copyright © 2008 CABI
Descriptors: zebrafish, chromosomes, cysteine, genes, gills, gonads, introns, kidneys, spleen, *Danio rerio*, fishes, Tetraodontidae.

Neurology

Alderman, S.L. and N.J. Bernier (2007). **Localization of corticotropin-releasing factor, urotensin I, and CRF-binding protein gene expression in the brain of the zebrafish, *Danio rerio*.** *Journal of Comparative Neurology* 502(5): 783-93. ISSN: 0021-9967.

Abstract: Our current understanding of the corticotropin-releasing factor (CRF) system distribution in the teleost brain is restricted by limited immunohistochemical studies and a lack of complete transcriptional distribution maps. The present study used in situ hybridization to localize and compare CRF, urotensin I (UI), and CRF-binding protein (CRF-BP) expression in the brain of adult zebrafish (*Danio rerio*). All three peptides were localized in the preoptic area, periventricular hypothalamic and tectal regions, and dorsal part of the trigeminal motor nucleus. CRF and UI were both expressed in several nuclei of the dorsal telencephalon, whereas CRF and CRF-BP were both expressed in the ventral nucleus of the ventral telencephalon. Sole expression of CRF and CRF-BP was apparent in the olfactory bulbs and superior raphe nucleus, respectively, whereas only UI was observed in the corpus mamillare, nucleus of the medial longitudinal fascicle, dorsal tegmental nucleus, nucleus lateralis valvulae, and nucleus interpeduncularis. A major finding of this study was the general regional overlapping of CRF-BP with its ligands and a tendency to be expressed in tandem with CRF rather than UI. Overall, the mRNA expression patterns outlined in this study support the stress-related neuroendocrine, autonomic, and behavioral functions generally ascribed to the vertebrate CRF system and suggest some unique functional roles for CRF and UI in the teleost brain.

Descriptors: zebrafish , brain metabolism, carrier corticotropin releasing hormone genetics, gene expression physiology, urotensins genetics, brain anatomy and histology, in situ hybridization methods.

Allison, W.T., T.J. Haimberger, C.W. Hawryshyn, and S.E. Temple (2004). **Visual pigment composition in zebrafish: Evidence for a rhodopsin-porphyropsin interchange system.** *Visual Neuroscience* 21(6): 945-52. ISSN: 0952-5238.

Abstract: Numerous reports have concluded that zebrafish (*Danio rerio*) possesses A1-based visual pigments in their rod and cone photoreceptors. In the present study, we investigated the possibility that zebrafish have a paired visual pigment system. We measured the spectral absorption characteristics of photoreceptors from zebrafish maintained in different temperature regimes and those treated with exogenous thyroid hormone using CCD-based microspectrophotometry. Rods from fish housed at 15 degrees C and 28 degrees C were not significantly different, having lambda max values of 503 +/- 5 nm (n = 106) and 504 +/- 6 nm (n = 88), respectively. Thyroid hormone treatment (held at 28 degrees C), however, significantly shifted the lambda max of rods from 503 +/- 5 nm (n = 194) to 527 +/- 8 nm (n = 212). Cone photoreceptors in fish housed at 28 degrees C (without thyroid hormone treatment) had lambda max values of 361 +/- 3 nm (n = 2) for ultraviolet-, 411 +/- 5 nm (n = 18) for short-, 482 +/- 6 nm (n = 9) for medium-, and 565 +/- 10 nm (n = 14) for long-wavelength sensitive cones. Thyroid hormone treatment of fish held at 28 degrees C significantly shifted the lambda max of long-wavelength sensitive cones to 613 +/- 11 nm (n = 20), substantially beyond that of the lambda max of the longest possible A1-based visual pigment (approximately 580 nm). Thyroid hormone treatment produced smaller shifts of lambda max in other cone types and increased the half-band width. All shifts in photoreceptor lambda max values resulting from thyroid hormone treatment matched predictions for an A1- to A2-based visual pigment system. We therefore conclude that zebrafish possess a rhodopsin-porphyropsin interchange system that functions to spectrally tune rod and cone photoreceptors. We believe that these observations should be carefully considered during analysis of zebrafish spectral sensitivity.

Descriptors: zebrafish eyes, photoreceptors, retinal pigments metabolism, rhodopsin metabolism, retina cones drug effects, retina cones metabolism, retinal rods drug effects and metabolism, spectrophotometry, thyroxine pharmacology.
Notes: Comments: Erratum In:*Vis Neurosci*.2005 Mar-Apr;22(2):249
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Alvarez, F.A., I. Rodriguez Martin, V. Gonzalez Nunez, E.M. de Velasco, R. Gonzalez Sarmiento, and R.E. Rodriguez (2006). **New kappa opioid receptor from zebrafish *Danio rerio*.** *Neuroscience Letters* 405(1-2): 94-9. ISSN: 0304-3940.
NAL Call Number: QP351.N3
Abstract: A cDNA that encodes a kappa opioid receptor like from zebrafish (ZFOR3) has been cloned and characterized. The encoded protein is 377 residues long and presents 70% identity with the mammalian kappa receptors, although less homology is found in the amino- and carboxyl-terminus as well as in the extracellular loops. In situ hybridization studies have revealed that ZFOR3 mRNA is highly expressed in particular brain areas that coincide with the expression of the kappa opioid receptor in other species. When ZFOR3 is stably expressed in HEK293 cells, [(3)H]-diprenorphine binds with high affinity (K(D)=1.05+/-0.26 nM), being this value on the same range as those reported for mammalian kappa opioid receptors. On the other hand, the selective agonist for mammalian kappa receptors U69,593 does not bind to ZFOR3. [(3)H]-diprenorphine binding is readily displaced by the peptidic ligand dynorphin A and by the non-endogenous compounds bremazocine, naloxone and morphine, although with different affinities. Our results demonstrate that ZFOR3 is a unique model to study the kappa opioid receptor functionality.
Descriptors: zebrafish, brain areas, kappa opioid receptor genetics, amino acid sequence, binding, competitive, brain anatomy and histology, brain metabolism, cell line, in situ hybridization, ligands, molecular sequence data, radio ligand assay, kappa opioid receptors biosynthesis, isolation and purification, amino acid sequence homology, proteins biosynthesis, isolation and purification.

Ampatzis, K. and C.R. Dermon (2007). **Sex differences in adult cell proliferation within the zebrafish (*Danio rerio*) cerebellum.** *European Journal of Neuroscience* 25(4): 1030-40. ISSN: 0953 816X.
Abstract: It has been reported that neurons generated in the adult brain show sex-specific differences in several brain regions of lower vertebrates and mammals. The present study questioned whether cell proliferation and survival in the adult zebrafish (*Danio rerio*) cerebellum, the most mitotically active area of adult teleost brain, is sexually differentiated. Adult zebrafish were treated with the thymidine analogue 5'-bromo-2'-deoxyuridine (BrdU) and allowed to survive for 24 h (short-term) and for 21 days (long-term). BrdU immunohistochemistry allowed visualization of cells incorporating BrdU at the S phase of mitosis. At short-term survival, male zebrafish had a higher number of labelled cells at proliferation sites of the molecular layer of corpus cerebelli (CCe) and the granular layer of the caudal lobe of the cerebellum (LCa) than did females. In long-term survival, BrdU-positive cells were found at their final destination, but only the granular layer of the medial division of the valvula cerebelli showed sex-specific differences in the number of labelled cells. This higher mitotic activity in male cerebellum might be related to sex-specific motor behaviour observed in male zebrafish. To investigate the role of programmed cell death, the terminal deoxynucleotidyl-mediated dUTP nick-end-labelling (TUNEL) method was applied. The vast majority of apoptotic figures occurred in the granular cell layer of valvula and CCe, only in a few cases within the BrdU-retaining cells. Apoptosis was found specifically at the sites of the final destination of proliferating cells, indicating that the close relation of cell birth and death might represent a possible plasticity mechanism in the adult zebrafish cerebellum.

Descriptors: zebrafish males, brains, cell proliferation, cerebellum cytology, neurons physiology, sex characteristics, bromodeoxyuridine metabolism, cell count methods, in situ nick end labeling methods, nerve tissue proteins metabolism, sex factors, time factors.

Anichtchik, O., V. Sallinen, N. Peitsaro, and P. Panula (2006). **Distinct structure and activity of monoamine oxidase in the brain of zebrafish (*Danio rerio*).** *Journal of Comparative Neurology* 498(5): 593-610. ISSN: 0021-9967.

Abstract: Monoamine oxidase (MAO) is a mitochondrial flavoprotein involved in the metabolism of, e.g., aminergic neurotransmitters and the parkinsonism-inducing neurotoxin 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP). We have reported earlier MPTP-related alterations of brain catecholaminergic system in zebrafish (Danio rerio) brain. Here we describe the structural and functional properties of zebrafish MAO and the distribution of MAO mRNA and activity in zebrafish brain. The gene is located in chromosome 9 and consists of 15 exons. The amino acid composition of the active center resembles both human MAO-A and MAO-B. The enzyme displayed the highest substrate specificity for tyramine, followed by serotonin, phenylethylamine, MPTP, and dopamine; isoform-specific antagonists blocked the activity of the enzyme with equal potency. Zebrafish MAO mRNA, which was present in several tissues, and enzyme displayed differential distribution in the brain; dopaminergic cell clusters had low to moderate levels of MAO activity, whereas the highest levels of MAO activity were detected in noradrenergic and serotonergic cell groups and the habenulointerpeduncular pathway, including its caudal projection to the medial ventral rhombencephalon. The results of this study confirm the presence of functionally active MAO in zebrafish brain and other tissues and characterize the neural systems that express MAO and areas of intense activity in the brain. They also suggest that MPTP toxicity not related to MAO may affect the zebrafish brain.

Descriptors: zebrafish, brain anatomy and histology, brain enzymology, monoamine oxidase metabolism, 1 methyl 4 phenyl 1,2,3,6 tetrahydropyridine pharmacology, analysis of variance, biogenic monoamines pharmacology, brain drug effects, molecular cloning methods , drug dose response relationship, enzyme activation drug effects, gene expression regulation drug effects, immunohistochemistry methods, in situ hybridization methods, molecular sequence data, monoamine oxidase chemistry and genetics, neurotoxins pharmacology, protein Conformation, messenger RNA metabolism, time factors.

Anichtchik, O.V., J. Kaslin, N. Peitsaro, M. Scheinin, and P. Panula (2004). **Neurochemical and behavioural changes in zebrafish *Danio rerio* after systemic administration of 6-hydroxydopamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.** *Journal of Neurochemistry* 88(2): 443-53. ISSN: 0022-3042.

NAL Call Number: QP351.J6

Abstract: Dopaminergic deficiency in the brain of zebrafish was produced by systemic administration of two catecholaminergic neurotoxins, 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and the neurochemical and behavioural changes were characterized. The levels of dopamine and noradrenaline decreased significantly after the injection of MPTP and 6-OHDA. Corresponding to these changes, fish exhibited characteristic changes in locomotor behaviour, i.e. the total distance moved and velocity decreased after both neurotoxins. Tyrosine hydroxylase and caspase 3 protein levels were not altered after MPTP or 6-OHDA injections, as studied by immunohistochemistry and western blotting. The catecholaminergic cell clusters suggested to correspond to the mammalian nigrostriatal cell group displayed normal tyrosine hydroxylase immunoreactivity after the toxin treatment and did not show signs of DNA fragmentation that would indicate activation of cascades that lead to cell death. The results show that single systemic injections of MPTP and 6-OHDA induce

both biochemical and behavioural changes in zebrafish, albeit failing to produce any significant morphological alteration in catecholaminergic cell clusters at the tested doses. This approach may be used for the screening of chemicals affecting the dopaminergic system. The model may be especially useful for evaluation of the role of novel genes in neurotoxicity, as a large number of zebrafish mutants are becoming available.
Descriptors: zebrafish, 1 methyl 4 phenyl 1,2,3,6 tetrahydropyridine pharmacology, brain drug effects, motor activity drug effects, oxidopamine pharmacology, metabolism, brain metabolism, dopamine metabolism, injections, intramuscular, motor activity physiology, norepinephrine metabolism, pilot projects.

Arata, N. and H. Nakayasu (2003). **A periaxonal net in the zebrafish central nervous system.** *Brain Research* 961(2): 179-89. ISSN: 0006 8993.
NAL Call Number: QP376
Abstract: We produced a monoclonal antibody, named A20, which specifically recognizes a 35 kDa protein and stains myelinated axons in zebrafish brain. The A20 antigen is located at the outside of the myelin layer of large axons, and comprises a fine meshwork composed of thin unit fibers about 1-2 microm in length and about 100-200 nm in thickness. The unit fibers form pentagonal and hexagonal structures, which further polymerize into an envelope structure on the axons. The A20 monoclonal antibody did not stain neuronal cell bodies nor synapses. Instead, the distribution of the A20 antigen was along axons, practically coincident with the distribution of myelin basic protein. The monoclonal antibody stained only axons in the central nervous system (CNS), and not the extracellular matrix surrounding Schwann cells. These results suggest that this antigenic meshwork (which we call the periaxonal net) is synthesized by oligodendrocytes. During the development of the zebrafish brain, the periaxonal net appeared after the formation of myelin on the axons. The periaxonal net developed first at the brain stem, then gradually appeared at the caudal end of the spinal cord. The thickness of the periaxonal net around the Mauthner axon changed during development. Although the thickness of the Mauthner axon continues to grow throughout life, the thickness of periaxonal net stopped growing at 6 months after fertilization.
Descriptors: zebrafish brains, surface antigens analysis, axons chemistry, central nervous system chemistry, growth and development, myelin proteins analysis, myelin sheath chemistry, monoclonal antibodies, surface antigens immunology, fluorescent antibody technique, immunoblotting, immunohistochemistry, oligodendroglia metabolism, optic nerve chemistry, spinal nerves chemistry.

Arenzana, F.J., D. Clemente, R. Sanchez Gonzalez , A. Porteros, J. Aijon, and R. Arevalo (2005). **Development of the cholinergic system in the brain and retina of the zebrafish.** *Brain Research Bulletin* 66(4-6): 421-5. ISSN: 0361 9230.
NAL Call Number: QP376
Abstract: We have analyzed the distribution pattern of choline acetyltransferase (ChAT) in the zebrafish brain and retina during ontogeny. ChAT-immunoreactive (ChAT-ir) neurons are observed in the prosencephalon from 60 h postfertilization (hpf) onwards, exclusively in the preoptic area (basal plate of p6) derived from the secondary prosencephalon. In the mesencephalon, ChAT-ir cells are observed in both the optic tectum and the tegmentum. Stained cells in the tegmentum are observed from 60 hpf onwards, while in the optic tectum they appear after hatching. In the rhombencephalon, ChAT-ir cells are first observed in the isthmic region (rh1) and in the medulla oblongata (rh5-rh7) at the end of embryonic life. The rhombencephalic cholinergic cell groups develop in a gradual caudorostral sequence. Motoneurons of the spinal cord are ChAT-ir from 48 hpf onwards. The retina displays ChAT-ir neuropil in both the inner and outer plexiform layers from embryonic life, whereas stained amacrine cells are only observed after hatching. The

staining in the outer plexiform layer gradually decreases during juvenile development. The optic nerve axons show a transient expression of ChAT at the end of embryonic development. The early presence of ChAT immunolabeling suggests an important neuromodulator role for acetylcholine in the first developmental stages.

Descriptors: zebrafish, brain growth and development, choline o acetyltransferase metabolism, developmental gene expression regulation physiology, retina growth and development, brain enzymology, immunohistochemistry methods, retina enzymology.

Argo, S., F. Weth, and S.I. Korsching (2003). **Analysis of penetrance and expressivity during ontogenesis supports a stochastic choice of zebrafish odorant receptors from predetermined groups of receptor genes.** *European Journal of Neuroscience* 17(4): 833-43. ISSN: 0953 816X.

Abstract: Olfactory receptor neurons select a single odourant receptor gene for expression out of a large gene family. The mechanisms of this extreme selectivity are largely unknown. We have determined in detail the developmental expression dynamics of a representative subset of the zebrafish odourant receptor repertoire, using in situ hybridization analysis. We have thus generated a dataset, which allows us to test hypotheses of odourant receptor gene regulation. The receptors chosen belong to four different groups with respect to ontogenetic onset of expression (onset groups). Statistical analysis of the data supports a model in which the final choice of an individual odourant receptor gene occurs stochastically from within a group of genes sharing a deterministically defined onset of expression. Genomic mapping revealed a pronounced correlation of onset of expression with genomic neighbourhood. During a protracted juvenile developmental period individual regulatory influences seem to modify the expression of odourant receptor genes, a notable example being a transient decrease in expressivity of two odourant receptor genes.

Descriptors: zebrafishbrain, developmental gene expression regulation, multigene family, penetrance, odorant receptors genetics, stochastic processes, molecular cloning, in situ hybridization, larva genetics, scanning electron microscopy, morphogenesis, olfactory receptor neurons cytology and embryology, polymorphism, restriction fragment length, odorant receptors ultrastructure, statistics, stem cells metabolism, time factors.

Ashworth, R. (2004). **Approaches to measuring calcium in zebrafish: focus on neuronal development.** *Cell Calcium* 35(5): 393-402. ISSN: 0143 4160.

NAL Call Number: QP772.V53C4

Abstract: Calcium ions are known to act as important cellular signals during nervous system development. In vitro studies have provided significant information on the role of calcium signals during neuronal development; however, the function of this messenger in nervous system maturation in vivo remains to be established. The zebrafish has emerged as a valuable model for the study of vertebrate embryogenesis. Fertilisation is external and the rapid growth of the transparent embryo, including development of internal organs, can be observed easily making it well suited for imaging studies. The developing nervous system is relatively simple and has been well characterised, allowing individual neurons to be identified. Using the zebrafish model, both intracellular and intercellular calcium signals throughout embryonic development have been characterised. This review summarises technical approaches to measure calcium signals in developing embryonic and larval zebrafish, and includes recent developments that will facilitate the study of calcium signalling in vivo. The application of calcium imaging techniques to investigate the action of this messenger during embryogenesis in intact zebrafish is illustrated by discussion of their contribution to our understanding of neuronal development in vivo.

Descriptors: zebrafish embryos, calcium analysis, calcium signaling, nervous system metabolism,

calcium metabolism, embryonic development, fluorescent dyes, confocal microscopy methods, nervous system embryology, weights and measures.

Avanesov, A. and J. Malicki (2004). **Approaches to study neurogenesis in the zebrafish retina.** *Methods in Cell Biology* 76: 333-84. ISSN: 0091 679X.
NAL Call Number: 442P92
Abstract: Similar to other vertebrate species, the zebrafish retina is simpler than other regions of the central nervous system (CNS). Relative simplicity, rapid development, and accessibility to genetic analysis make the zebrafish retina an excellent model system for the studies of neurogenesis in the vertebrate CNS. Numerous genetic screens have led to isolation of an impressive collection of mutations affecting the retina and the retinotectal projection in zebrafish. Mutant phenotypes are being studied using a rich variety of markers: antibodies, RNA probes, retrograde and anterograde tracers, as well as transgenic lines. Particularly impressive progress has been made in the characterization of the zebrafish genome. Consequently, positional and candidate cloning of mutant genes are now fairly easy to accomplish in zebrafish. Many mutant genes have, in fact, already been cloned and their analysis has provided important insights into the gene circuitry that regulates retinal neurogenesis. Genetic screens for visual system defects will continue in the future and progressively more sophisticated screening approaches will make it possible to detect a variety of subtle mutant phenotypes in retinal development. The remarkable evolutionary conservation of the vertebrate eye provides the basis for the use of the zebrafish retina as a model of human disorders. Some of the genetic defects of the zebrafish retina indeed resemble human retinopathies. As new techniques are being introduced and improved at a rapid pace, the zebrafish will continue to be an important organism for the studies of the vertebrate visual system.
Descriptors: zebrafish embryology, neurons cytology, retina embryology, alleles, amacrine cells chemistry, behavior physiology, biological markers analysis, cell differentiation physiology, cell proliferation, cell transplantation methods, electrophysiology, developmental gene expression regulation drug effects, genetic techniques, histological techniques methods, morphogenesis, mutagenesis genetics, mutation genetics and physiology, neuroglia chemistry, antisense oligonucleotides pharmacology, optic lobe chemistry, phenotype, photic stimulation, photoreceptors chemistry, retina cytology, growth and development, retinal ganglion cells chemistry, staining and labeling methods.

Avila, R.L., B.R. Tevlin, J.P. Lees, H. Inouye, and D.A. Kirschner (2007). **Myelin structure and composition in zebrafish.** *Neurochemical Research* 32(2): 197-209. ISSN: 0364-3190.
NAL Call Number: QP356.3 .N457
Abstract: To establish a standard for genotype/phenotype studies on the myelin of zebrafish *(Danio rerio*), an organism increasingly popular as a model system for vertebrates, we have initiated a detailed characterization of the structure and biochemical composition of its myelinated central and peripheral nervous system (CNS; PNS) tissues. Myelin periods, determined by X-ray diffraction from whole, unfixed optic and lateral line nerves, were approximately 153 and approximately 162 Angstrom, respectively. In contrast with the lability of PNS myelin in higher vertebrates, zebrafish lateral line nerve myelin exhibited structural stability when exposed to substantial changes in pH and ionic strength. Neither optic nor lateral line nerves showed swelling at the cytoplasmic apposition in CaCl(2)-containing Ringer's solution, in contrast with nerves from other teleost and elasmobranch fishes. Zebrafish optic nerve showed greater stability against changes in NaCl and CaCl(2) than lateral line nerve. The nerves from zebrafish having mutations in the gene for myelin basic protein (mbpAla2Thr and mbpAsp25Val) showed similar myelin periods as the wildtype (WT), but gave approximately 20% less compact myelin. Analysis of pro-

teins by SDS-PAGE and Western blotting identified in both CNS and PNS of WT zebrafish two orthologues of myelin P0 glycoprotein that have been characterized extensively in trout--intermediate protein 1 (24 kDa) and intermediate protein 2 (28 kDa). Treatment with endoglycosidase-F demonstrated a carbohydrate moiety of approximately 7 kDa, which is nearly threefold larger than for higher vertebrates. Thin-layer chromatography for lipids revealed a similar composition as for other teleosts. Taken together, these data will serve as a baseline for detecting changes in the structure and/or amount of myelin resulting from mutations in myelin-related genes or from exogenous, potentially cytotoxic compounds that could affect myelin formation or stability.
Descriptors: zebrafish nerve tissue, myelin sheath chemistry and genetics, central nervous system chemistry, cholesterol analysis, hydrogen ion concentration, hypotonic solutions pharmacology, lateral line system drug effects, myelin proteins analysis, optic nerve drug effects, peripheral nervous system chemistry, phosphatidylcholines analysis, phosphatidylethanolamines analysis, sodium chloride pharmacology, x ray diffraction.

Bahadori, R., O. Biehlmaier, C. Zeitz, T. Labhart, Y.V. Makhankov, U. Forster, M. Gesemann, W. Berger, and S.C. Neuhauss (2007). **Nyctalopin is essential for synaptic transmission in the cone dominated zebrafish retina.** *European Journal of Neuroscience* 25(5): 1618. ISSN: 0953 816X.
Descriptors: zebrafish eyes, nyctalopin, synaptic, transmission, retina cone.

Bahadori, R., O. Biehlmaier, C. Zeitz, T. Labhart, Y.V. Makhankov, U. Forster, M. Gesemann, W. Berger, and S.C. Neuhauss (2006). **Nyctalopin is essential for synaptic transmission in the cone dominated zebrafish retina.** *European Journal of Neuroscience* 24(6): 1664-74. ISSN: 0953 816X.
Abstract: The first synapse in the vertebrate visual system is the photoreceptor synapse between rod and cone photoreceptors and the second-order bipolar cells. Although mutations in the nyctalopin gene (NYX) in humans lead to congenital stationary night blindness (CSNB1), affecting synaptic transmission between both types of photoreceptors and ON-bipolar cells, the function of nyctalopin in cone-dominant animal models has not been studied. Because the larval zebrafish retina is cone-dominant, we isolated the zebrafish nyx ortholog and raised a polyclonal antibody against the protein. Nyctalopin is expressed postsynaptically in both synaptic layers of the retina. Functional disruption via morpholino antisense injection leads to characteristic defects in the electroretinogram and defects in visual contrast sensitivity. We therefore demonstrated that nyctalopin plays a similar role in retinal synapse function in the cone pathway as in the rod pathway, thereby creating a genetic model for CSNB1 and its effects on cone vision.
Descriptors: zebrafish eyes, retina cones metabolism, proteoglycans metabolism, retina cytology, synaptic transmission physiology, molecular cloning methods, retina cones drug effects, electroretinography methods, immunohistochemistry methods, in situ hybridization methods, intracellular signaling peptides and proteins metabolism, membrane glycoproteins genetics and metabolism, morpholines antagonists and inhibitors and chemistry, optokinetic nystagmus drug effects, optokinetic nystagmus physiology, oligodeoxyribonucleotides, antisense pharmacology, proteoglycans genetics, protein sequence analysis, transfection methods.

Bahadori, R., M. Huber, O. Rinner, M.W. Seeliger, S. Geiger Rudolph, R. Geisler, and S.C. Neuhauss (2003). **Retinal function and morphology in two zebrafish models of oculo-renal syndromes.** *European Journal of Neuroscience* 18(6): 1377-86. ISSN: 0953 816X.
Abstract: We characterized visual system defects in two recessive zebrafish mutants oval and elipsa. These mutants share the syndromic phenotype of outer retinal dystrophy in conjunction with cystic renal disorder. We tested the function of the larval visual system in a behavioural assay, eliciting optokinetic eye movements by high-contrast motion stimulation while recording eye movements in parallel. Visual stimulation did not elicit eye movements in mutant larvae,

while spontaneous eye movements could be observed. The retina proved to be unresponsive to light using electroretinography, indicative of a defect in the outer retina. Histological analysis of mutant retinas revealed progressive degeneration of photoreceptors, initiated in central retinal locations and spreading to more peripheral regions with increasing age. The inner retina remains unaffected by the mutation. Photoreceptors display cell type-specific immunoreactivity prior to apoptotic cell death, arguing for a dystrophic defect. Genomic mapping employing simple sequence-length polymorphisms located both mutations on different regions of zebrafish linkage group 9. These mutants may serve as accessible animal models of human outer retinal dystrophies, including oculo-renal diseases, and show the general usefulness of a behavioural genetic approach to study visual system development in the model vertebrate zebrafish.
Descriptors: zebrafish mutant larvae, visual system, polycystic kidney diseases physiopathology, retina physiopathology, retinal degeneration physiopathology, behavior, cell death, cell differentiation, chromosome mapping methods, disease models, electroretinography methods, eye movements, recessive genes, immunohistochemistry methods, in situ nick end labeling methods, light, mutation, optokinetic nystagmus physiology, photic stimulation, polycystic kidney diseases complications, genetics and pathology, retina cytology, retina growth and development, retinal degeneration complications, genetics and pathology, staining and labeling.

Baraban, S.C., M.R. Taylor, P.A. Castro, and H. Baier (2005). **Pentylenetetrazole induced changes in zebrafish behavior, neural activity and c-fos expression.** *Neuroscience* 131(3): 759-68. ISSN: 0306-4522.
NAL Call Number: QP351.N48
Abstract: Rodent seizure models have significantly contributed to our basic understanding of epilepsy. However, medically intractable forms of epilepsy persist and the fundamental mechanisms underlying this disease remain unclear. Here we show that seizures can be elicited in a simple vertebrate system e.g. zebrafish larvae *(Danio rerio*). Exposure to a common convulsant agent (pentylenetetrazole, PTZ) induced a stereotyped and concentration-dependent sequence of behavioral changes culminating in clonus-like convulsions. Extracellular recordings from fish optic tectum revealed ictal and interictal-like electrographic discharges after application of PTZ, which could be blocked by tetrodotoxin or glutamate receptor antagonists. Epileptiform discharges were suppressed by commonly used antiepileptic drugs, valproate and diazepam, in a concentration-dependent manner. Up-regulation of c-fos expression was also observed in CNS structures of zebrafish exposed to PTZ. Taken together, these results demonstrate that chemically-induced seizures in zebrafish exhibit behavioral, electrographic, and molecular changes that would be expected from a rodent seizure model. Therefore, zebrafish larvae represent a powerful new system to study the underlying basis of seizure generation, epilepsy and epileptogenesis.
Descriptors: zebrafish larvae, disease model, GABA antagonists pharmacology, gene expression drug effects, motor activity drug effects, neurons drug effects, pentylenetetrazole pharmacology, proto oncogene proteins C fos metabolism, anticonvulsants pharmacology, behavior drug effects, diazepam pharmacology, drug dose response relationship, drug interactions, excitatory amino acid antagonists pharmacology, in situ hybridization methods , membrane potentials drug effects, neurons physiology, proto oncogene proteins C fos genetics, messenger RNA biosynthesis, reaction time drug effects, reverse transcriptase PCR methods, seizures chemically induced, seizures drug therapy and physiopathology, superior colliculus cytology, superior colliculus drug effects, time factors.

Bass, S.L. and R. Gerlai (2008). **Zebrafish (*Danio rerio*) responds differentially to stimulus fish: the effects of sympatric and allopatric predators and harmless fish.** *Behavioural Brain Research* 186(1): 107-17. ISSN: 0166 4328.

Abstract: The zebrafish has been an excellent model organism of developmental biology and genetics. Studying its behavior will add to the already strong knowledge of its biology and will strengthen the use of this species in behavior genetics and neuroscience. Anxiety is one of the most problematic human psychiatric conditions. Arguably, it arises as a result of abnormally exaggerated natural fear responses. The zebrafish may be an appropriate model to investigate the biology of fear and anxiety. Fear responses are expressed by animals when exposed to predators, and these responses can be learned or innate. Here we investigated whether zebrafish respond differentially to a natural predator or other fish species upon their first exposure to these fish. Naive zebrafish were shown four species of fish chosen based on predatory status (predatory or harmless) and geographical origin (allopatric or sympatric). Our results suggest that naive zebrafish respond differentially to the stimulus fish. Particularly interesting is the antipredatory response elicited by the zebrafish's sympatric predator, the Indian Leaf Fish, and the fact that this latter species exhibited almost no predatory attacks. The findings obtained open a new avenue of research into what zebrafish perceive as "dangerous" or fear inducing. They will also allow us to develop fear and anxiety related behavioral test methods with which the contribution of genes to, or the effects of novel anxiolytic substances on these behaviors may be analyzed.
Descriptors: zebrafish, behavior, response, predator fish, harmless fish, genetics, anxiety model, neuroscience.

Beck, J.C., E. Gilland, D.W. Tank, and R. Baker (2004). **Quantifying the ontogeny of optokinetic and vestibulo ocular behaviors in zebrafish, *medaka*, and goldfish.** *Journal of Neurophysiology* 92(6): 3546-61. ISSN: 0022-3077.

Abstract: We quantitatively studied the ontogeny of oculomotor behavior in larval fish as a foundation for studies linking oculomotor structure and function with genetics. Horizontal optokinetic and vestibuloocular reflexes (OKR and VOR, respectively) were measured in three different species (goldfish, zebrafish, and *medaka*) during the first month after hatching. For all sizes of *medaka*, and most zebrafish, Bode plots of OKR (0.065-3.0 Hz, +/-10 degrees/s) revealed that eye velocity closely followed stimulus velocity (gain > 0.8) at low frequency but dropped sharply above 1 Hz (gain < 0.3 at 3 Hz). Goldfish showed increased gain proportional to size across frequencies. Linearity testing with steps and sinusoids showed excellent visual performance (gain > 0.8) in medaka almost from hatching; but zebrafish and goldfish exhibited progressive improvement, with only the largest equaling medaka performance. Monocular visual stimulation in zebrafish and goldfish produced gains of 0.5 versus <0.1 for the eye viewing a moving versus stationary stimulus pattern but 0.25 versus <0.1 in *medaka*. Angular VOR appeared much later than OKR, initially at only high accelerations (>200 degrees /s at 0.5 Hz), first in *medaka* followed by larger (8.11 mm) zebrafish; but it was virtually nonexistent in goldfish. Velocity storage was not observed except for an eye velocity build-up in the largest *medaka*. In summary, a robust OKR was achieved shortly after hatching in all three species. In contrast, larval fish seem to be unique among vertebrates tested in their lack of significant angular VOR at stages where active movement is required for feeding and survival.
Descriptors: zebrafish, behavior physiology, fishes physiology, vestibulo ocular reflex physiology, goldfish, nystagmus, physiologic physiology, *Oryzias*, *Saccades* physiology, monocular vision physiology.

Becker, C.G., B.C. Lieberoth, F. Morellini, J. Feldner, T. Becker, and M. Schachner (2004). **L1.1 is involved in spinal cord regeneration in adult zebrafish.** *Journal of Neuroscience* 24(36): 7837-42. ISSN: 0270-6474.

Abstract: Adult zebrafish, in contrast to mammals, regrow axons descending from the brainstem after spinal cord transection. L1.1, a homolog of the mammalian recognition molecule L1, is

upregulated by brainstem neurons during axon regrowth. However, its functional relevance for regeneration is unclear. Here, we show with a novel morpholino-based approach that reducing L1.1 protein expression leads to impaired locomotor recovery as well as reduced regrowth and synapse formation of axons of supraspinal origin after spinal cord transection. This indicates that L1.1 contributes to successful regrowth of axons from the brainstem and locomotor recovery after spinal cord transection in adult zebrafish.
Descriptors: zebrafish, nerve regeneration physiology, spinal cord physiology, spinal cord injuries physiopathology, axons metabolism, brain stem physiopathology, cordotomy, drug implants, gelatin sponge, absorbable, morpholines pharmacology, nerve regeneration drug effects, oligodeoxyribonucleotides pharmacology, recovery of function, single blind method, swimming.

Becker, C.G., J. Schweitzer, J. Feldner, T. Becker, and M. Schachner (2003). **Tenascin-R as a repellent guidance molecule for developing optic axons in zebrafish.** *Journal of Neuroscience* 23(15): 6232-7. ISSN: 0270-6474.
Abstract: To investigate the role of tenascin-R in nervous system development, we studied axon pathfinding in the developing optic system of zebrafish. Zebrafish tenascin-R has the same domain structure as tenascin-R in amniotes. Amino acid sequence identity with human tenascin-R is 60%. In 3-d-old larvae, tenascin-R mRNA is expressed in scattered cells throughout the periventricular cell layer of the diencephalon and tectum. Tenascin-R immunoreactivity is not detectable in the optic nerve, optic tract, or tectal optic neuropil but immediately borders the optic tract caudally. Reducing expression of tenascin-R in 3-d-old larvae in vivo by injecting morpholinos into fertilized eggs led to excessive branching of the optic tract in 86% of all injected larvae compared with 20-37% in controls. Branches were almost exclusively caudal, where tenascin-R immunoreactivity normally borders the optic tract, suggesting a role for tenascin-R in guiding optic axons in the ventral diencephalon by a contact-repellent mechanism.
Descriptors: zebrafish larvae, axons physiology, optic nerve growth and development, optic nerve physiology, tenascin physiology, axons drug effects, brain metabolism, molecular cloning, diencephalon cytology, diencephalon growth and development, fluorescent dyes, developmental gene expression regulation drug effects, developmental gene expression regulation physiology, immunohistochemistry, in situ hybridization, larva, molecular probe techniques, molecular sequence data, morpholines chemistry, nucleic acid probes chemistry, metabolism and pharmacology, optic nerve drug effects, messenger RNA biosynthesis, amino acid sequence homology, tenascin genetics and pharmacology, visual pathways cytology and drug effects.

Becker, T., B.C. Lieberoth, C.G. Becker, and M. Schachner (2005). **Differences in the regenerative response of neuronal cell populations and indications for plasticity in intraspinal neurons after spinal cord transection in adult zebrafish.** *Molecular and Cellular Neuroscience* 30(2): 265-78. ISSN: 1044-7431.
Abstract: In zebrafish, the capacity to regenerate long axons varies among different populations of axotomized neurons after spinal cord transection. In specific brain nuclei, 84-92% of axotomized neurons upregulate expression of the growth-related genes GAP-43 and L1.1 and 32-51% of these neurons regrow their descending axons. In contrast, 16-31% of spinal neurons with axons ascending to the brainstem upregulate these genes and only 2-4% regrow their axons. Dorsal root ganglion (DRG) neurons were not observed to regrow their ascending axons or to increase expression of GAP-43 mRNA. Expression of L1.1 mRNA is high in unlesioned and axotomized DRG neurons. In the lesioned spinal cord, expression of growth-related molecules is increased in a substantial population of non-axotomized neurons, suggesting morphological plasticity in the spinal-intrinsic circuitry. We propose that locomotor recovery in spinal-transected adult zebrafish is influenced less by recovery of ascending pathways, but more by regrowth of

descending tracts and rearrangement of intraspinal circuitry.
Descriptors: zebrafish, ganglia, spinal physiology, nerve regeneration physiology, neurons physiology, spinal cord physiology, biological markers, cell division, GAP 43 protein analysis and genetics, in situ hybridization, neurons cytology, messenger RNA.

Biehlmaier, O., J.M. Lampert, J.v. Lintig, and K. Kohler (2005). **Photoreceptor morphology is severely affected in the beta , beta -carotene-15,15'-oxygenase (bcox) zebrafish morphant**. *European Journal of Neuroscience* 21(1): 59-68. ISSN: 0953 816X.
Abstract: The retinoic acid molecule, a vitamin A derivative, is of key importance for eye and photoreceptor development in vertebrates. Several studies have provided evidence that the ventral part of the retina is particularly susceptible to impairment in retinoid signalling during the period of its development. In zebrafish, targeted gene knockdown of beta , beta -carotene-15,15'-oxygenase (bcox), the key enzyme for vitamin A formation, provokes a loss of retinoid signalling during early eye development that results in microphthalmia at larval stages. Using this model, we analysed the consequences of this for the retinal morphology of the fish larvae in structural details. Our analyses revealed that rods and cones do not express photoreceptor specific proteins (rhodopsin, peanut agglutinin, zpr1) in the peripheral retina. The photoreceptors in the central retina showed shortened outer segments, and electron dense debris in their intermembranal space. The number of phagosomes was increased, and cell death was frequently observed in the outer nuclear layer. Furthermore, the number of Muller cells was significantly reduced in the inner nuclear layer. Thus, we found that the lack of retinoid signalling strongly effects photoreceptor development in the ventral and dorsal retina. In addition, shortened outer segments and cell death of the remaining photoreceptors in the central retina indicate that there is an ongoing need for retinoid signalling for photoreceptor integrity and survival at later developmental stages. Copyright © 2008 CABI
Descriptors: zebrafish larva, animal models, beta carotene, biochemical receptors, biological development, enzymes, eyes, genes, laboratory animals, morphogenesis, organogenesis, photoreceptors, retina, retinoic acid, retinoids, signal transduction, *Danio rerio.*

Biehlmaier, O., S.C. Neuhauss, and K. Kohler (2003). **Synaptic plasticity and functionality at the cone terminal of the developing zebrafish retina.** *Journal of Neurobiology* 56(3): 222-36. ISSN: 0022-3034.
NAL Call Number: QP351.J55
Descriptors: zebrafish embryos, retina cones physiology, neuronal plasticity physiology, presynaptic terminals metabolism, retina physiology, retina cones embryology, growth, development and ultrastructure, developmental gene expression regulation physiology, lighting, presynaptic terminals ultrastructure, retina embryology, retina growth and development, retina ultrastructure, growth and development.

Bilotta, J., S.E. Trace, E.V. Vukmanic, and M.L. Risner (2005). **Ultraviolet- and short-wavelength cone contributions alter the early components of the ERG of young zebrafish.** *International Journal of Developmental Neuroscience* 23(1): 15-25. ISSN: 0736-5748.
NAL Call Number: QP363.5
Abstract: The electroretinogram (ERG) is a commonly used measure to examine retinal processing in both basic and clinical research. The purpose of this study was to determine the retinal mechanisms responsible for the developmental differences found in the zebrafish ERG waveform. The ERG of young zebrafish possesses a voltage-negative response to ultraviolet- and short-wavelength stimuli, but not to middle- and long-wavelength stimuli; the ERG of adult zebrafish does not possess this response component. ERGs were obtained from young zebrafish before and after

the introduction of either aspartate, or a combination of APB (DL-2-amino-4-phosphonobutyric acid) and PDA (cis-2,3-piperidinedicarboxylic acid) in order to suppress the responses of various types of retinal neurons. Log irradiance versus response amplitude functions of the ERG response to 200-ms stimuli of various wavelengths at various times following stimulus onset (70 and 120 ms) was derived as well as spectral sensitivity. Aspartate eliminated all voltage-positive responses regardless of stimulus wavelength; irradiance-response functions following aspartate were similar to the early responses of young control fish to ultraviolet- and short-wavelength stimuli. APB + PDA produced similar but not identical results as aspartate, suggesting that the combination of these agents does not completely eliminate all post-receptoral contributions to the ERG. Spectral sensitivity functions derived from aspartate-exposed subjects at various time measurements were dominated by contributions from ultraviolet- and short-wavelength-sensitive cone types. These wavelength-dependent ERG responses are similar to those found in humans with enhanced S-cone syndrome. Finally, ERG waveform differences across stimulus wavelength suggest that the circuitry of ultraviolet- and short-wavelength cone types is different to that of middle- and long-wavelength cone types in young zebrafish.

Descriptors: zebrafish, retina cones radiation effects, electroretinography radiation effects, radio waves, ultraviolet rays, age factors, aminobutyric acids pharmacology, analysis of variance, aspartic acid pharmacology, retina cones drug effects and physiology, radiation dose response relationship, drug combinations, excitatory amino acid agonists pharmacology, excitatory amino acid antagonists pharmacology, pipecolic acids pharmacology, time factors.

Bingham, S.M., G. Toussaint, and A. Chandrasekhar (2005). **Neuronal development and migration in zebrafish hindbrain explants.** *Journal of Neuroscience Methods* 149(1): 42-9. ISSN: 0165-0270.

NAL Call Number: RC346

Abstract: The zebrafish embryo is an excellent system for studying dynamic processes such as cell migration during vertebrate development. Dynamic analysis of neuronal migration in the zebrafish hindbrain has been hampered by morphogenetic movements in vivo, and by the impermeability of embryos. We have applied a recently reported technique of embryo explant culture to the analysis of neuronal development and migration in the zebrafish hindbrain. We show that hindbrain explants prepared at the somitogenesis stage undergo normal morphogenesis for at least 14 h in culture. Importantly, several aspects of hindbrain development such as patterning, neurogenesis, axon guidance, and neuronal migration are largely unaffected, inspite of increased cell death in explanted tissue. These results suggest that hindbrain explant culture can be employed effectively in zebrafish to analyze neuronal migration and other dynamic processes using pharmacological and imaging techniques.

Descriptors: zebrafish embryo, cytology and physiology, organ culture techniques methods, rhombencephalon cytology and embryology, body patterning physiology, cell movement physiology, cell proliferation, morphogenesis physiology, rhombencephalon physiology, zebrafish anatomy and histology.

Bladen, C.L., S. Navarre, W.S. Dynan, and D.J. Kozlowski (2007). **Expression of the Ku70 subunit (XRCC6) and protection from low dose ionizing radiation during zebrafish embryogenesis.** *Neuroscience Letters* 422(2): 97-102. ISSN: 0304-3940.

NAL Call Number: QP351.N3

Abstract: The Ku70 protein, a product of the XRCC6 gene, is a component of the nonhomologous end-joining (NHEJ) pathway of DNA repair, which protects cells from the effects of radiation-induced DNA damage. Although the spatial expression of Ku70 during vertebrate embryogenesis has not been described, DNA repair proteins are generally considered to be "housekeeping" genes, which are required for radioprotection in all cells. Here, we report the

cloning and characterization of the zebrafish Ku70 ortholog. In situ hybridization and RT-PCR analyses demonstrate that Ku70 mRNA is maternally provided and expressed uniformly among embryonic blastomeres. Later during embryogenesis, zygotically transcribed Ku70 mRNA specifically accumulates in neural tissue, including the retina and proliferative regions of the developing brain. In the absence of genotoxic stress, morpholino-mediated knockdown of Ku70 expression does not affect zebrafish embryogenesis. However, exposure of Ku70 morpholino-injected embryos to low doses of ionizing radiation leads to marked cell death throughout the developing brain, spinal cord, and tail. These results suggest that Ku70 protein plays a crucial role in protecting the developing nervous system from radiation-induced DNA damage during embryogenesis.
Descriptors: zebrafish embryos, antigens, nuclear genetics, DNA repair radiation effects, DNA binding proteins genetics, embryology, radiation effects, embryonic development radiation effects, antigens, nuclear isolation and purification, antigens, nuclear metabolism, cell death genetics, cell death radiation effects, cell differentiation genetics and radiation effects, cytoprotection genetics and radiation effects, DNA damage genetics and radiation effects, DNA repair genetics, DNA binding proteins genetics , proteins isolation and purification, DNA binding proteins genetics, proteins metabolism, embryonic development genetics, developmental gene expression regulation genetics, developmental gene expression regulation radiation effects, molecular sequence data, messenger RNAantagonists and inhibitors, messenger RNA metabolism, radiation dosage, ionizing radiation, amino acid sequence homology, nucleic acid sequence homology, species specificity, metabolism.

Bretaud, S., Q. Li, B.L. Lockwood, K. Kobayashi, E. Lin, and S. Guo (2007). **A choice behavior for morphine reveals experience-dependent drug preference and underlying neural substrates in developing larval zebrafish.** *Neuroscience* 146(3): 1109-16. ISSN: 0306-4522.
NAL Call Number: QP351.N48
Abstract: Transparent larval zebrafish offer the opportunity to unravel genetic and neuronal networks underlying behavior in a developing system. In this study, we developed a choice chamber paradigm to measure reward-associated behavior in larval zebrafish. In the chamber where larval zebrafish have a choice of spending their time in either a water- or morphine-containing compartment, larvae that have previously experienced morphine spend significantly more time in the compartment containing morphine. This behavior can be attentuated by pre-treatment with antagonists of the opioid receptor or the dopamine receptor, and furthermore, is impaired in the too few mutant, which has a genetic deficiency in the production of specific groups of dopaminergic and serotonergic neurons in the ventral forebrain. These results uncover a choice behavior for an addictive substance in larval zebrafish that is mediated through central opioid and monoaminergic neurotransmitter systems.
Descriptors: zebrafish, analgesics, opioid pharmacology, choice behavior drug effects, larva physiology, morphine pharmacology, biogenic amines physiology, liquid chromatography, molecular cloning, DNA mutational analysis, dopamine physiology, immunohistochemistry, in situ hybridization, mass spectrometry, motor activity physiology, naloxone pharmacology, narcotic antagonists pharmacology, nerve net physiology, neurotransmitter agents physiology, odorant receptors genetics and physiology, opioid receptors physiology, opioid receptors, mu genetics and physiology, reinforcement psychology, reverse transcriptase PCR, reward, signal transduction physiology.

Brockerhoff, S.E., F. Rieke, H.R. Matthews, M.R. Taylor, B. Kennedy, I. Ankoudinova, G.A. Niemi, C.L. Tucker, M. Xiao, M.C. Cilluffo, G.L. Fain, and J.B. Hurley (2003). **Light stimulates a transducin-independent increase of cytoplasmic Ca2+ and suppression of current in cones from the zebrafish mutant nof.** *Journal of Neuroscience* 23(2): 470-80. ISSN: 0270-6474.

Abstract: Transducins couple visual pigments to cGMP hydrolysis, the only recognized phototransduction pathway in vertebrate photoreceptors. Here we describe a zebrafish mutant, no optokinetic response f(w21) (nof), with a nonsense mutation in the gene encoding the alpha subunit of cone transducin. Retinal morphology and levels of phototransduction enzymes are normal in nof retinas, but cone transducin is undetectable. Dark current in nof cones is also normal, but it is insensitive to moderate intensity light. The nof cones do respond, however, to bright light. These responses are produced by a light-stimulated, but transducin-independent, release of Ca2+ into the cone cytoplasm. Thus, in addition to stimulating transducin, light also independently induces release of Ca2+ into the photoreceptor cytoplasm.
Descriptors: zebrafish larva mutant, calcium metabolism, retina cones radiation effects, cytoplasm metabolism, light, transducin metabolism, adaptation, ocular genetics, adaptation, ocular physiology, amino acid sequence, retina cones cytology and metabolism, cyclic gmp metabolism, GTP binding proteins metabolism, homozygote, in situ hybridization, larva, molecular sequence data, mutagenesis, organ specificity, phototransduction genetics and physiology, physical chromosome mapping, point mutation, messenger RNA biosynthesis, signal transduction physiology, transducin deficiency, transducin genetics.

Brustein, E., M. Chong, B. Holmqvist, and P. Drapeau (2003). **Serotonin patterns locomotor network activity in the developing zebrafish by modulating quiescent periods.** *Journal of Neurobiology* 57(3): 303-22. ISSN: 0022-3034.
NAL Call Number: QP351.J55
Descriptors: zebrafish embryos, nerve net physiology, neurons physiology, serotonin metabolism, swimming physiology, central nervous system physiology, physiology, immunohistochemistry, larva physiology, nerve net drug effects, neurons drug effects, patch clamp techniques, quipazine pharmacology, receptors, serotonin metabolism, serotonin agonists pharmacology.

Brustein, E. and P. Drapeau (2005). **Serotoninergic modulation of chloride homeostasis during maturation of the locomotor network in zebrafish.** *Journal of Neuroscience* 25(46): 10607-16. ISSN: 0270-6474.
Abstract: During development, neural networks progress through important functional changes such as the generation of spontaneous activity, the expression of a depolarizing chloride gradient, and the appearance of neuromodulation. Little is known about how these processes are integrated to yield mature behaviors. We showed previously that, during the maturation of the locomotor network of the zebrafish, endogenous serotonin (5HT) increased motor activity by reducing intervals of inactivity, without affecting the active swim periods that are the target of 5HT in other and more mature preparations. Because membrane properties were constant during the rest intervals, we examined here whether 5HT modulates chloride homeostasis. We compared the effects of blocking (inward) chloride cotransport with bumetanide to the effects of 5HT and its antagonists, both behaviorally by video imaging and cellularly by whole-cell and gramicidin-perforated patch recordings. Bumetanide mimicked the effects of 5HT antagonists, by prolonging rest intervals without affecting the properties of swim episodes (duration; frequency; extent of depolarization) either behaviorally or during fictive swimming. Furthermore, bumetanide and 5HT antagonists suppressed the amplitude of depolarizing responses evoked by ionophoresis of glycine onto spinal neurons in the presence of tetrodotoxin and transiently suppressed the amplitude of responses to glycine measured after fictive swimming. The effects of bumetanide contrasted with and occluded the effects of 5HT. We suggest that, during development, endogenous 5HT modulates chloride homeostasis during the quiescent intervals and thereby offsets the long periods of quiescence commonly observed in developing networks to allow expression of

sustained and behaviorally relevant activity.

Descriptors: zebrafish embryology, chlorides metabolism, homeostasis physiology, motor activity physiology, nerve net physiology, serotonin physiology, chlorides antagonists and inhibitors, chlorides physiology, homeostasis drug effects, motor activity drug effects, nerve net drug effects, nerve net embryology, serotonin agents pharmacology, sodium chloride symporters physiology.

Brustein, E., N. Marandi, Y. Kovalchuk, P. Drapeau, and A. Konnerth (2003). **"In vivo" monitoring of neuronal network activity in zebrafish by two-photon Ca(2+) imaging.** *Pflugers Archive European Journal of Physiology* 446(6): 766-73. ISSN: 0131-6768.

Abstract: The zebrafish larva is a powerful model for the analysis of behaviour and the underlying neuronal network activity during early stages of development. Here we employ a new approach of "in vivo" Ca(2+) imaging in this preparation. We demonstrate that bolus injection of membrane-permeable Ca(2+) indicator dyes into the spinal cord of zebrafish larvae results in rapid staining of essentially the entire spinal cord. Using two-photon imaging, we could monitor Ca(2+) signals simultaneously from a large population of spinal neurons with single-cell resolution. To test the method, Ca(2+) transients were produced by iontophoretic application of glutamate and, as observed for the first time in a living preparation, of GABA or glycine. Glycine-evoked Ca(2+) transients were blocked by the application of strychnine. Sensory stimuli that trigger escape reflexes in mobile zebrafish evoked Ca(2+) transients in distinct neurons of the spinal network. Moreover, long-term recordings revealed spontaneous Ca(2+) transients in individual spinal neurons. Frequently, this activity occurred synchronously among many neurons in the network. In conclusion, the new approach permits a reliable analysis with single-cell resolution of the functional organisation of developing neuronal networks.

Descriptors: zebrafish larva, calcium physiology, diagnostic imaging, nerve net physiology, calcium chemistry, calcium signaling drug effects and physiology, coloring agents, excitatory amino acids antagonists and inhibitors, excitatory amino acids pharmacology , fluorescent dyes, glycine agents pharmacology, larva physiology, nerve net drug effects, nerve net growth and development, neurons physiology, spinal cord cytology, growth, development and physiology, strychnine pharmacology.

Burgess, H.A. and M. Granato (2007). **Sensorimotor gating in larval zebrafish.** *Journal of Neuroscience* 27(18): 4984-94. ISSN: 0270-6474.

Abstract: Control of behavior in the natural environment where sensory stimuli are abundant requires superfluous information to be ignored. In part, this is achieved through selective transmission, or gating of signals to motor systems. A quantitative and clinically important measure of sensorimotor gating is prepulse inhibition (PPI) of the startle response, impairments in which have been demonstrated in several neuropsychiatric disorders, including schizophrenia. Here, we show for the first time that the acoustic startle response in zebrafish larvae is modulated by weak prepulses in a manner similar to mammalian PPI. We demonstrate that, like in mammals, antipsychotic drugs can suppress disruptions in zebrafish PPI induced by dopamine agonists. Because genetic factors underlying PPI are not well understood, we performed a screen and isolated mutant lines with reduced PPI. Analysis of Ophelia mutants demonstrates that they have normal sensory acuity and startle performance, but reduced PPI, suggesting that Ophelia is critical for central processing of sensory information. Thus, our results provide the first evidence for sensorimotor gating in larval zebrafish and report on the first unbiased screen to identify genes regulating this process.

Descriptors: zebrafish larva behavior, psychomotor performance physiology, reaction time physiology, startle reaction physiology, acoustic stimulation methods.

Buss, R.R., C.W. Bourque, and P. Drapeau (2003). **Membrane properties related to the firing behavior of zebrafish motoneurons.** *Journal of Neurophysiology* 89(2): 657-64. ISSN: 0022-3077.

Abstract: The physiological and pharmacological properties of the motoneuron membrane and action potential were investigated in larval zebrafish using whole cell patch current-clamp recording techniques. Action potentials were eliminated in tetrodotoxin, repolarized by tetraethylammonium (TEA) and 3,4-diaminopyridine (3,4-AP)-sensitive potassium conductances, and had a cobalt-sensitive, high-threshold calcium component. Depolarizing current injection evoked a brief (approximately 10-30 ms) burst of action potentials that was terminated by strong, outwardly rectifying voltage-activated potassium and calcium-dependent conductances. In the presence of intracellular cesium ions, a prolonged plateau potential often followed brief depolarizations. During larval development (hatching to free-swimming), the resting membrane conductance increased in a population of motoneurons, which tended to reduce the apparent outward rectification of the membrane. The conductances contributing to action potential burst termination are hypothesized to play a role in patterning the synaptically driven motoneuron output in these rapidly swimming fish.

Descriptors: zebrafish larva, 4 aminopyridine analogs and derivatives, action potentials physiology, motor neurons physiology, 4 aminopyridine pharmacology, action potentials drug effects, anesthetics, local pharmacology, calcium channels physiology, cobalt pharmacology, patch clamp techniques, potassium channel blockers pharmacology, sodium and potassium channels physiology, swimming physiology, tetraethylammonium pharmacology, tetrodotoxin pharmacology.

Cameron, D.A. and L.H. Carney (2004). **Cellular patterns in the inner retina of adult zebrafish: quantitative analyses and a computational model of their formation.** *Journal of Comparative Neurology* 471(1): 11-25. ISSN: 0021-9967.

Descriptors: adult zebrafish, body patterning, retina cytology, retina growth and development, cell differentiation, cluster analysis, afferent neurons cytology, vertebrate photoreceptors cytology.

Campbell, W.A., H. Yang, H. Zetterberg, S. Baulac, J.A. Sears, T. Liu, S.T. Wong, T.P. Zhong, and W. Xia (2006). **Zebrafish lacking Alzheimer presenilin enhancer 2 (Pen-2) demonstrate excessive p53-dependent apoptosis and neuronal loss.** *Journal of Neurochemistry* 96(5): 1423-40. ISSN: 0022-3042.

NAL Call Number: QP351.J6

Abstract: Gamma-secretase cleavage, mediated by a complex of presenilin, presenilin enhancer (Pen-2), nicastrin, and Aph-1, is the final proteolytic step in generating amyloid beta protein found in brains of Alzheimer's disease patients and Notch intracellular domain critical for proper neuronal development. Here, we employ the zebrafish model to study the role of Pen-2 in neuronal survival. We found that (i) knockdown of Pen-2 using antisense morpholino led to a reduction of islet-1 positive neurons, (ii) Notch signaling was reduced in embryos lacking Pen-2 or other gamma-secretase components, (iii) neuronal loss in Pen-2 knockdown embryos is not as a result of a lack of neuronal precursor cells or cell proliferation, (iv) absence of Pen-2 caused massive apoptosis in the whole animal, which could be suppressed by simultaneous knockdown of the tumor suppressor p53, (v) loss of islet-1 or acetylated tubulin positive neurons in Pen-2 knockdown embryos could be partially rescued by knockdown of p53. Our results demonstrate that knockdown of Pen-2 directly induces a p53-dependent apoptotic pathway that contributes to neuronal loss and suggest that Pen-2 plays an important role in promoting neuronal cell survival and protecting from apoptosis in vivo.

Descriptors: zebrafish, apoptosis genetics, fish, developmental gene expression regulation genetics, membrane, neurons metabolism, tumor suppressor protein p53 metabolism, alzheimer disease, genetically modified, Northern blotting, Western blotting methods, body patterning drug

effects, body patterning genetics, cell count methods, disease models, dose response relationship, drug fish proteins chemistry, fish proteins deficiency, developmental gene expression regulation drug effects, immunohistochemistry methods, in situ hybridization methods, in situ nick end labeling methods, indoles diagnostic use, membrane proteins chemistry, deficiency and metabolism, antisense oligonucleotides pharmacology, presenilin 2, messenger RNA metabolism, Notch receptors metabolism, reverse transcriptase PCR methods, tumor suppressor protein p53 chemistry, zebrafish, proteins deficiency and metabolism.

Castro, A., M. Becerra, M.J. Manso, and R. Anadon (2006). **Calretinin immunoreactivity in the brain of the zebrafish, *Danio rerio*: distribution and comparison with some neuropeptides and neurotransmitter-synthesizing enzymes. I. Olfactory organ and forebrain.** *Journal of Comparative Neurology* 494(3): 435-59. ISSN: 0021-9967.

Abstract: The distribution of calretinin (CR) in the forebrain and the olfactory system of the adult zebrafish was studied by using immunocytochemical techniques. Previous studies in trout forebrain have indicated that CR-immunoreactive neurons acquire this phenotype rather early in development (Castro et al., J. Comp. Neurol. 467:254-269, 2003). Thus, precise knowledge of CR-expressing neuronal populations in adult zebrafish may help to decipher late stages of forebrain morphogenesis. For analysis of some forebrain nuclei and regions, CR distribution was compared with that of various ancillary markers: choline acetyltransferase, glutamic acid decarboxylase, tyrosine hydroxylase, neuropeptide Y, thyrotropin-releasing hormone, and galanin. The results reveal that calretinin is a specific marker of olfactory receptor neurons and of various neuronal populations distributed throughout the telencephalon and diencephalon. In addition, CR immunocytochemistry revealed characteristic patterns of fibers and neuropil in several telencephalic and diencephalic regions, indicating that it is a useful marker for characterizing a number of neural centers, pathways, and neuronal subpopulations in the zebrafish forebrain. Some ancillary markers also showed a distinctive distribution in pallial and subpallial regions, revealing additional aspects of forebrain organization. Comparison of the distribution of CR observed in the forebrain of zebrafish with that reported in other teleosts revealed a number of similarities and also some interesting differences. This indicates that various neuronal populations have maintained the CR phenotype in widely divergent teleost lines and suggests that CR studies may prove very useful for comparative analysis.

Descriptors: zebrafish, calcium binding protein, vitamin D dependent metabolism, nerve tissue proteins metabolism, olfactory pathways metabolism, prosencephalon metabolism, choline o acetyltransferase metabolism, galanin metabolism, glutamate decarboxylase metabolism, immunohistochemistry, neuropeptide Ymetabolism, olfactory pathways cytology, organogenesis, prosencephalon cytology, thyrotropin releasing hormone metabolism, tissue distribution, tyrosine 3 monooxygenase metabolism, zebrafish anatomy and histology.

Castro, A., M. Becerra, M.J. Manso, and R. Anadon (2006). **Calretinin immunoreactivity in the brain of the zebrafish, *Danio rerio*: distribution and comparison with some neuropeptides and neurotransmitter-synthesizing enzymes. II. Midbrain, hindbrain, and rostral spinal cord.** *Journal of Comparative Neurology* 494(5): 792-814. ISSN: 0021-9967.

Abstract: The distribution of calretinin (CR) in the brainstem and rostral spinal cord of the adult zebrafish was studied by using immunocytochemical techniques. For analysis of some brainstem nuclei and regions, CR distribution was compared with that of complementary markers (choline acetyltransferase, glutamic acid decarboxylase, tyrosine hydroxylase, neuropeptide Y). The results reveal that CR is a marker of various neuronal populations distributed throughout the brainstem, including numerous cells in the optic tectum, torus semicircularis,

secondary gustatory nucleus, reticular formation, somatomotor column, gustatory lobes, octavolateral area, and inferior olive, as well as of characteristic tracts of fibers and neuropil. These results indicate that CR may prove useful for characterizing a number of neuronal subpopulations in zebrafish. Comparison of the distribution of CR observed in the brainstem of zebrafish with that reported in an advanced teleost (the gray mullet) revealed a number of similarities, and also some interesting differences. Our results indicate that many brainstem neuronal populations have maintained the CR phenotype in widely divergent teleost lines, so CR studies may prove very useful for comparative analysis.

Descriptors: zebrafish, brain stem metabolism, calcium binding protein, vitamin D dependent metabolism, neurons metabolism, spinal cord metabolism, brain stem cytology and enzymology, choline o acetyltransferase metabolism, glutamate decarboxylase metabolism, mesencephalon cytology, enzymology and metabolism, neurons cytology, neurons enzymology, neuropeptide Ymetabolism, rhombencephalon cytology, enzymology and metabolism, spinal cord cytology and enzymology , tissue distribution, tyrosine 3 monooxygenase metabolism, zebrafish anatomy and histology.

Catania, S., A. Germana, R. Laura, T. Gonzalez Martinez, E. Ciriaco, and J.A. Vega (2003). **The crypt neurons in the olfactory epithelium of the adult zebrafish express TrkA-like immunoreactivity.** *Neuroscience Letters* 350(1): 5-8. ISSN: 0304-3940.

NAL Call Number: QP351.N3

Abstract: The olfactory neurons of vertebrates, including teleosts, regenerate through adult life. This ability seems to depend partially on neurotrophic factors, which include some neurotrophins, mainly nerve growth factor (NGF), and their cognate Trk receptors. Zebrafish is an ideal model for studying neurogenesis in the olfactory system, but what kinds of olfactory sensory neurons (ciliated, microvillous or crypt neurons) are under the control of neurotrophins during adult life is unknown. Here we examined the expression of Trk proteins in the olfactory epithelium of adult zebrafish using immunohistochemistry. TrkA-like immunoreactivity was the only Trk detected, and it was restricted to the somata of crypt sensory neurons, their central processes being apparently unreactive. These results provide indirect evidence for a possible role of NGF-like molecules (i.e. neurotrophin-6 and neurotrophin-7) in the peripheral olfactory system of adult zebrafish, acting on TrkA-like receptor expressing crypt neurons. It remains to be established whether or not TrkA-like receptors and their ligands play a function in the neurogenesis or differentiation of these cells.

Descriptors: zebrafish, carrier proteins metabolism, membrane proteins metabolism, afferent neurons metabolism, olfactory mucosa cytology, receptor, TrkA, carrier proteins immunology, hu paraneoplastic encephalomyelitis antigens, immunohistochemistry, membrane proteins immunology, afferent neurons cytology, RNA binding proteins metabolism.

Cavodeassi, F., F. Carreira Barbosa, R.M. Young, M.L. Concha, M.L. Allende, C. Houart, M. Tada, and S.W. Wilson (2005). **Early stages of zebrafish eye formation require the coordinated activity of Wnt11, Fz5, and the Wnt/beta-catenin pathway.** *Neuron* 47(1): 43-56. ISSN: 0896-6273.

Abstract: During regional patterning of the anterior neural plate, a medially positioned domain of cells is specified to adopt retinal identity. These eye field cells remain coherent as they undergo morphogenetic events distinct from other prospective forebrain domains. We show that two branches of the Wnt signaling pathway coordinate cell fate determination with cell behavior during eye field formation. Wnt/beta-catenin signaling antagonizes eye specification through the activity of Wnt8b and Fz8a. In contrast, Wnt11 and Fz5 promote eye field development, at least in part, through local antagonism of Wnt/beta-catenin signal-

ing. Additionally, Wnt11 regulates the behavior of eye field cells, promoting their cohesion. Together, these results allow us to postulate a model in which Wnt11 and Fz5 signaling promotes early eye development through the coordinated antagonism of signals that suppress retinal identity and promotion of coherence of eye field cells.

Descriptors: zebrafish eye field, cytoskeletal proteins genetics and, eye growth and development, glycoproteins genetics and physiology, signal transduction genetics and physiology, trans activators genetics and physiology and physiology, brain embryology, growth and development, cell movement physiology, cell transplantation, molecular cloning, diencephalon embryology, growth, development and physiology, eye embryology, frizzled receptors, in situ hybridization, lithium chloride pharmacology, messenger RNAbiosynthesis genetics, G protein Coupled receptors, visual fields physiology, Wnt proteins , beta catenin.

Chandrasekar, G., G. Lauter, and G. Hauptmann (2007). **Distribution of corticotropin-releasing hormone in the developing zebrafish brain.** *Journal of Comparative Neurology* 505(4): 337-51. ISSN: 0021-9967.

Descriptors: zebrafish, brain embryology, brain metabolism, corticotropin releasing hormone metabolism, developmental gene expression regulation, base sequence, brain chemistry physiology, corticotropin releasing hormone genetics, immunohistochemistry, in situ hybridization, molecular sequence data, messenger RNA analysis, reverse transcriptase PCR.

Chapman, S.C., A.L. Sawitzke, D.S. Campbell, and G.C. Schoenwolf (2005). **A three-dimensional atlas of pituitary gland development in the zebrafish.** *Journal of Comparative Neurology* 487(4): 428-40. ISSN: 0021-9967.

Descriptors: zebrafish embryo, three dimensional imaging methods, pituitary gland cytology, pituitary gland embryology, pituitary gland growth and development.

Chapouton, P. and L. Bally-Cuif (2004). **Neurogenesis**. *Methods in Cell Biology* 76: 163-206. ISSN: 0091 679X.

NAL Call Number: 442 P92

Descriptors: zebrafish, development, cell biology, nervous system, morphogenesis, neurogenesis.

Chong, M. and P. Drapeau (2007). **Interaction between hindbrain and spinal networks during the development of locomotion in zebrafish.** *Developmental Neurobiology* 67(7): 933-47. ISSN: 1932 8451.

Abstract: Little is known about the role of the hindbrain during development of spinal network activity. We set out to identify the activity patterns of reticulospinal (RS) neurons of the hindbrain in fictively swimming (paralyzed) zebrafish larvae. Simultaneous recordings of RS neurons and spinal motoneurons revealed that these were coactive during spontaneous fictive swim episodes. We characterized four types of RS activity patterns during fictive swimming: (i) a spontaneous pattern of discharges resembling evoked high-frequency spiking during startle responses to touch stimuli, (ii) a rhythmic pattern of excitatory postsynaptic potentials (EPSPs) whose frequency was similar to the motoneuron EPSP frequency during swim episodes, (iii) an arrhythmic pattern consisting of tonic firing throughout swim episodes, and (iv) RS cell activity uncorrelated with motoneuron activity. Despite lesions to the rostral spinal cord that prevented ascending spinal axons from entering the hindbrain (normally starting at approximately 20 h), RS neurons continued to display the aforementioned activity patterns at day 3. However, removal of the caudal portion of the hindbrain prior to the descent of RS axons left the spinal cord network unable to generate the rhyth-

mic oscillations normally elicited by application of N-methyl-d-aspartate (NMDA), but in approximately 40% of cases chronic incubation in NMDA maintained rhythmic activity. We conclude that there is an autonomous embryonic hindbrain network that is necessary for proper development of the spinal central pattern generator, and that the hindbrain network can partially develop independently of ascending input.

Descriptors: zebrafish larva, locomotion physiology, nerve net growth and development, neural pathways growth and development, rhombencephalon growth and development, spinal cord growth and development, action potentials physiology, axons physiology, biological clocks physiology, cell differentiation physiology, excitatory postsynaptic potentials physiology, nerve net anatomy and histology, neural pathways anatomy and histology, neurons cytology, neurons physiology, periodicity, reticular formation anatomy and histology, reticular formation growth and development, rhombencephalon anatomy and histology, spinal cord anatomy and histology, synaptic transmission physiology, zebrafish anatomy and histology.

Cilluffo, M.C., H.R. Matthews, S.E. Brockerhoff, and G.L. Fain (2004). **Light-induced Ca2+ release in the visible cones of the zebrafish.** *Visual Neuroscience* 21(4): 599-609. ISSN: 0952-5238.

Abstract: We used suction-pipette recording and fluo-4 fluorescence to study light-induced Ca2+ release from the visible double cones of zebrafish. In Ringer, light produces a slow decrease in fluorescence which can be fitted by the sum of two decaying exponentials with time constants of 0.5 and 3.8 s. In 0Ca2+-0Na+ solution, for which fluxes of Ca2+ across the outer segment plasma membrane are greatly reduced, light produces a slow increase in fluorescence. Both the decrease and increase are delayed after incorporation of the Ca2+ chelator BAPTA, indicating that both are produced by a change in Ca2+. If the Ca2+ pool is first released by bright light in 0Ca2+-0Na+ solution and the cone returned to Ringer, the time course of Ca2+ decline is much faster than in Ringer without previous light exposure. This indicates that the time constants of 0.5 and 3.8 s actually reflect a sum of Na+/Ca2+-K+ exchange and light-induced release of Ca2+. The Ca2+ released by light appears to come from at least two sites, the first comprising 66% of the total pool and half-released by bleaching 4.8% of the pigment. Release of the remaining Ca2+ from the second site requires the bleaching of nearly all of the pigment. If, after release, the cone is maintained in darkness, a substantial fraction of the Ca2+ returns to the release pool even in the absence of pigment regeneration. The light-induced release of Ca2+ can produce a modulation of the dark current as large as 0.75 pA independently of the normal transduction cascade, though the rise time of the current is considerably slower than the normal light response. These experiments show that Ca2+ can be released within the cone outer segment by light intensities within the physiological range of photopic vision. The role this Ca2+ release plays remains unresolved.

Descriptors: zebrafish, calcium metabolism, retina cones metabolism, retina cones radiation effects, light and darkness, metabolism, aniline compounds, retina cones drug effects, retina cones physiology, electric conductivity, fluorescence, fluorescent dyes, isotonic solutions, retinal pigments radiation effects, sodium metabolism, time factors, xanthenes.

Clemente, D., A. Porteros, E. Weruaga, J.R. Alonso, F.J. Arenzana, J. Aijon, and R. Arevalo (2004). **Cholinergic elements in the zebrafish central nervous system: Histochemical and immunohistochemical analysis.** *Journal of Comparative Neurology* 474(1): 75-107. ISSN: 0021-9967.

Descriptors: zebrafish, central nervous system, acetylcholine metabolism, acetylcho-

linesterase metabolism, central nervous system metabolism, choline O acetyltransferase metabolism, central nervous system enzymology, histocytochemistry methods, immunohistochemistry methods.

Clemente, D., F.J. Arenzana, R. Sanchez-Gonzalez , A. Porteros, J. Aijon, and R. Arevalo (2005). **Comparative analysis of the distribution of choline acetyltransferase in the central nervous system of cyprinids**. *Brain Research Bulletin* 66(4-6): 546-549. ISSN: 0361-9230.
NAL Call Number: QP376
Descriptors: zebrafish , brain, central nervous system, choline, choline O-acetyltransferase, evolution, freshwater fish, neurons, reticular formation, thalamus, reticular nuclei, *Danio rerio.*

Connaughton, V.P., D. Graham, and R. Nelson (2004). **Identification and morphological classification of horizontal, bipolar, and amacrine cells within the zebrafish retina.** *Journal of Comparative Neurology* 477(4): 371-85. ISSN: 0021-9967.
Abstract: Horizontal, bipolar, and amacrine cells in the zebrafish retina were morphologically characterized using Diolistic techniques. In this method, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI)-coated microcarriers are shot at high speed onto the surfaces of living retinal slices where the DiI then delineates axons, somata, and dendrites of isolated neurons. Zebrafish retinal somata were 5-10 microm in diameter. Three horizontal cell types (HA-1, HA-2, and HB) were identified; dendritic tree diameters averaged 25-40 microm. HA somata were round. Cells classified as HA-2 were larger than HA-1 cells and possessed an axon. HB somata were flattened, without an axon, although short fusiform structure(s) projected from the soma. Bipolar cells were separated into 17 morphological types. Dendritic trees ranged from 10 to 70 microM. There were six B(on) types with axon boutons only in the ON sublamina of the inner plexiform layer (IPL), and seven B(off) types with axon boutons or branches only in the OFF sublamina. Four types of bistratified bipolar cells displayed boutons in both ON and OFF layers. Amacrine cells occurred in seven types. A(off) cells (three types) were monostratified and ramified in the IPL OFF sublamina. Dendritic fields were 60-150 microM. A(on) pyriform cells (three types) branched in the ON sublamina. Dendritic fields were 50-170 microM. A(diffuse) cells articulated processes in all IPL strata. Dendritic fields were 15-90 microM. These findings are important for studies examining signal processing in zebrafish retina and for understanding changes in function resulting from mutations and perturbations of retinal organization.
Descriptors: zebrafish retina, amacrine cells cytology, neurons classification, biolistics, carbocyanines, diagnostic imaging, computer assisted image processing, organ culture techniques.

Connor, R.M., C.L. Allen, C.A. Devine, C. Claxton, and B. Key (2005). **BOC, brother of CDO, is a dorsoventral axon-guidance molecule in the embryonic vertebrate brain**. *Journal of Comparative Neurology* 485(1): 32-42. ISSN: 0021 9967.
Abstract: The early axon scaffolding in the embryonic vertebrate brain consists of a series of ventrally projecting axon tracts that grow into a single major longitudinal pathway connected across the midline by commissures. We have investigated the role of Brother of CDO (BOC), an immunoglobulin (Ig) superfamily member distantly related to the Roundabout (Robo) family of axon-guidance receptors, in the development of this embryonic template of axon tracts in the zebrafish brain. A zebrafish homologue of BOC was isolated and shown to be expressed predominantly in the developing neural plate and later in the neural tube and developing brain. Zebrafish boc was initially highly localized to discrete bands in the

mid- and hindbrain, but, as the major brain subdivisions emerged, it became more evenly expressed along the rostrocaudal axis, particularly in dorsal regions. The function of zebrafish boc was examined by a loss-of-function approach. Analysis of embryos injected with antisense morpholinos designed against boc revealed highly selective defects in the development of dorsoventrally projecting axon tracts. Loss of boc caused ventrally projecting axons, particularly those arising from the presumptive telencephalon, to follow aberrant trajectories. These data indicate that boc is an axon-guidance molecule playing a fundamental role in pathfinding during the early patterning of the axon scaffold in the embryonic vertebrate brain. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, nervous system, development, biochemistry, neurodevelopment, brain function, immunoglobulin.

Cormie, P. and K.R. Robinson (2007). **Embryonic zebrafish neuronal growth is not affected by an applied electric field in vitro.** *Neuroscience Letters* 411(2): 128-32. ISSN: 0304-3940.

NAL Call Number: QP351.N3

Abstract: Naturally occurring electric fields (EFs) have been implicated in cell guidance during embryonic development and adult wound healing. Embryonic *Xenopus laevis* neurons sprout preferentially towards the cathode, turn towards the cathode, and migrate faster towards the cathode in the presence of an external EF in vitro. A recent Phase 1 clinical trial has investigated the effects of oscillating EFs on human spinal cord regeneration. The purpose of this study was to investigate whether embryonic zebrafish neurons respond to an applied EF, and thus extend this research into another vertebrate system. Neural tubes of zebrafish embryos (16-17 somites) were dissected and dissociated neuroblasts were plated onto laminin-coated glass. A 100 mV/mm EF was applied to cell cultures for 4 or 20 h and the responses of neurons to the applied EFs were investigated. After 4h in an EF neurites were significantly shorter than control neurites. No other statistically significant effects were observed. After 20 h, control and EF-exposed neurites were no different in length. No length difference was seen between cathodally- and anodally-sprouted neurites. Application of an EF did not affect the average number of neurons in a chamber. Growth cones did not migrate preferentially towards either pole of the EF and no asymmetry was seen in neurite sprout sites. We conclude that zebrafish neurons do not respond to a 100 mV/mm applied EF in vitro. This suggests that neurons of other vertebrate species may not respond to applied EFs in the same ways as *Xenopus laevis* neurons.

Descriptors: zebrafish embryos, electricity, cytology, neurites radiation effects, neurons radiation effects, cell polarity physiology, cell polarity radiation effects, cultured cells, radiation effects, neurons cytology.

Coutts, C.A., S.A. Patten, L.N. Balt, and D.W. Ali (2006). **Development of ionic currents of zebrafish slow and fast skeletal muscle fibers.** *Journal of Neurobiology* 66(3): 220-35. ISSN: 0022-3034.

NAL Call Number: QP351.J55

Descriptors: zebrafish larvae, action potentials physiology, ion transport physiology, fast twitch muscle fibers metabolism, skeletal muscle growth and development, electrophysiology, ion channels metabolism, patch clamp techniques.

Cui, W.W., L. Saint Amant, and J.Y. Kuwada (2004). **Shocked Gene is required for the function of a premotor network in the zebrafish CNS.** *Journal of Neurophysiology* 92(5): 2898-908. ISSN: 0022-3077.

Abstract: The analysis of behavioral mutations in zebrafish can be a powerful strategy for

identifying genes that regulate the function and development of neural circuits in the vertebrate CNS. A neurophysiological analysis of the shocked (sho) mutation that affects the initiation of swimming after mechanosensory stimulation was undertaken to identify the function of the sho gene product in the developing motor circuitry. The cutaneous Rohon-Beard (RB) mechanosensory neurons responded normally to stimulation, and muscle fibers were unaffected in sho embryos, suggesting that the output of the CNS is abnormal. Indeed whole cell patch recordings from mutant muscle cells showed normal spontaneous miniature endplate potentials, but abnormal touch-evoked endplate potentials. Furthermore, motor neuron recordings showed that bursts of rhythmic action potentials from synaptically dependent depolarizations are initiated in wild-type motor neurons after sensory stimulation or bath application of N-methyl-D-aspartate. These bursts presumably correspond to bouts of swimming. In sho motor neurons, the touch-evoked depolarizations were not sustained, resulting in an abbreviated burst of action potentials. The defective responses were not due to any obvious defect in sho motor neurons because their basic properties were normal. These results suggest that in sho embryos, there is aberrant motor processing within the CNS and that normal motor processing requires the sho gene product.
Descriptors: zebrafish embryos, genes physiology, motor neurons physiology, nerve net physiology, brain physiology, escape reaction physiology, motor activity physiology, motor cortex physiology, skeletal muscle innervation and physiology.

Das, T., B. Payer, M. Cayouette, and W.A. Harris (2003). **In vivo time-lapse imaging of cell divisions during neurogenesis in the developing zebrafish retina.** *Neuron* 37(4): 597-609. ISSN: 0896-6273.
Abstract: Two-photon excitation microscopy was used to reconstruct cell divisions in living zebrafish embryonic retinas. Contrary to proposed models for vertebrate asymmetric divisions, no apico-basal cell divisions take place in the zebrafish retina during the generation of postmitotic neurons. However, a surprising shift in the orientation of cell division from central-peripheral to circumferential occurs within the plane of the ventricular surface. In the sonic you (syu) and lakritz (lak) mutants, the shift from central-peripheral to circumferential divisions is absent or delayed, correlating with the delay in neuronal differentiation and neurogenesis in these mutants. The reconstructions here show that mitotic cells always remain in contact with the opposite basal surface by means of a thin basal process that can be inherited asymmetrically.
Descriptors: zebrafish embryos, neurons cytology, retina cytology, retina embryology, cell division physiology, fluorescent dyes, luminescent proteins genetics, video microscopy, mitosis, mutation, rats, recombinant fusion stem cells cytology, time factors.

Dethleffsen, K., G. Heinrich, M. Lauth, E.W. Knapik, and M. Meyer (2003). **Insert-containing neurotrophins in teleost fish and their relationship to nerve growth factor**. *Molecular and Cellular Neuroscience* 24(2): 380-394. ISSN: 1044-7431.
Descriptors: zebrafish, clones, DNA, evolution, genomes, growth, nerve growth factor, nerves, nucleotide sequence, phylogeny, *Danio rerio*, Fugu, Tetraodon, nerve growth factor, neurotrophins, swellfishes.

Devignot, V., L. Prado de Carvalho, P. Bregestovski, and C. Goblet (2003). **A novel glycine receptor alpha Z1 subunit variant in the zebrafish brain.** *Neuroscience* 122(2): 449-57. ISSN: 0306-4522.
NAL Call Number: QP351.N48
Abstract: Alpha subunits of the inhibitory glycine receptor (GlyR) display genetic hetero-

geneity in mammals and zebrafish. This diversity is increased in mammals by the alternative splicing mechanism. We report here in zebrafish, the characterization of a new alphaZ1 subunit likely arising from alphaZ1 gene by an alternative splice process (alphaZ1L). This novel cDNA possesses 45 supplementary nucleotides at the putative exon2/exon3 boundary. The corresponding protein contains 15 additional amino acids in the NH2-terminal domain. Heterologous expression of homomeric GlyRalphaZ1L in human embryonic kidney-293 cells generates glycine-gated strychnine-sensitive chloride channels with no obvious discrepancy with pharmacological properties of GlyRalphaZ1. Moreover, zinc modulation of glycine-induced currents is identical in alphaZ1 and alphaZ1L glycine receptors. During ontogenesis, simultaneous alphaZ1 and alphaZ1L mRNA synthesis have been observed. Embryonic and adult alphaZ1 and alphaZ1L mRNA expressions are restricted to the CNS. Embryonic alphaZ1L mRNA anatomical pattern of expression is, however, highly restrained and strictly limited to the rostral part of the brain revealing a highly regionalized function of alphaZ1L in the CNS. This report contributes to the characterization of the diversity of glycine receptor isoforms in zebrafish and emphasizes the common mechanism used among vertebrates for creating GlyR variety and specificity.

Descriptors: zebrafish, brain metabolism, glycine metabolism, nerve tissue proteins chemistry and genetics, glycine receptors chemistry and genetics, proteins chemistry, alternative splicing, amino acid and base sequence, brain drug effects, brain embryology, cell line, drug dose response relationship, glycine pharmacology, membrane potentials drug effects, membrane potentials physiology, molecular sequence data, nerve tissue proteins biosynthesis and physiology, organ specificity genetics, protein subunits biosynthesis, chemistry, genetics and physiology, glycine receptors biosynthesis and physiology, transfection, proteins biosynthesis and physiology.

Downes, G.B. and M. Granato (2006). **Supraspinal input is dispensable to generate glycine-mediated locomotive behaviors in the zebrafish embryo.** *Journal of Neurobiology* 66(5): 437-51. ISSN: 0022-3034.

NAL Call Number: QP351.J55

Abstract: The anatomy of the developing zebrafish spinal cord is relatively simple but, despite this simplicity, it generates a sequence of three patterns of locomotive behaviors. The first behavior exhibited is spontaneous movement, then touch-evoked coiling, and finally swimming. Previous studies in zebrafish have suggested that spontaneous movements occur independent of supraspinal input and do not require chemical neurotransmission, while touch-evoked coiling and swimming depend on glycinergic neurotransmission as well as supraspinal input. In contrast, studies in other vertebrate preparations have shown that spontaneous movement requires glycine and other neurotransmitters and that later behaviors do not require supraspinal input. Here, we use lesion analysis combined with high-speed kinematic analysis to re-examine the role of glycine and supraspinal input in each of the three behaviors. We find that, similar to other vertebrate preparations, supraspinal input is not essential for spontaneous movement, touch-evoked coiling, or swimming behavior. Moreover, we find that blockade of glycinergic neurotransmission decreases the rate of spontaneous movement and impairs touch-evoked coiling and swimming, suggesting that glycinergic neurotransmission plays critical yet distinct roles for individual patterns of locomotive behaviors.

Descriptors: zebrafish embryos, glycine metabolism, locomotion physiology, neural pathways embryology, spinal cord embryology, synaptic transmission physiology, cell differentiation physiology, growth cones metabolism and ultrastructure, movement physiology, nerve net cytology, embryology and metabolism, neural pathways cytology, neural pathways

metabolism, spinal cord cytology and metabolism, swimming physiology, touch physiology, metabolism.

Edwards, J.G., A. Greig, Y. Sakata, D. Elkin, and W.C. Michel (2007). **Cholinergic innervation of the zebrafish olfactory bulb.** *Journal of Comparative Neurology* 504(6): 631-45. ISSN: 0021-9967.

Abstract: A number of fish species receive forebrain cholinergic input but two recent reports failed to find evidence of cholinergic cell bodies or fibers in the olfactory bulbs (OBs) of zebrafish. In the current study we sought to confirm these findings by examining the OBs of adult zebrafish for choline acetyltransferase (ChAT) immunoreactivity. We observed a diffuse network of varicose ChAT-positive fibers associated with the nervus terminalis ganglion innervating the mitral cell/glomerular layer (MC/GL). The highest density of these fibers occurred in the anterior region of the bulb. The cellular targets of this cholinergic input were identified by exposing isolated OBs to acetylcholine receptor (AChR) agonists in the presence of agmatine (AGB), a cationic probe that permeates some active ion channels. Nicotine (50 microM) significantly increased the activity-dependent labeling of mitral cells and juxtaglomerular cells but not of tyrosine hydroxlase-positive dopaminergic neurons (TH(+) cells) compared to control preparations. The nAChR antagonist mecamylamine, an alpha7-nAChR subunit-specific antagonist, calcium-free artificial cerebrospinal fluid, or a cocktail of ionotropic glutamate receptor (iGluR) antagonists each blocked nicotine-stimulated labeling, suggesting that AGB does not enter the labeled neurons through activated nAChRs but rather through activated iGluRs following ACh-stimulated glutamate release. Deafferentation of OBs did not eliminate nicotine-stimulated labeling, suggesting that cholinergic input is primarily acting on bulbar neurons. These findings confirm the presence of a functioning cholinergic system in the zebrafish OB.

Descriptors: zebrafish, choline O acetyltransferase metabolism, olfactory bulb enzymology, olfactory pathways physiology, acetylcholine pharmacology, agmatine pharmacology, brain mapping, drug interactions, excitatory amino acid antagonists pharmacology, glutamic acid metabolism, mecamylamine pharmacology, neurons classification, neurons drug effects, physiology and ultrastructure, nicotinic antagonists pharmacology, olfactory bulb cytology, oxazines pharmacology, tyrosine 3 monooxygenase metabolism, gamma aminobutyric acid metabolism.

Edwards, J.G. and W.C. Michel (2003). **Pharmacological characterization of ionotropic glutamate receptors in the zebrafish olfactory bulb.** *Neuroscience* 122(4): 1037-47. ISSN: 0306-4522.

NAL Call Number: QP351.N48

Abstract: The distribution of N-methyl-D-aspartate- (NMDA) and kainic acid- (KA) sensitive ionotropic glutamate receptors (iGluR) in the zebrafish olfactory bulb was assessed using an activity-dependent labeling method. Olfactory bulbs were incubated with an ion channel permeant probe, agmatine (AGB), and iGluR agonists in vitro, and the labeled neurons containing AGB were visualized immunocytochemically. Preparations exposed to 250 microM KA in the presence of a NMDA receptor antagonist (D-2-amino-5-phosphono-valeric acid) and an alpha-amino-3-hydroxyl-5-methylisoxazole-4-propionic acid (AMPA) receptor antagonist (sym 2206), revealed KA receptor-mediated labeling of approximately 60-70% of mitral cells, juxtaglomerular cells, tyrosine hydroxylase-positive cells and granule cells. A higher proportion of ventral olfactory bulb neurons were KA-sensitive. Application of 333 microM NMDA in the presence of an AMPA/KA receptor antagonist (6-cyano-7-nitroquinoxaline-2,3-dione) resulted in NMDA receptor-mediated labeling of almost all neurons.

The concentrations eliciting 50% of the maximal response (effective concentration: EC(50) s) for NMDA-stimulated labeling of different cell types were not significantly different and ranged from 148 microM to 162 microM. These results suggest that while NMDA receptors with similar binding affinities are widely distributed in the neurons of the zebrafish olfactory bulb, KA receptors are heterogeneously expressed among these cells and may serve unique roles in different regions of the olfactory bulb.
Descriptors: zebrafish, olfactory bulb metabolism , kainic acid receptor metabolism, N methyl D aspartate receptor metabolism, drug dose response relationship, excitatory amino acid antagonists pharmacology, N methylaspartate pharmacology, olfactory bulb drug effects, receptors, kainic acid agonists, receptors, kainic acid antagonists and inhibitors, N methyl D aspartate receptor agonists, antagonists and inhibitors.

Ettl, A.K., J. Holzschuh, and W. Driever (2006). **The zebrafish mutation m865 affects formation of dopaminergic neurons and neuronal survival, and maps to a genetic interval containing the sepiapterin reductase locus.** *Anatomy and Embryology* 211(Suppl 1): 73-86. ISSN: 0340 2061.
NAL Call Number: QL951
Abstract: The zebrafish mutation m865 was isolated during a large-scale mutagenesis screen aimed at identifying genes involved in the development and maintenance of subgroups of neurons in the zebrafish central nervous system. The phenotype of m865 mutant embryos shows defects in the development of dopaminergic neurons in the pretectum and of retinal amacrine cells, as well as abnormal caudal dopaminergic cluster in the diencephalon. The effects of the mutation appear not to be restricted to dopaminergic neurons, as development of other neurotransmitter systems (serotonergic and cholinergic) is impaired as well. Furthermore, increased apoptosis is localized to the m865 mutant retina and in the optic tectum starting at 24hpf, and may lead to the observed reduced size of the mutant head and eye. Early patterning is not affected in m865 mutant embryos, and expression of genes known to play a role in dopaminergic cell differentiation is normal except for reduced expression of nurr1 in the mutant retina. Thus the m865 mutation does not specifically affect dopaminergic neuron development. m865 was genetically mapped to linkage group 5, and the critical genomic interval could be narrowed down to a region of 110 kb, containing four candidate genes. For one of these candidate genes, sepiapterin reductase (spr), a requirement for neuronal survival has previously been implicated, including dopaminergic neurons. Identification of the mutated gene should lead to a more detailed understanding of the defects observed in m865 mutant embryos, and potentially could enhance the understanding of the development and maintenance of specific dopaminergic neuronal populations.
Descriptors: zebrafish embryology, alcohol oxidoreductases genetics, brain embryology, mutation genetics, neurons physiology, apoptosis physiology, base sequence, cell differentiation genetics, chromosome mapping, DNA primers and binding proteins metabolism, in situ hybridization, in situ nick end labeling, molecular sequence data, mutagenesis, neurons metabolism, transcription factors metabolism, genetics.

Fame, R.M., C. Brajon, and A. Ghysen (2006). **Second-order projection from the posterior lateral line in the early zebrafish brain.** *Neural Development* 1: 4. ISSN: 1749-8104.
Abstract: ABSTRACT: BACKGROUND: Mechanosensory information gathered by hair cells of the fish lateral-line system is collected by sensory neurons and sent to the ipsilateral hindbrain. The information is then conveyed to other brain structures through a second-order projection. In the adult, part of the second-order projection extends to the contralateral hindbrain, while another part connects to a midbrain structure, the torus semicircularis.

RESULTS: In this paper we examine the second-order projection from the posterior lateral-line system in late embryonic/early larval zebrafish. At four days after fertilization the synaptic field of the sensory neurons can be accurately targeted, allowing a very reproducible labeling of second-order neurons. We show that second-order projections are highly stereotyped, that they vary according to rhombomeric identity, and that they are almost completely lateralized. We also show that the projections extend not only to the contralateral hindbrain and torus semicircularis but to many other brain centers as well, including gaze- and posture-controlling nuclei in the midbrain, and presumptive thalamic nuclei. CONCLUSION: We propose that the extensive connectivity observed in early brain development reveals a basic scaffold common to most vertebrates, from which different subsets are later reinforced in various vertebrate groups. The large repertoire of projection targets provides a promising system to study the genetic encoding of this differential projection capacity.
Descriptors: zebrafish, brain, hair cells, sensory neurons, rhombomeric identity, genetic encoding.

Fan, X., A. Majumder, S.S. Reagin, E.L. Porter, A.T. Sornborger, C.H. Keith, and J.D. Lauderdale (2007). **New statistical methods enhance imaging of cameleon fluorescence resonance energy transfer in cultured zebrafish spinal neurons.** *Journal of Biomedical Optics* 12(3): 034017. ISSN: 1083-3668.
Abstract: Cameleons are genetically encoded fluorescence resonance energy transfer (FRET)-based Ca(2+) indicators. Attempts to use cameleons to detect neural activity in vertebrate systems have been largely frustrated by the small FRET signal, in contradistinction to the higher signals seen in *Drosophila* and *Caenorhabditis elegans*. We have developed a statistical optimization method capable of detecting small ratiometric signals in noisy imaging data, called statistical optimization for the analysis of ratiometric signals. Using this method, we can detect and estimate anticorrelated ratiometric signals with subcellular resolution in cultured, dissociated zebrafish spinal neurons expressing cameleon or loaded with fluo-4 and fura-red. This method may make it possible to use yellow cameleons for measuring neural activity at high resolution in transgenic animals.
Descriptors: zebrafish, calcium metabolism, calcium binding proteins, fluorescence resonance energy transfer methods, fluorescent dyes, neurons metabolism, recombinant fusion proteins, metabolism, algorithms, calcium signaling physiology, cultured cells, statistical data interpretation, neurons cytology, zebrafish anatomy and histology.

Faraco, J.H., T. Yokogawa, S. Gaus, C. Renier, and E. Mignot (2004). **Developing novel transgenic zebrafish tools for studies of the hypocretin/orexin system**. *Sleep* 27(Suppl. S): 389. ISSN: 0161 8105.
Descriptors: transgenic zebrafish, behavior, nervous system, neural coordination, hypocretin, orexin.

Feldner, J., M.M. Reimer, J. Schweitzer, B. Wendik, D. Meyer, T. Becker, and C.G. Becker (2007). **PlexinA3 restricts spinal exit points and branching of trunk motor nerves in embryonic zebrafish.** *Journal of Neuroscience* 27(18): 4978-83. ISSN: 0270-6474.
Abstract: The pioneering primary motor axons in the zebrafish trunk are guided by multiple cues along their pathways. Plexins are receptor components for semaphorins that influence motor axon growth and path finding. We cloned plexinA3 in zebrafish and localized plexinA3 mRNA in primary motor neurons during axon outgrowth. Antisense morpholino knock-down led to substantial errors in motor axon growth. Errors comprised aberrant branching of primary motor nerves as well as additional exit points of axons from the spinal

cord. Excessively branched and supernumerary nerves were found in both ventral and dorsal pathways of motor axons. The trunk environment and several other types of axons, including trigeminal axons, were not detectably affected by plexinA3 knock-down. RNA overexpression rescued all morpholino effects. Synergistic effects of combined morpholino injections indicate interactions of plexinA3 with semaphorin3A homologs. Thus, plexinA3 is a crucial receptor for axon guidance cues in primary motor neurons.
Descriptors: zebrafish, plexinA3, membrane glyco, clones, motor neurons metabolism, spinal cord embryology and metabolism, axons metabolism, developmental gene expression regulation physiology, membrane glycoproteins genetics.

Feng, B., S. Bulchand, E. Yaksi, R.W. Friedrich, and S. Jesuthasan (2005). **The recombination activation gene 1 (Rag1) is expressed in a subset of zebrafish olfactory neurons but is not essential for axon targeting or amino acid detection.** *BioMed Central Neuroscience* 6: 46. ISSN: 1471 2202.
NAL Call Number: RC321
Abstract: BACKGROUND: Rag1 (Recombination activation gene-1) mediates genomic rearrangement and is essential for adaptive immunity in vertebrates. This gene is also expressed in the olfactory epithelium, but its function there is unknown. RESULTS: Using a transgenic zebrafish line and immunofluorescence, we show that Rag1 is expressed and translated in a subset of olfactory sensory neurons (OSNs). Neurons expressing GFP under the Rag1 promoter project their axons to the lateral region of the olfactory bulb only, and axons with the highest levels of GFP terminate in a single glomerular structure. A subset of GFP-expressing neurons contain Galphao, a marker for microvillous neurons. None of the GFP-positive neurons express Galphaolf, Galphaq or the olfactory marker protein OMP. Depletion of RAG1, by morpholino-mediated knockdown or mutation, did not affect axon targeting. Calcium imaging indicates that amino acids evoke chemotopically organized glomerular activity patterns in a Rag1 mutant. CONCLUSION: Rag1 expression is restricted to a subpopulation of zebrafish olfactory neurons projecting to the lateral olfactory bulb. RAG1 catalytic activity is not essential for axon targeting, nor is it likely to be required for regulation of odorant receptor expression or the response of OSNs to amino acids.
Descriptors: transgenic zebrafish, amino acids biosynthesis, axons metabolism, gene expression regulation physiology, homeodomain proteins biosynthesis, olfactory receptor neurons metabolism, proteins biosynthesis, amino acids genetics, genetically modified, gene expression regulation genetics, homeodomain olfactory nerve metabolism.

Fetcho, J.R., S.i. Higashijima, and D.L. McLean (2008). **Zebrafish and motor control over the last decade**. *Brain Research Reviews* 57(1): 86-93. ISSN: 0165-0173.
Descriptors: zebrafish, behavior, motor task performance, neural networks, reviews, *Danio rerio.*
Notes: Review article.

Fimbel, S.M., J.E. Montgomery, C.T. Burket, and D.R. Hyde (2007). **Regeneration of inner retinal neurons after intravitreal injection of ouabain in zebrafish.** *Journal of Neuroscience* 27(7): 1712-24. ISSN: 0270-6474.
Abstract: We examined the regenerative capacity of the adult zebrafish retina by intravitreal injection of a low ouabain concentration to rapidly damage the ganglion cell layer (GCL) and inner nuclear layer (INL) with minimal photoreceptor cell damage. By 24 h after ouabain injection, maximal numbers of terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling (TUNEL)-positive cells were detected in the INL and

GCL, with low numbers of TUNEL-positive cells in the outer nuclear layer. Immunolabeling revealed that approximately 85% of the HuC/D-positive amacrine and ganglion cells were lost by 7 d post-ouabain injection (dpi). This ganglion cell loss was consistent with the small, but statistically significant, decrease in the optic nerve diameter. The regeneration response began within 1 dpi with increased proliferating cell nuclear antigen (PCNA) expression in both the INL and GCL. By 3 dpi, PCNA expression is primarily restricted to the Muller glia. By 5 dpi, most of the PCNA expression was localized to neuronal progenitors expressing the olig2:egfp transgene rather than the Muller glia. By 7 dpi, the neuronal progenitors began committing to the ganglion cell fate based on the coexpression of the atoh7:EGFP transgene and the zn5 antigen. The regeneration of ganglion and amacrine cells continued until 60 dpi, when they reached 75% of their uninjected control number. This demonstrates that inner retinal damage, without extensive photoreceptor damage, is sufficient to induce a regeneration response that is marked by the Muller glial cells reentering the cell cycle to produce neuronal progenitor cells that regenerate INL and ganglion cells in the zebrafish retina.
Descriptors: zebrafish, retina, enzyme inhibitors toxicity, ouabain toxicity, regeneration physiology, retina pathology, retinal ganglion cells drug effects, analysis of variance, genetically modified, basic helix loop helix transcription factors genetics, Western blotting methods, drug dose response relationship, glial fibrillary acidic protein metabolism, green fluorescent hu paraneoplastic encephalomyelitis antigens metabolism, immunohistochemistry methods, in situ nick end labeling methods, nerve tissue neuroglia metabolism and pathology, proliferating cell nuclear antigen metabolism, time factors.

Finney, J.L., G.N. Robertson, C.A. McGee, F.M. Smith, and R.P. Croll (2006). **Structure and autonomic innervation of the swim bladder in the zebrafish (*Danio rerio*).** *Journal of Comparative Neurology* 495(5): 587-606. ISSN: 0021-9967.
Descriptors: zebrafish, swim bladder, air sacs anatomy, histology and innervation, autonomic nervous system anatomy and histology, acetylcholinesterase metabolism, autonomic nervous system physiology, immunohistochemistry, myocytes, smooth muscle cytology and metabolism, neuropeptide Ymetabolism, neurotransmitter agents metabolism, serotonin metabolism, tyrosine 3 monooxygenase metabolism, vasoactive intestinal peptide metabolism.

Friedrich, R.W., C.J. Habermann, and G. Laurent (2004). **Multiplexing using synchrony in the zebrafish olfactory bulb.** *Nature Neuroscience* 7(8): 862-71. ISSN: 1097-6256.
NAL Call Number: QP351
Abstract: In the olfactory bulb (OB) of zebrafish and other species, odors evoke fast oscillatory population activity and specific firing rate patterns across mitral cells (MCs). This activity evolves over a few hundred milliseconds from the onset of the odor stimulus. Action potentials of odor-specific MC subsets phase-lock to the oscillation, defining small and distributed ensembles within the MC population output. We found that oscillatory field potentials in the zebrafish OB propagate across the OB in waves. Phase-locked MC action potentials, however, were synchronized without a time lag. Firing rate patterns across MCs analyzed with low temporal resolution were informative about odor identity. When the sensitivity for phase-locked spiking was increased, activity patterns became progressively more informative about odor category. Hence, information about complementary stimulus features is conveyed simultaneously by the same population of neurons and can be retrieved selectively by biologically plausible mechanisms, indicating that seemingly alternative coding strategies operating on different time scales may coexist.

Descriptors: zebrafish, brain mapping, cortical synchronization, neurons physiology, olfactory bulb physiology, action potentials physiology, nose innervation, patch clamp techniques.

Friedrich, R.W. and G. Laurent (2004). **Dynamics of olfactory bulb input and output activity during odor stimulation in zebrafish.** *Journal of Neurophysiology* 91(6): 2658-69. ISSN: 0022-3077.

Abstract: The processing of odor-evoked activity in the olfactory bulb (OB) of zebrafish was studied by extracellular single unit recordings from the input and output neurons, i.e., olfactory receptor neurons (ORNs) and mitral cells (MCs), respectively. A panel of 16 natural amino acid odors was used as stimuli. Responses of MCs, but not ORNs, changed profoundly during the first few hundred milliseconds after response onset. In MCs, but not ORNs, the total evoked excitatory activity in the population was initially odor-dependent but subsequently converged to a common level. Hence, the overall population activity is regulated by network interactions in the OB. The tuning widths of both ORN and MC response profiles were similar and, on average, stable over time. However, when analyzed for individual neurons, MC response profiles could sharpen (excitatory response to fewer odors) or broaden (excitatory response to more odors), whereas ORN response profiles remained nearly unchanged. Several observations indicate that dynamic inhibition plays an important role in this remodeling. Finally, the reliability of odor identification based on MC population activity patterns improved over time, whereas odor identification based on ORN activity patterns was most reliable early in the odor response. These results demonstrate that several properties of MC, but not ORN, activity change during the initial phase of the odor response with important consequences for odor-encoding activity patterns. Furthermore, our data indicate that inhibitory interactions in the OB are important in dynamically shaping the activity of OB output neurons.

Descriptors: zebrafish, action potentials physiology, odors, olfactory bulb physiology, olfactory receptor neurons physiology, smell physiology.

Fuller, C.L. and C.A. Byrd (2005). **Ruffed cells identified in the adult zebrafish olfactory bulb.** *Neuroscience Letters* 379(3): 190-4. ISSN: 0304-3940.

NAL Call Number: QP351.N3

Abstract: The morphology and distribution of ruffed cells was examined in the olfactory bulb of adult zebrafish, *Danio rerio*, using retrograde tract tracing and Golgi-Kopsch techniques. The neurons had variable-shaped soma that ranged in size from 7 to 15 microm in diameter. There was an obvious protrusion of the membrane, a ruff, near the initial portion of the axon, and the cells appeared to be distributed primarily in the glomerular layer and superficial internal cell layer. This cell type has been described for a number of teleosts, but not for other animal groups. While the presence of ruffed cells in all teleosts has been suggested, the existence of this cell type in zebrafish was uncertain until now. This new evidence may provide additional insight into olfactory coding and processing in this key model system.

Descriptors: zebrafish, biotin analogs and derivatives, neurons cytology, olfactory bulb cytology, biotin metabolism, cell count, cell size, dextrans metabolism, immunohistochemistry methods, neurons classification, neurons metabolism, phylogeny.

Fuller, C.L., H.K. Yettaw, and C.A. Byrd (2006). **Mitral cells in the olfactory bulb of adult zebrafish (*Danio rerio*): morphology and distribution.** *Journal of Comparative Neurology* 499(2): 218-30. ISSN: 0021-9967.

Descriptors: zebrafish, neurons physiology, olfactory bulb cytology, anatomy and histol-

ogy, biotin analogs and derivatives, biotin metabolism, cell count methods, cell size, dextrans metabolism, fmrfamide metabolism, functional laterality, immunohistochemistry methods, anatomic models system, nerve net ultrastructure, neurons ultrastructure, pyridinium compounds metabolism, silver staining methods, tyrosine 3 monooxygenase metabolism.

Gabriel, J.P., R. Mahmood, A.M. Walter, A. Kyriakatos, G. Hauptmann, R.L. Calabrese, and A. El Manira (2008). **Locomotor pattern in the adult zebrafish spinal cord in vitro**. *Journal of Neurophysiology* 99(1): 37-48. ISSN: 0022-3077.
Descriptors: zebrafish, embryos, locomotor activity, motor neurons, muscles, N-methyl-D-aspartic acid, neurons, rhythms, spinal cord, synaptic transmission, ventral roots, strychnine, *Danio rerio*.

Gahtan, E. and D.M. O'Malley (2003). **Visually guided injection of identified reticulospinal neurons in zebrafish: a survey of spinal arborization patterns.** *Journal of Comparative Neurology* 459(2): 186-200. ISSN: 0021-9967.
Descriptors: zebrafish larva, brain stem anatomy, histology, growth and development, neurons cytology, spinal nerves cytology, axonal transport physiology, brain stem chemistry and enzymology, fluorescent dyes analysis, immunohistochemistry, neurons chemistry, pyramidal tracts anatomy, histology, chemistry, embryology, growth and development, spinal nerves chemistry and embryology.

Gahtan, E., P. Tanger, and H. Baier (2005). **Visual prey capture in larval zebrafish is controlled by identified reticulospinal neurons downstream of the tectum.** *Journal of Neuroscience* 25(40): 9294-303. ISSN: 0270-6474.
Abstract: Many vertebrates are efficient hunters and recognize their prey by innate neural mechanisms. During prey capture, the internal representation of the prey's location must be constantly updated and made available to premotor neurons that convey the information to spinal motor circuits. We studied the neural substrate of this specialized visuomotor system using high-speed video recordings of larval zebrafish and laser ablations of candidate brain structures. Seven-day-old zebrafish oriented toward, chased, and consumed paramecia with high accuracy. Lesions of the retinotectal neuropil primarily abolished orienting movements toward the prey. Wild-type fish tested in darkness, as well as blind mutants, were impaired similarly to tectum-ablated animals, suggesting that prey capture is mainly visually mediated. To trace the pathway further, we examined the role of two pairs of identified reticulospinal neurons, MeLc and MeLr, located in the nucleus of the medial longitudinal fasciculus of the tegmentum. These two neurons extend dendrites into the ipsilateral tectum and project axons into the spinal cord. Ablating MeLc and MeLr bilaterally impaired prey capture but spared several other behaviors. Ablating different sets of reticulospinal neurons did not impair prey capture, suggesting a selective function of MeLr and MeLc in this behavior. Ablating MeLc and MeLr neurons unilaterally in conjunction with the contralateral tectum also mostly abolished prey capture, but ablating them together with the ipsilateral tectum had a much smaller effect. These results suggest that MeLc and MeLr function in series with the tectum, as part of a circuit that coordinates prey capture movements.
Descriptors: zebrafish larva, wild type, blind mutants, corpora quadrigemina cytology, motion perception physiology, neurons physiology, predatory behavior physiology, psychomotor performance physiology, vision physiology, exploratory behavior radiation effects, functional laterality physiology, green fluorescent proteins biosynthesis, Hedgehog proteins, larva, motor activity physiology, orientation physiology, reaction time physiology, superior

colliculus physiology, trans activators metabolism, transcription factors biosynthesis and genetics, visual pathways cytology and physiology.

Germana, A., F. Abbate, T. Gonzalez Martinez, M.E. del Valle, F. de Carlos, G. Germana, and J.A. Vega (2004). **S100 protein is a useful and specific marker for hair cells of the lateral line system in postembryonic zebrafish.** *Neuroscience Letters* 365(3): 186-9. ISSN: 0304-3940.
NAL Call Number: QP351.N3
Abstract: The neuromast of the lateral line system of zebrafish has become an ideal model for the study of both developmental genetics and the vertebrate auditory system. Interestingly, the hair cells of this system have been found to selectively display immunoreactivity for S100 protein in some teleosts. In order to provide a selective marker for the sensory cells of the lateral line system, we have analyzed immunohistochemically the expression of S100 protein in zebrafish from the larval to the adult stage. In larval and adult animals S100 protein immunoreactivity was detected restricted to the hair cells of both superficial and canal neuromasts. Apparently the expression of S100 protein by hair cells was independent of the age, but it was expressed heterogeneously in the hair cells of canal neuromasts. The results of this work provide a feasible method to easily identify sensory cells in the neuromasts, and may be of interest in studies regarding development, differentiation or turnover of hair cells.
Descriptors: zebrafish larva, neuromast, auditory system, hair cells metabolism, S100 proteins biosynthesis, biological markers analysis, immunohistochemistry.

Germana, A., T. Gonzalez Martinez, S. Catania, R. Laura, J. Cobo, E. Ciriaco, and J. Vega (2004). **Neurotrophin receptors in taste buds of adult zebrafish (*Danio rerio*).** *Neuroscience Letters* 354(3): 189-192. ISSN: 0304 3940.
NAL Call Number: QP351.N3
Abstract: TrkB plays crucial roles in the development and maintenance of taste buds in mammals. In this study we investigated the presence and cell localization of Trks (TrkA, TrkB and TrkC) in taste buds of the zebrafish (*Danio rerio*) using Western blot and immunohistochemistry. Proteins of 140 and 145 kDa, identified as full-length TrkA and TrkB, were detected. Conversely, the anti-TrkC antibody recognized a protein lower than expected (100 kDa). In agreement with these results the sensory cells of taste buds, displayed TrkA- and TrkB-like, but not TrkC-like, immunoreactivity. TrkA and TrkB co-existed in the same taste buds, but remains to be clarified whether or not they are coexpressed in the same cells. Present results demonstrate that as for mammals neurotrophins might play a role in sensory cells of the teleostean taste buds.
Descriptors: zebrafish, *Danio rerio*, chemoreception, taste buds, neurotrophin receptor presence and cell localization, immunohistochemical analysis.

Germana, A., G. Montalbano, F. de Carlos, M.B. Levanti, F. Abbate, J.A. Vega, and E. Ciriaco (2006). **Epidermal growth factor (EGF) expression in lateral line system and in taste buds of adult zebrafish (*Brachidanio rerio*).** *Neuroscience Letters* 397(3): 210-3. ISSN: 0304-3940.
NAL Call Number: QP351.N3
Abstract: The mechano and chemosensory organs of adult teleosts undergoes a continuous cell renewal and turnover which is regulated in part by growth factors. Here, we investigated the occurrence and the cell localization of epidermal growth factor (EGF) in the lateral line system and taste bud of adult zebrafish, using Western blot and immunohistochemistry associated to a polyclonal antibody against mammalian EGF. Furthermore, the distribution of S100 protein was studied in parallel to label hair sensory cells in the lateral line system.

Western blot revealed one unique protein band with an estimated molecular weight of about 13 kDa, equivalent to the EGF of mammals. Specific immunoreactivity for EGF was observed in the epithelial basal and/or supporting cells of the neuromasts of the lateral line system and taste buds. Conversely, the sensory cells in both sensory structures were devoid of immunostaining. Present results demonstrate the occurrence of EGF in mechano and sensory system of adult zebrafish, suggesting a role for this molecule in the cell renewal and turnover of these structures.

Descriptors: zebrafish, epidermal growth factor biosynthesis, sense organs metabolism, immunohistochemistry, taste buds metabolism.

Germana, A., G. Montalbano, R. Laura, E. Ciriaco, M.E. del Valle, and J.A. Vega (2004). **S100 protein-like immunoreactivity in the crypt olfactory neurons of the adult zebrafish.** *Neuroscience Letters* 371(2-3): 196-8. ISSN: 0304-3940.

NAL Call Number: QP351.N3

Abstract: The olfactory epithelium of some teleosts, including zebrafish, contains three types of olfactory sensory neurons. Because zebrafish has become an ideal model for the study of neurogenesis in the olfactory system, it is of capital importance the identification of specific markers for different neuronal populations. In this study we used immunohistochemistry to analyze the distribution of S100 protein-like in the adult zebrafish olfactory epithelium. Surprisingly, specific S100 protein-like immunostaining was detected exclusively in crypt neurons, whereas ciliated and microvillous neurons were not reactive, and the supporting glial cells as well. The pattern of immunostaining was exclusively cytoplasmic without apparent polarity within the soma, and the intensity of immunostaining was not related with the maturative stage of the neurons. The role of S100 protein in crypt olfactory neurons is unknown, although it is probably associated with the capacity of these cells to respond to chemical stimuli. In any case, it represents an excellent marker to identify crypt olfactory neurons in zebrafish.

Descriptors: zebrafish, neurons chemistry, olfactory mucosa chemistry, S100 proteins analysis, immunochemistry, neurons metabolism, olfactory mucosa metabolism, S100 proteins biosynthesis.

Ghysen, A. and C. Dambly Chaudiere (2004). **Development of the zebrafish lateral line.** *Current Opinion in Neurobiology* 14(1): 67-73. ISSN: 0959 4388.

NAL Call Number: QP351.C885

Abstract: The lateral line system is simple (comprising six cell types), its sense organs form according to a defined and reproducible pattern, and its neurons are easily visualized. In the zebrafish, these advantages can be combined with a wealth of genetic tools, making this system ideally suited to a combined molecular, cellular and genetic analysis. Recent progress has taken advantage of these various qualities to elucidate the mechanism that drives the migration from head to tail of the sense organ precursor cells, and to approach the questions surrounding axonal guidance and target recognition.

Descriptors: zebrafish embryology, functional laterality physiology, nervous system embryology, cell movement physiology, embryonic induction physiology, nervous system cytology, growth and development, sense organs cytology, embryology, growth and development.

Ghysen, A., C. Dambly Chaudiere, and D. Raible (2007). **Making sense of zebrafish neural development in the Minervois.** *Neural Development* 2: 15. ISSN: 1749-8104.

Abstract: ABSTRACT: The meeting 'From sensory perception to motor output: genetic bases of behavior in the zebrafish embryo' was held at Minerve (South of France) on March

16-18, 2007. The meeting site was beautifully situated in the heart of the Minervois wine country, and its remoteness promoted conversations and interaction over the course of the program. The meeting covered neurogenesis and eye development on day 1, ear and lateral line development on day 2, and brain connectivity and behavior on day 3. Underlying all sessions, however, ran the growing importance of live imaging, an approach that takes full advantage of the transparency of fish embryos and early larvae, as illustrated by several movies and links in this report.
Descriptors: zebrafish embryos, genetic bases, neural development, motor output, neurogenesis.
Notes: A meeting held in Minervois, France in 2007.

Gilmour, D., H. Knaut, H.M. Maischein, and C. Nuesslein-Volhard (2004). **Towing of sensory axons by their migrating target cells in vivo**. *Nature Neuroscience* 7(5): 491-492. ISSN: 1097-6256.
NAL Call Number: QP351
Descriptors: zebrafish, axon guidance, cell migration, cells, methodology, neurons, sense organs, sensory neurons, target cells, *Danio rerio*.

Gleason, M.R., S.I. Higashijima, J. Dallman, K. Liu, G. Mandel, and J.R. Fetcho (2003). **Translocation of CaM kinase II to synaptic sites in vivo**. *Nature Neuroscience* 6(3): 217-218. ISSN: 1097-6256.
NAL Call Number: QP351
Descriptors: zebrafish, biochemistry, ca super(2+), calmodulin-dependent protein kinase II, calcium , electrical stimuli, enzymes, glutamic acid, neurons, postsynapse, protein transport, sensory stimuli, translocation, *Danio rerio.*

Gloriam, D.E., T.K. Bjarnadottir, Y.L. Yan, J.H. Postlethwait, H.B. Schioth, and R. Fredriksson (2005). **The repertoire of trace amine G-protein-coupled receptors: large expansion in zebrafish.** *Molecular PhyloGenetics and Evolution* 35(2): 470-482. ISSN: 1055-7903.
NAL Call Number: QH367.5.M56
Descriptors: zebrafish, *Danio rerio*, neurotransmitter receptors, trace amine G protein Coupled receptors, diversity , phylogenetic comparisons and implications for molecular evolutionary history and mechanisms, evolutionary adaptation, phylogeny, biochemical variation.

Gonzalez-Nunez, V., R. Gonzalez-Sarmiento, and R.E. Rodriguez (2003). **Characterization of zebrafish proenkephalin reveals novel opioid sequences**. *Molecular Brain Research* 114(1): 31-39. ISSN: 0169-328X.
Descriptors: zebrafish, enkephalins, evolution, freshwater fish, genomes, nucleotide sequence, opioids, peptides, proenkephalin, *Danio rerio*, met-enkephalin-gly-tyr, amino acid sequence prediction, cDNA, proenkephalin.

Gonzalez Nunez, V., R. Gonzalez-Sarmiento, and R.E. Rodriguez (2003). **Identification of two proopiomelanocortin genes in zebrafish (*Danio rerio*)**. *Molecular Brain Research* 120(1): 1-8. ISSN: 0169-328X.
Descriptors: zebrafish, amino acid sequence, DNA, endorphins, gene expression, genomes, nervous system, nucleotide sequence, pituitary, pituitary gland, proopiomelanocortin, cyprinidae, *Danio rerio*, *Teleostei*, carps, endorphins, minnows.

Gonzalez Nunez, V., E. Marron Fernandez de Velasco, G. Arsequell, G. Valencia, and R.E. Rodriguez (2007). **Identification of dynorphin a from zebrafish: a comparative study with mammalian dynorphin A.** *Neuroscience* 144(2): 675-84. ISSN: 0306-4522.

NAL Call Number: QP351.N48

Abstract: We report the cloning and molecular characterization of the zfPDYN. The complete open reading frame for this propeptide is comprised in two exons that are localized on chromosome 23. zfPDYN cDNA codes for a polypeptide of 252 amino acids that contains the consensus sequences for four opioid peptides: an Ile-enkephalin, the neo-endorphins, dynorphin A and dynorphin B. Upon comparison between zebrafish (zfDYN A) and mammalian dynorphin A (mDYN A) it has been stated that these two peptides only differ in two amino acids: the Leu(5) is replaced by Met(5) and the Lys(13) by Arg(13). Taking into consideration that mDYN A is able to bind to the three mammalian opioid receptors, we have compared the pharmacological profile of zfDYN A and mDYN A on the zebrafish opioid receptors. By means of radioligand binding techniques, we have established that these two dynorphins bind and activate all of the cloned opioid receptors from zebrafish (delta-, mu- and kappa-like), although with different affinities. zfDYN A and mDYN A displace [(3) H]-diprenorphine binding with K(i) values on the nanomolar range, showing greater affinity for zebrafish opioid receptor (ZFOR) 3 (kappa) receptor. ZFOR1 (delta) and ZFOR4 (delta) present higher affinity for zfDYN A than for mDYN A, while the opposing behavior is observed in ZFOR2 (mu). Functional [(35)S]guanosine 5'-[gamma-thio]triphosphate (GTPgammaS) stimulation experiments indicate that these two peptides fully activate the zebrafish opioid receptors, although the mean effective dose (EC(50)) values obtained for ZFOR2 and ZFOR3 receptors are lower than those seen for ZFOR1 and ZFOR4. A comparative study indicates that mammalian and zebrafish opioid receptors might bind their corresponding dynorphin A in a similar fashion, hence suggesting an important role of the opioid system through the vertebrate evolution.

Descriptors: zebrafish, dynorphins metabolism, binding, competitive, molecular cloning methods, drug dose response relationship, dynorphins genetics and pharmacology, guanosine 5' O 3 thiotriphosphate pharmacokinetics, radioligand assay, reverse transcriptase PCR methods, sequence alignment, sulfur isotopes.

Goodrich, L.V. (2005). **Hear, hear for the zebrafish.** *Neuron* 45(1): 3-5. ISSN: 0896-6273.

Abstract: Our senses of hearing and balance rely on the function of specialized receptor neurons called "hair cells." In this issue of Neuron, Grant et al. report a series of elegant zebrafish experiments that reveal a previously unappreciated role for glia in the regulation of hair cell proliferation and differentiation. This work is a beautiful example of how zebrafish are particularly useful model systems for studying hair cell development and function.

Descriptors: zebrafish embryos, cell differentiation physiology, hair cells embryology, neuroglia physiology, peripheral nervous system embryology, cell communication physiology, cell movement physiology, hair cells cytology, hair cells physiology, mechanotransduction, cellular physiology, research models, neuroglia cytology, peripheral nervous system cytology and physiology, .

Notes:

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Granato, M. (2004). **Motor axon guidance in the zebrafish embryo**. *Federation of American Societies for Experimental Biology Journal* 18(4-5): Abst. 304.7. ISSN: 0892 6638.

NAL Call Number: QH301.F3

Abstract: During embryogenesis spinal motor axons navigate through a complex and

dynamic environment. While studies have shown that extrinsic cues are important for directing motor axons, very little is known about the identity of such cues. We have previously shown that a small subpopulation of mesodermal derived adaxial cells play a crucial role in the guided migration of zebrafish motor axons. In mutants lacking adaxial cells, motor axons fail to exit the spinal cord. Moreover, adaxial cells initially decorate the axonal path, but migrate away as the first growth cones approach, suggesting that adaxial cells provide motor axons with contact independent cues. We have previously identified two genes that function as adaxial derived cues, diwanka and unplugged, as they are required in adaxial cells. Mutations in the zebrafish diwanka gene prevent motor axons from exiting the spinal cord and mutations in the unplugged gene disrupt pathway selection at a somitic choice point. We have now cloned both genes by positional cloning. We will present molecular cloning of both genes and report on the molecular mechanisms by which these genes direct motor axons.
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Descriptors: zebrafish, development, molecular genetics, nervous system, neural coordination, embryogenesis.

Grant, K.A., D.W. Raible, and T. Piotrowski (2005). **Regulation of latent sensory hair cell precursors by glia in the zebrafish lateral line.** *Neuron* 45(1): 69-80. ISSN: 0896-6273.
Abstract: The lateral line is a placodally derived mechanosensory organ in anamniotes that detects the movement of water. In zebrafish embryos, a migrating primordium deposits seven to nine clusters of sensory hair cells, or neuromasts, at intervals along the trunk. Postembryonically, neuromasts continue to be added. We show that some secondary neuromasts arise from a pool of latent precursors that are deposited by the primordium between primary neuromasts. Interneuromast cells lie adjacent to the lateral line nerve and associated glia. These cells remain quiescent while they are juxtaposed with the glia; however, when they move away from the nerve they increase proliferation and form neuromasts. If glia are manually removed or genetically ablated by mutations in cls/sox10, hypersensitive (hps), or rowgain (rog), neuromasts precociously differentiate. Transplantation of wt glia into mutants rescues the appropriate temporal differentiation of interneuromast cells. Our studies reveal a role for glia in regulating sensory hair cell precursors.
Descriptors: mutant zebrafish embryos, cell communication physiology, cell differentiation physiology, mechanoreceptors embryology, neuroglia metabolism, stem cells metabolism, genetically modified, carrier cell proliferation, cytology, embryology, metabolism, green fluorescent proteins, high mobility group mechanoreceptors cytology and metabolism, mechanotransduction, cellular physiology, mutation genetics, neuroglia cytology and transplantation, peripheral nervous system embryology and metabolism, stem cell transplantation and cytology.

Halpern, M.E., J.O. Liang, and J.T. Gamse (2003). **Leaning to the left: laterality in the zebrafish forebrain.** *Trends in Neurosciences* 26(6): 308-13. ISSN: 0166-2236.
NAL Call Number: RC321.T74
Abstract: How the brain becomes lateralized is poorly understood. By contrast, much is known about molecular cues that specify the left-right axis of the body, fashioning the asymmetric morphology and positioning of the visceral organs. In zebrafish, the Nodal signaling pathway functions in visceral asymmetry and also in the embryonic brain, to bias laterality of the epithalamus. Formation of an asymmetric pineal complex differentially influences adjacent diencephalic nuclei, the left and right habenulae, which acquire distinctive molecular and cellular features. Results from the genetically tractable zebrafish system provide a promising entry point for exploring how left-right biases are established and propagated in the

developing vertebrate brain.
Descriptors: zebrafish brain, body patterning genetics, epithalamus growth and development, functional laterality physiology, body patterning physiology, diencephalon growth and development, diencephalon metabolism, epithalamus metabolism, functional laterality genetics, developmental gene expression regulation physiology, learning physiology, organogenesis genetics, organogenesis physiology, signal transduction genetics, signal transduction physiology, transforming growth factor beta genetics.

Halpern, M.E., O. Gunturkun, W.D. Hopkins, and L.J. Rogers (2005). **Lateralization of the vertebrate brain: taking the side of model systems**. *Journal of Neuroscience* 25(45): 10351-10357. ISSN: 0270 6474.
Descriptors: zebrafish brain, molecular genetics, nervous system, brain imaging, imaging and microscopy techniques, neuroanatomy, neural processing, behavioral specialization, genetic specialization, anatomical specialization, brain laterality.

Heinrich, G. and C.J. Pagtakhan (2004). **Both 5' and 3' flanks regulate zebrafish brain-derived neurotrophic factor gene expression.** *BioMed Central Neuroscience* 5: 19. ISSN: 1471 2202.
NAL Call Number: RC321
Abstract: BACKGROUND: Precise control of developmental and cell-specific expression of the brain-derived neurotrophic factor (BDNF) gene is essential for normal neuronal development and the diverse functions of BDNF in the adult organism. We previously showed that the zebrafish BDNF gene has multiple promoters. The complexity of the promoter structure and the mechanisms that mediate developmental and cell-specific expression are still incompletely understood. RESULTS: Comparison of pufferfish and zebrafish BDNF gene sequences as well as 5' RACE revealed three additional 5' exons and associated promoters. RT-PCR with exon-specific primers showed differential developmental and organ-specific expression. Two exons were detected in the embryo before transcription starts. Of the adult organs examined, the heart expressed a single 5' exon whereas the brain, liver and eyes expressed four of the seven 5' exons. Three of the seven 5' exons were not detectable by RT-PCR. Injection of promoter/GFP constructs into embryos revealed distinct expression patterns. The 3' flank profoundly affected expression in a position-dependent manner and a highly conserved sequence (HCS1) present in 5' exon 1c in a dehancer-like manner. CONCLUSIONS: The zebrafish BDNF gene is as complex in its promoter structure and patterns of differential promoter expression as is its murine counterpart. The expression of two of the promoters appears to be regulated in a temporally and/or spatially highly circumscribed fashion. The 3' flank has a position-dependent effect on expression, either by affecting transcription termination or post-transcriptional steps. HCS1, a highly conserved sequence in 5' exon 1c, restricts expression to primary sensory neurons. The tools are now available for detailed genetic and molecular analyses of zebrafish BDNF gene expression.
Descriptors: zebrafish embryos, brains, 3' flanking region physiology, 5' flanking region physiology, brain derived neurotrophic factor genetics, developmental gene expression regulation physiology, 3' flanking region genetics, 5' flanking region genetics, alternative splicing, genetically modified, brain derived neurotrophic factor biosynthesis, molecular cloning, conserved sequence genetics exons genetics, developmental gene expression regulation genetics, gene transfer techniques, reporter genes, molecular sequence data, organ specificity, promoter regions genetics and physiology, recombinant reverse transcriptase PCR, sequence homology, tetraodontiformes genetics.

Heinrich, G. (2003). **A novel BDNF gene promoter directs expression to skeletal muscle.** *BioMed Central Neuroscience* 4 ISSN: 1471 2202.
NAL Call Number: RC321
Abstract: Background:Cell-specific expression of the gene that encodes brain-derived neurotrophic factor (BDNF) is required for the normal development of peripheral sensory neurons and efficient synaptic transmission in the mature central and peripheral nervous system. The control of BDNF gene expression involves multiple tissue and cell-specific promoters that are differentially regulated. The molecular mechanisms that are responsible for tissue and cell-specific expression of these promoters are still incompletely understood. Results:The cloning and analysis of three additional zebrafish (*Danio rerio*) BDNF gene exons and two associated promoters, is reported. Among them are two exons that generate a novel tripartite mature transcript. The exons were located on the transcription unit, whose overall organization was determined by cloning, Southern blot hybridization and sequence analysis, and compared with the pufferfish (*Fugu rubripes*) and mammalian BDNF loci, revealing a conserved but more compact organization. Structural and functional analysis of the exons, their adjacent promoters and 5' flanks, showed that they are expressed cell-specifically. The promoter associated with the 5' exon of the tripartite transcript is GC-rich, TATA-less and the 5' flank adjacent to it contains multiple Sp1, Mef2, and AP1 elements. A fusion gene containing the promoter and 1.5 KB of 5' flank is directed exclusively to skeletal muscle of transiently transfected embryos. The second promoter, whose associated 5' exon contains a 25-nucleotide segment of identity with a mammalian BDNF gene exon, was transiently expressed in yolk of the early embryo. RT-PCR analysis of total RNA from whole juvenile fish and adult female skeletal muscle revealed tissue-specific expression of the 5' exons but the novel exon could not be detected even after two rounds of nested PCR. Conclusion:The zebrafish BDNF gene is as complex as the mammalian gene yet much more compact. Its exons are expressed in an independently regulated and cell-specific fashion. An initial structural and functional analysis has shown that the regions controlling zebrafish BDNF gene expression have been cloned and identified. They can now be subjected to detailed molecular and genetic analyses to identify the cellular mechanisms by which the transcription factors that act on these regions control BDNF gene expression. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics, skeletal system, reverse transcriptase PCR, Southern blot hybridization, cloning, nested PCR, sequence analysis, transient transfection, genetic techniques, laboratory techniques, molecular mechanisms, pufferfish.

Hendricks, M. and S. Jesuthasan (2007). **Asymmetric innervation of the habenula in zebrafish.** *Journal of Comparative Neurology* 502(4): 611-619. ISSN: 0021-9967.
Descriptors: zebrafish, brains, afferent pathways anatomy and histology, axons ultrastructure, habenula anatomy and histology, afferent pathways physiology, axons physiology, brain mapping, carbocyanines, diencephalon anatomy and histology and physiology, functional laterality physiology, habenula physiology, telencephalon anatomy and histology and physiology.

Hendricks, M. and S. Jesuthasan (2007). **Electroporation-based methods for in vivo, whole mount and primary culture analysis of zebrafish brain development.** *Neural Development* 2: 6. ISSN: 1749-8104.
Abstract: ABSTRACT: BACKGROUND: Electroporation is a technique for the introduction of and other into cells. In chick embryos it has been a particularly powerful technique for the spatial and temporal control of gene expression in developmental studies. Electroporation methods have also been reported for *Xenopus*, zebrafish, and mouse. RESULTS: We

present a new protocol for zebrafish brain electroporation. Using a simple set-up with fixed spaced electrodes and microinjection equipment, it is possible to electroporate 50 to 100 embryos in 1 hour with no lethality and consistently high levels of transgene expression in numerous cells. Transfected cells in the zebrafish brain are amenable to in vivo time lapse imaging. Explants containing transfected neurons can be cultured for in vitro analysis. We also present a simple enzymatic method to isolate whole brains from fixed zebrafish for immunocytochemistry. CONCLUSION: Building on previously described methods, we have optimized several parameters to allow for highly efficient unilateral or of a large number of cells in the zebrafish brain. This method is simple and provides consistently high levels of transgenesis for large numbers of embryos.
Descriptors: zebrafish embryos brain, electropration, nucleic acids, macromolecules , bilateral transgenesis.

Hernandez, P.P., F.A. Olivari, A.F. Sarrazin, P.C. Sandoval, and M.L. Allende (2007). **Regeneration in zebrafish lateral line neuromasts: expression of the neural progenitor cell marker sox2 and proliferation-dependent and-independent mechanisms of hair cell renewal.** *Developmental Neurobiology* 67(5): 637-54. ISSN: 1932 8451.
Abstract: Mechanosensory hair cells are essential for audition in vertebrates, and in many species, have the capacity for regeneration when damaged. Regeneration is robust in the fish lateral line system as new hair cells can reappear after damage induced by waterborne aminoglycoside antibiotics, platinum-based drugs, and heavy metals. Here, we characterize the loss and reappearance of lateral line hair cells induced in zebrafish larvae treated with copper sulfate using diverse molecular markers. Transgenic fish that express green fluorescent protein in different cell types in the lateral line system have allowed us to follow the regeneration of hair cells after different damage protocols. We show that conditions that damage only differentiated hair cells lead to reappearance of new hair cells within 24 h from nondividing precursors, whereas harsher conditions are followed by a longer recovery period that is accompanied by extensive cell division. In order to characterize the cell population that gives rise to new hair cells, we describe the expression of a neural stem cell marker in neuromasts. The zebrafish sox2 gene is strongly expressed in neuromast progenitor cells, including those of the migrating lateral line primordium, the accessory cells that underlie the hair cells in neuromasts, and in interneuromastic cells that give rise to new neuromasts. Moreover, we find that most of the cells that proliferate within the neuromast during regeneration express this marker. Thus, our results describe the dynamics of hair cell regeneration in zebrafish and suggest the existence of at least two mechanisms for recovery of these cells in neuromasts.
Descriptors: zebrafish, DNA binding proteins genetics, proteins biosynthesis, hmgb proteins biosynthesis, hair cells physiology , lateral line system physiology, neurons physiology, stem cells physiology, transcription factors biosynthesis, genetically modified, antimetabolites diagnostic use, bromodeoxyuridine diagnostic use, cell proliferation drug effects, copper toxicity, DNA binding proteins genetics, green fluorescent proteins biosynthesis and genetics, hmgb hair cells drug effects, computer assisted image processing, immunohistochemistry, in situ hybridization, mitosis physiology, nerve regeneration physiology, stem cells drug effects, transcription factors genetics.

Higashijima, S., G. Mandel, and J.R. Fetcho (2004). **Distribution of prospective glutamatergic, glycinergic, and GABAergic neurons in embryonic and larval zebrafish.** *Journal of Comparative Neurology* 480(1): 1-18. ISSN: 0021-9967.
Abstract: Zebrafish are an excellent model for studies of the functional organization of neuronal circuits, but little is known regarding the transmitter phenotypes of the neurons in

their nervous system. We examined the distribution in spinal cord and hindbrain of neurons expressing markers of transmitter phenotype, including the vesicular glutamate transporter (VGLUT) genes for glutamatergic neurons, the neuronal glycine transporter (GLYT2) for glycinergic neurons, and glutamic acid decarboxylase (GAD65/67) for GABAergic neurons. All three markers were expressed in a large domain in the dorsal two-thirds of spinal cord, with additional, more ventral expression domains for VGLUT2 and GAD/GABA. In the large dorsal domain, dual in situ staining showed that GLYT2-positive cells were intermingled with VGLUT2 cells, with no dual-stained neurons. Many of the neurons in the dorsal expression domain that were positive for GABA markers at embryonic stages were also positive for GLYT2, suggesting that the cells might use both GABA and glycine, at least early in their development. The intermingling of neurons expressing inhibitory and excitatory markers in spinal cord contrasted markedly with the organization in hindbrain, where neurons expressing a particular marker were clustered together to form stripes that were visible running from rostral to caudal in horizontal sections and from dorsomedial to ventrolateral in cross sections. Dual labeling showed that the stripes of neurons labeled with one transmitter marker alternated with stripes of cells labeled for the other transmitter phenotypes. The differences in the distribution of excitatory and inhibitory neurons in spinal cord versus hindbrain may be tied to differences in their patterns of development and functional organization.

Descriptors: zebrafish embryos, central nervous system embryology, glutamic acid metabolism, glycine metabolism, neurons metabolism, gamma aminobutyric acid metabolism, amino acid transport systems, neutral metabolism, biological markers, central nervous system growth and development and metabolism, glutamate decarboxylase metabolism, glycine plasma membrane transport proteins, immunohistochemistry, isoenzymes metabolism, larva growth and development, larva metabolism, membrane transport proteins metabolism, molecular sequence data, neural inhibition physiology, neurons cytology , rhombencephalon embryology, growth, development and metabolism, amino acid sequence homology, nucleic acid sequence homology, spinal cord embryology, growth, development and metabolism, synaptic transmission physiology, vesicular glutamate transport protein 1.

Higashijima, S., M.A. Masino, G. Mandel, and J.R. Fetcho (2003). **Imaging neuronal activity during zebrafish behavior with a genetically encoded calcium indicator.** *Journal of Neurophysiology* 90(6): 3986-97. ISSN: 0022-3077.

Abstract: Genetically encoded calcium indicators, such as cameleon, have offered the promise of noninvasively monitoring activity of neurons, but no one has demonstrated whether these indicators can report calcium transients in neurons of behaving vertebrates. We show that cameleon can be expressed at high levels in sensory and spinal cord neurons in zebrafish by using neural-specific promoters in both transient expression experiments and in a stable transgenic line. Using standard confocal microscopy, calcium transients in identified motoneurons and spinal interneurons could be detected during escape behaviors produced by a touch on the head of the fish. Small movements of the restrained fish during the behavior did not represent a major problem for analyzing the calcium responses because of the ratiometric nature of cameleon. We conclude that cameleon can be used to noninvasively study the activity of neurons in an intact, behaving vertebrate. The ability to introduce an indicator genetically allows for studies of the functional roles of local interneurons that cannot easily be monitored with other approaches. Transgenic lines such as the one we generated can also be crossed into mutant lines of fish to study both structural and functional consequences of the mutations.

Descriptors: zebrafish spinal cord neurons, behavior physiology, calcium signaling physiol-

ogy, calcium binding proteins diagnostic use, *Drosophila* proteins diagnostic use, neurons physiology, genetically modified, calcium binding proteins biosynthesis, complementary DNA biosynthesis, complementary DNA genetics, *Drosophila* proteins biosynthesis, electric stimulation, electrophysiology escape reaction physiology, interneurons physiology, confocal microscopy, motor neurons physiology, patch clamp techniques, plasmids genetics.

Higashijima, S., M. Schaefer, and J.R. Fetcho (2004). **Neurotransmitter properties of spinal interneurons in embryonic and larval zebrafish.** *Journal of Comparative Neurology* 480(1): 19-37. ISSN: 0021-9967.

Abstract: Many classes of spinal interneurons in zebrafish have been described based on morphology, but their transmitter phenotypes are largely unknown. Here we combine back-filling or genetic labeling of spinal interneurons with in situ staining for markers of neurotransmitter phenotypes, including the vesicular glutamate transporter (VGLUT) genes for glutamatergic neurons, the neuronal glycine transporter (GLYT2) for glycinergic neurons, and glutamic acid decarboxylase (GAD) for GABAergic neurons. Neurons positive for VGLUT include the commissural CoPA, MCoD, UCoD, and some of the CoSA neurons. The CiD interneurons, which have ipsilateral descending axons, were also VGLUT-positive, as were the ventrally located VeMe interneurons, whose descending axonal trajectory has not been clearly revealed. Cells positive for GLYT2 include the commissural CoLAs as well as some of the CoBL and CoSA neurons. The CiA cells were the only GLYT2-positive cells with an ipsilateral axon. Cells staining for GAD included, most notably, the dorsal longitudinal ascending (DoLA) and KA interneurons. Our approach allowed us to define the likely transmitter phenotypes of most of the known classes of spinal interneurons. These data provide a foundation for understanding the functional organization of the spinal networks in zebrafish.

Descriptors: zebrafish embryology, interneurons metabolism, neurotransmitter agents metabolism, spinal cord embryology, amino acid transport systems, neutral metabolism, axons metabolism, axons ultrastructure, biological markers, functional laterality physiology, glutamate decarboxylase metabolism, glutamic acid metabolism, glycine metabolism, glycine plasma membrane transport proteins, green fluorescent proteins, immunohistochemistry, interneurons cytology, isoenzymes metabolism, larva cytology, larva growth and development, larva metabolism, membrane transport proteins metabolism, neural pathways cytology, embryology, growth and development, neurotransmitter agents genetics, messenger RNA metabolism, spinal cord cytology, spinal cord growth and development, vesicular glutamate transport protein 1, gamma aminobutyric acid metabolism.

Higashijima, S., M.A. Masino, G. Mandel, and J. Fetcho (2004). **Engrailed-1 expression marks a primitive class of inhibitory spinal interneuron**. *Journal of Neuroscience* 24(25): 5827-5839. ISSN: 0270-6474.

Descriptors: zebrafish embryos, developmental stages, engrailed-1 protein, evolution, fish larvae, freshwater fish, gene expression, glycine, inhibitors, interneurons, nervous system, neurons, spinal cord, transcription, transcription factors, *Danio rerio*, engrailed-1 expression, interneurons.

Hinsch, K. and G.K. Zupanc (2007). **Generation and long-term persistence of new neurons in the adult zebrafish brain: a quantitative analysis.** *Neuroscience* 146(2): 679-96. ISSN: 0306-4522.

NAL Call Number: QP351.N48

Abstract: Zebrafish, like other teleosts, are distinguished by their enormous potential to

produce new neurons in many parts of the adult brain. By labeling S-phase cells with the thymidine analog 5-bromo-2'-deoxyuridine (BrdU), quantitative analysis demonstrated that, on average, 6000 new cells were generated in the entire adult brain within any 30 min period. This corresponds to roughly 0.06% of the total number of brain cells. Part of these cells underwent a second round of cell division a few days after their generation so that 10 days post-BrdU administration, when the cells have exited the mitotic cycle, approximately 10,000 BrdU-labeled cells were present in the entire brain. At post-BrdU survival times of 446-656 days, on average 4600 BrdU-labeled cells were found, suggesting that approximately 46% of the cells present at 10 days persisted in the adult zebrafish brain. Combination of BrdU-labeling of mitotic cells with immunostaining against Hu showed that roughly 47% of the BrdU-labeled cells that persisted in the brain expressed this neuronal marker protein. Taken together, the results of this investigation demonstrate that at least half of the cells generated in the adult zebrafish brain develop into neurons and are likely to persist for the rest of the fish's life.

Descriptors: zebrafish, brain cytology, neurons cytology, neurons physiology, brain anatomy and histology, bromodeoxyuridine metabolism, cell count, cell differentiation, cell proliferation, hu paraneoplastic encephalomyelitis antigens metabolism, time factors.

Hong, S.K., C.E. Haldin, N.D. Lawson, B.M. Weinstein, I.B. Dawid, and N.A. Hukriede (2005). **The zebrafish kohtalo/trap230 gene is required for the development of the brain, neural crest, and pronephric kidney.** *Proceedings of the National Academy of Sciences of the United States of America* 102(51): 18473-8. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: Mutation of the gene encoding the Mediator component thyroid hormone receptor-associated protein (TRAP)230/MED12 affects the development of multiple systems in zebrafish embryogenesis. We isolated two ethylnitrosourea-induced alleles in the gene encoding this protein and named the locus kohtalo (kto) after the homologous locus in *Drosophila*. Homozygous kto mutant zebrafish embryos show defects in brain, neural crest, and kidney development and die at approximately 6 days postfertilization. In the affected tissues, differentiation is initiated and many cell type-specific genes are expressed, but there is a failure of morphogenesis and failure to complete differentiation. These results suggest that critical targets of TRAP230 function may include proteins important for cell mobility, cell sorting, and tissue assembly.

Descriptors: zebrafish embryos, brain embryology, *Drosophila* proteins metabolism, eye proteins metabolism, kidney embryology, neural crest embryology, transcription factors metabolism, base sequence, brain metabolism, molecular cloning, *Drosophila* eye developmental gene expression regulation, kidney metabolism, molecular sequence data, mutation genetics, neural crest metabolism, phenotype, transcription factors genetics.

Hua, J.Y., M.C. Smear, H. Baier, and S. Smith (2005). **Regulation of axon growth in vivo by activity-based competition**. *Nature* 434(7036): 1022-1026. ISSN: 0028-0836.

NAL Call Number: 472 N21

Descriptors: zebrafish, axon guidance, competition, development, electrophysiology, embryonic development, ganglia, growth regulators, neural networks, neurogenesis , neurons, neurosecretion, retinal ganglion cells, retinas, SNAp receptors, synaptogenesis , *Danio rerio.*

Hua, J.Y. and S.J. Smith (2004). **Neural activity and the dynamics of central nervous system development**. *Nature Neuroscience* 7(4): 327-332. ISSN: 1097 6256.

NAL Call Number: QP351

Abstract: Recent imaging studies show that the formation of neural connections in the central nervous system is a highly dynamic process. The iterative formation and elimination of synapses and neuronal branches result in the formation of a much larger number of trial connections than is maintained in the mature brain. Neural activity modulates development through biasing this process of formation and elimination, promoting the formation and stabilization of appropriate synaptic connections on the basis of functional activity patterns.
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Descriptors: zebrafish brain, nervous system development, neural activity, neuroimaging , imaging and microscopy techniques, synaptic connections.

Huang, L. and L. Li (2006). **Differential expression of voltage-activated calcium currents in zebrafish retinal ganglion cells.** *Journal of Neuroscience Research* 84(3): 497-504. ISSN: 0360-4012.
Abstract: We report a study on the characterization of voltage-activated calcium currents (I(Ca)) in retinal ganglion cells (RGCs) and the topographic distribution of RGCs that express different types of I(Ca) in zebrafish retinas. In acutely isolated zebrafish RGCs, both high-voltage-activated (HVA; peak activation potential +7.4 +/- 1.1 mV) and low-voltage-activated (LVA; peak activation potential -33.0 +/- 1.2 mV) I(Ca) were recorded. HVA I(Ca) were recorded in all of the tested RGCs, whereas LVA I(Ca) were recorded in approximately one-third of the tested cells. In RGCs that expressed both HVA and LVA I(Ca), the two currents were readily separated by depolarizing the cell membrane to different voltages from different holding potentials. Among RGCs that expressed LVA I(Ca), some cells expressed large LVA I(Ca) (up to 130 pA), whereas others expressed small LVA I(Ca) (approximately 20 pA). RGCs that expressed large and small LVA I(Ca) were designated as class I and class II cells, respectively, and RGCs that expressed only HVA I(Ca) were designated as class III cells. The topographic distribution of cell classes was similar in various areas of the retina. In the nasal-ventral retina, for example, class III cells outnumbered class I and class II cells by 10.8- and 2.6-fold, respectively. In the temporal and dorsal retinas, the density of class III cells slightly decreased, whereas the density of class I and class II cells increased. The differential expression of I(Ca) in RGCs may correlate with the development and function of the retina.
Descriptors: zebrafish retinas, action potentials physiology, calcium channels metabolism, calcium signaling physiology, cell membrane physiology, retinal ganglion cells metabolism, cultured cells, patch clamp techniques, retinal ganglion cells classification and cytology, visual pathways cytology, visual pathways physiology.

Huang, Y.Y., O. Rinner, P. Hedinger, S.C. Liu, and S.C. Neuhauss (2006). **Oculomotor instabilities in zebrafish mutant belladonna: a behavioral model for congenital nystagmus caused by axonal misrouting.** *Journal of Neuroscience* 26(39): 9873-80. ISSN: 0270-6474.
Abstract: A large fraction of homozygous zebrafish mutant belladonna (bel) larvae display a reversed optokinetic response (OKR) that correlates with failure of the retinal ganglion cells to cross the midline and form the optic chiasm. Some of these achiasmatic mutants display strong spontaneous eye oscillations (SOs) in the absence of motion in the surround. The presentation of a stationary grating was necessary and sufficient to evoke SO. Both OKR reversal and SO depend on vision and are contrast sensitive. We built a quantitative model derived from bel fwd (forward) eye behaviors. To mimic the achiasmatic condition, we reversed the sign of the retinal slip velocity in the model, thereby successfully reproducing both reversed OKR and SO. On the basis of the OKR data, and with the support of the quantitative model, we hypothesize that the reversed OKR and the SO can be completely attributed to RGC misrouting. The strong resemblance between the SO and congenital nystagmus (CN)

seen in humans with defective retinotectal projections implies that CN, of so far unknown etiology, may be directly caused by a projection defect.
Descriptors: zebrafish mutant larva, disease models, nerve tissue proteins deficiency, optokinetic nystagmus physiology, nystagmus, pathologic genetics, optic chiasm pathology, retinal ganglion cells pathology, proteins deficiency, axons pathology, computer simulation, contrast sensitivity genetics, contrast sensitivity physiology, genetic crosses, eye movements genetics and physiology, neurological models, morphogenesis genetics, motion perception physiology, nerve tissue optokinetic nystagmus genetics, nystagmus, pathologic congenital, nystagmus, pathologic pathology, photic stimulation.

Ichijo, H. (2004). **Chondroitin sulfate proteoglycans as axonal guidance cues in formation of retinotectal pathway**. *Anatomical Science International* 79(August): 205. ISSN: 1447 6959.
Descriptors: zebrafish, cell biology, development, nervous system, sense organs, retinotectal pathway, chrondroitin sulfate.

Jeong, J.Y., Z. Einhorn, S. Mercurio, S. Lee, B. Lau, M. Mione, S.W. Wilson, and S. Guo (2006). **Neurogenin1 is a determinant of zebrafish basal forebrain dopaminergic neurons and is regulated by the conserved zinc finger protein Tof/Fezl.** *Proceedings of the National Academy of Sciences of the United States of America* 103(13): 5143-8. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: The development of vertebrate basal forebrain dopaminergic (DA) neurons requires the conserved zinc finger protein Too Few (Tof/Fezl) in zebrafish. However, how Tof/Fezl regulates the commitment and differentiation of these DA neurons is not known. Proneural genes encoding basic helix-loop-helix transcription factors regulate the development of multiple neuronal lineages, but their involvement in vertebrate DA neuron determination is unclear. Here we show that neurogenin 1 (ngn1), a vertebrate proneural gene related to the *Drosophila* atonal, is expressed in and required for specification of DA progenitor cells, and when overexpressed leads to supernumerary DA neurons in the forebrain of zebrafish. Overexpression of ngn1 is also sufficient to induce tyrosine hydroxylase expression in addition to the pan-neuronal marker Hu in nonneural ectoderm. We further show that Tof/Fezl is required to establish basal forebrain ngn1-expressing DA progenitor domains. These findings identify Ngn1 as a determinant of brain DA neurons and provide insights into how Tof/Fezl regulates the development of these clinically important neuronal types.
Descriptors: zebrafish, basic helix loop helix transcription factors metabolism, carrier proteins metabolism, dopamine metabolism, nerve tissue proteins metabolism, neurons metabolism, prosencephalon cytology and metabolism, basic helix loop helix transcription factors genetics, carrier proteins chemistry and genetics, cell shape, conserved sequence, dopamine biosynthesis, ectoderm cytology and metabolism, developmental gene expression regulation, mutation genetics, nerve tissue neurons cytology, prosencephalon embryology, stem cells metabolism, tyrosine 3 monooxygenase metabolism, zinc fingers.

Jin, Z.G., X.H. Jing, J.W. Li, L. Zhang, and J. Jiang (2007). **Preliminary observation of acupoint-like and meridian-like structures on the body surface of the zebrafish**. *Zhongguo Zhen Jiu* 27(2): 117-9. ISSN: 0255-2930.
Abstract: OBJECTIVE: To probe whether there are acupoint-like and meridian-like structures on the body surface of the zebrafish. METHODS: Adult zebrafish were put into a 10 micromol/L DiA water solution and then anaesthetized for observation under a fluorescent microscope. RESULTS: The labeled substance appeared in round bright spots and some of

them gather together to form a cluster which distributed through the body surface orderly. The labeled clusters along the long axis of the body distributed in four lines, dorsal line, dorsal lateral line, lateral line and ventral line. The lateral line could be divided into two lines, lateral superior line and lateral inferior line; and the ventral line into ventral lateral line and ventral line based on their nerve connection under high power microscope. CONCLUSION: Sensory organs of the zebrafish distributed orderly and formed clusters appearing orderly in lines throughout the body surface, which are much like acupoints and meridians described in traditional Chinese medicine.
Descriptors: zebrafish, acupuncture points, meridians, zebrafish anatomy and histology.
Language of Text: Chinese.

Jontes, J.D. and S.J. Smith (2003). **Delivery of n - cadherin to developing synapses studied by in vivo 2 - photon time - lapse in the developing zebrafish spinal cord.** *Society for Neuroscience Abstract Viewer and Itinerary Planner* ISSN: 1558-3635.
Abstract: N-cadherin is a classical cadherin that is widely expressed in the central nervous system. It has been shown to be important in an array of morphogenetic processes, such as neural tube closure, as well as being implicated in others, such as neurite extension and synapse specificity. However, a number of basic questions about the function and behavior of N-cadherin remain unanswered, including the timing and mechanism of N-cadherin delivery to developing synapses and the importance of this delivery for synaptogenesis. To address these questions, we have cloned the zebrafish N-cadherin, made a number of deletion mutants that were fused to GFP and expressed these in developing zebrafish embryo using an islet-1 enhancer, which directs expression in Rohon-Beard neurons, primary sensory neurons present in the dorsal spinal cord. Specifically, we generated full-length N-cadherin-GFP, a GFP fusion that lacks the entire cytoplasmic domain, a deletion that lacks the beta-catenin binding site (while retaining the p120ctn binding site), and a deletion that lacks extracellular repeats 1-4, while retaining the signal sequence pre-pro region, transmembrane domain and cytoplasmic domains. We find that, along developing Rohon-Beard axons, N-cadherin is observed in 3 states: large, stable aggregates; small, discrete and mobile transport organelles; and large, elongated, transport structures that seem to be primarily targeted to the growth cone. In addition, most stable N-cadherin aggregates appear to originate from the growth cone and grow by capture of the small, discrete, transport packets. Our preliminary data also suggest that beta-catenin binding and, presumably, coupling to the actin cytoskeleton is not required for aggregation of N-cadherin at synapses or synaptic stability.
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Descriptors: zebrafish, cell biology, nervous system, spinal cord, N-cadherin, zebrafish.
Notes: AbstractNo.783.1.

Jontes, J.D., M.R. Emond, and S.J. Smith (2004). **In vivo trafficking and targeting of N-cadherin to nascent presynaptic terminals**. *Journal of Neuroscience* 24(41): 9027-9034. ISSN: 0270 6474.
Abstract: N-cadherin is a prominent component of developing and mature synapses, yet very little is known about its trafficking within neurons. To investigate N-cadherin dynamics in developing axons, we used in vivo two-photon time-lapse microscopy of N-cadherin - green fluorescent protein (Ncad - GFP), which was expressed in Rohon - Beard neurons of the embryonic zebrafish spinal cord. Ncad - GFP was present as either stable accumulations or highly mobile transport packets. The mobile transport packets were of two types: tubulovesicular structures that moved preferentially in the anterograde direction and discrete - punctate structures that exhibited bidirectional movement. Stable puncta of Ncad - GFP

accumulated in the wake of the growth cone with a time course. Colocalization of Ncad - GFP puncta with synaptic markers suggests that N-cadherin is a very early component of nascent synapses. Expression of deletion mutants revealed a potential role of the extracellular domain in appropriate N-cadherin trafficking and targeting. These results are the first to characterize the trafficking of a synaptic cell-adhesion molecule in developing axons in vivo. In addition, we have begun to investigate the cell biology of N-cadherin trafficking and targeting in the context of an intact vertebrate embryo. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, nerve synapse, N-cadherin, development, nervous system, two photon time lapse microscopy, imaging and microscopy techniques.

Jonz, M.G. and C.A. Nurse (2006). **Epithelial mitochondria-rich cells and associated innervation in adult and developing zebrafish.** *Journal of Comparative Neurology* 497(5): 817-32. ISSN: 0021-9967.
Abstract: Studies of ion regulation by mitochondria-rich cells (MRCs) of transport epithelia in fish have revealed many processes by which ion homeostasis is achieved. However, the control of these mechanisms and, particularly, the extent of nervous system involvement are not completely understood. We characterized the potential innervation of MRCs in various gill and extrabranchial tissues involved in ion transport in the model vertebrate the zebrafish. Confocal and conventional microscopy of whole-mount preparations were combined with immunofluorescence techniques to label MRCs with antibodies against a subunit of the enzyme Na(+)/K(+)-ATPase and nerve fibers with a zebrafish neuronal marker, zn-12. MRCs of the gill filaments were identified by their morphology and migration out to the lamellae in response to ion-poor water acclimation. Gill MRCs were intimately associated with nerve fibers originating from outside the filaments. MRCs of the opercular epithelium resembled those of the gill and were also located adjacent to nerve fibers. Mitochondria-rich "pseudobranch cells" were identified in the pseudobranch by immunofluorescence and labeling of dissociated cells with the mitochondrial marker DASPEI. Pseudobranch MRCs resembled gill MRCs and received innervation from a dense network of nerve fibers. In larvae, MRCs were distributed across the surface of the skin. These cells were situated among a dense network of varicose nerve fibers, and some MRCs of the skin displayed extensive cytoplasmic processes. Evidence is presented suggestive of widespread association of MRCs with the nervous system in transport epithelia and the neural control of MRC-mediated ion regulation in teleost fish.
Descriptors: zebrafish, epithelium innervation, gills innervation, ion transport physiology, skin innervation, epithelial cells cytology and metabolism, epithelium metabolism, gills cytology and metabolism, mitochondria metabolism, Na+ K+ exchanging ATPase metabolism, skin cytology and metabolism, zebrafish growth and development.

Kanungo, J., B.S. Li, M. Goswami, Y.L. Zheng, R. Ramchandran, and H.C. Pant (2007). **Cloning and characterization of zebrafish (*Danio rerio*) cyclin-dependent kinase 5.** *Neuroscience Letters* 412(3): 233-8. ISSN: 0304-3940.
NAL Call Number: QP351.N3
Abstract: Cyclin-dependent kinase 5 (cdk5) is a ubiquitous protein activated by neuron-specific activators, p35 and p39. Cdk5 regulates neuronal migration, differentiation, axonogenesis, synaptic transmission and apoptosis. However, its role in primary neurogenesis remains unexplored. Here, we have cloned and characterized the zebrafish cdk5 ortholog. Zebrafish cdk5 is 96% identical to its human counterpart. In situ hybridization analyses demonstrated that zebrafish cdk5 transcripts are ubiquitously expressed as early as the blastula stage. At 11.5h of development, cdk5 transcripts were present in the neural plate at the

domains where primary neurons begin to be specified. RT-PCR analyses showed equal levels of cdk5 transcripts up to 72 h of development. SiRNA-mediated cdk5 knockdown resulted in a reduction in primary sensory neurons of the trigeminal ganglia of the peripheral nervous system, suggesting that cdk5 plays a crucial role in the development of the peripheral nervous system.

Descriptors: zebrafish embryos, cyclin dependent kinase 5 genetics and metabolism, developmental gene expression regulation physiology, molecular cloning, embryonic development drug effects and physiology, developmental gene expression regulation drug effects, in situ hybridization methods, mice, small interfering RNA pharmacology.

Kanungo, J., B.S. Li, Y. Zheng, and H.C. Pant (2006). **Cyclin-dependent kinase 5 influences Rohon-Beard neuron survival in zebrafish.** *Journal of Neurochemistry* 99(1): 251-9. ISSN: 0022-3042.

NAL Call Number: QP351.J6

Abstract: Cyclin-dependent kinase 5 (cdk5), a member of the cyclin-dependent kinase family, is expressed predominantly in post-mitotic cell populations. Unlike the other cdks, cdk5 is abundant and most active in differentiated neurons. Here, we describe the function of a cdk5 ortholog in zebrafish. Cdk5 catalytic activity is meager but present in early stages of development. However, at 24 h post-fertilization (hpf), the activity is remarkably higher and continues to be high through 48 and 72 hpf. Knocking down cdk5 by micro-injection of a specific siRNA resulted in decreased cdk5 protein level accompanied by reduced kinase activity. In the cdk5 siRNA-injected embryos, the number of primary sensory Rohon-Beard (RB) neurons was significantly reduced and there were more apoptotic cells in the brain. These phenotypes were rescued by co-injection of cdk5 mRNA. Within the first two days of development, RB neurons undergo apoptosis in zebrafish. To examine whether cdk5 has a role in RB neuron survival, cdk5 mRNA was injected into the one- to two-cell embryos. In these embryos, RB neuron apoptosis was inhibited compared with the uninjected control embryos. These results suggest that in zebrafish, cdk5 influences RB neuron survival and potentially regulates early neuronal development.

Descriptors: zebrafish embryos, cyclin dependent kinase 5 genetics and metabolism, neurons cytology, neurons enzymology, cell death and survival, cytology, embryonic development, developmental gene expression regulation, immunohistochemistry, small interfering RNA genetics.

Kashyap, B., L.C. Frederickson, and D.L. Stenkamp (2007). **Mechanisms for persistent microphthalmia following ethanol exposure during retinal neurogenesis in zebrafish embryos.** *Visual Neuroscience* 24(3): 409-21. ISSN: 0952-5238.

Abstract: The exposure of the developing human embryo to ethanol results in a spectrum of disorders involving multiple organ systems, including the visual system. One common phenotype seen in humans exposed to ethanol in utero is microphthalmia. The objective of this study was to describe the effects of ethanol during retinal neurogenesis in a model organism, the zebrafish, and to pursue the potential mechanisms by which ethanol causes microphthalmia. Zebrafish embryos were exposed to 1% or 1.5% ethanol from 24 to 48 h after fertilization, a period during which the retinal neuroepithelium undergoes rapid proliferation and differentiation to form a laminated structure composed of different retinal cell types. Ethanol exposure resulted in significantly reduced eye size immediately following the treatment, and this microphthalmia persisted through larval development. This reduced eye size could not entirely be accounted for by the accompanying general delay in embryonic development. Retinal cell death was only slightly higher in ethanol-exposed embryos, although

cell death in the lens was extensive in some of these embryos, and lenses were significantly reduced in size as compared to those of control embryos. The initiation of retinal neurogenesis was not affected, but the subsequent waves of cell differentiation were markedly reduced. Even cells that were likely generated after ethanol exposure--rod and cone photoreceptors and Muller glia--were delayed in their expression of cell-specific markers by at least 24 h. We conclude that ethanol exposure over the time of retinal neurogenesis resulted in persistent microphthalmia due to a combination of an overall developmental delay, lens abnormalities, and reduced retinal cell differentiation.

Descriptors: zebrafish, cell proliferation drug effects, central nervous system depressants toxicity, drug effects, ethanol toxicity, neurons drug effects, retina pathology, thalamus abnormalities, thalamus drug effects, thalamus embryology, drug induced abnormalities, cell count methods, drug dose response relationship, pathology, embryonic development drug effects, eye proteins metabolism, developmental gene expression regulation drug effects, histones metabolism, homeodomain proteins metabolism, in situ nick end labeling methods, neurons physiology, paired box transcription factors metabolism, repressor proteins metabolism, time factors.

Kaslin, J., J.M. Nystedt, M. Ostergard, N. Peitsaro, and P. Panula (2004). **The orexin/hypocretin system in zebrafish is connected to the aminergic and cholinergic systems.** *Journal of Neuroscience* 24(11): 2678-89. ISSN: 0270-6474.

Abstract: The orexin/hypocretin (ORX) system is involved in physiological processes such as feeding, energy metabolism, and the control of sleep and wakefulness. The ORX system may drive the aminergic and cholinergic activities that control sleep and wakefulness states because of the ORX fiber projections to the aminergic and cholinergic cell clusters. The biological mechanisms and relevance of the interactions between these neurotransmitter systems are poorly understood. We studied these systems in zebrafish, a model organism in which it is possible to simultaneously study these systems and their interactions. We cloned a zebrafish prepro-ORX gene that encodes for the two functional neuropeptides orexin-A (ORX-A) and orexin-B (ORX-B). The prepro-ORX gene of the zebrafish consisted of one exon in contrast to mammals. The sequence of the ORX-A peptide of the zebrafish was less conserved than the ORX-B peptide compared with other vertebrates. By using in situ hybridization and immunohistochemistry, we found that the organization of the ORX system of zebrafish was similar to the ORX system in mammals, including a hypothalamic cell cluster and widespread fiber projections. The ORX system of the zebrafish showed a unique characteristic with an additional putatively ORX-containing cell group. The ORX system innervated several aminergic nuclei, raphe, locus ceruleus, the mesopontine-like area, dopaminergic clusters, and histaminergic neurons. A reciprocal relationship was found between the ORX system and several aminergic systems. Our results suggest that the architecture of these neurotransmitter systems is conserved in vertebrates and that these neurotransmitter systems in zebrafish may be involved in regulation of states of wakefulness and energy homeostasis by similar mechanisms as those in mammals.

Descriptors: zebrafish, acetylcholine metabolism, carrier proteins metabolism, dopamine metabolism , histamine metabolism, intracellular signaling peptides and proteins, neuropeptides metabolism, protein precursors metabolism, amino acid sequence, antibody specificity, carrier cholinergic fibers metabolism, molecular cloning, hypothalamus metabolism, immunohistochemistry, models, molecular sequence data, neural pathways metabolism, neurons cytology and metabolism, neuropeptides genetics, neurotransmitter agents metabolism, norepinephrine metabolism, protein precursors genetics, messenger RNA metabolism, serotonin metabolism, tyrosine 3 monooxygenase biosynthesis.

Kato, S., T. Nakagawa, M. Ohkawa, K. Muramoto, O. Oyama, A. Watanabe, H. Nakashima, T. Nemoto, and K. Sugitani (2004). **A computer image processing system for quantification of zebrafish behavior.** *Journal of Neuroscience Methods* 134(1): 1-7. ISSN: 0165-0270.
NAL Call Number: RC346
Abstract: The tropical zebrafish (*Danio rerio*) has frequently been used for investigating developmental biology. Here, we developed a computer image processing system for quantifying zebrafish behavior. We could acquire an image of zebrafish freely moving in an aquarium using a CCD camera through a graphic I/O board. To acquire the image of moving zebrafish in real time, we required high spatial (256 x 256 pixels) and temporal (10 frames/s) resolution. Such a high speed of data analysis was accomplished using a skipping search method. By using a small aquarium, trackings of newborn zebrafish could be traced. The velocity of adult zebrafish (7.2 cm/s) was far faster than that of newborn zebrafish (1.8 cm/s). Furthermore, by separation of occluded images of two fish, we could acquire images of the two zebrafish. They behaved as in a school in which one fish chased the other. The chasing was defined by the distance, angle and approach of the two fishes. The chasing ratio of pairs of zebrafish was 37%, whereas those of pairs of different fish were significantly reduced to less than 20%. The present image processing system is a very useful tool for quantitatively scoring the schooling behavior of multiple fish.
Descriptors: zebrafish, behavior physiology, computer assisted image processing methods, swimming physiology, spatial behavior physiology.

Kennedy, M.J., F.A. Dunn, and J.B. Hurley (2004). **Visual pigment phosphorylation but not transducin translocation can contribute to light adaptation in zebrafish cones.** *Neuron* 41(6): 915-28. ISSN: 0896-6273.
Abstract: The ability of cone photoreceptors to adapt to light is extraordinary. In this study we evaluated two biochemical processes, visual pigment phosphorylation and transducin translocation, for their ability to contribute to light adaptation in zebrafish cones. Since cytoplasmic Ca2+ regulates light adaptation, the sensitivities of these processes to both light and Ca2+ were examined. Cytoplasmic Ca2+ regulates the sites of light-stimulated phosphorylation. Unexpectedly, we found that Ca2+ also regulates the extent of phosphorylation of unbleached cone pigments. Immunocytochemical analyses revealed that neither light nor cytoplasmic Ca2+ influences the localization of transducin in zebrafish cones.
Descriptors: zebrafish, adaptation, ocular physiology, retina cones metabolism, phototransduction physiology, retinal pigments metabolism, transducin metabolism, adaptation, ocular drug effects, calcium metabolism and pharmacology, calcium signaling drug effects, calcium signaling physiology, retina cones cytology and drug effects, cytoplasm drug effectsand metabolism, immunohistochemistry, light, mutation genetics, opsin genetics and metabolism, organ culture techniques, phosphorylation drug effects and radiation effects, photic stimulation, phototransduction radiation effects, protein transport physiology and radiation effects, retinal pigments genetics.

Kim, M.J., I.H. Liu, Y. Song, J.A. Lee, W. Halfter, R.J. Balice Gordon, E. Linney, and G.J. Cole (2007). **Agrin is required for posterior development and motor axon outgrowth and branching in embryonic zebrafish.** *Glycobiology* 17(2): 231-47. ISSN: 0959-6658.
NAL Call Number: QP552.G59G593
Abstract: Although recent studies have extended our understanding of agrin's function during development, its function in the central nervous system (CNS) is not clearly understood. To address this question, zebrafish agrin was identified and characterized. Zebrafish agrin is expressed in the developing CNS and in nonneural structures such as somites and

notochord. In agrin morphant embryos, acetylcholine receptor (AChR) cluster number and size on muscle fibers at the choice point were unaffected, whereas AChR clusters on muscle fibers in the dorsal and ventral regions of the myotome were reduced or absent. Defects in the axon outgrowth by primary motor neurons, subpopulations of branchiomotor neurons, and Rohon-Beard sensory neurons were also observed, which included truncation of axons and increased branching of motor axons. Moreover, agrin morphants exhibit significantly inhibited tail development in a dose-dependent manner, as well as defects in the formation of the midbrain-hindbrain boundary and reduced size of eyes and otic vesicles. Together these results show that agrin plays an important role in both peripheral and CNS development and also modulates posterior development in zebrafish.
Descriptors: zebrafish embryos, agrin physiology, motor neurons physiology, nervous system embryology, agrin analysis and genetics, axons chemistry and physiology, cell differentiation embryonic development genetics, motor neurons chemistry and cytology, skeletal muscle embryology and muscle innervation, nervous system chemistry, messenger RNA analysis and metabolism, cholinergic receptors analysis and metabolism, zebrafish abnormalities , proteins analysis.

Kim, Y.J., R.H. Nam, Y.M. Yoo, and C.J. Lee (2004). **Identification and functional evidence of GABAergic neurons in parts of the brain of adult zebrafish (*Danio rerio*).** *Neuroscience Letters* 355(1-2): 29-32. ISSN: 0304-3940.
NAL Call Number: QP351.N3
Abstract: The distribution of GABA-containing neurons was studied in the brain of the adult zebrafish by Nissl staining and immunohistochemistry. GABA immunoreactivity (GABA-IR) was demonstrated in parts of the brain such as olfactory bulb (OB), telencephalon, tectum stratum, and in the hypothalamus. GABA-IR appeared in the area where Nissl-stained cell bodies were abundant. The internal cellular layer of the OB was most densely stained by Nissl staining, and also showed a high level of GABA-IR. The telencephalon and the hypothalamus revealed a similar pattern to the OB in terms of Nissl staining and GABA-IR. However, the distribution and shape of stained cells of the tectum stratum were distinct from those in other regions: Nissl-stained neurons were ubiquitously present throughout all cellular layers including the stratum griseum centrale, the stratum album centrale (SAC), and the stratum periventriculare (SP). However, GABA-IR was weakly expressed in a limited number of neurons only in the SAC and SP. Whether GABA serves as an inhibitory neurotransmitter was also tested in the isolated telencephalon preparation by using extracellular field potential recordings. The synaptic activity recorded in the posterior dorsal telencephalon in response to the electrical stimulation of the anterior dorsal telencephalon was increased in the presence of the GABAA receptor antagonist, BMI, suggesting an inhibitory role for GABA-immunoreactive neurons in the adult brain of the zebrafish.
Descriptors: zebrafish embryos, brain metabolism, neurons metabolism, gamma aminobutyric acid metabolism, action potentials drug effects, action potentials physiology, brain cytology, GABA antagonists pharmacology, immunohistochemistry, neural inhibition drug effects, neural inhibition physiology, neurons cytology, GABA A receptors antagonists, inhibitors and metabolism, superior colliculus cytology and metabolism, synaptic transmission drug effects and physiology, zebrafish anatomy and histology.

Kirby, B.B., N. Takada, A.J. Latimer, J. Shin, T.J. Carney, R.N. Kelsh, and B. Appel (2006). **In vivo time-lapse imaging shows dynamic oligodendrocyte progenitor behavior during zebrafish development.** *Nature Neuroscience* 9(12): 1506-11. ISSN: 1097-6256.
NAL Call Number: QP351

Abstract: Myelinating oligodendrocytes arise from migratory and proliferative oligodendrocyte progenitor cells (OPCs). Complete myelination requires that oligodendrocytes be uniformly distributed and form numerous, periodically spaced membrane sheaths along the entire length of target axons. Mechanisms that determine spacing of oligodendrocytes and their myelinating processes are not known. Using in vivo time-lapse confocal microscopy, we show that zebrafish OPCs continuously extend and retract numerous filopodium-like processes as they migrate and settle into their final positions. Process remodeling and migration paths are highly variable and seem to be influenced by contact with neighboring OPCs. After laser ablation of oligodendrocyte-lineage cells, nearby OPCs divide more frequently, orient processes toward the ablated cells and migrate to fill the unoccupied space. Thus, process activity before axon wrapping might serve as a surveillance mechanism by which OPCs determine the presence or absence of nearby oligodendrocyte-lineage cells, facilitating uniform spacing of oligodendrocytes and complete myelination.
Descriptors: zebrafish, cell differentiation physiology, cell movement physiology, oligodendroglia cytology, spinal cord cytology, stem cells cytology, genetically modified, cell lineage physiology, fluorescence microscopy, myelin sheath physiology, spinal cord growth and development.

Koester, R.W. and S.E. Fraser (2006). **FGF signaling mediates regeneration of the differentiating cerebellum through repatterning of the anterior hindbrain and reinitiation of neuronal migration**. *Journal of Neuroscience* 26(27): 7293-7304. ISSN: 0270 6474.
Abstract: To address the regenerative capability of the differentiating hindbrain, we ablated the cerebellum in wild-type and transgenic zebrafish embryos. These larvae showed no obvious locomotive malfunction several days after the ablation. Expression analysis and in vivo time-lapse recording in GFP (green fluorescent protein)-transgenic embryos indicate that cerebellar neuronal cells can regenerate from the remaining anterior hindbrain. The onset of regeneration is accompanied by repatterning within the anterior hindbrain. Inhibition of FGF signaling immediately after cerebellar ablation results in the lack of regenerating cerebellar neuronal cells and the absence of cerebellar structures several days later. Moreover, impaired FGF signaling inhibits the repatterning of the anterior hindbrain and the reexpression of rhombic lip marker genes soon after cerebellar ablation. This demonstrates that the hindbrain is highly plastic in recapitulating early embryonic differentiation mechanisms during regeneration. Moreover, the regenerating system offers a means to uncouple cerebellar differentiation from complex morphogenetic tissue rearrangements. Copyright©Thomson Reuters 2009
Descriptors: transgenic zebrafish embryo larva, molecular genetics, nervous system, neural coordination, GFP, neuronal migration, FGF signaling.

Korsching, S. (2005). **Selective imaging of the receptor neuron population in the olfactory bulb of zebrafish and mice.** *Chemical Senses* 30(Suppl 1): I101-I102. ISSN: 0379 864X.
NAL Call Number: QP456.C5
Descriptors: zebrafish, senses, smell, neurons, methods, imaging, mice.

Kotradi, S.M. and Q. Liu (2003). **Functional analysis of R-cadherin in developing zebrafish (*Danio rerio*) retinal ganglion cells**. *Ohio Journal of Science* 103(1): A7. ISSN: 0030-0950.
Descriptors: zebrafish embryos, adhesion, antibodies, body size, central nervous system, embryonic development, freshwater fish, functional analysis, ganglia, retinas, *Danio rerio.*

Krueckl, S.L., E.A. Fradinger, and N.M. Sherwood (2003). **Developmental changes in the expression of growth hormone-releasing hormone and pituitary adenylate cyclase-activating polypeptide in zebrafish.** *Journal of Comparative Neurology* 455(3): 396-405. ISSN: 0021-9967.

Descriptors: zebrafish, brain growth and development, growth hormone releasing hormone metabolism, neuropeptides metabolism, base sequence, blastula metabolism, brain embryology, gastrula metabolism, growth hormone releasing hormone chemistry, isolation and purification, in situ hybridization, molecular sequence data, neuropeptides chemistry, neuropeptides isolation and purification, pituitary adenylate cyclase activating polypeptide, vasoactive intestinal peptide receptors, reverse transcriptase PCR.

Kucenas, S., Z. Li, J.A. Cox, T.M. Egan, and M.M. Voigt (2003). **Molecular characterization of the zebrafish P2X receptor subunit gene family**. *Neuroscience Oxford* 121(4): 935-945. ISSN: 0306 4522.

NAL Call Number: QP351.N48

Abstract: P2X receptors are non-selective cation channels gated by extracellular ATP and are encoded by a family of seven subunit genes in mammals. These receptors exhibit high permeabilities to calcium and in the mammalian nervous system they have been linked to modulation of neurotransmitter release. Previously, three complementary DNAs (cDNAs) encoding members of the zebrafish gene family have been described. We report here the cloning and characterization of an additional six genes of this family. Sequence analysis of all nine genes suggests that six are orthologs of mammalian genes, two are paralogs of previously described zebrafish subunits, and one remains unclassified. All nine subunits were physically mapped onto the zebrafish genome using radiation hybrid analysis. Of the nine gene products, seven give functional homo-oligomeric receptors when recombinantly expressed in human embryonic kidney cell line 293 cells. In addition, these subunits can form hetero-oligomeric receptors with phenotypes distinct from the parent subunits. Analysis of gene expression patterns was carried out using in situ hybridization, and seven of the nine genes were found to be expressed in embryos at 24 and 48 h post-fertilization. Of the seven that were expressed, six were present in the nervous system and four of these demonstrated considerable overlap in cells present in the sensory nervous system. These results suggest that P2X receptors might play a role in the early development and/or function of the sensory nervous system in vertebrates. Copyright © 2008 CABI

Descriptors: zebrafish, biochemical receptors, characterization, complementary DNA, DNA cloning, gene expression, genes, genomes, nervous system, nucleotide sequences, *Danio rerio.*

Kucenas, S., F. Soto, J.A. Cox, and M.M. Voigt (2006). **Selective labeling of central and peripheral sensory neurons in the developing zebrafish using P2X(3) receptor subunit transgenes.** *Neuroscience* 138(2): 641-52. ISSN: 0306-4522.

NAL Call Number: QP351.N48

Abstract: The two paralogous P2X receptor subunit genes p2rx3.1 and p2rx3.2 are selectively expressed in overlapping, but unique, patterns of sensory neurons in the developing zebrafish. We constructed a series of transgenes derived from both genes using the recombineering technique. Transgenes utilizing either enhanced green fluorescent protein or monomeric red fluorescent protein-1 were shown to be expressed with the same spatial and temporal patterns as the native genes. The p2rx3.1-derived transgenes labeled the vast majority of the Rohon-Beard neurons in the spinal cord and neurons of the trigeminal ganglia. The p2rx3.2-derived transgene labeled fewer Rohon-Beard and trigeminal neurons than what was observed for the p2rx3.1-derived transgenes, but was also detected in neurons of the epibran-

chial ganglia. Three distinct populations of sensory neurons were detected: those expressing only one or the other paralog, and those expressing both paralogs. The fluorescent proteins encoded by the transgenes allowed for visualization of the neuronal somas as well as their peripheral and central projections. These reagents should prove extremely useful in providing the basis for future studies aimed at elucidating the developmental and physiological attributes of sensory neurons.
Descriptors: zebrafish embryos, central nervous system physiology, neurons physiology, peripheral nerves physiology, purinergic p2 receptor genetics, spinal cord physiology, genetically modified, physiology, developmental gene expression regulation, genetic engineering, genetic markers, green fluorescent larva, promoter regions genetics.

Kucenas, S., N. Takada, H.-C. Park, E. Woodruff, K. Broadie, and B. Appel (2008). **CNS-derived glia ensheath peripheral nerves and mediate motor root development**. *Nature Neuroscience* 11(2): 143-151. ISSN: 1097-6256.
NAL Call Number: QP351
Descriptors: zebrafish, axon guidance, central nervous system, computed tomography, development, embryos, glia, motor neurons, peripheral nerves, Schwann cells, spinal cord, *Danio rerio*.

Lam, C.S., V. Korzh, and U. Strahle (2005). **Zebrafish embryos are susceptible to the dopaminergic neurotoxin MPTP.** *European Journal of Neuroscience* 21(6): 1758-62. ISSN: 0953 816X.
Abstract: The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces selective loss of dopaminergic neurons in the mammalian midbrain, eliciting symptoms characteristic of Parkinson's disease. By exploiting the advantages of zebrafish embryos, we report here that dopaminergic neurons in this species are specifically perturbed when exposed to MPTP. In contrast to mammals, the zebrafish does not possess a midbrain dopaminergic system. Instead, the main population of neurons expressing the dopamine transporter is located in the posterior tuberculum of the diencephalon. Exposure of embryos to MPTP led to a pronounced reduction in the number of dopaminergic cells in the diencephalon. This effect can be reversed by deprenyl, a specific inhibitor of monoamine oxidase B that catalyses the conversion of MPTP to its active metabolite, MPP+. Similarly, direct treatment of embryos with MPP+ abolished the diencephalic dopaminergic neurons. These larvae also demonstrated behavioural defects in swimming responses. Thus, dopaminergic neurons in the posterior tuberculum of the zebrafish may be homologous to the midbrain dopaminergic system of mammals. In addition, the mechanism behind the loss of dopaminergic neurons following pharmacological perturbation may be conserved among vertebrates and suggest that the zebrafish can be used as a convenient and economical system to study the pathogenesis of Parkinson's disease and for testing potential therapeutic strategies.
Descriptors: zebrafish embryos, 1 methyl 4 phenyl 1,2,3,6 tetrahydropyridine pharmacology, dopamine metabolism, drug effects, motor activity drug effects, disease susceptibility chemically induced, metabolism, MPTP poisoning metabolism, motor activity physiology.

Lam, C.S., S. Rastegar, and U. Strahle (2006). **Distribution of cannabinoid receptor 1 in the CNS of zebrafish.** *Neuroscience* 138(1): 83-95. ISSN: 0306-4522.
NAL Call Number: QP351.N48
Abstract: The cannabinoid receptor 1 (Cb1) mediates the psychoactive effect of marijuana. In mammals, there is abundant evidence advocating the importance of cannabinoid signaling; activation of Cb1 exerts diverse functions, chiefly by its ability to modulate neurotransmission. Thus, much attention has been devoted to understand its role in health and

disease and to evaluate its therapeutic potential. Here, we have cloned zebrafish cb1 and investigated its expression in developing and adult zebrafish brain. Sequence analysis showed that there is a high degree of conservation, especially in residues demonstrated to be critical for function in mammals. In situ hybridization revealed that zebrafish cb1 appears first in the preoptic area at 24 hours post-fertilization. Subsequently, transcripts are detected in the dorsal telencephalon, hypothalamus, pretectum and torus longitudinalis. A similar pattern of expression is recapitulated in the adult brain. While cb1 is intensively stained in the medial zone of the dorsal telencephalon, expression elsewhere is weak by comparison. In particular, localization of cb1 in the telencephalic periventricular matrix is suggestive of the involvement of Cb1 in neurogenesis, bearing strong resemblance in terms of expression and function to the proliferative mammalian hippocampal formation. In addition, a gradient-like expression of cb1 is detected in the torus longitudinalis, a teleost specific neural tissue. In relation to dopaminergic neurons in the diencephalic posterior tuberculum (considered to be the teleostean homologue of the mammalian midbrain dopaminergic system), both cb1 and tyrosine hydroxylase-expressing cells occupy non-overlapping domains. However there is evidence that they are co-localized in the caudal zone of the hypothalamus, implying a direct modulation of dopamine release in this particular region. Collectively, our data indicate the propensity of zebrafish cb1 to participate in multiple neurological processes.

Descriptors: zebrafish, central nervous system metabolism, cannabinoid receptor, cb1 metabolism, amino acid sequence, brain chemistry drug effects and physiology, complementary DNA biosynthesis and genetics, diencephalon metabolism and physiology, in situ hybridization, larva, mesencephalon metabolism, molecular sequence data, open reading frames physiology, cannabinoid receptor, Cb1 genetics, telencephalon metabolism and physiology.

Larson, E.T., D.M. O'Malley, and R.H.J. Melloni (2006). **Aggression and vasotocin are associated with dominant-subordinate relationships in zebrafish.** *Behavioural Brain Research* 167(1): 94-102. ISSN: 0166 4328.

Abstract: Agonistic interactions are present throughout the animal kingdom as well as in humans. In this report, we present a model system to study neurological correlates of dominant-subordinate relationships. Zebrafish, *Danio rerio*, has been used as a model system for developmental biology for decades. We propose here that it is also an excellent model for studying social behavior. Adult male zebrafish were separated for 5 days and then pairs were formed and allowed to interact for 5 days. Under these conditions, aggression is prevalent and dominant-subordinate relationships are quickly established. Dominant behavior is characterized by a repeated pattern of chasing and biting, whereas subordinates engage in retreats. By day 5, the dominant-subordinate relationship was firmly established and there were differences in behavior over time. Chases, bites and retreats were all less frequent on day 5 of the social interaction than on day 1. Arginine vasotocin is the teleostean homologue of arginine vasopressin, a neuropeptide whose expression has been linked to aggression and social position in mammals. Immunohistochemistry indicated differences in vasotocin staining between dominant and subordinate individuals. Dominant individuals express vasotocin in one to three pairs of large cells in the magnocellular preoptic area whereas subordinate individuals express vasotocin in 7-11 pairs of small cells in the parvocellular preoptic area. These results suggest that the vasotocinergic system may play a role in shaping dominant-subordinate relationships and agonistic behavior in this model organism.

Descriptors: zebrafish, aggression physiology, dominance subordination, social behavior, vasotocin physiology, analysis of variance, arginine vasopressin metabolism, behavior, cell

count, chi square distribution, gene expression regulation physiology, immunohistochemistry methods, neurons metabolism, telencephalon cytology and metabolism, time factors.

Lecaudey, V., I. Anselme, R. Dildrop, U. Ruther, and S. Schneider Maunoury (2005). **Expression of the zebrafish Iroquois genes during early nervous system formation and patterning.** *Journal of Comparative Neurology* 492(3): 289-302. ISSN: 0021-9967.
Descriptors: zebrafish, body patterning, central nervous system anatomy and histology and embryology, developmental gene expression regulation, homeodomain morphogenesis, molecular cloning, embryonic structures anatomy and histology and metabolism, homeodomain proteins metabolism, in situ hybridization, multigene family, neurons cytology, neurons metabolism, protein isoforms genetics and metabolism, stem cells cytology and metabolism.

Lee, J.S., S. Von der Hardt, M.A. Rusch, S.E. Stringer, H.L. Stickney, W.S. Talbot, R. Geisler, C. NUsslein-Volhard, S.B. Selleck, C.B. Chien, and H. Roehl (2004). **Axon sorting in the optic tract requires HSPG synthesis by ext2 (dackel) and extl3 (boxer)**. *Neuron* 44(6): 947-960. ISSN: 0896-6273.
Descriptors: zebrafish, axon guidance, biosynthesis, freshwater fish, ganglia, glycosyltransferase, heparan sulfate proteoglycans, molecular modelling, neurons, optic tectum, optic tract, optics, phenotypes, retinal ganglion cells, retinas, robo protein, *Danio rerio*.

Lefebvre, J.L., L. Jing, S. Becaficco, C. Franzini-Armstrong, and M. Granato (2007). **Differential requirement for MuSK and dystroglycan in generating patterns of neuromuscular innervation**. *Proceedings of the National Academy of Sciences, USA* 104(7): 2483-2488. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, dystroglycan, genetic diversity, innervation, muscles, neuromuscular junctions, neurophysiology, signal transduction, synapses, synaptogenesis, *Danio rerio.*

Levine, J., K. Panchalingam, R.J. McClure, S. Gershon, and J.W. Pettegrew (2003). **Effects of acetyl-L-carnitine and myo-inositol on high-energy phosphate and membrane phospholipid metabolism in zebra fish: a 31P-NMR-spectroscopy study.** *Neurochemical Research* 28(5): 687-90. ISSN: 0364-3190.
NAL Call Number: QP356.3 .N457
Abstract: Acetyl-L-carnitine (ALCAR) and myo-inositol are reported to enhance motor activity in animal models; modulate membrane phospholipid metabolism (ALCAR and myo-inositol) and high-energy phosphate metabolism (ALCAR) back to normal; and be effective treatments of major depression in humans. Fish in general and zebra fish in particular present unique animal models for the in vivo study of high-energy phosphate and membrane phospholipid metabolism by noninvasive in vivo 31P NMR. This 31P NMR study of free-swimming zebra fish showed that both ALCAR and myo-inositol decreased levels of phosphodiesters and inorganic orthophosphate and increased levels of PCr in the fish. These findings demonstrate both ALCAR and myo-inositol modulate membrane phospholipid and high-energy phosphate metabolism in free-swimming zebra fish.
Descriptors: zebrafish, acetylcarnitine pharmacology, inositol pharmacology, membrane lipids metabolism, phosphates metabolism, phospholipids metabolism, acetylcarnitine therapeutic use, depressive disorder drug therapy, magnetic resonance spectroscopy, motor activity drug effects, phosphocreatine metabolism, phosphoric acid esters metabolism, phosphorus, reference values.

Lewis, K.E. and J.S. Eisen (2003). **From cells to circuits: development of the zebrafish spinal cord.** *Progress in Neurobiology* 69(6): 419-49. ISSN: 0301-0082.

NAL Call Number: QP361.P7

Abstract: The ability of an animal to carry out its normal behavioral repertoire requires generation of an enormous diversity of neurons and glia. The relative simplicity of the spinal cord makes this an especially attractive part of the nervous system for addressing questions about the development of vertebrate neural specification and function. The last decade has witnessed an explosion in our understanding of spinal cord development and the functional interactions among spinal cord neurons and glia. Cellular, genetic, molecular, physiological and behavioral studies in zebrafish have all been important in providing insights into questions that remained unanswered by studies from other vertebrate model organisms. This is the case because many zebrafish spinal neurons can be individually identified and followed over time in living embryos and larvae. In this review, we discuss what is currently known about the cellular, genetic and molecular mechanisms involved in specifying distinct cell types in the zebrafish spinal cord and how these cells establish the functional circuitry that mediates particular behaviors. We start by describing the early signals and morphogenetic movements that form the nervous system, and in particular, the spinal cord. We then provide an overview of the cell types within the spinal cord and describe how they are specified and patterned. We begin ventrally with floor plate and proceed dorsally, through motoneurons and oligodendrocytes, interneurons, astrocytes and radial glia, spinal sensory neurons and neural crest. We next describe axon pathfinding of spinal neurons. Finally, we discuss the roles of particular spinal cord neurons in specific behaviors.

Descriptors: zebrafish embryos, cell communication physiology, nerve net embryology, spinal cord embryology, motor activity physiology, nerve net physiology, nervous system physiology, neural crest physiology, neuroglia physiology, neurons physiology, spinal cord physiology, zebrafish growth and development.

Li, J., J.A. Mack, M. Souren, E. Yaksi, S. Higashijima, M. Mione, J.R. Fetcho, and R.W. Friedrich (2005). **Early development of functional spatial maps in the zebrafish olfactory bulb.** *Journal of Neuroscience* 25(24): 5784-95. ISSN: 0270-6474.

Abstract: In the adult olfactory bulb (OB), particular chemical classes of odorants preferentially activate glomeruli within loosely defined regions, resulting in a coarse and fractured "chemotopic" map. In zebrafish, amino acids and bile acids predominantly stimulate glomeruli in the lateral and medial OB, respectively. We studied the development of these spatial response maps in zebrafish. At 3 d postfertilization (dpf), the OB contained protoglomerular structures that became refined and more numerous during subsequent days. In a transgenic zebrafish line expressing the Ca2+ indicator protein inverse pericam, mainly in mitral cells, odor responses in the OB were first detected at 2.5-3 dpf. Already at this stage, amino acids and bile acids evoked activity predominantly in the lateral and medial OB, respectively. Two-photon Ca2+ imaging using a synthetic indicator was used to reconstruct activity patterns at higher resolution in three dimensions. Responses to amino acids and bile acids were detected predominantly in the lateral and medial OB, respectively, with little overlap. Between 2.5 and 6 dpf, the number of odor-responsive units increased, but the overall spatial organization of activity persisted. Hence, a coarse spatial organization of functional activity maps is established very early during OB development when glomeruli are not yet differentiated. This spatial organization is maintained during development and growth of neuronal circuits and may have important functions for odor processing in larvae, for the differentiation of glomeruli, and for the refinement of activity maps at later developmental stages.

Descriptors: zebrafish, olfactory bulb anatomy and histology and physiology, genetically

modified, green fluorescent proteins analysis and genetics, odors, olfactory bulb growth and development, smell.

Li, Q., K. Shirabe, C. Thisse, B. Thisse, H. Okamoto, I. Masai, and J.Y. Kuwada (2005). **Chemokine signaling guides axons within the retina in zebrafish.** *Journal of Neuroscience* 25(7): 1711-17. ISSN: 0270-6474.

Abstract: Chemokines are a large family of secreted proteins that play an important role in the migration of leukocytes during hematopoiesis and inflammation. Chemokines and their receptors are also widely distributed in the CNS. Although recent investigations are beginning to elucidate chemokine function within the CNS, relatively little is known about the CNS function of this important class of molecules. To better appreciate the CNS function of chemokines, the role of signaling by stromal cell-derived factor-1 (SDF-1) through its receptor, chemokine (CXC motif) receptor 4 (CXCR4), was analyzed in zebrafish embryos. The SDF-1/CXCR4 expression pattern suggested that SDF-1/CXCR4 signaling was important for guiding retinal ganglion cell axons within the retina to the optic stalk to exit the retina. Antisense knockdown of the ligand and/or receptor and a genetic CXCR4 mutation both induced retinal axons to follow aberrant pathways within the retina. Furthermore, retinal axons deviated from their normal pathway and extended to cells ectopically expressing SDF-1 within the retina. These data suggest that chemokine signaling is both necessary and sufficient for directing retinal growth cones within the retina.

Descriptors: zebrafish embryos, Cxc chemokines physiology, growth cones physiology, optic nerve cytology, Cxcr4 receptors physiology, retina ultrastructure, retinal ganglion cells cytology, signal transduction physiology, genetically modified, Cxc chemokines genetics, gene targeting, growth cones ultrastructure, mosaicism, oligodeoxyribonucleotides, antisense pharmacology, optic nerve embryology, phenotype, Cxcr4 receptors genetics, recombinant fusion, retina embryology.

Li, W., F. Ono, and P. Brehm (2003). **Optical measurements of presynaptic release in mutant zebrafish lacking postsynaptic receptors.** *Journal of Neuroscience* 23(33): 10467-74. ISSN: 0270-6474.

Abstract: Differentiation of presynaptic nerve terminals is mediated, in part, through contact with the appropriate postsynaptic target cell. In particular, studies using dissociated nerve and muscle derived from Xenopus embryos have indicated that the properties of transmitter release from motor neurons are altered after contact with skeletal muscle. This maturation of presynaptic function has further been linked to retrograde signaling from muscle that involves activation of postsynaptic ACh receptors. Using FM1-43 optical determinants of exocytosis, we now compare calcium-mediated exocytosis at neuromuscular junctions of wild-type zebrafish to mutant fish lacking postsynaptic ACh receptors. In response to either high-potassium depolarization or direct electrical stimulation, we observed no differences in the rate or extent of FM1-43 destaining. These data indicate that the acquisition of stimulus-evoked exocytosis at early developmental stages occurs independent of both postsynaptic receptor and synaptic responses in zebrafish.

Descriptors: mutant zebrafish, neuromuscular junction physiology, presynaptic terminals metabolism, cholinergic receptors deficiency, synaptic transmission physiology, calcium metabolism , cell differentiation physiology, electric stimulation, exocytosis physiology, fluorescent dyes pharmacokinetics, mutation, neuromuscular junction drug effects and metabolism, optics, potassium pharmacology, cholinergic receptors genetics, synaptic transmission drug effects, synaptic vesicles metabolism.

Lieberoth, B.C., C.G. Becker, and T. Becker (2003). **Double labeling of neurons by retrograde axonal tracing and non-radioactive in situ hybridization in the CNS of adult zebrafish.** *Methods in Cell Science* 25(1-2): 65-70. ISSN: 1381-5741.

NAL Call Number: QH573.T52

Abstract: A number of genes affecting axonal projections are currently being identified in zebrafish mutant screens. Analyzing the expression of these genes in the adult brain in relation to specific neuronal populations could yield insights into new functional contexts, such as the successful axonal regeneration in adult zebrafish. Here, we provide a relatively simple procedure for non-radioactive in situ hybridization in sections of adult zebrafish brains in combination with retrograde axonal tracing using the fluorescent neuronal tracer rhodamine dextran amine (RDA). A lesion is inflicted on the spinal cord of adult zebrafish and a crystal of RDA is then applied to the lesion site resulting in retrograde labeling of neurons in the brain through their spinal axons. Six to eighteen days later fish are perfusion-fixed, and in situ hybridization is carried out on vibratome-cut floating sections using a protocol simplified from that used for whole-mounted zebrafish embryos. This procedure leads to robust double labeling of axotomized neurons with RDA and an in situ hybridization signal for the growth-associated protein 43 (GAP-43). This method can be used to identify gene expression in specific populations of projection neurons and to detect changes in gene expression in axotomized neurons in the CNS of adult zebrafish.

Descriptors: zebrafish, axons metabolism, brain metabolism, gap 43 protein metabolism, in situ hybridization, rhodamines chemistry.

Liu, K.S., M. Gray, S.J. Otto, J.R. Fetcho, and C.E. Beattie (2003). **Mutations in deadly seven/ Notch1a reveal developmental plasticity in the escape response circuit**. *Journal of Neuroscience* 23(22): 8159-8166. ISSN: 0270-6474.

Descriptors: zebrafish, calcium imaging, escape behavior, escapement, evolution, fish larvae, genes, kinematics, Mauthner cells, mutations, neurophysiology, plasticity, plasticity (behavioral), protective behavior, startle response, *Danio rerio.*

Liu, Q. and R.L. Londraville (2003). **Using the adult zebrafish visual system to study cadherin-2 expression during central nervous system regeneration.** *Methods in Cell Science* 25(1-2): 71-8. ISSN: 1381-5741.

NAL Call Number: QH573.T52

Abstract: Zebrafish is a good model organism for the study of vertebrate development due to numerous experimental advantages. Here we show that zebrafish are also suitable for the study of cellular and molecular mechanisms underlying tissue regeneration in the vertebrate central nervous system. In this report we describe simple protocols to introduce injuries to the adult zebrafish visual structures and analyze cadherin-2 expression in the regenerating visual system using immunocytochemistry and immunoblotting.

Descriptors: zebrafish, cadherins metabolism, eye metabolism, nerve regeneration physiology, retina metabolism, brain metabolism, eye physiopathology, optic nerve metabolism and physiology.

Liu, Q., J.A. Marrs, E. Azodi, A.E. Kerstetter, S.G. Babb, and L. Hashmi (2004). **Differential expression of cadherins in the developing and adult zebrafish olfactory system.** *Journal of Comparative Neurology* 478(3): 269-81. ISSN: 0021-9967.

Descriptors: zebrafish larva, embryo, cadherins metabolism, developmental gene expression regulation physiology, olfactory mucosa metabolism, olfactory nerve metabolism, olfactory pathways metabolism, age factors, cadherins classification and genetics immunohistochem-

istry methods, in situ hybridization methods, larva, olfactory mucosa embryology, growth and development, olfactory nerve embryology, growth and development, olfactory pathways embryology and growth and development, messenger RNA metabolism, telencephalon growth and development and metabolism.

Liu, T., J. Lu, Y. Wang, W.A. Campbell, L. Huang, J. Zhu, W. Xia, and S.T. Wong (2006). **Computerized image analysis for quantitative neuronal phenotyping in zebrafish.** *Journal of Neuroscience Methods* 153(2): 190-202. ISSN: 0165-0270.
NAL Call Number: RC346
Abstract: An integrated microscope image analysis pipeline is developed for automatic analysis and quantification of phenotypes in zebrafish with altered expression of Alzheimer's disease (AD)-linked genes. We hypothesize that a slight impairment of neuronal integrity in a large number of zebrafish carrying the mutant genotype can be detected through the computerized image analysis method. Key functionalities of our zebrafish image processing pipeline include quantification of neuron loss in zebrafish embryos due to knockdown of AD-linked genes, automatic detection of defective somites, and quantitative measurement of gene expression levels in zebrafish with altered expression of AD-linked genes or treatment with a chemical compound. These quantitative measurements enable the archival of analyzed results and relevant meta-data. The structured database is organized for statistical analysis and data modeling to better understand neuronal integrity and phenotypic changes of zebrafish under different perturbations. Our results show that the computerized analysis is comparable to manual counting with equivalent accuracy and improved efficacy and consistency. Development of such an automated data analysis pipeline represents a significant step forward to achieve accurate and reproducible quantification of neuronal phenotypes in large scale or high-throughput zebrafish imaging studies.
Descriptors: zebrafish, computer assisted image processing methods, neurons cytology, cell count methods gene expression physiology, developmental gene expression regulation, immunohistochemistry methods, in situ hybridization methods, neurons classification, antisense oligonucleotides, RNA administration and dosage.

Locati, J., M. Nehl, and C. Lamb (2004). **Primary gustatory centers in the zebrafish brain**. *Proceedings of the South Dakota Academy of Science* 83: 271. ISSN: 0096-378X.
Descriptors: zebrafish, brain, evolution, freshwater fish, nerves, neurons, taste, *Carassius auratus*, *Cyprinidae*, *Danio rerio*.

Lopez Schier, H. and A.J. Hudspeth (2005). **Supernumerary neuromasts in the posterior lateral line of zebrafish lacking peripheral glia.** *Proceedings of the National Academy of Sciences of the United States of America* 102(5): 1496-501. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: The lateral line and its associated sensory nerves develop from cephalic epithelial thickenings called neurogenic placodes. In the zebrafish, the transcription factor neurogenin 1 is essential for the generation of the sensory ganglion from the placode, but is dispensable for the migration of the primordium and the initial development of neuromasts. We find that inactivation of the gene encoding neurogenin 1 leads to the development of over twice the normal number of neuromasts along the posterior lateral line of zebrafish larvae. Mutation of the gene encoding another transcription factor, sox10, has a similar effect. After a normal number of proneuromasts is initially deposited by the migrating primordia, interneuromast cells divide and differentiate to form the extra neuromasts. Our results indicate that the development of these intercalary neuromasts occurs principally because of the absence of

neural crest-derived peripheral glia, which evidently inhibit the assembly of interneuromast cells into neuromasts.
Descriptors: zebrafish, physiology, neuroglia cytology, afferent neurons cytology, morphogenesis, growth and development.

Luna, V.M., M. Wang, F. Ono, M.R. Gleason, J.E. Dallman, G. Mandel, and P. Brehm (2004). **Persistent electrical coupling and locomotory dysfunction in the zebrafish mutant shocked.** *Journal of Neurophysiology* 92(4): 2003-9. ISSN: 0022-3077.
Abstract: On initial formation of neuromuscular junctions, slow synaptic signals interact through an electrically coupled network of muscle cells. After the developmental onset of muscle excitability and the transition to fast synaptic responses, electrical coupling diminishes. No studies have revealed the functional importance of the electrical coupling or its precisely timed loss during development. In the mutant zebrafish shocked (sho) electrical coupling between fast muscle cells persists beyond the time that it would normally disappear in wild-type fish. Recordings from sho indicate that muscle depolarization in response to motor neuron stimulation remains slow due to the low-pass filter characteristics of the coupled network of muscle cells. Our findings suggest that the resultant prolonged muscle depolarizations contribute to the premature termination of swimming in sho and the delayed acquisition of the normally rapid touch-triggered movements. Thus the benefits of gap junctions during early synapse development likely become a liability if not inactivated by the time that muscle would normally achieve fast autonomous function.
Descriptors: mutant zebrafish, locomotion genetics and physiology, mutation physiology, nervous system diseases genetics and physiopathology, connexin 43 genetics and physiology, connexins genetics and physiology, electrophysiology, evoked potentials physiology, patch clamp techniques, phenotype.

Ma, P.M. (2003). **Catecholaminergic systems in the zebrafish. IV. Organization and projection pattern of dopaminergic neurons in the diencephalon.** *Journal of Comparative Neurology* 460(1): 13-37. ISSN: 0021-9967.
Descriptors: zebrafish , catecholamines metabolism, diencephalon cytology, dopamine metabolism, neurons cytology and metabolism, cerebral ventricles cytology, dopamine beta hydroxylase biosynthesis, immunohistochemistry, neurons classification, tyrosine 3 monooxygenase biosynthesis.

Ma, P.M. and M. Lopez (2003). **Consistency in the number of dopaminergic paraventricular organ-accompanying neurons in the posterior tuberculum of the zebrafish brain.** *Brain Research* 967(1-2): 267-72. ISSN: 0006 8993.
NAL Call Number: QP376
Abstract: The teleostean diencephalon contains a relatively large number of dopaminergic neurons compared to other vertebrates. In the zebrafish, 17 groups of such neurons have been distinguished. One of the most unusual among these is the group of paraventricular organ-accompanying cells, which are easily distinguished by their large somal size, high tyrosine hydroxylase content, and characteristic dendritic architecture. This cell group is also heterogeneous-subsets of neurons can be identified on the bases of dendritic orientation. In this study, the number of paraventricular organ-accompanying neurons is counted in adult brain sections stained with anti-tyrosine hydroxylase antibodies. There is an average of 7.2+/-1.0 neurons on each side of the brain, and an average sum of 14+/-1.1 neurons on both sides. The average difference between the left and the right sides is 0.8+/-0.5 neurons. Neuron numbers between the two sides of the same brain are highly correlated. These results suggest

that there is a relatively stringent regulation of paraventricular organ-accompanying neuron number in the zebrafish brain. The correlated left-right numbers suggests that genetic factors may play a major role in this regulation. The consistent and low cell number should be helpful in elucidating the number of subsets of these neurons, the anatomical and functional organization of some of the dopaminergic neurons along the paraventricular organ, as well as factors that play a role in regulating neuron numbers.

Descriptors: zebrafish, brain cytology, dopamine analysis, neurons chemistry and cytology, anatomy and histology, cell count methods.

Mack Bucher, J.A., J. Li, and R.W. Friedrich (2007). **Early functional development of interneurons in the zebrafish olfactory bulb.** *European Journal of Neuroscience* 25(2): 460-70. ISSN: 0953 816X.

Abstract: In the adult olfactory bulb (OB) of vertebrates, local GABAergic interneurons (INs) mediate recurrent and lateral inhibition between the principal neurons of the OB, the mitral cells (MCs), and play pivotal roles in the processing of odor-evoked activity patterns. The properties and functions of INs in the developing OB are, however, not well understood. We studied the functional development of INs in the OB of living zebrafish larvae 3-6 days postfertilization using anatomical techniques and in-vivo two-photon Ca2+ imaging. We identified MCs and INs by cell-type-specific expression of transgenic fluorescent markers and found that the IN:MC ratio was lower than in the adult fish. Moreover, the fraction of INs responding with Ca2+ signals to a set of natural odors was substantially lower than in adults. Odors of different chemical classes evoked overlapping patterns of Ca2+ signals that were concentrated in the center of the IN layer. The GABA(A) receptor agonists GABA and muscimol strongly suppressed odor responses, whereas a GABA(A) receptor antagonist enhanced responses and altered the spatial distribution of odor-evoked activity. These results indicate that IN odor responses at early developmental stages are sparse and exhibit no obvious chemotopic organization. Nevertheless, GABAergic signaling is already inhibitory at early stages of OB development and strongly influences odor-evoked activity patterns. Hence, INs already participate in the processing of odor information at very early stages of OB development even though the majority of INs emerge only at later stages.

Descriptors: zebrafish, developmental gene expression regulation physiology, interneurons physiology, odors, olfactory bulb cytology, embryology, growth and development, calcium metabolism, drug interactions, GABA agonists pharmacology, green fluorescent proteins metabolism, homeodomain proteins, hu paraneoplastic encephalomyelitis antigens metabolism, three dimensional imaging methods, interneurons drug effects, muscimol pharmacology , neural inhibition drug effects, pyridazines pharmacology, metabolism, gamma aminobutyric acid pharmacology.

Masino, M.A. and J.R. Fetcho (2005). **Fictive swimming motor patterns in wild type and mutant larval zebrafish.** *Journal of Neurophysiology* 93(6): 3177-88. ISSN: 0022-3077.

Abstract: Larval zebrafish provide a unique model for investigating the mechanisms involved in generating rhythmic patterns of behavior, such as swimming, due to the array of techniques available including genetics, optical imaging, and conventional electrophysiology. Because electrophysiological and imaging studies of rhythmic motor behaviors in paralyzed preparations depend on the ability to monitor the central motor pattern, we developed a fictive preparation in which the activity of axial motor neurons was monitored using extracellular recordings from peripheral nerves. We examined spontaneous and light induced fictive motor patterns in wild type and mutant larval zebrafish (4-6 days post-fertilization) paralyzed with curare. All spontaneous and light-induced preparations produced alternation of motor

activity from side-to-side (mean contralateral phase = 50.7 +/- 7.0%; mean burst frequency = 35.6 +/- 4.7 Hz) and a progression of activity from head-to-tail (mean ipsilateral rostrocaudal delay = 0.8 +/- 0.5 ms per segment), consistent with lateral undulation and forward propulsion during swimming, respectively. The basic properties of the motor pattern were similar in spontaneous and light-induced swimming. This fictive preparation can be used in combination with conventional electrophysiological and imaging methods to investigate normal circuit function as well as to elucidate functional deficits in mutant lines. Toward this end, we show that two accordion class mutants, accordion and bandoneon, have alternating activity on opposite sides of the body, contradicting the hypothesis that their deficit results from the absence of the reciprocal glycinergic inhibition that is typically found in the spinal cord of swimming vertebrates.

Descriptors: zebrafish mutant and wildtype larvae, action potentials physiology, motor neurons physiology, mutation, periodicity, swimming physiology, action potentials radiation effects, diagnostic imaging, functional laterality physiology, larva genetics, motor neurons classification and radiation effects, neural inhibition genetics and physiology, peripheral nerves cytology, physiology and radiation effects, photic stimulation methods, proteins genetics.

Mathieu, M., M. Ciarlo, N. Trucco, F. Griffero, G. Damonte, A. Salis, and M. Vallarino (2004). **Pituitary adenylate cyclase-activating polypeptide in the brain, spinal cord and sensory organs of the zebrafish, *Danio rerio*, during development.** *Brain Research* 151(1-2): 169-85. ISSN: 0006 8993.

NAL Call Number: QP376

Abstract: The distribution of Pituitary adenylate cyclase-activating polypeptide (PACAP) was investigated in the brain, pituitary and sensory organs of the zebrafish, *Danio rerio*, during development, in juvenile and adult specimens, using the immunofluorescence method. In 24 h post fertilization (hpf) embryos, PACAP immunoreactive cells appeared in the rostral telencephalon, dorsal diencephalon, caudal and medial rhombencephalon, spinal cord and retina. At 48 hpf stage, positive cells were present in the dorsal diencephalon, medial rhombencephalon, spinal cord, retina and olfactory placode (Op). At 72 hpf stage, additional immunoreactive elements appeared in the medial telencephalon, hypothalamus, mesencephalic tegmentum, retina and otic sensory epithelium (Ose). At day 5, new immunoreactive cells were found in the anterior rhombencephalon and pituitary pars distalis. At day 13, positive cells were mainly concentrated in the mesencephalic tegmentum and spinal cord. In the telencephalon, diencephalon, rhombencephalon and pituitary, the distribution of positive cells was similar to that previously reported. At 1 month stage, positive cells were detected in the hypothalamus, nucleus of the medial longitudinal fascicle (nMlf), rhombencephalic griseum centrale (Gc) and pituitary pars distalis. At 2-3 month stages, immunoreactive elements were found in several hypothalamic nuclei, in the mesencephalic nucleus isthmi, cerebellum and pituitary. In adults, PACAP immunoreactivity was confined to a few brain regions and the pituitary. PACAP immunoreactivity was transiently expressed in several regions suggesting that the peptide may have a role in the control of cells differentiation and proliferation during zebrafish ontogeny. The finding of positive fibers in the pituitary from day 5 onward indicates that PACAP may function from this stage as a hypophysiotropic peptide.

Descriptors: zebrafish, brain metabolism, neuropeptides metabolism, sense organs metabolism, spinal cord metabolism, age factors, brain growth and development, metabolism, fluorescent antibody technique methods, larva metabolism, pituitary adenylate cyclase

activating polypeptide, vasoactive intestinal peptide receptors, sense organs growth and development, spinal cord growth and development.

McDearmid, J.R. and P. Drapeau (2006). **Rhythmic motor activity evoked by NMDA in the spinal zebrafish larva.** *Journal of Neurophysiology* 95(1): 401-17. ISSN: 0022-3077.

Abstract: We have examined the localization and activity of the neural circuitry that generates swimming behavior in developing zebrafish that were spinalized to isolate the spinal cord from descending brain inputs. We found that addition of the excitatory amino acid agonist N-methyl-d-aspartate (NMDA) to spinalized zebrafish at 3 days in development induced repeating episodes of rhythmic tail beating activity reminiscent of slow swimming behavior. The neural correlate of this activity, monitored by extracellular recording comprised repeating episodes of rhythmic, rostrocaudally progressing peripheral nerve discharges that alternated between the two sides of the body. Motoneuron recordings revealed an activity pattern comprising a slow oscillatory and a fast synaptic component that was consistent with fictive swimming behavior. Pharmacological and voltage-clamp analysis implicated glycine and glutamate in generation of motoneuron activity. Contralateral alternation of motor activity was disrupted with strychnine, indicating a role for glycine in coordinating left-right alternation during NMDA-induced locomotion. At embryonic stages, while rhythmic synaptic activity patterns could still be evoked in motoneurons, they were typically lower in frequency. Kinematic recordings revealed that prior to 3 days in development, NMDA was unable to reliably generate rhythmic tail beating behavior. We conclude that NMDA induces episodes of rhythmic motor activity in spinalized developing zebrafish that can be monitored physiologically in paralyzed preparations. Therefore as for other vertebrates, the zebrafish central pattern generator is intrinsic to the spinal cord and can operate in isolation provided a tonic source of excitation is given.

Descriptors: zebrafish, biological clocks physiology, motor activity physiology, motor neurons physiology, skeletal muscle physiology, N-methylaspartate pharmacology, spinal cord physiology, swimming physiology, biological clocks drug effects, larva physiology, motor activity drug effects, motor neurons drug effects, skeletal muscle drug effects and innervation.

McDowell, A.L., L.J. Dixon, J.D. Houchins, and J. Bilotta (2004). **Visual processing of the zebrafish optic tectum before and after optic nerve damage.** *Visual Neuroscience* 21(2): 97-106. ISSN: 0952-5238.

Abstract: Although the zebrafish has become an important model in visual neuroscience, little has been done to examine the processing of its higher visual centers. The purpose of this work was twofold. The first purpose was to examine the physiology of the zebrafish retinotectal system and its relationship to retinal physiology. Spectral sensitivity functions were derived from visually evoked tectal responses and these functions were compared to the functions of electroretinogram (ERG) responses obtained using the same stimulus conditions. The second purpose was to examine the recovery of visual functioning of the tectum following optic nerve damage. The optic nerves of adult zebrafish were damaged (crushed), and tectal visual processing was assessed following damage. The results showed that the spectral sensitivity functions based on the On-responses of the tectum and ERG were qualitatively similar. The functions based on each response type received similar cone contributions including both nonopponent and opponent contributions. However, the spectral sensitivity functions based on the Off-responses of the tectum and ERG differed. The results also showed that the zebrafish visual system is capable of neural regeneration. By 90 days following an optic nerve crush, the spectral sensitivity function based on the tectal On-response

was similar to functions obtained from normal zebrafish. Although the tectal Off-response did recover, the spectral sensitivity based on the Off-response was not the same as the function of normal zebrafish. These results support the notion that different levels of the visual system process information differently and that the zebrafish visual system, like those of other lower vertebrates, is capable of functional regeneration.
Descriptors: zebrafish, nerve regeneration physiology, optic nerve physiology, retina physiology, superior colliculus physiology, visual pathways physiology, color perception physiology, electroretinography, evoked potentials, visual physiology, nerve crush.

McKinley, E.T., T.C. Baranowski, D.O.C.C. Blavo, T.N. Doan, and A.L. Rubinstein (2005). **Neuroprotection of MPTP-induced toxicity in zebrafish dopaminergic neurons.** *Brain Research. Molecular Brain Research* 141(2): 128-37. ISSN: 0169 328X.
Abstract: Parkinson's disease is characterized by a severe loss of dopaminergic neurons resulting in a range of motor deficits. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is known to cause a similar loss of dopaminergic neurons in the human midbrain with corresponding Parkinsonian symptoms. Several animal species have also shown sensitivity to MPTP, including primates, mice, goldfish, and, most recently, zebrafish. This study demonstrates that the effect of MPTP on dopaminergic neurons in zebrafish larvae is mediated by the same pathways that have been demonstrated in mammalian species. MPTP-induced neurodegeneration was prevented by co-incubation with either the monoamine oxidase-B (MAO-B) inhibitor l-deprenyl or the dopamine transporter (DAT) inhibitor nomifensine. Furthermore, targeted inactivation of the DAT gene by antisense morpholinos also protected neurons from MPTP damage. Thus, the mechanism for MPTP-induced dopaminergic neuron toxicity in mammals is conserved in zebrafish larvae. Effects on swimming behavior and touch response that result from MPTP damage are partially ameliorated by both l-deprenyl and DAT knockdown.
Descriptors: zebrafish embryos, larva, 1 methyl 4 phenyl 1,2,3,6 tetrahydropyridine antagonists and inhibitors, MPTP poisoning prevention and control, monoamine oxidase inhibitors therapeutic use, nerve degeneration prevention and control, neurons drug effects, neuroprotective agents therapeutic use, nomifensine therapeutic use, oligodeoxyribonucleotides, antisense therapeutic use, selegiline therapeutic use, brain drug effects, brain pathology, dopamine physiology, dopamine plasma membrane transport proteins antagonists, inhibitors, biosynthesis, deficiency and genetics, gene targeting, mptp poisoning pathology, monoamine oxidase, monoamine oxidase inhibitors pharmacology, morpholines pharmacology and therapeutic use, chemically induced nerve degeneration, nerve degeneration pathology, nerve tissue proteins biosynthesis and genetics, neuroprotective agents pharmacology, nomifensine pharmacology, oligodeoxyribonucleotides, antisense pharmacology, selegiline pharmacology, species specificity, swimming, tyrosine 3 monooxygenase biosynthesis and genetics.

McLachlan, E., T.W. White, C. Ugonabo, C. Olson, J.I. Nagy, and G. Valdimarsson (2003). **Zebrafish Cx35: cloning and characterization of a gap junction gene highly expressed in the retina.** *Journal of Neuroscience Research* 73(6): 753-64. ISSN: 0360-4012.
Descriptors: zebrafish , connexins genetics, eye gap junctions genetics, retina metabolism, proteins, Northern blotting, Western blotting, brain metabolism , brain chemistry, molecular cloning, connexins metabolism, electrophysiology, molecular evolution, eye proteins metabolism, gap junctions metabolism, immunohistochemistry, liver metabolism, mice, molecular sequence data, oocytes, messenger RNA analysis, retina cytology, retina embryology, reverse transcriptase PCR, amino acid sequence homology, time factors, *Xenopus*.

McLean, D.L. and J.R. Fetcho (2004). **Ontogeny and innervation patterns of dopaminergic, noradrenergic, and serotonergic neurons in larval zebrafish.** *Journal of Comparative Neurology* 480(1): 38-56. ISSN: 0021-9967.

Abstract: We report the development of aminergic neurons from 0-10 days postfertilization (dpf) in zebrafish (*Danio rerio*). This study was prompted by the lack of information regarding patterns of spinal aminergic innervation at early stages, when the fish are accessible to optical, genetic, and electrophysiological approaches toward understanding neural circuit function. Our findings suggest that aminergic populations with descending processes are among the first to appear during development. Descending aminergic fibers, revealed by antibodies to tyrosine hydroxylase (TH) and serotonin (5-hydroxytryptamine; 5-HT), innervate primarily the ventral (TH, 5-HT), but also the dorsal (5-HT) aspects of the spinal cord by 4 dpf, with the extent of innervation not changing markedly up to 10 dpf. By tracking the spatiotemporal expression of TH, 5-HT, and dopamine beta hydroxylase reactivity, we determined that these fibers likely originate from neurons in the posterior tuberculum (dopamine), the raphe region (5-HT) and, possibly, the locus coeruleus (noradrenaline). In addition, spinal neurons positive for 5-HT emerge between 1-2 dpf, with processes that appeared to descend along the ventrolateral cord for only 1-2 muscle segments. Their overall morphology distinguished these cells from previously described "VeMe" (ventromedial) interneurons, which are also located ventromedially, but have long, multisegmental descending processes. We confirmed the distinction between spinal serotonergic and VeMe interneurons using fish genetically labeled with green fluorescent protein. Our results suggest that the major aminergic systems described in adults are in place shortly after hatching, at a time when zebrafish are accessible to a battery of techniques to test neuronal function during behavior.

Descriptors: zebrafish, central nervous system embryology, efferent pathways embryology, neurons metabolism, neurotransmitter agents metabolism, aging physiology, axons metabolism and ultrastructure, cell differentiation physiology, central nervous system cytology, growth and development, dopamine metabolism, efferent pathways cytology, growth and development, cytology, embryology, metabolism, fluorescent antibody technique, interneurons cytology, metabolism, larva cytology, larva growth, development and metabolism, locus coeruleus cytology, embryology, growth and development, neurons cytology, norepinephrine metabolism, raphe nuclei cytology, embryology, growth and development, serotonin metabolism, spinal cord cytology and embryology, growth and development, tyrosine 3 monooxygenase metabolism.

McLean, D.L. and J.R. Fetcho (2004). **Relationship of tyrosine hydroxylase and serotonin immunoreactivity to sensorimotor circuitry in larval zebrafish.** *Journal of Comparative Neurology* 480(1): 57-71. ISSN: 0021-9967.

Abstract: Our previous study tracked the ontogeny of aminergic systems in zebrafish (*Danio rerio*). Here we use tyrosine hydroxylase (TH) and serotonin (5-hydroxytryptamine; 5-HT) immunoreactivity, in conjunction with retrograde and genetic labeling techniques, to provide a more refined examination of the potential synaptic contacts of aminergic systems. Our focus was on different levels of the sensorimotor circuit for escape, from sensory inputs, through identified descending pathways, to motor output. We observed 5-HT reactivity in close proximity to the collaterals of the Rohon-Beard sensory neurons in spinal cord. In the brainstem we found TH and 5-HT reactivity closely apposed to the dendritic processes of the nucleus of the medial longitudinal fascicle (nMLF), in addition to the ventral dendrites of the Mauthner neuron and its serial homologs MiD2cm and MiD3cm. Only TH reactivity was observed near the lateral dendrites of the Mauthner cell. TH and 5-HT reactivity

were also positioned near the outputs of reticulospinal cells in spinal cord. Finally, both TH and 5-HT reactivity were detected close to the dendritic processes of primary and secondary spinal motor neurons. We also confirmed, using dual TH and 5-HT staining and retrograde labeling, that the sources of spinal aminergic reactivity include the posterior tuberculum (dopamine) and inferior raphe region (5-HT). Our data indicate that aminergic systems may interact at all levels of the sensorimotor pathways involved in escape. The identification of some of these likely sites of aminergic action will allow for directed studies of their functional roles using the powerful combination of techniques available in zebrafish.
Descriptors: zebrafish, brain metabolism, catecholamines metabolism, neural pathways metabolism, serotonin metabolism, tyrosine 3 monooxygenase metabolism, afferent pathways cytology and metabolism, behavior physiology, brain cytology, brain stem cytology and metabolism, dendrites physiology and ultrastructure, efferent pathways cytology and metabolism, fluorescent dyes, immunohistochemistry, locomotion physiology, confocal microscopy, neural pathways cytology, presynaptic terminals metabolism and ultrastructure, raphe nuclei cytology and metabolism, reticular formation cytology and metabolism, spinal cord cytology and metabolism, synaptic transmission physiology, zebrafish anatomy and histology.

McLean, D.L., J. Fan, S. Higashijima, M.E. Hale, and J.R. Fetcho (2007). **A topographic map of recruitment in spinal cord**. *Nature* 446(7131): 71-75. ISSN: 0028-0836.
NAL Call Number: 472 N21
Descriptors: zebrafish, ablation, electrophysiology, imaging techniques, neurons, spinal cord, *Danio rerio.*

Menger, G.J., J.R. Koke, and G.M. Cahill (2005). **Diurnal and circadian retinomotor movements in zebrafish.** *Visual Neuroscience* 22(2): 203-9. ISSN: 0952-5238.
Abstract: Key indicators of circadian regulation include the persistence of physiological rhythmicity in the absence of environmental time cues and entrainment of this rhythmicity by the ambient light cycle. In some teleosts, the inner segments of rod and cone photoreceptors contract and elongate according to changes in ambient lighting and the circadian cycle. Pigment granules in the retinal pigment epithelium (RPE) disperse and aggregate in a similar manner. Collectively, these movements are known as retinomotor movements. We report the histological characterization of diurnal and circadian retinomotor movements in zebrafish, *Danio rerio*. Adult fish subjected to a 14:10 light:dark (LD) cycle, constant darkness (DD), or constant light (LL) were sacrificed at 1-13 h intervals and processed for semithin sectioning of the retina. Using bright-field microscopy, 15 measurements of pigment granule position and the inner segment lengths of 30 rods and 30-45 cones were collected from the central third of the dorso-optic retina per time point. In LD, rods and cones followed a clear diurnal rhythm in their inner segment movements. Short-single, UV-sensitive cones were found to contract significantly 1 h before light onset in LD conditions. In DD conditions, the inner segments movements of short-single and double cones displayed statistically significant rhythms. RPE pigment granule movements are rhythmically regulated in both LD and DD although fluctuations are damped in the absence of photic cues. No significant retinomotor movements were observed in LL. These findings indicate retinomotor movements in zebrafish are differentially regulated by an endogenous oscillator and by light-dependent mechanisms.
Descriptors: zebrafish, circadian rhythm physiology, vertebrate photoreceptors physiology, cell movement physiology, pigment epithelium of eye physiology.

Menuet, A., E. Pellegrini, F. Brion, M.M. Gueguen, I. Anglade, F. Pakdel, and O. Kah (2005). **Expression and estrogen-dependent regulation of the zebrafish brain aromatase gene.** *Journal of Comparative Neurology* 485(4): 304-20. ISSN: 0021-9967.

Descriptors: zebrafish, aromatase biosynthesis, brain drug effects, brain enzymology, estradiol analogs and derivatives, estrogens pharmacology, enzymologic gene expression regulation drug effects, metabolism, aromatase genetics, brain embryology, cell line, cricetinae, drug dose response relationship, estradiol pharmacology, enzymologic gene expression regulation physiology, mice, rats.

Meyers, J.R. and P.A. Raymond (2005). **Hindbrain progenitors produce retinal cell types when transplanted into developing zebrafish retina**. *Investigative Ophthalmology & Visual Science* 46(Suppl. S): 2442. ISSN: 0146 0404.

Abstract: Purpose: To test the lineage restriction and specification of retinal progenitors. The neural retina develops from the eye field -- a region of the anteriorneural tube delineated by the overlapping expression of several transcriptionfactors implicated in retinal determination. In order to test the capacity ofneural progenitors that do not normally express retinal determination factorsto differentiate into retinal cell types, we have examined the fates of hindbrain progenitors transplanted into the developing zebrafish retina. Methods: Donor zebrafish embryos were injected with the lineage tracer FluoRuby at the 2-16 cell stage to uniformly label all cells. Hindbrain progenitors were removed from donor embryos at 14, 24, 48 or 72 hours post fertilization and injectedinto the developing retina of unlabeled host embryos at 14, 24, 48 or 72 hours post fertilization. After allowing 4-6 days for cellular differentiation, host retinas were sectioned and labeled with markers for differentiated retinal cell types. Results: Hindbrain progenitors survived in the host retinas and were capable of producing cells in all layers of the retina. Both calretinin-positive neurons and GFAP-positive Muller glia derived from the donor cells couldbe found in the appropriate layers of the retina of the host fish when 14- or24-hour progenitors were transplanted isochronically. In addition, when transplanted isochronically 14- or 24-hour hindbrain progenitors were able to produce cells in the photoreceptor layer expressing zpr-1, a specific marker of zebrafish double cone photoreceptors. When hindbrain cells from 72-hour fish weretransplanted into 72-hour retinas, which were laminated and differentiated, few transplanted cells survived. Conclusions: Progenitors from young hindbrain (14 or 24 hours post fertilization) retain the capacity to respecify as retinal progenitors and produce neurons, glia, and photoreceptors in the appropriate positions in the retina. Few cells from older hindbrain (72 hpf) survived in the retina, suggesting that the window for conversion of hindbrain into retina may be limited to early stages prior to retinal differentiation. Heterochronic transplants will be used to further dissect the timing of specification ofhindbrain and retinal progenitors. These experiments suggest that cell fate as retina is not determined early, and that cells transplanted into the developing retina can respond to environmental cues therein to change their fates andproduce retinal-specific cell types. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, developing rertina, nervous system, neural coordination, sense organs, sensory reception, cell differentiation.

Mikula, P., R. Dobsikova, Z. Svobodova, and J. Jarkovsky (2006). **Evaluation of xenoestrogenic potential of propylparaben in zebrafish (*Danio rerio*).** *Neuroendocrinology Letters* 27(Suppl 2): 104-7. ISSN: 0172-780X.

Abstract: OBJECTIVES: Xenoestrogenic potential of propylparaben (PP), one of the most commonly used preservatives in drugs, cosmetics and food, was investigated in vivo using zebrafish (*Danio rerio*). METHODS: Juvenile zebrafish (20 days post hatching) were exposed

to three different concentrations of propylparaben (PP) dissolved in ethanol and added into the water. After 20 days of exposure the fish were euthanized and vitellogenin concentrations in their whole body homogenates were measured by the method of direct sandwich ELISA. Simultaneously, vitellogenin concentrations in either fish from the control group (exposed to solvent without the substance tested) and in fish from the positive control group (exposed to natural estrogen 17beta-estradiol) were measured. RESULTS: Vitellogenin concentration in whole body homogenates of control fish was 400 (396-540) ng/ml(-1) (geometric mean (95% CI)). Zebrafish exposure to propylparaben at the concentrations of 0.1; 0.4 and 0.9 mg/l(-1) elicited statistically significant decline (P<0.001) of vitellogenin production, i.e. geometric means of vitellogenin concentrations in whole body homogenates were 240 (186-311); 218 (175-270) and 270 (234-311) ng/ml(-1), respectively. Conversely, the geometric mean of vitellogenin concentration in whole body homogenates of zebrafish exposed to 100 ng/ml(-1) of 17beta-estradiol (positive control) was significantly higher (P<0.001) than values in all other groups, i.e. 35,553 (16,860-74,968) ng/ml(-1). CONCLUSIONS: Our results suggest an antiestrogenic potential of propylparaben tested in vivo in juvenile zebrafish (*Danio rerio*). The estrogenic effect of 17beta-estradiol was confirmed.
Descriptors: juvenile zebrafish, estrogens toxicity, parabens toxicity, preservatives, pharmaceutical toxicity, toxicity tests, vitellogenins analysis.

Miller, N. and R. Gerlai (2007). **Quantification of shoaling behaviour in zebrafish .** *Behavioural Brain Research* 184(2): 157-66. ISSN: 0166 4328.
Abstract: Zebrafish has been a favourite of developmental biologists and numerous genetic tools have been developed for this species. In recent years, zebrafish has become an increasingly popular subject of neuroscientists and behavioural scientists. One of the typical characteristics of zebrafish is shoaling, individuals forming a tight group in which fish swim together. The biological mechanisms of social behaviours are complex and not well understood in vertebrates, and zebrafish, due to its highly social nature and the genetic tools developed for it, may represent an excellent animal model with which these mechanisms may be studied. Improvement of behavioural quantification methods would facilitate research in this area. We describe a custom software application that allows the precise quantification of several parameters of group cohesion in zebrafish. We also present three experimental examples to illuminate the use of our methodology, and show how group cohesion changes in response to manipulations of the environment.
Descriptors: zebrafish, grouping, schooling, group behavior.

Mione, M., Z. Lele, C.T. Kwong, M.L. Concha, and J.D. Clarke (2006). **Expression of pcp4a in subpopulations of CNS neurons in zebrafish.** *Journal of Comparative Neurology* 495(6): 769-87. ISSN: 0021-9967.
Abstract: The molecular organization of the zebrafish brain and its relation to neuroanatomical divisions are still largely unknown. In this study we have analyzed the expression of a small transcript encoding for the IQ containing polypeptide Pcp4a in developing and juvenile zebrafish. The transcript is exclusively expressed in neural structures with a pattern that is highly specific for restricted domains and cell populations throughout development, and it allows us to follow the development of these structures at different times. The expression of pcp4a characterizes the dorsocaudal telencephalon, dorsal habenula, pretectal nuclei, preglomerular complex, mammillary bodies, and deep layers of the optic tectum and is a hallmark of a subpopulation of reticulospinal neurons. In the telencephalon, comparison of the expression of pcp4a with other pallial markers showed a rostrocaudal gradient in the expression of these genes, which suggests that the dorsal telencephalon of zebrafish may

be organized in distinct areas with different molecular natures. Pcp4 has been involved in modulating calcium signals and in binding to calmodulin, but its precise role in neuronal functions is not known. The analysis of pcp4a expression and localization in the zebrafish brain suggests that pcp4a may be a useful marker for sensory and some motor neuronal circuitries and for telencephalic areas processing sensory inputs.
Descriptors: juvenile zebrafish, brain metabolism, nerve tissue proteins metabolism, neural pathways metabolism, neurons metabolism, afferent pathways cytology andmetabolism, brain cytology, growth and development, efferent pathways cytology and metabolism, developmental gene expression regulation physiology, molecular sequence data, neural pathways cytology, neurons cytology, messenger RNA metabolism, amino acid sequence homology, telencephalon cytology, growth, development and metabolism, zebrafish anatomy, histology, proteins genetics, proteins isolation and purification.

Miyasaka, N., Y. Sato, and Y. Yoshihara (2005). **Axon guidance of olfactory sensory neurons in zebrafish.** *Chemical Senses* 30(Suppl 1): I92-I93. ISSN: 0379 864X.
NAL Call Number: QP456.C5
Descriptors: zebrafish, axon, olfactory, sensory, neurons.

Moutsaki, P., D. Whitmore, J. Bellingham, K. Sakamoto, Z.K. David Gray, and R.G. Foster (2003). **Teleost multiple tissue (tmt) opsin: a candidate photopigment regulating the peripheral clocks of zebrafish?** *Brain Research. Molecular Brain Research* 112(1-2): 135-45. ISSN: 0169 328X.
Abstract: Isolated organs and cell lines from zebrafish exhibit circadian oscillations in clock gene expression that can be entrained to a 24-h light/dark cycle. The mechanism underlying this cellular photosensitivity is unknown. We report the identification of a novel opsin family, tmt-opsin, that has a genomic structure characteristic of vertebrate photopigments, an amino acid identity equivalent to the known photopigment opsins, and the essential residues required for photopigment function. Significantly, tmt-opsin is expressed in a wide variety of neural and non-neural tissues, including a zebrafish embryonic cell line that exhibits a light entrainable clock. Collectively the data suggest that tmt-opsin is a strong candidate for the photic regulation of zebrafish peripheral clocks.
Descriptors: zebrafish, biological clocks physiology, cell line metabolism, gene expression regulation genetics, opsin isolation and purification, photoperiod, takifugu metabolism, amino acid sequence genetics, base sequence genetics, brain metabolism, cell line cytology, complementary DNA analysis, complementary DNA genetics, molecular evolution, genomic library, molecular sequence data, opsin genetics, phylogeny, amino acid sequence homology, takifugu genetics, viscera metabolism, .

Mueller, T., P. Vernier, and M.F. Wullimann (2004). **The adult central nervous cholinergic system of a neurogenetic model animal, the zebrafish *Danio rerio*.** *Brain Research* 1011(2): 156-69. ISSN: 0006 8993.
NAL Call Number: QP376
Descriptors: zebrafish, cholinergic system, central nervous system cytology, choline O acetyltransferase genetics, neurons enzymology, central nervous system enzymology, choline O acetyltransferase metabolism, immunohistochemistry methods, research models, neurons physiology.

Mueller, T., P. Vernier, and M.F. Wullimann (2006). **A phylotypic stage in vertebrate brain development: GABA cell patterns in zebrafish compared with mouse.** *Journal of Comparative Neurology* 494(4): 620-34. ISSN: 0021-9967.

Descriptors: zebrafish, diencephalon metabolism, developmental gene expression regulation physiology, telencephalon metabolism, gamma aminobutyric acid metabolism, Anura, cerebellum cytology, cerebellum growth and development, cerebellum metabolism, diencephalon cytology, growth and development , ferrets, lampreys, mice, rhombencephalon cytology, growth and development, metabolism, stem cells cytology, metabolism, telencephalon cytology, growth and development, tissue distribution, zebrafish growth and development.

Mueller, T. and M.F. Wullimann (2003). **Anatomy of neurogenesis in the early zebrafish brain.** *Brain Research* 140(1): 137-55. ISSN: 0006 8993.

NAL Call Number: QP376

Abstract: The detailed architecture of postembryonic (i.e. secondary, as opposed to primary) neurogenesis in the zebrafish brain at 2 days postfertilization was investigated by studying expression domains of various proneural basic helix-loop-helix genes (i.e. neuroD=nrd, neurogenin1=ngn1, Zash-1b) and neurogenic genes (i.e. Notch-1a, deltaA) on the level of in situ-hybridized sectioned material and compared with brain sections of the same age immunostained for PCNA (a proliferation marker) or for Hu-proteins (marker for early neuronal differentiation). Whereas both Notch-1a and deltaA domains are present in all proliferative zones of the brain, only the more diffuse and scattered deltaA expression appears to extend additionally into the adjacent postmitotic gray matter. Zash-1b is the first achaete-scute orthologue shown here to be expressed exclusively in all proliferative central nervous zones (except for the eye). The ngn1 and nrd genes are typically both expressed in overlapping fashion in many-but not all-brain regions, with ngn1 being more restricted towards the ventricular proliferative zones and nrd extending more laterally. This fact as well as comparisons with PCNA- and Hu-immunostains indicate that a great proportion of ngn1-positive cells are mitotic, but some appear to extend into the postmitotic gray matter where the nrd-domains lie. A comparison of the relative extents of PCNA- (proliferative), nrd- (freshly determined) and Hu-positive (differentiating) cell populations allows to determine the relative maturation state of a given brain part. Similar to findings in late embryonic amniote brains, expression of nrd is absent (from 2 to 5 days) in the zebrafish subpallium, ventral preoptic region, ventral thalamus and hypothalamus. These four regions are also free of ngn1 expression (with the exception of an unusual peripheral ngn1 domain in the preoptic region), indicating that a neurogenetic network not involving nrd and ngn1 is at work there. Our characterization of locally distinct patterns and dynamics of secondary neurogenesis in the entire early (2 dpf) postembryonic zebrafish brain delivers the blueprint for more specialized functional studies.

Descriptors: zebrafish embryos, brain growth and development, neurons physiology, aging, brain cytology, cell differentiation, cell division, developmental gene expression regulation, immunohistochemistry, in situ hybridization, membrane neurons cytology, proliferating cell nuclear antigen genetics.

Mumm, J.S., P.R. Williams, L. Godinho, A. Koerber, A.J. Pittman, T. Roeser, C.B. Chien, H. Baier, and R.O. Wong (2006). **In vivo imaging reveals dendritic targeting of laminated afferents by zebrafish retinal ganglion cells.** *Neuron* 52(4): 609-21. ISSN: 0896-6273.

Abstract: Targeting of axons and dendrites to particular synaptic laminae is an important mechanism by which precise patterns of neuronal connectivity are established. Although axons target specific laminae during development, dendritic lamination has been thought to

occur largely by pruning of inappropriately placed arbors. We discovered by in vivo time-lapse imaging that retinal ganglion cell (RGC) dendrites in zebrafish show growth patterns implicating dendritic targeting as a mechanism for contacting appropriate synaptic partners. Populations of RGCs labeled in transgenic animals establish distinct dendritic strata sequentially, predominantly from the inner to outer retina. Imaging individual cells over successive days confirmed that multistratified RGCs generate strata sequentially, each arbor elaborating within a specific lamina. Simultaneous imaging of RGCs and subpopulations of presynaptic amacrine interneurons revealed that RGC dendrites appear to target amacrine plexuses that had already laminated. Dendritic targeting of prepatterned afferents may thus be a novel mechanism for establishing proper synaptic connectivity.

Descriptors: zebrafish retinal ganglion cells, dendrites ultrastructure, presynaptic terminals ultrastructure, retina cytology and embryology, retinal ganglion cells cytology, afferent pathways cytology, embryology and physiology, amacrine cells cytology and physiology, genetically modified, cell communication genetics, cell differentiation physiology, cell shape physiology, dendrites physiology, developmental gene expression regulation genetics, image cytometry, luminescent confocal microscopy, presynaptic terminals physiology, retina physiology and ganglion cells physiology, time factors.

Nakayama, H. and Y. Oda (2004). **Common sensory inputs and differential excitability of segmentally homologous reticulospinal neurons in the hindbrain**. *Journal of Neuroscience* 24(13): 3199-3209. ISSN: 0270-6474.

Descriptors: zebrafish, freshwater fish, hindbrain, Mauthner cells, nerves, neurons, potassium, projections, *Carassius auratus*, *Danio rerio*.

Nam, R.H., W. Kim, and C.J. Lee (2004). **NMDA receptor-dependent long-term potentiation in the telencephalon of the zebrafish.** *Neuroscience Letters* 370(2-3): 248-51. ISSN: 0304-3940.

NAL Call Number: QP351.N3

Abstract: In Mg2+ -free aCSF, bursting discharges were induced in the posterior telencephalon of zebrafish following an electrical stimulation of the anterior telencephalon. The bursting discharges were partially reduced by CNQX (10 microM), an AMPA receptor antagonist, and the remaining activity was completely blocked by an additional treatment of APV (50 microM), an NMDA receptor antagonist. Long-term potentiation that lasted more than 1 h was also induced after 20 min of perfusion with KCl (10 mM). The degree of KCl-induced long-term potentiation (K-LTP) was reduced when a concomitant electrical stimulation was not delivered during a KCl perfusion. K-LTP was blocked by APV (50 microM) but not by nifedipine (1 microM), an L-type Ca2+ channel blocker. Furthermore, K-LTP was not induced in the presence of a broad spectrum inhibitor for protein kinases, H-7 (10 microM). These results suggest that NMDA receptors and protein kinases play important roles in the synaptic plasticity of the zebrafish brain.

Descriptors: zebrafish brain, long term potentiation physiology, N methyl D aspartate receptors physiology, telencephalon physiology, valine analogs and derivatives, 1 5 isoquinolinesulfonyl 2 methylpiperazine pharmacology, 6 cyano 7 nitroquinoxaline 2,3 dione pharmacology , calcium channel blockers pharmacology, drug interactions, electric stimulation methods, enzyme inhibitors pharmacology, excitatory amino acid agonists pharmacology and antagonists pharmacology, long term potentiation drug effects and radiation effects, magnesium metabolism, nifedipine pharmacology, potassium chloride pharmacology, N methyl D aspartate receptors drug effects and radiation effects, telencephalon drug effects and radiation effects, time factors, valine pharmacology.

Niell, C.M., M.P. Meyer, and S.J. Smith (2004). **In vivo imaging of synapse formation on a growing dendritic arbor**. *Nature Neuroscience* 7(3): 254-260. ISSN: 1097-6256.
NAL Call Number: QP351
Descriptors: zebrafish, biological development, dendritic branching, imaging techniques, larvae, marine fish, neurons, optic tectum, Pseudopodia, synapses, synaptogenesis, *Danio rerio*, axons, dendrites, filopodia.

Niell, C.M. and S.J. Smith (2005). **Functional imaging reveals rapid development of visual response properties in the zebrafish tectum.** *Neuron* 45(6): 941-51. ISSN: 0896-6273.
Abstract: The visual pathway from the retina to the optic tectum in fish and frogs has long been studied as a model for neural circuit formation. Although morphological aspects, such as axonal and dendritic arborization, have been well characterized, less is known about how this translates into functional properties of tectal neurons during development. We developed a system to provide controlled visual stimuli to larval zebrafish, while performing two-photon imaging of tectal neurons loaded with a fluorescent calcium indicator, allowing us to determine visual response properties in intact fish. In relatively mature larvae, we describe receptive field sizes, visual topography, and direction and size selectivity. We also characterize the onset and development of visual responses, beginning when retinal axons first arborize in the tectum. Surprisingly, most of these properties are established soon after dendrite growth and synaptogenesis begin and do not require patterned visual experience or a protracted period of refinement.
Descriptors: zebrafish larvae, cell differentiation physiology, retinal ganglion cells physiology, superior colliculus physiology, vision physiology, visual pathways physiology, calcium signaling physiology, dendrites physiology and ultrastructure, fluorescent dyes, growth cones physiology and ultrastructure, image cytometry methods, larva anatomy, histology, growth and development, fluorescence microscopy, photic stimulation, retinal ganglion cells cytology, superior colliculus cytology growth and development, synapses physiology and ultrastructure, time factors, visual fields physiology, visual pathways cytology, growth and development, zebrafish anatomy, histology growth and development.

Novak, C.M., X. Jiang, C. Wang, J.A. Teske, C.M. Kotz, and J.A. Levine (2005). **Caloric restriction and physical activity in zebrafish (*Danio rerio*).** *Neuroscience Letters* 383(1-2): 99-104. ISSN: 0304-3940.
NAL Call Number: QP351.N3
Abstract: Understanding the mechanism of energy flux may be critical for explaining how obesity has emerged as a public health epidemic. It is known that changes in caloric intake predictably alter physical activity levels (PA) in mammals. Here, our goal was to test the hypothesis that fasting induces a biphasic pattern of change in PA by measuring PA before and after long-term food deprivation in zebrafish. Compared to control-fed fish, food-deprived fish showed a significant increase in PA levels during the first 2 days of food deprivation. Subsequently, however, fasted fish showed a significant chronic decrease in PA compared to fish fed at weight-maintenance levels. These data are comparable to those seen with mammals, which also show a biphasic response of PA to caloric restriction. In a separate group of fish, long-term food deprivation, associated with decreases in PA, induced a significant increase in brain preproorexin mRNA levels compared to fed controls. No change in orexin mRNA was seen after 2 days of food deprivation. The finding that orexin mRNA expression is altered only after long-term starvation suggests that orexin may be coupled with the changes in PA seen at this time. Thus, the association between negative energy balance and reductions in PA occurs across genera in biology and is associated with predictable neu-

rological changes in brain gene expression.
Descriptors: zebrafish, behavior physiology, caloric restriction, motor activity physiology, Northern blotting, body weight physiology, brain metabolism, gene expression physiology, intracellular signaling peptides and intracellular signaling peptides and proteins metabolism, neuropeptides genetics and metabolism, messenger RNA biosynthesis, random allocation, reverse transcriptase PCR methods, starvation metabolism, time factors.

O'Malley, D.M., Q. Zhou, and E. Gahtan (2003). **Probing neural circuits in the zebrafish: a suite of optical techniques.** *Methods* 30(1): 49-63. ISSN: 0882-4010.
NAL Call Number: QP519.7.M47
Abstract: The ability to image neural activity in populations of neurons inside an intact animal, while obtaining single-cell or subcellular spatial resolution, has led to several advances in our understanding of vertebrate locomotor control. This result, first reported in a 1995 study of motoneurons in larval zebrafish, was the beginning of a series of technical developments that exploited the transparency and simplicity of the larval CNS. Presented here, in chronological fashion, is a suite of imaging techniques that have extended the ability to probe and optically dissect neural control systems. Included are methodological details pertaining to: (1). the in vivo optical recording of neural activity, (2). the optical dissection of complex neural architectures, and (3). additional fluorescence imaging-based techniques for the anatomical and physiological characterization of these systems. These approaches have provided insights into the descending neural control of escape and other locomotive behaviors, such as swimming and prey capture. The methods employed are discussed in relation to complementary and alternative imaging techniques, including, for example, the Nipkow disk confocal. While these methodologies focus on descending motor control in the larval zebrafish, the extension of such approaches to other neural systems is viewed as a promising and necessary step if neurobiologists are to bridge the gap between synaptic and brain region levels of analysis. The efficiency of optical techniques for surveying the cellular elements of intricate neural systems is of particular relevance because a comprehensive description of such elements is deemed necessary for a precise understanding of vertebrate neural architectures.
Descriptors: zebrafish, nerve net physiology, neurons physiology, confocal microscopy methods, nerve net chemistry and cytology, neurons chemistry and cytology, optics.

Ochocinska, M.J. and P.F. Hitchcock (2007). **Dynamic expression of the basic helix-loop-helix transcription factor neuroD in the rod and cone photoreceptor lineages in the retina of the embryonic and larval zebrafish.** *Journal of Comparative Neurology* 501(1): 1-12. ISSN: 0021-9967.
Abstract: NeuroD is a basic helix-loop-helix (bHLH) transcription factor critical for determining neuronal cell fate and regulating withdrawal from the cell cycle. We showed previously that, in goldfish, neuroD is expressed in the rod photoreceptor lineage, and we inferred that neuroD is also expressed in a subset of amacrine cells and nascent cone photoreceptors. Here we extended that study by examining the temporal and spatial expression pattern of neuroD in the embryonic and larval zebrafish and by identifying the cell types that express this gene. NeuroD expression in the developing zebrafish retina is dynamic, spanning early retinogenesis and the maturation of cone photoreceptors. In early retinogenesis neuroD expression expands from a small patch in the ventronasal retina, through the remaining retinal neuroepithelium. As retinogenesis progresses, neuroD expression becomes restricted to amacrine cells, immature cones, and cells of rod and cone lineages. This expression achieves an adult pattern by 96 hours postfertilization (hpf), whereupon the temporal pattern of neuroD expression in central retina is spatially recapitulated at the germinative margin. The

cellular pattern of expression suggests that neuroD regulates aspects of rod and cone genesis, but through separate cellular lineages. Furthermore, neuroD is coexpressed with the cone-rod-homeobox transcription factor (Crx) in putative cone progenitors and nascent cone photoreceptors, suggesting that, in the zebrafish retina, as in other vertebrate retinas, similar genetic cascades regulate photoreceptor genesis and maturation.
Descriptors: zebrafish embryos and larvae, basic helix loop helix transcription factors metabolism, retina cones metabolism, nerve tissue proteins metabolism, retina embryology and metabolism, retinal rods metabolism, cell lineage, retina cones cytology, metabolism, developmental gene expression regulation, homeodomain larva metabolism, mitosis, retina growth and development, retinal rodscytology, tissue distribution, trans activators genetics, zebrafish growth and development.

Ono, F., G. Mandel, and P. Brehm (2004). **Acetylcholine receptors direct rapsyn clusters to the neuromuscular synapse in zebrafish.** *Journal of Neuroscience* 24(24): 5475-81. ISSN: 0270-6474.
Abstract: Clustering of nicotinic muscle acetylcholine receptors (AChRs) requires association with intracellular rapsyn, a protein with an intrinsic ability to self-cluster. Previous studies on sofa potato (sop), an AChR null line of zebrafish, have suggested that AChRs may play an active role in subsynaptic localization of rapsyn clusters. To test this proposal directly, we identified and cloned the gene responsible for the sop phenotype and then attempted to rescue subsynaptic localization of the receptor-rapsyn complex in mutant fish. sop contains a leucine to proline mutation at position 28, near the N terminus of the zebrafish AChR delta subunit. Transient expression of mutant delta subunit in sop fish was unable to restore surface expression of muscle AChRs. In contrast, expression of wild-type delta subunit restored the ability of muscle to assemble surface receptors along with the ability of fish to swim. Most importantly, the ability of rapsyn clusters to localize effectively to subsynaptic sites also was rescued in large part. Our results point to direct involvement of the AChR molecule in restricting receptor-rapsyn clusters to the synapse.
Descriptors: zebrafish , muscle proteins metabolism, neuromuscular junction metabolism, cholinergic receptors physiology, molecular sequence data, skeletal muscle metabolism, mutation , protein subunits, cholinergic receptors biosynthesis, cholinergic receptors genetics, swimming.

Orger, M.B. and H. Baier (2005). **Channeling of red and green cone inputs to the zebrafish optomotor response.** *Visual Neuroscience* 22(3): 275-81. ISSN: 0952-5238.
Abstract: Visual systems break scenes down into individual features, processed in distinct channels, and then selectively recombine those features according to the demands of particular behavioral tasks. In primates, for example, there are distinct pathways for motion and form processing. While form vision utilizes color information, motion pathways receive input from only a subset of cone photoreceptors and are generally colorblind. To explore the link between early channeling of visual information and behavioral output across vertebrate species, we measured the chromatic inputs to the optomotor response of larval zebrafish. Using cone-isolating gratings, we found that there is a strong input from both red and green cones but not short-wavelength cones, which nevertheless do contribute to another behavior, phototaxis. Using a motion-nulling method, we measured precisely the input strength of gratings that stimulated cones in combination. The fish do not respond to gratings that stimulate different cone types out of phase, but have an enhanced response when the cones are stimulated together. This shows that red and green cone signals are pooled at a stage before motion detection. Since the two cone inputs are combined into a single 'luminance' channel,

the response to sinusoidal gratings is colorblind. However, we also find that the relative contributions of the two cones at isoluminance varies with spatial frequency. Therefore, natural stimuli, which contain a mixture of spatial frequencies, are likely to be visible regardless of their chromatic composition.

Descriptors: zebrafish embryos, larva, color perception physiology, retina cones physiology, motion perception physiology, visual pathways physiology, color, contrast sensitivity, visual pattern recognition, photic stimulation methods, reaction time, sensory thresholds, time factors.

Orger, M.B., A.R. Kampff, K.E. Severi, J. Bollmann, and F. Engert (2008). **Control of visually guided behavior by distinct populations of spinal projection neurons**. *Nature Neuroscience* 11(3): 327-333. ISSN: 1097-6256.

NAL Call Number: QP351

Descriptors: zebrafish, calcium imaging, circuits, computed tomography, functional morphology, motor systems and task performance, neurons, spinal cord, visual pathways and stimuli.

Ott, M., B.C. Walz, U.J. Paulsen, A.F. Mack, and H.J. Wagner (2007). **Retinotectal ganglion cells in the zebrafish, *Danio rerio*.** *Journal of Comparative Neurology* 501(4): 647-58. ISSN: 0021-9967.

Descriptors: zebrafish, retina cytology, retinal ganglion cells physiology, superior colliculus cytology, fluorescent dyes metabolism, numerical analysis, computer assisted, retina anatomy and histology, retinal ganglion cells classification.

Owens, K.N., D.E. Cunningham, G. MacDonald, E.W. Rubel, D.W. Raible, and R. Pujol (2007). **Ultrastructural analysis of aminoglycoside-induced hair cell death in the zebrafish lateral line reveals an early mitochondrial response.** *Journal of Comparative Neurology* 502(4): 522-43. ISSN: 0021-9967.

Descriptors: zebrafish, hair cells drug effects, lateral line system drug effects, mitochondria drug effects, neomycin toxicity, cell death drug effects and physiology, drug dose response relationship, hair cells pathology and physiopathology, lateral line system pathology and system physiopathology, electron microscopy, mitochondria pathology and ultrastructure, protein synthesis inhibitors toxicity, reaction time drug effects and physiology, time factors.

Page-McCaw, P.S., S.C. Chung, A. Muto, T. Roeser, W. Staub, K.C. Finger-Baier, J.I. Korenbrot, and H. Baier (2004). **Retinal network adaptation to bright light requires tyrosinase**. *Nature Neuroscience* 7(12): 1329-1336. ISSN: 1097-6256.

NAL Call Number: QP351

Descriptors: zebrafish, acclimatization, adaptations, electroretinograms, enzymes, levodopa, light intensity, melanin, neural networks, photoreceptors, retina, retinas, vision, visual pigments, visual system, retinal pigment epithelium, tyrosinase, *Danio rerio*.

Palevitch, O., K. Kight, E. Abraham, S. Wray, Y. Zohar, and Y. Gothilf (2007). **Ontogeny of the GnRH systems in zebrafish brain: in situ hybridization and promoter-reporter expression analyses in intact animals.** *Cell and Tissue Research* 327(2): 313-22. ISSN: 0302 766X.

NAL Call Number: QH581.A1Z4

Abstract: The ontogeny of two gonadotropin-releasing-hormone (GnRH) systems, salmon GnRH (sGnRH) and chicken GnRH-II (cGnRH-II), was investigated in zebrafish (*Danio rerio*). In situ hybridization (ISH) first detected sGnRH mRNA-expressing cells at 1 day

post-fertilization (pf) anterior to the developing olfactory organs. Subsequently, cells were seen along the ventral olfactory organs and the olfactory bulbs, reaching the terminal nerve (TN) ganglion at 5-6 days pf. Some cells were detected passing posteriorly through the ventral telencephalon (10-25 days pf), and by 25-30 days pf, sGnRH cells were found in the hypothalamic/preoptic area. Continuous documentation in live zebrafish was achieved by a promoter-reporter expression system. The expression of enhanced green fluorescent protein (EGFP) driven by the sGnRH promoter allowed the earlier detection of cells and projections and the migration of sGnRH neurons. This expression system revealed that long leading processes, presumably axons, preceded the migration of the sGnRH neuron somata. cGnRH-II mRNA expressing cells were initially detected (1 day pf) by ISH analysis at lateral aspects of the midbrain and later on (starting at 5 days pf) at the midline of the midbrain tegmentum. Detection of red fluorescent protein (DsRed) driven by the cGnRH-II promoter confirmed the midbrain expression domain and identified specific hindbrain and forebrain cGnRH-II-cells that were not identified by ISH. The forebrain DsRed-expressing cells seemed to emerge from the same site as the sGnRH-EGFP-expressing cells, as revealed by co-injection of both constructs. These studies indicate that zebrafish TN and hypothalamic sGnRH cell populations share a common embryonic origin and migratory path, and that midbrain cGnRH-II cells originate within the midbrain.

Descriptors: zebrafish larva, olfactory organs, brain growth and development, gonadotropin releasing hormone metabolism, neural pathways growth and development, brain cytology and metabolism, gene expression, gonadotropin releasing hormone analogs and derivatives and genetics, green fluorescent proteins genetics and metabolism, hypothalamus cytology and metabolism, in situ hybridization, larva growth, development and metabolism, mesencephalon cytology and metabolism, neural pathways metabolism, neurons cytology and metabolism, olfactory pathways cytology and metabolism, preoptic area cytology and metabolism, messenger RNA genetics and metabolism, recombinant fusion proteins genetics and metabolism, telencephalon cytology and metabolism, transfection.

Panzer, J.A., Y. Song, and R.J. Balice Gordon (2006). **In vivo imaging of preferential motor axon outgrowth to and synaptogenesis at prepatterned acetylcholine receptor clusters in embryonic zebrafish skeletal muscle.** *Journal of Neuroscience* 26(3): 934-47. ISSN: 0270-6474.

Abstract: Little is known about the spatial and temporal dynamics of presynaptic and postsynaptic specializations that culminate in synaptogenesis. Here, we imaged presynaptic vesicle clusters in motor axons and postsynaptic acetylcholine receptor (AChR) clusters in embryonic zebrafish to study the earliest events in synaptogenesis in vivo. Prepatterned AChR clusters are present on muscle fibers in advance of motor axon outgrowth from the spinal cord. Motor axon growth cones and filopodia are selectively extended toward and contact prepatterned AChR clusters, followed by the rapid clustering of presynaptic vesicles and insertion of additional AChRs, hallmarks of synaptogenesis. All initially formed neuromuscular synapses contain AChRs that were inserted into the membrane at the time the prepattern is present. Examination of embryos in which AChRs were blocked or clustering is absent showed that neither receptor activity or receptor protein is required for these events to occur. Thus, during initial synaptogenesis, postsynaptic differentiation precedes presynaptic differentiation, and prepatterned neurotransmitter clusters mark sites destined for synapse formation.

Descriptors: zebrafish motor axons, axons physiology, motor neurons physiology, skeletal muscle embryology, cholinergic receptors physiology, synapses physiology, genetically modi-

fied , cytology, embryology, physiology, growth cones physiology, confocal microscopy, motor neurons cytology, skeletal muscle physiology.

Park, H.C., J. Boyce, J. Shin, and B. Appel (2005). **Oligodendrocyte specification in zebrafish requires notch-regulated cyclin-dependent kinase inhibitor function.** *Journal of Neuroscience* 25(29): 6836-44. ISSN: 0270-6474.

Abstract: Cyclin-dependent kinase inhibitors (Cdkis) influence both cell-cycle progression and differentiation of neural cells. However, the precise roles of Cdkis in coordinating formation of neurons and glia and the mechanisms that regulate expression of genes that encode Cdkis in the vertebrate CNS remain unknown. Here, we report that, in zebrafish, expression of the Cdki gene cyclin-dependent kinase inhibitor 1c (cdkn1c), a p57 homolog, is negatively regulated by Delta-Notch signaling and that Cdkn1c function is required for neural plate cells to stop dividing and differentiate as neurons on schedule, even in the absence of Notch signaling activity. Furthermore, Cdkn1c function is required for specification of oligodendrocytes from ventral spinal cord precursors. We propose that levels of cdkn1c expression are an important factor in regulating neural development: high levels of Cdkn1c promote cell-cycle exit and neuronal development, whereas, during late embryogenesis, neural cells that have low but functional levels of Cdkn1c, regulated by Notch activity, are specified for oligodendrocyte fate.

Descriptors: zebrafish, cyclin dependent kinase inhibitor p57 metabolism, homeodomain proteins metabolism, nerve tissue proteins metabolism, oligodendroglia cytology , and physiology, Notch1 receptor metabolism, proteins metabolism, genetically modified, cell differentiation physiology, cell division physiology, cyclin dependent kinase inhibitor p57 genetics, developmental gene expression regulation, homeodomain nerve tissue neurons cytology, neurons physiology, Notch1 receptor genetics, signal transduction physiology, spinal cord cytology, embryology and physiology.

Peitsaro, N., J. Kaslin, O.V. Anichtchik, and P. Panula (2003). **Modulation of the histaminergic system and behaviour by alpha-fluoromethylhistidine in zebrafish.** *Journal of Neurochemistry* 86(2): 432-41. ISSN: 0022-3042.

NAL Call Number: QP351.J6

Abstract: The functional role of histamine (HA) in zebrafish brains was studied. Zebrafish did not display a clear circadian variation in brain HA levels. Loading of zebrafish with l-histidine increased HA concentration in the brain. A single injection of the histidine decarboxylase (HDC) inhibitor, alpha-fluoromethylhistidine (alpha-FMH), gave rise to a rapid reduction in zebrafish brain HA. Low HDC activity in the brain after injections verified the effect of alpha-FMH. A reduction in the number of histaminergic fibres but not neurones and an increased expression of HDC mRNA was evident after alpha-FMH. Automated behavioural analysis after alpha-FMH injection showed no change in swimming activity, but abnormalities were detected in exploratory behaviour examined in a circular tank. No significant behavioural changes were detected after histidine loading. The time spent for performance in the T-maze was significantly increased in the first trial 4 days after alpha-FMH injections, suggesting that lack of HA may impair long-term memory. The rostrodorsal telencephalon, considered to correspond to the mammalian amygdala and hippocampus in zebrafish, is densely innervated by histaminergic fibres. These results suggest that low HA decreases anxiety and/or affects learning and memory in zebrafish, possibly through mechanisms that involve the dorsal forebrain.

Descriptors: zebrafish, behavior drug effects, brain metabolism, histamine metabolism, methylhistidines pharmacology, brain drug effects, circadian rhythm physiology, enzyme

induction drug effects, enzyme inhibitors pharmacology, histidine pharmacology, histidine decarboxylase antagonists and inhibitors, histidine decarboxylase metabolism, maze learning drug effects, swimming.

Pellegrini, E., K. Mouriec, I. Anglade, A. Menuet, Y. Le Page, M.M. Gueguen, M.H. Marmignon, F. Brion, F. Pakdel, and O. Kah (2007). **Identification of aromatase-positive radial glial cells as progenitor cells in the ventricular layer of the forebrain in zebrafish.** *Journal of Comparative Neurology* 501(1): 150-67. ISSN: 0021-9967.
Abstract: Compared with other vertebrates, the brain of adult teleost fish exhibits two unique features: it exhibits unusually high neurogenic activity and strongly expresses aromatase, a key enzyme that converts aromatizable androgens into estrogens. Until now, these two features, high neurogenic and aromatase activities, have never been related to each other. Recently, it was shown that aromatase is expressed in radial glial cells of the forebrain and not in neurons. Here, we further document that Aromatase B is never detected in cells expressing the markers of postmitotic neurons, Hu and acetylated tubulin. By using a combination of bromodeoxyuridine (BrdU) treatment and immunohistochemical techniques, we demonstrate for the first time to our knowledge that aromatase-positive radial cells actively divide to generate newborn cells in many forebrain regions. Such newborn cells can further divide, as shown by BrdU-proliferating cell nuclear antigen double staining. We also demonstrate that, over time, newborn cells move away from the ventricles, most likely by migrating along the radial processes. Finally, by using antisera to Hu and acetylated tubulin, we further document that some of the newborn cells derived from radial glia differentiate into neurons. These data provide new evidence for the mechanism of neurogenesis in the brain of adult fish. In addition, given that estrogens are well-known neurotrophic and neuroprotective factors affecting proliferation, apoptosis, migration, and differentiation, the expression of aromatase in the neural stem cells of the adult strongly demonstrates that the fish brain is an outstanding model for studying the effects of estrogens on adult neurogenesis and brain repair.
Descriptors: zebrafish, aromatase metabolism, neuroglia enzymology, prosencephalon cytology and enzymology, stem cells enzymology, metabolism, cell differentiation, division, movement and proliferation, cerebral ventricles, isoenzymes metabolism, neuroglia cytology, neuroglia physiology, neurons cytology, prosencephalon metabolism, stem cells cytology and physiology, tubulin metabolism.

Petit, A., A. Pasini, C.A. DaCosta, E. Ayral, J.F. Hernandez, C. Dumanchin-Njock, C.J. Phiel, P. Marambaud, S. Wilk, M. Farzan, P. Fulcrand, J. Martinez, D. Andrau, and F. Checler (2003). **Jlk isocoumarin inhibitors: selective gamma-secretase inhibitors that do not interfere with notch pathway in vitro or in vivo.** *Journal of Neuroscience Research* 74(3): 370-377. ISSN: 0360 4012.
Descriptors: zebrafish, enzymology, pharmacology, Alzheimer's disease, behavioral and mental disorders, nervous system disease, Notch pathway, somitogenesis.

Pineda, R.H., R.A. Heiser, and A.B. Ribera (2005). **Developmental, molecular, and genetic dissection of INa in vivo in embryonic zebrafish sensory neurons.** *Journal of Neurophysiology* 93(6): 3582-93. ISSN: 0022-3077.
Abstract: The presence of multiple Nav1 isotypes within a neuron and the lack of specific blockers hamper identification of the in vivo roles of sodium current (INa) components, especially during embryonic stages. To identify the functional properties of INa components in vivo in developing neurons, we took a molecular genetic approach. Embryonic zebrafish Rohon-Beard (RB) mechanosensory neurons express two different sodium channel isotypes:

Nav1.1 and Nav1.6. To examine the properties of Nav1.1- and Nav1.6-encoded currents in RB cells at different developmental stages, we eliminated the contribution of Nav1.6 and Nav1.1 channels, respectively, using an antisense morpholino (MO) approach. MOs were injected into one-cell stage embryos, and RB sodium currents were recorded using patch-clamp techniques in both conventional whole cell mode as well from nucleated patches. Only a subset of RB cells appeared to be affected by the Nav1.1MO. Overall, the effect of the Nav1.1MO was a small 25% average reduction in current amplitude. Further, Nav1.1MO effects were most pronounced in RB cells of younger embryos. In contrast, the effects of the Nav1.6 MO were observed in all cells and increased as development proceeded. These results indicated that developmental upregulation of RB INa entailed an increase in the number of functional Nav1.6 channels. In addition, analysis of voltage-dependent steady-state activation and inactivation parameters revealed that specific functional properties of channels were also developmentally regulated. Finally, analysis of macho mutants indicated that developmental upregulation of INa was absent in RB cells. These results indicate that MOs are a useful tool for the molecular dissection and analysis of ion channel function in vivo.

Descriptors: zebrafish embryos, developmental gene expression regulation physiology, afferent neurons physiology, sodium channels genetics, spinal cord cytology, age factors, analysis of variance, cell nucleus drug effects and physiology, embryo, gene expression physiology, developmental gene expression regulation drug effects, ion channel gating drug effects and physiology, ion channel gating radiation effects, membrane potentials drug effects and physiology, membrane potentials radiation effects, microinjections, molecular biology methods, mutation physiology, afferent neurons cytology and drug effects, oligodeoxyribonucleotides, antisense pharmacology, patch clamp techniques methods, reaction time physiology, sodium channel blockers pharmacology and metabolism, tetrodotoxin pharmacology.

Placzek, M. and J. Briscoe (2005). **The floor plate: multiple cells, multiple signals**. *Nature Reviews Neuroscience* 6(3): 230-240. ISSN: 1471 003X.

NAL Call Number: RC321

Descriptors: zebrafish embryos, nervous system, neural coordination, anteroposterior axis.

Prober, D.A., J. Rihel, A.A. Onah, R.J. Sung, and A.F. Schier (2006). **Hypocretin/orexin overexpression induces an insomnia-like phenotype in zebrafish.** *Journal of Neuroscience* 26(51): 13400-10. ISSN: 0270-6474.

Abstract: As many as 10% of humans suffer chronic sleep disturbances, yet the genetic mechanisms that regulate sleep remain essentially unknown. It is therefore crucial to develop simple and cost-effective vertebrate models to study the genetic regulation of sleep. The best characterized mammalian sleep/wake regulator is hypocretin/orexin (Hcrt), whose loss results in the sleep disorder narcolepsy and that has also been implicated in feeding behavior, energy homeostasis, thermoregulation, reward seeking, addiction, and maternal behavior. Here we report that the expression pattern and axonal projections of embryonic and larval zebrafish Hcrt neurons are strikingly similar to those in mammals. We show that zebrafish larvae exhibit robust locomotive sleep/wake behaviors as early as the fifth day of development and that Hcrt overexpression promotes and consolidates wakefulness and inhibits rest. Similar to humans with insomnia, Hcrt-overexpressing larvae are hyperaroused and have dramatically reduced abilities to initiate and maintain rest at night. Remarkably, Hcrt function is modulated by but does not require normal circadian oscillations in locomotor activity. Our zebrafish model of Hcrt overexpression indicates that the ancestral function of Hcrt is to promote locomotion and inhibit rest and will facilitate the discovery of neural circuits, genes, and drugs that regulate Hcrt function and sleep.

Descriptors: zebrafish, intracellular signaling peptides and neuropeptides biosynthesis and genetics, phenotype, sleep initiation and maintenance disorders genetics and metabolism, proteins biosynthesis and genetics, circadian rhythm genetics, gene expression regulation physiology, intracellular signaling peptides and, motor activity genetics, neuropeptides physiology, sleep genetics.

Puppala, D., H. Maaswinkel, B. Mason, S.J. Legan, and L. Li (2004). **An in vivo microdialysis study of light/dark-modulation of vitreal dopamine release in zebrafish.** *Journal of Neurocytology* 33(2): 193-201. ISSN: 0030-4864.

Abstract: Dopamine (DA) is an important neuromodulator in the visual system. The release of DA in the retina largely depends on environmental lighting conditions. Most previous studies have assessed the effect of illumination on retinal DA or its metabolites using homogenates or in vitro preparations. This study was designed to investigate the effect of transitions between lighting conditions--from dark to steady or flickering light and vice versa--on retinal DA release in zebrafish using in vivo microdialysis. The transition from dark to flickering light increased DA release, whereas the transition from flickering light to dark decreased it. This latter effect depended on time of day within the light period, e.g., it was strongest in the late afternoon. When using steady light, none of these effects were seen. Our study also demonstrates that in vivo microdialysis can successfully be applied to the investigation of retinal DA release in zebrafish.

Descriptors: zebrafish, dopamine secretion, neurons secretion, retina secretion, vitreous body metabolism, adaptation, ocular physiology, circadian rhythm physiology and radiation effects, dark adaptation physiology, dopamine metabolism, light, microdialysis methods, neurons metabolism and radiation effects, photic stimulation, photoperiod, retina metabolism and radiation effects.

Raible, D.W., J.L. Lewis, and J.W. Ragland (2005). **Combinatorial signaling in zebrafish neural crest development**. *Federation of American Societies for Experimental Biology Journal* 19(5, Suppl. S, Part 2): A1688. ISSN: 0892 6638.

NAL Call Number: QH301.F3

Abstract: Both Writ and Bmp signals have been implicated in the formation of neural crest. We have explored the roles of these signals in zebrafish neural crest induction by several approaches. Study of embryos lacking involuted mesoderm demonstrates that mesoderm-derived signals, including Bmp inhibitors, are dispensable for the formation of neural crest. Inactivation of Wnt8 using antisense morpholino oligonucleotides demonstrates that this molecule is critical for neuralcrest formation. We have used conditional inactivation of the Wnt and Bmp signaling pathways to determine where and when these signals are needed in the formation of neural crest. Dominant inhibitors of Writ or Bmp signaling were fused to GFP and placed under the control of the zebrafish hsp70 promoter. To deter-mine the critical period for each signaling pathway, transgenic lines carrying either construct were subjected to heat shock at different times. These experiments demonstrate that Bmps are needed at a period earlier than Writs, suggesting that these signals act sequentially. We are currently testing whether signals must be received directly by neural crest precursors by generating mosaic embryos. Together these experiments should clarify how combinations of signals are used for the formation of the neural crest. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, nervous system, neural coordination, reproductive system, combinatorial signaling.

Ramdya, P., B. Reiter, and F. Engert (2006). **Reverse correlation of rapid calcium signals in the zebrafish optic tectum in vivo.** *Journal of Neuroscience Methods* 157(2): 230-7. ISSN: 0165-0270.

NAL Call Number: RC346

Abstract: Reverse correlation techniques provide a quantitative means of computing neuronal input/output relationships. Until now these methods have been limited to electrically recorded responses since unprocessed optical signals generally lack necessary temporal characteristics. We sought to overcome this barrier since combining reverse correlation with calcium imaging would afford a powerful alternative to current methods of measuring response properties of neurons non-invasively in vivo. We labeled zebrafish optic tecta with a calcium indicator and measured responses to a whole-field random flicker light stimulus. Although calcium signals exhibited slow decay kinetics, we could use computational modeling to show that the positive differential of these traces extracts high frequency information. Experimentally, we found that calcium signals processed in this way were synchronous with simultaneously measured synaptic responses and could be used with reverse correlation to determine temporal filters of neurons in the zebrafish optic tectum. These findings demonstrate that calcium responses to physiological stimulation can be processed to obtain rapid signal information and consequently to determine linear filter properties in vivo.

Descriptors: zebrafish , calcium signaling physiology, diagnostic imaging, evoked potentials, visual physiology, neurological models, superior colliculus metabolism, neurons metabolism, patch clamp techniques, photic stimulation.

Ren, J.Q. and L. Li (2004). **Rod and cone signaling transmission in the retina of zebrafish: An ERG study** . *International Journal of Neuroscience* 114(2): 259-270. ISSN: 0020-7454.

Descriptors: zebrafish, adaptations, bipolar cells, cones, cornea, electroretinograms, freshwater fish, light effects, neurotransmission, retina, retinas, signal transduction, *Danio rerio*, Teleostei.

Rigo, J.M. and P. Legendre (2006). **Frequency-dependent modulation of glycine receptor activation recorded from the zebrafish larvae hindbrain.** *Neuroscience* 140(2): 389-402. ISSN: 0306-4522.

NAL Call Number: QP351.N48

Abstract: In vertebrates, most glycinergic inhibitory neurons discharge phasically at a relatively low frequency. Such a pattern of glycine liberation from presynaptic terminals may affect the kinetics of post-synaptic glycine receptors. To examine this influence, we have analyzed the behavior of glycine receptors in response to repetitive stimulation at frequencies at which consecutive outside-out currents did not superimpose (0.5-4 Hz). Neurotransmitter release was mimicked on outside-out patches from zebrafish hindbrain Mauthner cells using fast flow application techniques. The amplitude of outside-out currents evoked by short (1 ms) repetitive applications of a saturating concentration (3 mM) of glycine remained unchanged for application frequencies<or=1 Hz. When the application frequency was increased from 1 to 4 Hz, the amplitude of the outside-out currents decreased with time to reach a steady state level. This decrease in current amplitude was larger and occurred faster with increasing application frequencies. Recovery occurred when the stimulation frequency was decreased back to 1 Hz. The recovery time constant was independent on the application frequency. This frequency-dependent inhibition was also observed for non-saturating glycine concentrations. Our results indicate that glycine receptor activity is down-regulated when the stimulation frequency increases to values>1 Hz. Glycine-evoked current simulations using a simple Markov model describing zebrafish glycine receptor kinetic behavior, indicates that

this down-regulation of glycine receptor efficacy is due to a progressive accumulation of the receptors in a long lasting desensitization state. Our simulations suggest that this down-regulation can occur even when spontaneous inhibitory currents were generated randomly at a frequency>1 Hz.

Descriptors: zebrafish, glycine metabolism, neural inhibition physiology, neurons metabolism, glycine receptors metabolism, rhombencephalon metabolism, synaptic transmission physiology, drug dose response relationship, down regulation drug effects and physiology, efferent pathways cytology, drug effects and metabolism, electric stimulation, glycine pharmacology, Markov chains, membrane potentials drug effects, membrane potentials physiology, neurological models, neural inhibition drug effects, neurons drug effects, patch clamp techniques, glycine receptors drug effects, reticular formation cytology, drug effects and metabolism , rhombencephalon cytology and drug effects, synaptic transmission drug effects.

Rink, E. and S. Guo (2004). **The too few mutant selectively affects subgroups of monoaminergic neurons in the zebrafish forebrain.** *Neuroscience* 127(1): 147-54. ISSN: 0306-4522.

NAL Call Number: QP351.N48

Abstract: Monoaminergic neurons are present in small numbers and in multiple distinct locations of the vertebrate CNS. They are involved in important functions such as movement coordination, motivation, and the response to environmental stress. However, the mechanisms involved in their subtype specification are not well understood. In this study, we examined the states of forebrain dopaminergic (DA) and serotonergic (5HT) neurons in larval and adult zebrafish of wild type and the too few mutant. The majority of DA and 5HT neuronal subgroups that were found in adults were established in the 6-day old larval zebrafish. Rather than affecting all monoaminergic neurons in the forebrain, selective subgroups of these neurons are reduced in the too few mutant, starting from the larval stage. Taken together, our study establishes that similar to DA neurons, distinct subtypes of 5HT neurons exist in larval as well as adult zebrafish. The development of a subset of these monoaminergic neurons is dependent on the too few gene product. Thus, this mutant is potentially important for understanding the development as well as the function of forebrain DA and 5HT neurons.

Descriptors: zebrafish larvae, biogenic monoamines biosynthesis, nervous system malformations genetics, neurons metabolism, prosencephalon abnormalities, growth and development, cell differentiation genetics, dopamine biosynthesis, immunohistochemistry, larva cytology, growth, development and metabolism, mutation genetics, nervous system malformations metabolism, nervous system malformations physiopathology, neurons cytology, prosencephalon cytology, serotonin biosynthesis, tyrosine 3 monooxygenase biosynthesis.

Rink, E. and M.F. Wullimann (2004). **Connections of the ventral telencephalon (subpallium) in the zebrafish (*Danio rerio*).** *Brain Research* 1011(2): 206-20. ISSN: 0006 8993.

NAL Call Number: QP376

Descriptors: zebrafish larvae, lysine analogs and derivatives, neural pathways anatomy and histology, telencephalon anatomy and histology, amino acids metabolism, lysine metabolism, neural pathways physiology, telencephalon physiology.

Rinner, O., Y.V. Makhankov, O. Biehlmaier, and S.C. Neuhauss (2005). **Knockdown of cone-specific kinase GRK7 in larval zebrafish leads to impaired cone response recovery and delayed dark adaptation.** *Neuron* 47(2): 231-42. ISSN: 0896-6273.

Abstract: Phosphorylation of rhodopsin by rhodopsin kinase GRK1 is an important desen-

sitization mechanism in scotopic vision. For cone vision GRK1 is not essential. However, cone opsin is phosphorylated following light stimulation. In cone-dominant animals as well as in humans, but not in rodents, GRK7, a cone-specific homolog of GRK1, has been identified in cone outer segments. To investigate the function of GRK7 in vivo, we cloned two orthologs of grk7 in zebrafish and knocked down gene expression of grk7a in zebrafish larvae by morpholino antisense nucleotides. Photoresponse recovery in Grk7a-deficient larvae was delayed in electroretinographic measurements, and temporal contrast sensitivity was reduced, particularly under bright-light conditions. These results show that function of a cone-specific kinase is essential for cone vision in the zebrafish retina and argue that pigment bleaching and spontaneous decay alone are not sufficient for light adaptation and rapid cone response inactivation.

Descriptors: zebrafish, retina cones physiology, dark adaptation genetics, larva genetics, protein serine threonine kinases physiology, genetically modified, arrestin metabolism, Western blotting, molecular cloning methods, retina cones embryology and metabolism, contrast sensitivity genetics and physiology, electroretinography methods, evoked potentials genetics and radiation effects, eye metabolism and pathology, developmental gene expression regulation, immunohistochemistry methods, in situ hybridization methods, larva physiology, photic stimulation methods, pineal gland embryology and metabolism, protein serine threonine kinases deficiency, psychophysics, rhodopsin metabolism, time factors, .

Rodriguez Martin, I., E.M. de Velasco, and R.E. Rodriguez (2007). **Characterization of cannabinoid-binding sites in zebrafish brain.** *Neuroscience Letters* 413(3): 249-54. ISSN: 0304-3940.

NAL Call Number: QP351.N3

Abstract: We present here the pharmacological characterization of cannabinoid-binding sites in zebrafish brain homogenates using radiolabeled binding techniques. The nonselective agonist [3H]-CP55940 binds with high affinity (KD = 0.50+/-0.06 nM and a Bmax = 1047+/-36.01 fmol/mg protein), displaying one binding site. The slightly CB2 selective agonist [3H]-WIN55212-2 also binds with high affinity to zebrafish brain membranes displaying two different binding sites with affinities KD1 = 0.35+/-0.09 nM and KD2 = 105.81+/-66.36 nM. Competition binding assays using [3H]-WIN55212-2 and several unlabeled ligands were performed. WIN55212-2 significantly displaced the tritiated ligand binding showing the two binding sites observed with its tritiated homologous, while the slightly selective CB1 cannabinoid ligand HU-210, the nonselective cannabinoid ligand CP55940 and the endogenous cannabinoid ligand anandamide presented one binding site. Also, the functionality of these cannabinoid sites was analyzed using the known [35S] GTPgammaS assay. All the agonist used presented an agonist profile and the rank order for potency was HU-210 > WIN55212-2 > CP55940 >anandamide. Our results provide evidence that, although some of the typical cannabinoid ligands for mammalian receptors do not fully recognize the cannabinoid-binding sites in zebrafish brain, the activity of the endogenous zebrafish cannabinoid system might not significantly differ from that displayed by the cannabinoid system described in other species. Hence the study of zebrafish cannabinoid activity may contribute to an understanding of the endogenous cannabinoid system in higher vertebrates.

Descriptors: zebrafish, cannabinoid activity, competitive drug effects, brain metabolism, cannabinoids pharmacokinetics, cyclohexanols pharmacokinetics, drug dose response relationship, drug interactions, excitatory amino acid antagonists pharmacology, guanosine 5' O 3 thiotriphosphate metabolism, morpholines pharmacokinetics, naphthalenes pharmacoki-

netics, protein bindingdrug effects, radioligand assay methods, tetrahydrocannabinol analogs and derivatives, tetrahydrocannabinol pharmacology, tritium pharmacokinetics.

Roeser, T. and H. Baier (2003). **Visuomotor behaviors in larval zebrafish after GFP-guided laser ablation of the optic tectum.** *Journal of Neuroscience* 23(9): 3726-34. ISSN: 0270-6474.
Abstract: The optic tectum is the largest visual center in most vertebrates and the main target for retinal ganglion cells (RGCs) conveying visual information from the eye to the brain. The retinotectal projection has served as an important model in many areas of developmental neuroscience. However, knowledge of the function of the tectum is limited. We began to address this issue using laser ablations and subsequent behavioral testing in zebrafish. We used a transgenic zebrafish line that expresses green-fluorescent protein in RGCs projecting to the tectum. By aiming a laser beam at the labeled retinal fibers demarcating the tectal neuropil, the larval tectum could be selectively destroyed. We tested whether tectum-ablated zebrafish larvae, when presented with large-field movements in their surroundings, displayed optokinetic responses (OKR) or optomotor responses (OMR), two distinct visuomotor behaviors that compensate for self-motion. Neither OKR nor OMR were found to be dependent on intact retinotectal connections. Also, visual acuity remained unaffected. Tectum ablation, however, slowed down the OKR by reducing the frequency of saccades but left tracking velocity, gain, and saccade amplitude unaffected. Removal of the tectum had no effect on the processing of second-order motion, to which zebrafish show both OKR and OMR, suggesting that the tectum is not an integral part of the circuit that extracts higher-order cues in the motion pathway.
Descriptors: zebrafish larva, tectum ablation, behavior physiology, eye movements physiology, luminescent proteins biosynthesis, motion perception physiology, superior colliculus physiology, genetically modified, cues, green fluorescent proteins, larva, laser surgery, luminescent retinal ganglion cells physiology, saccades physiology, superior colliculus surgery, visual acuity physiology, visual pathways physiology.

Sakata, Y., J.K. Olson, and W.C. Michel (2003). **Assessment of neuronal maturation and acquisition of functional competence in the developing zebrafish olfactory system.** *Methods in Cell Science* 25(1-2): 39-48. ISSN: 1381 5741.
NAL Call Number: QH573.T52
Abstract: Olfactory coding at the level of the olfactory bulb is thought to depend upon an ensemble response of mitral cells receiving input from chemotopically-organized projections of olfactory sensory neurons and regulated by lateral inhibitory circuits. Immunocytochemical methods are described to metabolically classify neurons in the developing zebrafish olfactory system based on the relative concentrations of taurine, glutamate, GABA (and potentially other small biogenic amines) and a small guanidium-based cation, agmatine, which labels NMDA-sensitive cells by permeating through active ionotropic glutamate receptor channels. Using metabolic profiling in conjunction with activity dependent labeling we demonstrate that neuronal differentiation in the developing olfactory bulb, as assessed by acquisition of a mature neurochemical profile, and sensitivity to an ionotropic glutamate receptor agonist, NMDA, occurs during the second day of development. This experimental approach is likely to be useful in studies concerned with the development of glutamatergic signaling pathways.
Descriptors: zebrafish, olfactory bulb metabolism, olfactory receptor neurons metabolism, glutamate receptors metabolism, gamma aminobutyric acid metabolism, agmatine metabolism, amino acids metabolism, cytology, metabolism, developmental gene expression

regulation physiology, glutamic acid metabolism, n methylaspartate metabolism, olfactory bulb cytology, embryology, olfactory receptor neurons cytology and embryology.

Sato, Y., N. Miyasaka, and Y. Yoshihara (2007). **Hierarchical regulation of odorant receptor gene choice and subsequent axonal projection of olfactory sensory neurons in zebrafish.** *Journal of Neuroscience* 27(7): 1606-15. ISSN: 0270-6474.

Abstract: In both *Drosophila* and mice, olfactory sensory neurons (OSNs) expressing a given odorant receptor (OR) project axons to specific glomeruli in the antennal lobe or olfactory bulb (OB), developing a topographic odor map. To gain insights into the modes of OR expression and axonal projection in zebrafish, we generated a bacterial artificial chromosome transgenic line carrying an OR gene cluster in which two OR-coding sequences, OR111-7 and OR103-1, were replaced with yellow fluorescent protein (YFP) and cyan fluorescent protein (CFP), respectively. In the transgenic embryos, YFP and CFP signals appear in small populations of OSNs at an early stage of development when OR expression is first observed. Time-lapse imaging of living embryos revealed that both YFP- and CFP-expressing OSNs project axons to the medial portion of the OB. This pattern of axonal projection is maintained in the adult transgenic fish, in which fluorescently labeled OSN axons target a topographically fixed cluster of glomeruli in the medial OB. Because the OR-coding sequences were replaced with fluorescent reporter genes, we examined which OR genes are expressed in YFP/CFP-expressing OSNs and found that the OR choice is mostly restricted to OR members within the same subfamily of the cluster. Furthermore, we found that the one receptor-one neuron rule is not always applicable to zebrafish OSNs and that multiple receptors-one neuron is true for a subpopulation of OSNs in both wild-type and transgenic fish. These data demonstrate two distinct modes of OR expression and suggest a model of the hierarchical regulation of OR gene choice and subsequent axonal projection in the zebrafish olfactory system.

Descriptors: zebrafish, axons physiology, developmental gene expression regulation physiology, olfactory pathways cytology, olfactory receptor neurons cytology, odorant receptors genetics and metabolism, age factors, genetically modified, chromosomes, bacterial physiology immunohistochemistry methods, luminescent proteins genetics and metabolism, olfactory receptor neurons physiology.

Sato, Y., N. Miyasaka, and Y. Yoshihara (2005). **Mutually exclusive glomerular innervation by two distinct types of olfactory sensory neurons revealed in transgenic zebrafish.** *Journal of Neuroscience* 25(20): 4889-97. ISSN: 0270-6474.

Abstract: The olfactory epithelium of fish contains two major types of olfactory sensory neurons (OSNs) that are distinct morphologically (ciliated vs microvillous) and possibly functionally. Here, we found that these OSNs express different sets of signal transduction machineries: the ciliated OSNs express OR-type odorant receptors, cyclic nucleotide-gated channel A2 subunit, and olfactory marker protein (OMP), whereas the microvillous OSNs express V2R-type receptors and transient receptor potential channel C2 (TRPC2). To visualize patterns of axonal projection from the two types of OSNs to the olfactory bulb (OB), we generated transgenic zebrafish in which spectrally distinct fluorescent proteins are expressed in the ciliated and microvillous OSNs under the control of OMP and TRPC2 gene promoters, respectively. An observation of whole-mount OB in adult double-transgenic zebrafish revealed that the ciliated OSNs project axons mostly to the dorsal and medial regions of the OB, whereas the microvillous OSNs project axons to the lateral region of the OB. A careful histological examination of OB sections clarified that the axons from the two distinct types of OSNs target different glomeruli in a mutually exclusive manner. This segregation is already

established at very early developmental stages in zebrafish embryos. These findings clearly demonstrate the relationships among cell morphology, molecular signatures, and axonal terminations of the two distinct types of OSNs and suggest that the two segregated neural pathways are responsible for coding and processing of different types of odor information in the zebrafish olfactory system.

Descriptors: zebrafish, axons metabolism, olfactory bulb cytology, olfactory mucosa innervation, olfactory pathways anatomy and histology, olfactory receptor neurons cytology, genetically modified metabolism, axons physiology enzymologic gene expression regulation physiology, immunohistochemistry methods, in situ hybridization methods, luminescent proteins classification and metabolism, molecular biology methods, olfactory marker protein genetics and metabolism, olfactory mucosa metabolism, olfactory pathways metabolism, olfactory receptor neurons metabolism, messenger RNA metabolism, reverse transcriptase PCR methods, time factors, transient receptor potential channels genetics and metabolism.

Schmidt, J.T., M.R. Fleming, and B. Leu (2004). **Presynaptic protein kinase C controls maturation and branch dynamics of developing retinotectal arbors: Possible role in activity-driven sharpening**. *Journal of Neurobiology* 58(3): 328-340. ISSN: 0022-3034.

NAL Call Number: QP351.J55

Descriptors: zebrafish, arachidonic acid, biochemical analysis, dendrites, development, eyes, fish larvae, freshwater fish, ganglia, larval development, nervous system, neurons, optic tectum, photoreceptors, protein kinase C, retina, retinas, sense organs, stabilizing, vision, *Danio rerio*, *Xenopus*, protein kinase C, retinotectal arbors.

Schneider, H. and B. Sulner (2006). **Innervation of dorsal and caudal fin muscles in adult zebrafish *Danio rerio*.** *Journal of Comparative Neurology* 497(5): 702-16. ISSN: 0021-9967.

Abstract: The organization of the neuromuscular system of the dorsal and caudal fin of zebrafish, *Danio rerio,* was studied, including the anatomy of fin motoneurons as revealed by neurobiotin backfills and differential staining using fluorescent markers. The musculature of the dorsal fin consists of one pair of protractor and retractor muscles and 10 sets of muscles attaching to the bases of dorsal fin rays. Each set consists of one pair of erector, depressor, and inclinator muscles. The supplying nerves of the dorsal fin musculature originate from spinal segments 9-17 and form a dorsal fin plexus at the base of the muscles. Dorsal and caudal fin motoneurons have small cell bodies and ipsilateral dendritic branching patterns, thus resembling secondary motoneurons of the axial musculature. As shown by differential staining using fluorescent-labeled dextrans, cell bodies of dorsal fin motoneurons and axial motoneurons seem to be located in separate motor columns. The musculature of the caudal fin is composed of 12 muscles that are arranged in a superficial and a deep muscle layer. The nerves that supply the caudal fin musculature arise from the last five caudal segments of the spinal cord and form the caudal plexus. Neurobiotin backfills were performed on the dorsal caudal muscles, the medial caudal muscles, and the ventral caudal muscles. Most cell bodies of caudal fin motoneurons are small and are located in a ventral motor column. The organization of dorsal and caudal fin motoneurons is compared with the innervation of fins in other fish.

Descriptors: zebrafish, motor neurons cytology, skeletal muscle innervation, neural pathways cytology, spinal cord cytology, tail innervation, biotin analogs, derivatives and metabolism, motor neurons metabolism, skeletal muscle anatomy, histology and metabolism, neural pathways metabolism, spinal cord metabolism, swimming physiology, tail metabolism.

Schonthaler, H.B., J.M. Lampert, A. Isken, O. Rinner, A. Mader, M. Gesemann, V. Oberhauser, M. Golczak, O. Biehlmaier, K. Palczewski, S.C. Neuhauss, and J. von Lintig (2007). **Evidence for RPE65-independent vision in the cone-dominated zebrafish retina.** *European Journal of Neuroscience* 26(7): 1940-9. ISSN: 0953-816X.

Abstract: An enzyme-based cyclic pathway for trans to cis isomerization of the chromophore of visual pigments (11-cis-retinal) is intrinsic to vertebrate cone and rod vision. This process, called the visual cycle, is mostly characterized in rod-dominated retinas and essentially depends on RPE65, an all-trans to 11-cis-retinoid isomerase. Here we analysed the role of RPE65 in zebrafish, a species with a cone-dominated retina. We cloned zebrafish RPE65 and showed that its expression coincided with photoreceptor development. Targeted gene knockdown of RPE65 resulted in morphologically altered rod outer segments and overall reduced 11-cis-retinal levels. Cone vision of RPE65-deficient larvae remained functional as demonstrated by behavioural tests and by metabolite profiling for retinoids. Furthermore, all-trans retinylamine, a potent inhibitor of the rod visual cycle, reduced 11-cis-retinal levels of control larvae to a similar extent but showed no additive effects in RPE65-deficient larvae. Thus, our study of zebrafish provides in vivo evidence for the existence of an RPE65-independent pathway for the regeneration of 11-cis-retinal for cone vision.

Descriptors: zebrafish, in vivo, rpE65-independent pathway, regeneration , 11-cis-retinal, cone vision.

Schroeter, E.H., R.O. Wong, and R.G. Gregg (2006). **In vivo development of retinal ON-bipolar cell axonal terminals visualized in nyx::MYFP transgenic zebrafish.** *Visual Neuroscience* 23(5): 833-43. ISSN: 0952-5238.

Abstract: Axonal differentiation of retinal bipolar cells has largely been studied by comparing the morphology of these interneurons in fixed tissue at different ages. To better understand how bipolar axonal terminals develop in vivo, we imaged fluorescently labeled cells in the zebrafish retina using time-lapse confocal and two photon microscopy. Using the upstream regulatory sequences from the nyx gene that encodes nyctalopin, we constructed a transgenic fish in which a subset of retinal bipolar cells express membrane targeted yellow fluorescent protein (MYFP). Axonal terminals of these YFP-labeled bipolar cells laminated primarily in the inner half of the inner plexiform layer, suggesting that they are likely to be ON-bipolar cells. Transient expression of MYFP in isolated bipolar cells indicates that two or more subsets of bipolar cells, with one or two terminal boutons, are labeled. Live imaging of YFP-expressing bipolar cells in the nyx::MYFP transgenic fish at different ages showed that initially, filopodial-like structures extend and retract from their primary axonal process throughout the inner plexiform layer (IPL). Over time, filopodial exploration becomes concentrated at discrete foci prior to the establishment of large terminal boutons, characteristic of the mature form. This sequence of axonal differentiation suggests that synaptic targeting by bipolar cell axons may involve an early process of trial and error, rather than a process of directed outgrowth and contact. Our observations represent the first in vivo visualization of axonal development of bipolar cells in a vertebrate retina.

Descriptors: zebrafish, presynaptic terminals metabolism, proteoglycans metabolism, retina cytology, retina growth and development, retinal bipolar cells cytology, genetically modified, behavior, exploratory behavior physiology, gene expression physiology, immunohistochemistry methods, luminescent proteins biosynthesis and genetics, mice, protein kinase C metabolism, proteoglycans genetics, reverse transcriptase PCR methods, zebrafish anatomy and histology.

Senut, M.C., A. Gulati Leekha, and D. Goldman (2004). **An element in the alpha1-tubulin promoter is necessary for retinal expression during optic nerve regeneration but not after eye injury in the adult zebrafish.** *Journal of Neuroscience* 24(35): 7663-73. ISSN: 0270-6474.

Abstract: We have shown previously that a 1.696 kb upstream fragment of the goldfish alpha1-tubulin promoter was capable of driving green fluorescent protein (GFP) expression in the developing and regenerating zebrafish CNS in a pattern closely mimicking the endogenous alpha1-tubulin gene. Comparison of fish and rat alpha1-tubulin promoters identified a 64 bp region with a conserved repetitive homeodomain (HD) consensus sequence core (TAAT) and a nearby basic helix-loop-helix binding E-box sequence (CANNTG), which led us to speculate that it could be of importance for regulating alpha1-tubulin gene transcription. To address this issue, we examined the ability of deletion mutants of the 1.696 kb promoter to drive expression of GFP in zebrafish retinal cells under normal conditions and after injury. Interestingly, although wild-type 1.696 kb and mutant promoters, lacking the E-box and/or HD sequences, exhibited rather similar patterns of GFP expression in the developing retina, significant differences were noticed in the mature retina. First, although the 1.696 kb promoter directed transgene expression to retinal neurons and progenitor cells, the activity of mutant promoters was drastically reduced. Second, we found that the E-box and HD sequences were necessary for transgene reinduction during optic nerve regeneration, but were not as important for transgene expression in regenerating retinal neurons after eye injury. In this latter lesion model, remarkably, both 1.696 kb and mutant promoters targeted GFP expression to Muller glia-like cells, some of which re-entered the cell cycle. These new findings will be useful for identifying the molecular signals necessary for successful CNS regeneration.

Descriptors: zebrafish, rat, E box elements genetics, eye gene expression regulation genetics, nerve regeneration genetics, nerve tissue optic nerve injuries genetics, promoter regions genetics , regeneration genetics, retina growth and development, tubulin genetics, genetically modified, axotomy, cell cycle, eye proteins biosynthesis, developmental gene expression regulation genetics, reporter genes, green fluorescent proteins biosynthesis and genetics, introns genetics, nerve crush, nerve tissue proteins biosynthesis, neuroglia metabolism, optic nerve injuries metabolism, recombinant fusion proteins biosynthesis, retina embryology, injuries and physiology, retinal ganglion cells metabolism, sequence deletion, genetic transcription, tubulin biosynthesis.

Shields, C.R., J. Klooster, Y. Claassen, M. Ul Hussain, G. Zoidl, R. Dermietzel, and M. Kamermans (2007). **Retinal horizontal cell-specific promoter activity and protein expression of zebrafish connexin 52.6 and connexin 55.5.** *Journal of Comparative Neurology* 501(5): 765-79. ISSN: 0021-9967.

Descriptors: zebrafish, connexins metabolism, gap junctions metabolism, promoter regions genetics, retinal horizontal cells metabolism, biological markers analysis and metabolism, cell communication genetics, cell membrane genetics, metabolism and ultrastructure, cultured cells, connexins genetics, gap junctions genetics and ultrastructure, green fluorescent proteins, immunohistochemistry, membrane potentials genetics, immunoelectron microscopy, patch clamp techniques, photoreceptors metabolism and ultrastructure, presynaptic terminals metabolism and ultrastructure, retinal horizontal cells cytology.

Sidi, S., E. Busch Nentwich, R. Friedrich, U. Schoenberger, and T. Nicolson (2004). **Gemini encodes a zebrafish L-type calcium channel that localizes at sensory hair cell ribbon synapses.** *Journal of Neuroscience* 24(17): 4213-23. ISSN: 0270-6474.

Abstract: L-type Ca2+ channels (LTCCs) drive the bulk of voltage-gated Ca2+ entry in vertebrate inner ear hair cells (HCs) and are essential for mammalian auditory processing. LTCC currents have been implicated in neurotransmitter release at the HC afferent active zone, the ribbon synapse. It is likely that LTCCs play a direct role in vesicle fusion; however, the subcellular localization of the channels in HCs has not been fully resolved. Via positional cloning, we show that mutations in a zebrafish LTCC encoding gene, cav1.3a, underlie the auditory-vestibular defects of gemini (gem) circler mutants. gem homozygous receptor mutant HCs display normal cell viability, afferent synaptogenesis, and peripheral innervation, yet exhibit strongly reduced extracellular potentials (approximately 50% of wild-type potentials). Apical FM1-43 uptake, however, is unaffected in gem mutant HCs, suggesting that mechanotransduction channels are functional. Using a Gem-specific antibody, we show that the bulk of Gem/Ca(v)1.3a immunoreactivity in HCs is restricted to basally located focal spots. The number and location of focal spots relative to nerve terminals, and their remarkable ring-shaped structure, which is reminiscent of synaptic dense bodies, are consistent with Gem/Ca(v)1.3a channels clustering at HC ribbon synapses.
Descriptors: zebrafish larvae, calcium channels, l type biosynthesis, hair cells metabolism, synapses metabolism, amino acid sequence, calcium channels, l type genetics, cell membrane metabolism and ultrastructure, deafness genetics and physiopathology, hair cells cytology, molecular sequence data, phenotype, phylogeny, point mutation, protein isoforms biosynthesis and genetics, synapses ultrastructure, vestibular diseases genetics and physiopathology.

Song, P.I., J.I. Matsui, and J.E. Dowling (2008). **Morphological types and connectivity of horizontal cells found in the adult zebrafish (*Danio rerio*) retina.** *Journal of Comparative Neurology* 506(2): 328-38. ISSN: 0021-9967.
Descriptors: zebrafish, retina eyes, photoreceptors, horizontal cells, blue-sensitive, ultraviolet-sensitive.

Sovrano, V.A. and R.J. Andrew (2006). **Eye use during viewing a reflection: behavioural lateralisation in zebrafish larvae.** *Behavioural Brain Research* 167(2): 226-31. ISSN: 0166 4328.
Abstract: Development of visual lateralisation was studied in zebrafish larvae of an outbred strain when examining their own reflection in a mirror. There was significant left eye preference at all ages studied. A decrease in left eye use around 14 days, followed by a later increase at 21 days, parallels similar but differently timed shifts in the domestic chick. Age-dependent shifts in the likelihood of control by one or other eye system may be responsible. Larvae tested at 26 days of age with unfamiliar conspecifics of similar age also used the left eye (LE). Larvae of another strain (TupLF) also LE viewing of their reflection, although this was confined to the first or first and second minutes of the test rather than lasting through 10 min. The reflection differs from prior experience of fellows in the timing and character of its movements relative to those of the larva. Specialisation of the left eye system for the assessment of novelty, which is present in zebrafish, just as in many other vertebrates, probably therefore explains left eye use in the mirror test. Reasons why strains might differ in the length of time that they devote to assessment of the reflection are discussed.
Descriptors: zebrafish larva, behavior, functional laterality physiology, recognition psychology, visual perception physiology, analysis of variance, inbred strains, species specificity.

Stadler, J.A., A. Shkumatava, and C.J. Neumann (2004). **The role of Hedgehog signaling in the development of the zebrafish visual system.** *Developmental Neuroscience* 26(5-6): 346-51. ISSN: 0378 5866.
Abstract: The vertebrate visual system is a region of the nervous system that is characterized

by relative simplicity, and its development has hence been studied intensively, to serve as a paradigm for the rest of the central nervous system. The zebrafish model organism offers an impressive array of tools to dissect this process experimentally, and in recent years has helped to significantly deepen our understanding of the development of the visual system. A number of these studies have focused on the role of the Hedgehog family of secreted signaling molecules in eye development, and this is the main topic of this review. Hedgehog signaling plays an important role in all major steps of visual system development, starting with the regionalization of the eye primordium into proximal and distal territories, continuing with the control of cellular differentiation in the retina, and ending with the guidance of axonal projections from the retina to the optic centers of the brain.
Descriptors: zebrafish embryos, brain embryology, retina embryology, signal transduction physiology, trans activators metabolism, visual pathways embryology, brain physiology, cell communication, cell differentiation physiology, Hedgehog proteins, research models, retina physiology, trans activators genetics, visual pathways physiology, .

Stewart, R.A., A.T. Look, J.P. Kanki, and P.D. Henion (2004). **Development of the peripheral sympathetic nervous system in zebrafish.** *Methods in Cell Biology* 76: 237-60. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish embryos, sympathetic nervous system embryology, cell differentiation physiology, cell movement physiology, sympathetic ganglia embryology, growth and development, developmental gene expression regulation, immunohistochemistry, in situ hybridization, fluorescence microscopy, mutation, neural crest embryology, growth and development, neuroblastoma etiology and genetics, neurons cytology and physiology, norepinephrine physiology, peripheral nervous system embryology, growth and development, stem cells cytology and physiology, sympathetic nervous system growth and development, tyrosine 3 monooxygenase metabolism.

Tabor, R., E. Yaksi, J.M. Weislogel, and R.W. Friedrich (2004). **Processing of odor mixtures in the zebrafish olfactory bulb.** *Journal of Neuroscience* 24(29): 6611-20. ISSN: 0270-6474.
Abstract: Components of odor mixtures often are not perceived individually, suggesting that neural representations of mixtures are not simple combinations of the representations of the components. We studied odor responses to binary mixtures of amino acids and food extracts at different processing stages in the olfactory bulb (OB) of zebrafish. Odor-evoked input to the OB was measured by imaging Ca2+ signals in afferents to olfactory glomeruli. Activity patterns evoked by mixtures were predictable within narrow limits from the component patterns, indicating that mixture interactions in the peripheral olfactory system are weak. OB output neurons, the mitral cells (MCs), were recorded extra- and intracellularly and responded to odors with stimulus-dependent temporal firing rate modulations. Responses to mixtures of amino acids often were dominated by one of the component responses. Responses to mixtures of food extracts, in contrast, were more distinct from both component responses. These results show that mixture interactions can result from processing in the OB. Moreover, our data indicate that mixture interactions in the OB become more pronounced with increasing overlap of input activity patterns evoked by the components. Emerging from these results are rules of mixture interactions that may explain behavioral data and provide a basis for understanding the processing of natural odor stimuli in the OB.
Descriptors: zebrafish, odors, olfactory bulb physiology , smell physiology, action potentials, calcium metabolism, neurons physiology, olfactory bulb cytology, olfactory pathways metabolism, patch clamp techniques.

Takeda, H. (2003). **Roles of Fgf signal in induction and patterning of zebrafish neural tissues.** *Zoological Science* 20(12): 1514. ISSN: 0289 0003.
NAL Call Number: 442P92
Descriptors: zebrafish embryos, development, genetics, nervous system, neural coordination, fate maps, gene expression, neural development, signaling pathways.

Thorsen, D.H. and M.E. Hale (2007). **Neural development of the zebrafish (*Danio rerio*) pectoral fin.** *Journal of Comparative Neurology* 504(2): 168-84. ISSN: 0021-9967.
Descriptors: zebrafish embryos, forelimb innervation, locomotion physiology, motor neurons cytology, spinal cord cytology, forelimb embryology and metabolism, motor neurons metabolism, spinal cord embryology and metabolism.

Thorsen, D.H., J.J. Cassidy, and M.E. Hale (2004). **Swimming of larval zebrafish: fin-axis coordination and implications for function and neural control.** *Journal of Experimental Biology* 207(24): 4175-4183. ISSN: 0022 0949.
NAL Call Number: 442.8B77
Abstract: Adult actinopterygian fishes typically perform steady forward swimming using either their pectoral fins or their body axis as the primary propulsor. In most species, when axial undulation is employed for swimming, the pectoral fins are tucked (i.e. adducted) against the body; conversely, when pectoral fins are beating, the body axis is held straight. In contrast to adults, larval fishes can combine their pectoral fin and body-axis movements during locomotion; however, little is known about how these locomotor modes are coordinated. With this study we provide a detailed analysis of the coordinated fin and axial movements during slow and fast swimming by examining forward locomotion in larval zebrafish (*Danio rerio* L.). In addition, we describe the musculature that powers pectoral fin movement in larval zebrafish and discuss its functional implications. As larvae, zebrafish alternate their pectoral fins during slow swimming (0.011 [plus or minus] 0.001 mm ms-1) in conjunction with axial undulations of the same frequency (18-28 Hz). During fast swimming (0.109 [plus or minus] 0.030 mm ms-1; 36-67 Hz), the fins are tucked against the body and propulsion occurs by axial undulation alone. We show that during swimming, larval fishes can use a similar limb-axis coordination pattern to that of walking and running salamanders. We suggest that the fin-axis coordination observed in larval zebrafish may be attributed to a primitive neural circuit and that early terrestrial vertebrates may have gained the ability to coordinate limbs and lateral bending by retaining a larval central pattern generator for limb-axis coordination in the adult life history stage.
Descriptors: zebrafish larva, *Danio rerio*, fins, biomechanics, skeletal musculature, nervous system, larval development, swimming, fin axis coordination, implications for function and neural control.

Todd, K.J., C.A. Slatter, and D.W. Ali (2004). **Activation of ionotropic glutamate receptors on peripheral axons of primary motoneurons mediates transmitter release at the zebrafish NMJ.** *Journal of Neurophysiology* 91(2): 828-40. ISSN: 0022-3077.
Abstract: The development and function of the vertebrate neuromuscular junction (NMJ) is continually being redefined. Previous studies have indicated that glutamate may play a role in the development or function of the NMJ by associating with presynaptic receptors. We have used larval zebrafish *(Danio rerio*) to investigate the presence of presynaptic ionotropic glutamate receptors (iGluRs) at the NMJ in vivo. In whole-mount zebrafish larvae, antibody staining directed to NR2A subunits colocalized with specific staining of motoneuron axon tracts. Whole cell voltage-clamp recordings of miniature endplate currents (mEPCs) from

axial white muscle were performed during application of iGluR agonists and antagonists. Local perfusion of the NMJ with iGluR agonists resulted in significant increases in the frequency of spontaneous acetylcholine (ACh) release. These increases were blocked by the N-methyl-d-aspartate (NMDA) receptor antagonist d-(-)-2-amino-5-phosphonopentanoic acid (50 microM) and by the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxalene-2,3-dione (50 microM). Further pharmacological investigation revealed no effect of the kainate receptor-specific antagonist (2S,4R)-4-methylglutamate (10 microM) on kainate-induced rises in the frequency of spontaneous ACh release. However, these were blocked with the AMPA receptor-specific antagonist 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (50 microM). Application of glutamate (1 mM) in the presence of the glutamate uptake inhibitor d-threo-beta-benzyloxyaspartate(200 microM) resulted in a significant increase in the frequency of mEPCs. These results suggest the presence of AMPA and NMDA receptors in association with motoneuron axons of larval zebrafish.
Descriptors: zebrafish larvae, acetylcholine secretion, axons secretion, motor neurons secretion, neuromuscular junction secretion, glutamate receptors metabolism, acetylcholine metabolism, axons drug effects and metabolism, dimethyl sulfoxide pharmacology, motor neurons drug effects and metabolism, N methylaspartate pharmacology, neuromuscular junction drug effects and metabolism, peripheral nerves drug effects, metabolism and secretion, N methyl D aspartate receptors agonists and metabolism, alpha amino 3 hydroxy 5 methyl 4 isoxazolepropionic acid pharmacology.

Tomchik, S.M. (2006). **Appetite for destruction: neuron ablations, prey capture, and sensorimotor integration in larval zebrafish.** *Journal of Neuroscience* 26(5): 1329-30. ISSN: 0270-6474.
Descriptors: zebrafish larva, afferent neurons physiology, predatory behavior physiology, visual pathways physiology, motor neurons physiology, nerve net physiology, superior colliculus cytology and physiology , visual pathways cytology, zebrafish growth and development.

Tsujikawa, M. and J. Malicki (2004). **Intraflagellar transport genes are essential for differentiation and survival of vertebrate sensory neurons**. *Neuron* 42(5): 703-716. ISSN: 0896-6273.
Descriptors: zebrafish, cilia, freshwater fish, genes, hair cells, neurodegeneration, neurons, olfactory receptor neurons, phenotypes, photoreceptors, polypeptides, retina, retinas, sense organs, sensory neurons, survival , opsins, *Danio rerio*.

Van Epps, H.A., M. Hayashi, L. Lucast, G.W. Stearns, J.B. Hurley, P. De Camilli, and S.E. Brockerhoff (2004). **The zebrafish nrc mutant reveals a role for the polyphosphoinositide phosphatase synaptojanin 1 in cone photoreceptor ribbon anchoring.** *Journal of Neuroscience* 24(40): 8641-50. ISSN: 0270-6474.
Abstract: Visual, vestibular, and auditory neurons rely on ribbon synapses for rapid continuous release and recycling of synaptic vesicles. Molecular mechanisms responsible for the properties of ribbon synapses are mostly unknown. The zebrafish vision mutant nrc has unanchored ribbons and abnormal synaptic transmission at cone photoreceptor synapses. We used positional cloning to identify the nrc mutation as a premature stop codon in the synaptojanin1 (synj1) gene. Synaptojanin 1 (Synj1) is undetectable in nrc extracts, and biochemical activities associated with it are reduced. Furthermore, morpholinos directed against synj1 phenocopy the nrc mutation. Synj1 is a polyphosphoinositide phosphatase important at conventional synapses for clathrin-mediated endocytosis and actin cytoskeletal rearrangement. In the nrc cone photoreceptor pedicle, not only are ribbons unanchored, but synaptic vesicles are reduced in number, abnormally distributed, and interspersed within a

dense cytoskeletal matrix. Our findings reveal a new role for Synj1 and link phosphoinositide metabolism to ribbon architecture and function at the cone photoreceptor synapse.
Descriptors: NRC mutant zebrafish larvae, retina cones enzymology and ultrastructure, phosphoric monoester hydrolases physiology, synaptic vesicles ultrastructure, actins analysis , amino acid sequence, retina cones chemistry, larva anatomy, histology and enzymology, molecular sequence data, mutation, nerve tissue nerve tissue, phenotype, phosphoric monoester hydrolases antagonists and inhibitors and genetics, retina anatomy, histology, growth and development, proteins antagonists and inhibitors.

Vankirk, A.M. and C.A. Byrd (2003). **Apoptosis following peripheral sensory deafferentation in the olfactory bulb of adult zebrafish.** *Journal of Comparative Neurology* 455(4): 488-98. ISSN: 0021-9967.
Descriptors: zebrafish olfactory bulb, apoptosis, neurodegenerative diseases pathology, olfactory bulb pathology, cell count, denervation, disease models, disease progression, immunohistochemistry, in situ nick end labeling, interneurons pathology, neuroglia pathology, olfactory mucosa physiology, olfactory pathways physiology.

Von Philipsborn, A.C., A. Ferrer Vaquer, E. Rivera Milla, C.A. Stuermer, and E. Malaga Trillo (2005). **Restricted expression of reggie genes and proteins during early zebrafish development.** *Journal of Comparative Neurology* 482(3): 257-72. ISSN: 0021-9967.
Descriptors: zebrafish, developmental gene expression regulation physiology, membrane proteins metabolism, brain cytology, brain embryology and metabolism, cell membrane metabolism, embryonic development genetics and physiology , eye cytology, embryology and metabolism, developmental gene expression regulation genetics, membrane mesoderm cytology, mesoderm metabolism, neurons cytology and metabolism, olfactory mucosa cytology and embryology, olfactory mucosa metabolism, messenger RNA analysis, spinal cord cytology, spinal cord embryology and metabolism, tissue distribution.

Wada, N., Y. Javidan, and T. Schilling (2005). **Morphogenesis and chondrogenesis of cranial neural crest at the midline in the zebrafish neurocranium**. *Zoological Science* 22(12): 1462. ISSN: 0289 0003.
NAL Call Number: 442P92
Descriptors: zebrafish brain, skeletal system, movement and support, nervous system, neural coordination, morphogenesis, neurocranium, chondrogenesis.

Wagle, M., B. Grunewald, S. Subburaju, C. Barzaghi, S. Le Guyader, J. Chan, and S. Jesuthasan (2004). **EphrinB2a in the zebrafish retinotectal system.** *Journal of Neurobiology* 59(1): 57-65. ISSN: 0022-3034.
NAL Call Number: QP351.J55
Descriptors: zebrafish, ephrin A2 metabolism, developmental gene expression regulation, retina metabolism, superior colliculus metabolism, visual pathways metabolism, axons metabolism and virology , Baculoviridae physiology, carbocyanines metabolism, embryo, ephrin A2 genetics, eye metabolism and transplantation, in situ hybridization methods, mutation, organ transplantation methods, retina cytology and embryology, retinal ganglion cells metabolism, superior colliculus cytology and embryology, visual pathways embryology.

Wang, X., N. Yang, E. Uno, R.G. Roeder, and S. Guo (2006). **A subunit of the mediator complex regulates vertebrate neuronal development**. *Proceedings of the National Academy of Sciences, USA* 103(46): 17284-17289. ISSN: 0027-8424.

NAL Call Number: 500N21P
Descriptors: zebrafish embryo, brain, cofactors, cranial nerves, differentiation, embryos, evolution, freshwater fish, ganglia, gene expression, genetic screening, motor neurons, mutation, mutations, neurons, peripheral nervous system, RNA, sensory neurons, Sox9 protein, gamma -aminobutyric acid, *Danio rerio*.

Watkins, J., A. Miklosi, and R.J. Andrew (2004). **Early asymmetries in the behaviour of zebrafish larvae.** *Behavioural Brain Research* 151(1-2): 177-83. ISSN: 0166 4328.
Descriptors: zebrafish larvae, functional laterality physiology, locomotion physiology, ocular physiology, visual perception physiology, avoidance learning physiology, photic stimulation, startle reaction physiology, nonparametric statistics, swimming physiology, visual fields physiology.

Weber, S.A. and L.S. Ross (2003). **Gap junctional coupling in the olfactory organ of zebrafish embryos**. *Developmental Brain Research* 143(1): 25-31. ISSN: 0165-3806.
Descriptors: zebrafish, brain, cell division, coupling, development, dyes, embryonic development, embryos, freshwater fish, gap junctions, neurogenesis, olfaction, olfactory organs, receptors, *Danio rerio*, pisces.

Wen, H. and P. Brehm (2005). **Paired motor neuron-muscle recordings in zebrafish test the receptor blockade model for shaping synaptic current.** *Journal of Neuroscience* 25(35): 8104-11. ISSN: 0270-6474.
Abstract: The transparent spinal cord and electrically compact fast muscle of zebrafish offer the first opportunity to perform simultaneous whole-cell patch-clamp recordings from both motor neuron and target skeletal muscle in situ. Our paired recordings reveal the fastest reported kinetics for both spontaneous and evoked synaptic currents at any synapse and a large quantal size that facilitates the resolution of spontaneous synaptic currents. We used this preparation to test the recent proposal that open channel block of the acetylcholine receptor by acetylcholine modulates the kinetics and timing of transmission between nerve and muscle in larval zebrafish (Legendre et al., 2000). Contrary to the predictions of this model, we find similar delay and onset kinetics of synaptic current at positive and negative muscle membrane potentials, even after inhibition of acetylcholinesterase. In contrast, blockade of motor neuron K channels by 4-aminopyridine prolonged the action potential and introduced a significant delay and slowing of evoked synaptic currents, demonstrating our ability to measured altered transmitter release with this system. We conclude that the kinetics of neuromuscular synaptic currents in zebrafish is not governed by receptor block.
Descriptors: zebrafish larvae, motor neurons physiology, neuromuscular junction physiology, cholinergic receptors physiology, synaptic transmission physiology, acetylcholine pharmacology, action potentials drug effects, action potentials physiology, motor neurons drug effects, neuromuscular junction drug effects, synaptic transmission drug effects.

Whitlock, K.E. (2003). **Gonadotropin releasing hormone (GnRH) cells of the hypothalamus and terminal nerve have distinct, non - olfactory origins in the developing zebrafish, *Danio rerio.*** *Society for Neuroscience Abstract Viewer and Itinerary Planner* ISSN: 1558-3635.
Abstract: The olfactory placode generates the olfactory sensory neurons and is also thought to generate a group of neuroendocrine cells containing gonadotropin-releasing hormone (GnRH). The location of the GnRH cells within the nervous system is correlated with their function, where those acting as a neuromodulator lie in the terminal nerve, a cranial nerve, and those acting as a releasing hormone lie in the hypothalamus.Our previous work has

shown that the olfactory placodes do not give rise to GnRH cells (Whitlock and Westerfield, 2000). Subsequently we have found that the endocrine and neuromodulatory GnRH cells arise from two separate, non-olfactory regions in the developing neural plate. Specifically, the neuromodulatory GnRH cells of the terminal nerve arise from the cranial neural crest, and the endocrine GnRH cells of the hypothalamus arise from the adenohypophyseal region of the developing anterior neural plate (Whitlock et. al., 2003). Both the adenohypophyseal and neural crest populations of the developing embryo give rise to endocrine/neuroendocrine cells, thus our reported origins of GnRH cells are consistent with the developmental potential of these regions. We are currently using morpholino technology to knock-down the c-ret, fkh6 and sox10 gene products, known to be involved in neural crest development, to selectively disrupt development of the terminal nerve GnRH cells. Our preliminary data indicate that the loss of c-ret protein results in the disruption of the terminal nerve GnRH cells, and the co-injection of the sox10 and fkh6 morpholinos results in the loss of the terminal nerve GnRH cells. Our findings draw attention to the value of understanding early fate maps for they reveal the developmental relationship of adult tissues, relationships no longer evident after the extensive morphogenesis of the developing neural tube is complete.
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Descriptors: zebrafish embryology, nervous system , GnRH, hypothalamus.
Notes: AbstractNo.333.13.

Wullimann, M.F. and T. Mueller (2004). **Identification and morphogenesis of the eminentia thalami in the zebrafish.** *Journal of Comparative Neurology* 471(1): 37-48. ISSN: 0021-9967.
Descriptors: zebrafish embryology, entopeduncular nucleus embryology, developmental gene expression regulation, nerve tissue proteins metabolism, neurons metabolism, thalamus embryology, proteins metabolism, basic helix loop helix transcription factors, body patterning physiology, cell differentiation physiology, cell movement physiology, entopeduncular nucleus growth, development and metabolism, eye proteins, gene expression profiling, homeodomain proteins metabolism, hu paraneoplastic encephalomyelitis antigens, immunohistochemistry, morphogenesis, paired box transcription factors, proliferating cell nuclear antigen metabolism, RNA binding proteins metabolism, repressor proteins, thalamus growth and development and metabolism, transcription factors metabolism.

Xiao, T. and H. Baier (2007). **Lamina-specific axonal projections in the zebrafish tectum require the type IV collagen Dragnet.** *Nature Neuroscience* 10(12): 1529-37. ISSN: 1097-6256.
NAL Call Number: QP351
Abstract: The mechanisms underlying the precise targeting of tectal layers by ingrowing retinal axons are largely unknown. In zebrafish, individual axons choose one of four retinorecipient layers upon entering the tectum and remain restricted to this layer, despite continual remodeling and shifting of their terminal arbors. In dragnet mutants, by contrast, a large fraction of retinal axons aberrantly trespass between layers or form terminal arbors that span two layers. The dragnet gene, drg, encodes collagen IV(alpha5) (Col4a5), a basement membrane component lining the surface of the tectum. Heparan sulfate proteoglycans (HSPGs) are normally associated with the tectal basement membrane but are dispersed in the dragnet mutant tectum. Zebrafish boxer (extl3) mutants, which are deficient in HSPG synthesis, show laminar targeting defects similar to those in dragnet. Our results show that the collagen IV sheet anchors secreted factors at the surface of the tectum, which serve as guidance cues for retinal axons.

Descriptors: dragnet and boxer mutant zebrafish, tectum, axonal projections, type IV collagen, heparan sulfate proteoglycans.

Xiao, T., W. Shoji, W. Zhou, F. Su, and J.Y. Kuwada (2003). **Transmembrane sema4E guides branchiomotor axons to their targets in zebrafish.** *Journal of Neuroscience* 23(10): 4190-8. ISSN: 0270-6474.

Abstract: Class 4 semaphorins are a large class of transmembrane proteins that contain a sema domain and that are expressed in the CNS, but their in vivo neural function is unknown. In zebrafish, the epithelial cells that line the pharyngeal arches express Sema4E. Extension of branchiomotor axons along the mesenchymal cells bounded by these epithelial cells suggests that Sema4E may act as a repulsive guidance molecule to restrict the branchiomotor axons to the mesenchymal cells. To test this hypothesis, Sema4E was misexpressed in hsp70 promoter-regulated transgenic zebrafish in which sema4E was heat-inducible, and Sema4E was knocked down by injection of antisense morpholino oligonucleotides that acted specifically against Sema4E. Ubiquitous induction of Sema4E retarded outgrowth by the facial and gill branchiomotor axons significantly. Furthermore, outgrowth by gill motor axons was specifically inhibited when Sema4E-expressing transgenic cells were transplanted to their pathway in nontransgenic host embryos. Morpholino knockdown of Sema4E caused facial motor axons to defasciculate and follow aberrant pathways. These results show that Sema4E is repulsive for facial and gill motor axons and functions as a barrier for these axons within the pharyngeal arches.

Descriptors: transgenic zebrafish, axons physiology, gills innervation, membrane, motor neurons physiology, semaphorins physiology, genetically modified, axons drug effects, axons metabolism, body patterning drug effects, body patterning genetics, body patterning physiology, facial nerve drug effects, metabolism and physiology, gene expression regulation drug effects and genetics, genetic vectors, gills physiology, HSP70 heat shock membrane proteins biosynthesis and genetics, motor neurons drug effects and metabolism, neural inhibition genetics, antisense oligonucleotides pharmacology, phenotype, promoter regions genetics drug effects, genetics and genetics physiology, recombinant fusion proteins biosynthesis, recombinant fusion rhombencephalon chemistry, rhombencephalon drug effects, metabolism and physiology, semaphorins biosynthesis and genetics.

Xu, X., T. Scott Scheiern, L. Kempker, and K. Simons (2007). **Active avoidance conditioning in zebrafish (*Danio rerio*).** *Neurobiology of Learning and Memory* 87(1): 72-7. ISSN: 1074-7427.

Abstract: The zebrafish represents a potentially useful organism for studying genes involved in learning and memory function in vertebrates, because a number of genetic techniques in zebrafish have been developed to produce a wide variety of genetic mutants. While zebrafish mutants are being developed, behavioral studies on learning and memory function in zebrafish are in urgent need. The present study investigated active avoidance conditioning in normal zebrafish. Zebrafish were trained to swim from a lighted (CS) compartment to a dark compartment to avoid an electrical body shock (US) in a shuttle-box that consisted of a water-filled tank separated by an opaque barrier into two equal compartments. By varying the number of trials per training session and the duration of the intertrial interval, Experiments 1 and 2 showed that, with the CS, US, and intertrial interval being 12s, zebrafish learned avoidance responses within a training session consisting of 30 trials and retained the avoidance responses. Experiment 3 showed that zebrafish learned avoidance responses following the association between the CS of light and the US of shock in the avoidance conditioning paradigm. Using the avoidance conditioning paradigm, Experiment 4 investigated

the amnestic effects of N-methyl-D-aspartate receptor antagonist MK-801 and nitric oxide synthase inhibitor L-NAME in zebrafish. Experiment 4 showed that post-training injection of L-NAME significantly impaired retention of avoidance responses while MK-801 did not, confirming previous results with other vertebrates. The results of the present study suggest the similar involvements of neurochemicals in learning and memory among vertebrates. Thus, future studies with zebrafish mutants may identify genes involved in learning and memory in vertebrates.

Descriptors: zebrafish, avoidance learning physiology, conditioning, classical physiology, nitric oxide metabolism, N methyl D aspartate receptors metabolism, avoidance learning drug effects, conditioning, classical drug effects, dizocilpine maleate pharmacology, enzyme inhibitors pharmacology, excitatory amino acid antagonists pharmacology, ng nitroarginine methyl ester pharmacology, N methyl D aspartate receptors drug effects, reference values, time factors.

Yeo, S.-Y. and A.B. Chitnis (2007). **Jagged-mediated Notch signaling maintains proliferating neural progenitors and regulates cell diversity in the ventral spinal cord**. *Proceedings of the National Academy of Sciences, USA* 104(14): 5913-5918. ISSN: 0027-8424.

NAL Call Number: 500N21P

Descriptors: zebrafish, delta protein, differentiation, motor neurons, nervous system, neural stem cells, neural tube, neurogenesis, neurons, Notch protein, Olig2 protein, serrate protein, signal transduction, species diversity, spinal cord, gamma-aminobutyric acid, *Danio rerio*.

Yoshida, M. and W.B. Macklin (2005). **Oligodendrocyte development and myelination in GFP-transgenic zebrafish.** *Journal of Neuroscience Research* 81(1): 1-8. ISSN: 0360-4012.

Descriptors: zebrafish embryos, brain embryology, green fluorescent proteins metabolism, myelin p0 protein metabolism, myelin proteolipid protein metabolism and physiology, nerve tissue proteins metabolism, oligodendroglia cytology, genetically modified, biological markers metabolism, brain metabolism, cell differentiation genetics and physiology, metabolism, gene expression profiling, developmental gene expression regulation, reporter green fluorescent research models, myelin p0 protein genetics, myelin proteolipid protein genetics, nerve tissue oligodendroglia metabolism, promoter regions genetics.

Yoshida, Y., K. Saitoh, Y. Aihara, S. Okada, T. Misaka, and K. Abe (2007). **Transient receptor potential channel M5 and phospholipaseC-beta2 colocalizing in zebrafish taste receptor cells.** *Neuroreport* 18(15): 1517-20. ISSN: 0959-4965.

Abstract: In mammals, transient receptor potential (TRP) channel M5 (TRPM5) is coexpressed with phospholipaseC-beta2 (PLC-beta2) in the taste receptor cells, and both PLC-beta2 and TRPM5 are essential elements in the signal transduction of sweet, bitter and umami stimuli. In this study, we identified the zebrafish homologue of TRPM5 (zfTRPM5) and examined its expression in the gustatory system by in-situ hybridization. Using a transgenic zebrafish line that expressed green fluorescent protein under the control of the PLC-beta2 promoter, we showed that zfTRPM5 is expressed in green fluorescent protein-labeled cells of the taste buds. These results demonstrate that zfTRPM5 and PLC-beta2 colocalize in zebrafish taste receptor cells, suggesting their crucial roles in taste signaling via the fish taste receptors.

Descriptors: zebrafish taste receptor cells, chemoreceptors metabolism, phospholipase C beta metabolism, TRPMcation channels metabolism, taste buds metabolism, genetically

modified, DNA genetics, genetic databases, green fluorescent in situ hybridization, phospholipase C beta genetics, TRPMcation channels genetics.

Yu, C.J., Y. Gao, C.L. Willis, P. Li, J.P. Tiano, P.A. Nakamura, D.R. Hyde, and L. Li (2007). **Mitogen-associated protein kinase- and protein kinase A-dependent regulation of rhodopsin promoter expression in zebrafish rod photoreceptor cells.** *Journal of Neuroscience Research* 85(3): 488-96. ISSN: 0360-4012.

Abstract: Mitogen-associated protein kinase (MAPK)- and protein kinase A (PKA)-dependent signal transductions play important roles in the regulation of gene expression. Both MAPK and PKA pathways can be activated by light exposure. In this study, we investigated the effect of light on MAPK and PKA signal transduction and their roles in the regulation of rhodopsin promoter expression by using transgenic zebrafish [Tg(rhod::GFP)]. The Tg(rhod::GFP) fish express short half-life GFP that is under the transcriptional control of the zebrafish rhodopsin promoter and can therefore be used for in vivo studies of rhodopsin gene transcription in live cells. Blue light plays a role in the regulation of rhodopsin promoter expression via an MAPK-mediated signal transduction cascade. Blue light excites cryptochromes (CRY), which activate the downstream PKC-dependent MAPK signal pathway. White light, on the other hand, regulates rhodopsin promoter expression via a G-protein-coupled cAMP-dependent PKA pathway. White light promotes dopamine release in the retina, which activates dopamine receptors and the downstream PKA pathway. Blocking MAPK signaling diminishes the blue light-induced increases in rhodopsin promoter expression, but this treatment has no effect on white light-mediated rhodopsin promoter expression. Conversely, blocking the PKA pathway diminishes the white light-induced rhodopsin promoter expression but does not affect rhodopsin promoter expression regulated by blue light. Together, the data suggest that MAPK and PKA regulate rhodopsin transcription through parallel signal transduction pathways.

Descriptors: zebrafish, cyclic AMP dependent protein kinases metabolism, mitogen activated protein kinases metabolism, rhodopsin genetics, retinal rodsphysiology, gene expression regulation, kinetics, light, promoter regions genetics radiation effects, genetic transcription.

Yu, C.J. and L. Li (2005). **Dopamine modulates voltage-activated potassium currents in zebrafish retinal on bipolar cells.** *Journal of Neuroscience Research* 82(3): 368-76. ISSN: 0360-4012.

Descriptors: zebrafish eyes, dopamine metabolism, potassium channels, voltage gated metabolism , dopamine D1 receptors, metabolism, G protein Coupled receptors metabolism, retina metabolism, retinal bipolar cells metabolism, 8 bromo cyclic adenosine monophosphate pharmacology, cultured cells, cyclic AMP metabolism, dopamine pharmacology, dopamine agonists pharmacology, guanosine 5' o 3 thiotriphosphate pharmacology, membrane potentials drug effects, membrane potentials physiology, patch clamp techniques, potassium channels, voltage gated drug effects, reaction time drug effects and physiology, dopamine D1 receptorsagonists , G protein Coupled receptors agonists, retina drug effects, retinal bipolar cells drug effects.

Yurco, P. and D.A. Cameron (2007). **Cellular correlates of proneural and Notch-delta gene expression in the regenerating zebrafish retina.** *Visual Neuroscience* 24(3): 437-43. ISSN: 0952-5238.

Abstract: Fish can regenerate retinal neurons following ocular injury. Evidence is mounting that astrocytic glia function as inducible, regenerative stem cells in this process, but the underlying molecular events that enable neuronal regeneration are comparatively unclear. In the current study gene array, quantitative real-time PCR, in situ hybridization, and immu-

nohistochemical approaches were used to identify, in the damaged retina of adult zebrafish, correlations between transcriptional events and entry into the cell cycle by Muller cells, a type of astrocytic cell present in all vertebrate retinas that is a candidate 'stem cell' of regenerated neurons. A proneural gene (achaete-scute homolog 1a, ash1a) and neurogenic components of the Notch signaling pathway, including notch3 and deltaA, were implicated. An injury-induced, enhanced expression of ash1a was observed in Muller cells, which is hypothesized to contribute to the transition of these cells, or their cellular progeny, into a notch3-expressing, regenerative progenitor. A model of vertebrate retinal repair is suggested in which damage-induced expression of proneural genes, plus canonical Notch-Delta signaling, could contribute to retinal stem cell promotion and subsequent regenerative neurogenesis.
Descriptors: zebrafish, eye proteins metabolism, gene expression regulation physiology, nerve regeneration physiology, retina metabolism, basic helix loop helix transcription factors metabolism, eye injuries physiopathology, eye homeodomain proteins metabolism, in situ hybridization methods , nerve tissue proteins metabolism, oligonucleotide array sequence analysis methods, Notch1 receptor metabolism, retina pathology, reverse transcriptase PCR methods, stem cells physiology, zebrafish anatomy and histology.

Yurco, P. and D.A. Cameron (2005). **Responses of Muller glia to retinal injury in adult zebrafish.** *Vision Research* 45(8): 991-1002. ISSN: 0042-6989.
Abstract: In an effort to identify the cellular events that enable neuronal regeneration in the vertebrate retina, the identity and characteristics of mitotic and apoptotic cells were examined in lesioned retinas of adult zebrafish. Following lesion a complex spatiotemporal pattern of mitosis was observed, including a delayed entry of Muller glia into the cell cycle. Characteristics of these proliferative Muller glia indicated they might serve as a stem/precursor cell of regenerated retina. The results suggested a model of retinal regeneration in which lesions are filled, in part, by a localized en place cytogenesis within intact retina surrounding the lesion site.
Descriptors: zebrafish, nerve regeneration, neuroglia physiology, retina injuries, apoptosis, cell division, mitosis, neuroglia cytology, retina cytology, stem cells cytology.

Zhang, J., J.L. Lefebvre, S. Zhao, and M. Granato (2004). **Zebrafish unplugged reveals a role for muscle-specific kinase homologs in axonal pathway choice.** *Nature Neuroscience* 7(12): 1303-9. ISSN: 1097-6256.
NAL Call Number: QP351
Abstract: En route to their target, pioneering motor growth cones repeatedly encounter choice points at which they make pathway decisions. In the zebrafish mutant unplugged, two of the three segmental motor axons make incorrect decisions at a somitic choice point. Using positional cloning, we show here that unplugged encodes a homolog of muscle-specific kinase (MuSK) and that, unlike mammalian MuSK, unplugged has only a limited role in neuromuscular synaptogenesis. We demonstrate that unplugged is transiently expressed in cells adjacent to the choice point and that unplugged signaling before the arrival of growth cones induces changes in the extracellular environment. In addition, we find that the unplugged locus generates three different transcripts. The splice variant 1 (SV1) isoform lacks the extracellular modules essential for agrin responsiveness, and signaling through this isoform mediates axonal pathfinding, independent of the MuSK downstream component rapsyn. Our results demonstrate a new role for MuSK homologs in axonal pathway selection.
Descriptors: zebrafish, axons physiology, homeodomain, receptor protein tyrosine kinases physiology, cholinergic receptors physiology, amino acid sequence, homeodomain molecu-

lar sequence data, mutation, neural pathways physiology, receptor protein tyrosine kinases genetics, cholinergic receptors genetics, amino acid sequence homology.

Zhdanova, I.V., L. Yu, M. Lopez-Patino, E. Shang, S. Kishi, and E. Guelin (2008). **Aging of the circadian system in zebrafish and the effects of melatonin on sleep and cognitive performance**. *Brain Research Bulletin* 75(2-4): 433-441. ISSN: 0361-9230.
NAL Call Number: QP376
Descriptors: zebrafish, aging, circadian system, melatonin, sleep, cognitive performance.

Ziv, L., S. Levkovitz, R. Toyama, J. Falcon, and Y. Gothilf (2005). **Functional development of the zebrafish pineal gland: light-induced expression of period2 is required for onset of the circadian clock.** *Journal of Neuroendocrinology* 17(5): 314-20. ISSN: 0953-8194.
Abstract: In zebrafish, the pineal gland is a photoreceptive organ that contains an intrinsic circadian oscillator and exhibits rhythmic arylalkylamine-N-acetyltransferase (zfaanat2) mRNA expression. In the present study, we investigated the role of light and of a clock gene, zperiod2 (zper2), in the development of this rhythm. Analysis of zfaanat2 mRNA expression in the pineal gland of 3-day-old zebrafish embryos after exposure to different photoperiodic regimes indicated that light is required for proper development of the circadian clock-controlled rhythmic expression of zfaanat2, and that a 1-h light pulse is sufficient to initiate this rhythm. Analysis of zper2 mRNA expression in zebrafish embryos exposed to different photoperiodic regimes indicated that zper2 expression is transiently up-regulated by light but is not regulated by the circadian oscillator. To establish the association between light-induced zper2 expression and light-induced clock-controlled zfaanat2 rhythm, zPer2 knock-down experiments were performed. The zfaanat2 mRNA rhythm, induced by a 1-h light pulse, was abolished in zPer2 knock-down embryos. These experiments indicated that light-induced zper2 expression is crucial for establishment of the clock-controlled zfaanat2 rhythm in the zebrafish pineal gland.
Descriptors: zebrafish embryos, pineal gland, arylalkylamine N acetyltransferase metabolism, circadian rhythm physiology, eye proteins metabolism, developmental gene expression regulation radiation effects, pineal gland enzymology, metabolism, arylalkylamine n acetyltransferase genetics and radiation effects, biological markers metabolism , circadian rhythm genetics and radiation effects, eye proteins genetics and radiation effects, fish proteins genetics and radiation effects, light, pineal gland embryology and radiation effects, messenger RNA analysis.

Ziv, L., R. Toyama, and Y. Gothilf (2003). **Photoreception is required for the onset of the circadian clock in the pineal gland of zebrafish embryo**. *Neural Plasticity* 10(3): 241. ISSN: 0792 8483.
Descriptors: zebrafish, biosynchronization, development, endocrine system, chemical coordination.

Zupanc, G.K., K. Hinsch, and F.H. Gage (2005). **Proliferation, migration, neuronal differentiation, and long-term survival of new cells in the adult zebrafish brain.** *Journal of Comparative Neurology* 488(3): 290-319. ISSN: 0021-9967.
Descriptors: zebrafish, brain cytology, cell differentiation physiology, cell movement physiology, cell proliferation, neurons cytology, brain mapping, bromodeoxyuridine metabolism, calcium binding protein, vitamin D dependent metabolism, cell count methods, cell survival physiology, glial fibrillary acidic protein metabolism, hu paraneoplastic encephalomyelitis

antigens, immunohistochemistry methods, nerve tissue proteins metabolism, RNA binding proteins metabolism, s100 proteins metabolism, time factors.

Pharmacology

Airhart, M.J., B. Miller, B. Turner, D. Lee, and R.G. Skalko (2004). **Adverse effect of paroxetine (paxil) on motor development in zebrafish.** *Birth Defects Research* 70(5): 305. ISSN: 1542 0752.
NAL Call Number: QL991.T4
Descriptors: zebrafish, neural coordination, paroxetine, pharmacology, toxicology, motor development.

Anonymous (2006). **Conference Report: Eicosanoids meeting report and abstracts**. *Prostaglandins and Other Lipid Mediators* 79(1-2): 141-143. ISSN: 1098-8823.
NAL Call Number: QP801.P68P729
Descriptors: zebrafish, pharmacology, biochemistry, molecular biophysics, human medicine, colon cancer, digestive system disease, neoplastic disease, Alzheimer's disease, nervous system disease, behavioral and mental disorders, drug therapy, lipidomics.

Bayliss, P.E., K.L. Bellavance, G.G. Whitehead, J.M. Abrams, S. Aegerter, H.S. Robbins, D.B. Cowan, M.T. Keating, T. O'Reilly, J.M. Wood, T.M. Roberts, and J. Chan (2006). **Chemical modulation of receptor signaling inhibits regenerative angiogenesis in adult zebrafish.** *Nature Chemical Biology* 2(5): 265-73. ISSN: 1552-4450.
Abstract: We examined the role of angiogenesis and the need for receptor signaling using chemical inhibition of the vascular endothelial growth factor receptor in the adult zebrafish tail fin. Using a small-molecule inhibitor, we were able to exert precise control over blood vessel regeneration. An angiogenic limit to tissue regeneration was determined, as avascular tissue containing skin, pigment, neuronal axons and bone precursors could regenerate up to about 1 mm. This indicates that tissues can regenerate without direct interaction with endothelial cells and at a distance from blood supply. We also investigated whether the effects of chemical inhibition could be enhanced in zebrafish vascular mutants. We found that adult zebrafish, heterozygous for a mutation in the critical receptor effector phospholipase Cgamma1, show a greater sensitivity to chemical inhibition. This study illustrates the utility of the adult zebrafish as a new model system for receptor signaling and chemical biology.
Descriptors: zebrafish, tail fin, angiogenesis inhibitors pharmacology, endothelial cells drug effects, vascular endothelial growth factor receptor antagonists, inhibitors and metabolism, regeneration drug effects , axons drug effects and physiology, bone regeneration drug effects and physiology , endothelial cells physiology, guided tissue regeneration methods, heterozygote, fluorescence microscopy, mutation, neurons metabolism, phospholipase C gamma pharmacology, pigment epithelium of eye drug effects and physiology, regeneration physiology, skin physiology drug effects, tissue engineering.

Becker, T., M.A. McLane, and C.G. Becker (2003). **Integrin antagonists affect growth and pathfinding of ventral motor nerves in the trunk of embryonic zebrafish.** *Molecular and Cellular Neuroscience* 23(1): 54-68. ISSN: 1044-7431.
Abstract: Integrins are thought to be important receptors for extracellular matrix (ECM) components on growing axons. Ventral motor axons in the trunk of embryonic zebrafish grow in a midsegmental pathway through an environment rich in ECM components. To test the role of integrins in this process, integrin antagonists (the disintegrin echistatin in native and recombinant form, as well as the Arg-Gly-Asp-Ser peptide) were injected into embryos just prior to axon outgrowth at 14-16 h postfertilization (hpf). All integrin antagonists affected growth of ventral motor nerves in a similar way and native echistatin was most effective. At 24 hpf, when only the three primary motor axons per trunk hemisegment had grown out, 80% (16 of 20) of the

embryos analyzed had abnormal motor nerves after injection of native echistatin, corresponding to 19% (91 of 480) of all nerves. At 33 hpf, when secondary motor axons were present in the pathway, 100% of the embryos were affected (24 of 24), with 20% of all nerves analyzed (196 of 960) being abnormal. Phenotypes comprised abnormal branching (64% of all abnormal nerves) and truncations (36% of all abnormal nerves) of ventral motor nerves at 24 hpf and mostly branching of the nerves at 33 hpf (94% of all abnormal nerves). Caudal branches were at least twice as frequent as rostral branches. Surrounding trunk tissue and a number of other axon fascicles were apparently not affected by the injections. Thus integrin function contributes to both growth and pathfinding of axons in ventral motor nerves in the trunk of zebrafish in vivo.
Descriptors: zebrafish, axons physiology, integrins antagonists and inhibitors, motor neurons ultrastructure, nervous system embryology, motor neurons drug effects, skeletal muscle innervation, nervous system cytology, oligopeptides pharmacology, peptides pharmacology , platelet aggregation inhibitors pharmacology, somites cytology.

Berghmans, S., J.P.4. Morris, J.P. Kanki, and A.T. Look (2004). **Zebrafish sperm cryopreservation.** *Methods in Cell Biology* 77: 645-59. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, cryopreservation methods, semen preservation methods, acetamides pharmacology, cryoprotective agents pharmacology, sperm capacitation, sperm motility, spermatozoa drug effects, spermatozoa metabolism.

Bhatt, D.H., S.J. Otto, B. Depoister, and J.R. Fetcho (2004). **Cyclic AMP-induced repair of zebrafish spinal circuits.** *Science* 305(5681): 254-8. ISSN: 0036-8075.
NAL Call Number: 470Sci2
Abstract: Neurons in the human central nervous system (CNS) are unable to regenerate, as a result of both an inhibitory environment and their inherent inability to regrow. In contrast, the CNS environment in fish is permissive for growth, yet some neurons still cannot regenerate. Fish thus offer an opportunity to study molecules that might surmount the intrinsic limitations they share with mammals, without the complication of an inhibitory environment. We show by in vivo imaging in zebrafish that post-injury application of cyclic adenosine monophosphate can transform severed CNS neurons into ones that regenerate and restore function, thus overcoming intrinsic limitations to regeneration in a vertebrate.
Descriptors: zebrafish, axons physiology, bucladesine pharmacology, nerve regeneration, neurons physiology, spinal cord cytology and injuries drug therapy and physiopathology, axons drug effects, calcium metabolism, cyclic AMP analogs and derivatives and pharmacology, electroporation, escape reaction, fluorescent dyes, interneurons physiology, confocal microscopy, neurons drug effects, regeneration, rhodamines.

Braida, D., V. Limonta, S. Pegorini, A. Zani, C. Guerini Rocco, E. Gori, and M. Sala (2007). **Hallucinatory and rewarding effect of salvinorin A in zebrafish: kappa-opioid and CB1-cannabinoid receptor involvement.** *Psychopharmacology* 190(4): 441-8. ISSN: 0269-8811.
Abstract: RATIONALE: The hallucinatory effect and potential abuse of salvinorin A, the major ingredient of *Salvia divinorum*, has not been documented in animals. OBJECTIVE: The effects of salvinorin A on the zebrafish *(Danio rerio)* model, through its swimming behavior and conditioned place preference (CPP) task, was studied. MATERIALS AND METHODS: Swimming activity was determined in a squared observational chamber after an i.m. treatment of salvinorin A (0.1-10 microg/kg). For the CPP test, zebrafish were given salvinorin A (0.2 and 1 microg/kg) or vehicle and evaluated in a two-compartment chamber. RESULTS: Salvinorin A (0.1 and 0.2 microg/kg) induced accelerated swimming behavior in comparison with vehicle, whereas a

"trance-like" effect, at doses as 5 and 10 microg/kg, was obtained. Pretreatment with the kappa-opioid antagonist, nor-binaltorphimine (nor-BNI; 10 mg/kg) and the cannabinoid type 1 (CB(1)) antagonist, rimonabant (1 mg/kg), blocked salvinorin A-induced both stimulating and depressive effects obtained at a dose of 0.2 and 10 microg/kg, respectively. In the CPP test, salvinorin A (0.2 and 0.5 microg/kg) produced an increase in the time spent in the drug-associated compartment. A dose of 1 microg/kg produced no effect, whereas a dose of 80 microg/kg induced aversion. Pretreatment with nor-BNI or rimonabant fully reversed the reinforcing properties of salvinorin A (0.5 microg/kg). CONCLUSIONS: Taken together, these results indicate that salvinorin A, as is sometimes reported in humans, exhibits rewarding effects, independently from its motor activity, suggesting the usefulness of the zebrafish model to study addictive behavior. These effects appear mediated by activation of both kappa-opioid and cannabinoid CB(1) receptors.
Descriptors: zebrafish, salvinorin A, addictive behavior metabolism, behavior drug effects, diterpenes pharmacology, hallucinogens pharmacology, cannabinoid receptor, cb1 drug effects, kappa opioid receptors drug effects, metabolism, conditioning psychology drug effects, drug dose response relationship, motor activity drug effects, naltrexone analogs and derivatives and pharmacology, narcotic antagonists pharmacology, piperidines pharmacology, pyrazoles pharmacology, cannabinoid receptor, CB1 metabolism, kappa opioid receptors metabolism, reinforcement psychology, reproducibility of results, reward, swimming, time factors.

Bromee, T., J.P. Kukkonen, P. Andersson, J.M. Conlon, and D. Larhammar (2005). **Pharmacological characterization of ligand-receptor interactions at the zebrafish bradykinin receptor.** *British Journal of Pharmacology* 144(1): 11-6. ISSN: 0007 1188.
Abstract: Ligand interactions of a piscine bradykinin (BK) receptor expressed in vitro have been characterized for the first time by measuring inositol phosphate accumulation.The ligands were analogues of zebrafish BK with serial substitutions by D-amino acids or alanine. Substitutions at residues Arg(1), Gly(4), Ser(6), Pro(7), Leu(8) and Arg(9) caused greatly reduced potency and maximum response. The Pro(3) --> Ala analogue had higher potency but lower maximum response.The peptide HOE140 was a weak partial agonist although it is an antagonist at the human B2 receptor and a potent agonist at chicken B2.Thus, cloned zebrafish BK receptor reveals a ligand-interaction profile that is distinct from mammalian B1 and B2 receptors and from the previously characterized BK receptor in trout stomach, but similar to the receptor in cod intestine. These results increase our understanding of the evolution of BK receptors and the functions of the kallikrein-kinin system.
Descriptors: zebrafish BK receptors, bradykinin analogs, derivatives and pharmacology, bradykinin receptors antagonists, inhibitors and drug effects, alanine chemistry, amino acid substitution, amino acids chemistry, bradykinin chemistry and metabolism, cell line, inositol phosphates analysis and biosynthesis, ligands, molecular structure, bradykinin receptors genetics, species specificity, transfection.

Cardona Costa, J., J. Roig, M. Perez Camps, and F. Garcia Ximenez (2006). **Vitrification of caudal fin explants from zebrafish adult specimens.** *Cryo Letters* 27(5): 329-32. ISSN: 0143 2044.
NAL Call Number: QC278.C72
Abstract: No data on vitrification of tissue samples are available in fishes. Three vitrification solutions were compared: V1: 20% ethylene glycol and 20% dimethyl sulphoxide; V2: 25% propylene glycol and 20% dimethyl sulphoxide, and; V3: 20% propylene glycol and 13% methanol, all three prepared in Hanks' buffered salt solution plus 20 percent FBS, following the same one step vitrification procedure developed in mammals. Caudal fin tissue pieces were vitrified into 0.25 ml plastic straws in 30s and stored in liquid nitrogen for 3 days minimum, warmed (10s in nitrogen vapour and 5s in a 25 degree C water bath) and cultured (L-15 plus 20% FBS at 28.5

degree C). At the third day of culture, both attachment and outgrowing rates were recorded. V3 led to the worst results (8% of attachment rate). V1 and V2 allow higher attachment rates (V1: 63% vs V2: 50%. $P < 0.05$) but not significantly different outgrowing rates (83% to 94%). Vitrification of caudal fin pieces is advantageous in fish biodiversity conservation, particularly in the wild, due to the simplicity of procedure and equipment.
Descriptors: zebrafish, cryopreservation methods, agents pharmacology and standards, dimethyl sulfoxide pharmacology, drug combinations, methanol pharmacology, organ culture techniques, ethylene and propylene glycol pharmacology.

Carney, S.A., J. Chen, C.G. Burns, K.M. Xiong, R.E. Peterson, and W. Heideman (2006). **Aryl hydrocarbon receptor activation produces heart-specific transcriptional and toxic responses in developing zebrafish.** *Molecular Pharmacology* 70(2): 549-61. ISSN: 0026-895X.
NAL Call Number: QP901.M6
Abstract: Proper regulation of the aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor, is required for normal vertebrate cardiovascular development. AHR hyperactivation by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) during zebrafish (*Danio rerio*) development results in altered heart morphology and function, culminating in death. To identify genes that may cause cardiac toxicity, we analyzed the transcriptional response to TCDD in zebrafish hearts. Zebrafish larvae were exposed to TCDD for 1 h at 72 h after fertilization (hpf), and the hearts were extracted for microarray analysis at 1, 2, 4, and 12 h after exposure (73, 74, 76, and 84 h postfertilization). The remaining body tissue was also collected at each time for comparison. TCDD rapidly induced expression in 42 genes within 1 to2hof exposure. These genes function in xenobiotic metabolism, proliferation, heart contractility, and pathways that regulate heart development. Furthermore, these expression changes preceded signs of cardiovascular toxicity, characterized by decreased stroke volume, peripheral blood flow, and a halt in heart growth. This identifies strong candidates for important AHR target genes. It is noteworthy that the TCDD-induced transcriptional response in the hearts of zebrafish larvae was substantially different from that induced in the rest of the body tissues. One of the biggest differences included a cluster of genes that were down-regulated 12 h after exposure in heart tissue, but not in the body samples. More than 70% of the transcripts in this heart-specific cluster promote cellular growth and proliferation. Thus, the developing heart stands out as being responsive to TCDD at both the level of toxicity and gene expression.
Descriptors: zebrafish heart development, heart drug effects, myocardium metabolism, aryl hydrocarbon receptors physiology, tetrachlorodibenzodioxin toxicity, genetic transcription drug effects, aryl hydrocarbon receptor nuclear translocator physiology, gene expression profiling, oligonucleotide array sequence analysis, research design, xenobiotics metabolism.

Carney, S.A., R.E. Peterson, and W. Heideman (2004). **2,3,7,8-Tetrachlorodibenzo-p-dioxin activation of the aryl hydrocarbon receptor/aryl hydrocarbon receptor nuclear translocator pathway causes developmental toxicity through a CYP1A-independent mechanism in zebrafish.** *Molecular Pharmacology* 66(3): 512-21 . ISSN: 0026 895X.
NAL Call Number: QP901.M6
Abstract: The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that dimerizes with ARNT to mediate responses to compounds such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD and other AHR agonists cause toxic responses in early life stages of fish, including the zebrafish, *Danio rerio*. The most well characterized target gene for the AHR/aryl hydrocarbon receptor nuclear translocator (ARNT) dimer is a cytochrome P450, CYP1A. Induction of CYP1A by TCDD has been correlated with certain toxic responses in developing zebrafish and has been postulated to mediate these responses. To determine whether CYP1A is the

important downstream effector enzyme for the AHR/ARNT pathway, we used morpholino oligonucleotides (MOs) to block induction of CYP1A in response to TCDD in zebrafish embryos. Although the zfcyp1a-MO effectively prevented CYP1A up-regulation, it did not prevent the signs of developmental toxicity, including pericardial edema, slowed blood flow, craniofacial malformation, and defects in erythropoiesis. We conclude that the important target for the AHR/ARNT pathway in developing zebrafish exposed to TCDD is not zfcyp1a. This suggests an alternative model in which TCDD-activated AHR/ARNT disrupts the normal process of growth and development by altering programs of gene expression or function.
Descriptors: zebrafish early development, cytochrome P450 CYPA1A1 deficiency, DNA binding proteins genetics, proteins metabolism, drug effects, aryl hydrocarbon receptors metabolism, teratogens toxicity, tetrachlorodibenzodioxin toxicity, transcription factors metabolism, aryl hydrocarbon receptor nuclear translocator, cytochrome P450 CYPA1A1 genetics.

Chang, W.N., J.N. Tsai, B.H. Chen, H.S. Huang, and T.F. Fu (2007). **Serine hydroxymethyltransferase isoforms are differentially inhibited by leucovorin: characterization and comparison of recombinant zebrafish serine hydroxymethyltransferases.** *Drug Metabolism and Disposition* 35(11): 2127-37. ISSN: 0090 9556.
Abstract: Serine hydroxymethyltransferase (SHMT) provides activated one-carbon units required for the biosynthesis of nucleotides, protein, and methyl group by converting serine and tetrahydrofolate to glycine and N(5),N(10)-methylenetetrahydrofolate. It is postulated that SHMT activity is associated with the development of methotrexate resistance and the in vivo activity of SHMT is regulated by the binding of N(5)-CHO-THF, the rescue agent in high-dose methotrexate chemotherapy. The aim of this study is to advance our understanding of the folate-mediated one-carbon metabolism in zebrafish by characterizing zebrafish mitochondrial SHMT. The cDNA encoding zebrafish mitochondrial SHMT was cloned, overexpressed in *Escherichia coli*, and purified with a three-step purification protocol. Similarities in structural, physical, and kinetic properties were revealed between the recombinant zebrafish mitochondrial SHMT and its mammalian orthologs. Surprisingly, leucovorin significantly inhibits the aldol cleavage of serine catalyzed by zebrafish cytosolic SHMT but inhibits to a lesser extent the reaction catalyzed by the mitochondrial isozyme. This is, to our knowledge, the first report on zebrafish mitochondrial folate enzyme as well as the differential inhibition of leucovorin on these two SHMT isoforms. Western blot analysis revealed tissue-specific distribution with the highest enrichment present in liver for both cytosolic and mitochondrial SHMTs. Intracellular localization was confirmed by confocal microscopy for both mitochondrial and cytosolic SHMTs. Unexpectedly, the cytosolic isoform was observed in both nucleus and cytosol. Together with the previous report on zebrafish cytosolic SHMT, we suggest that zSHMTs can be used in in vitro assays for folate-related investigation and antifolate drug discovery.
Descriptors: zebrafish, serine hydroxymethyltransferase, isoforms, cytosolic and mitochondrial SHMT, inhibition, leucovorin, characterization, comparison.

Craig, M.P., S.D. Gilday, and J.R. Hove (2006). **Dose-dependent effects of chemical immobilization on the heart rate of embryonic zebrafish.** *Lab Animal* 35(9): 41-7. ISSN: 0093-7375.
Abstract: The small size and optical transparency of zebrafish embryos and larvae greatly facilitate modern intravital microscopic phenotyping of these experimentally tractable laboratory animals. Neither the experimentally derived dose-response relationships for chemicals commonly used in the mounting of live fish larvae, nor their effect on the stress of the animal, are currently available in the research literature. This is particularly problematic for IACUCs attempting to maintain the highest ethical standards of animal care in the face of a recent spate in investigator-initiated requests to use embryonic zebrafish as experimental models. The authors address this

issue by describing the dose-dependent efficacy of several commonly used chemical mounting treatments and their effect on one stress parameter, embryo heart rate. The results of this study empirically define, for the first time, effective, minimally stressful treatments for immobilization and in vivo visualization during early zebrafish development.
Descriptors: zebrafish embryos heart rates, heart rate drug effects, aminobenzoic acids pharmacology, anesthetics pharmacology, IACUC concerns, immobilization techniques, drug dose response relationship, enzyme inhibitors pharmacology, heart rate physiology, immobilization methods, phenylthiourea pharmacology, pronase pharmacology, sepharose pharmacology.

Cross, L.M., M.A. Cook, S. Lin, J.N. Chen, and A.L. Rubinstein (2003). **Rapid analysis of angiogenesis drugs in a live fluorescent zebrafish assay.** *Arteriosclerosis Thrombosis and Vascular Biology* 23(5): 911-2. ISSN: 1079 5642.
Descriptors: live zebrafish assay, angiogenesis inhibitors pharmacology, biological assay methods, preclinical drug evaluation methods , fluorescent dyes analysis, luminescent proteins analysis and genetics, physiologic neovascularization drug effects, genetically modified, biological markers, blood supply, drug effects, recombinant fusion proteins analysis and biosynthesis, vascular endothelial growth factor receptor 2 analysis, biosynthesis and genetics.

Damodaran, S., C.A. Dlugos, T.D. Wood, and R.A. Rabin (2006). **Effects of chronic ethanol administration on brain protein levels: A proteomic investigation using 2-D DIGE system**. *European Journal of Pharmacology* 547(1-3): 75-82. ISSN: 0014-2999.
Descriptors: zebrafish strain, brain, desorption, drug abuse, drug tolerance, ethanol, exposure tolerance, fluorescence, freshwater fish, gel electrophoresis, lasers, mass spectroscopy, pharmacology , proteins, strains, swimming behavior, proteomics, *Danio rerio.*

Dlugos, C.A. and R.A. Rabin (2003). **Ethanol effects on three strains of zebrafish: model system for genetic investigations.** *Pharmacology, Biochemistry, and Behavior* 74(2): 471-80. ISSN: 0091-3057.
NAL Call Number: 0091-3057
Abstract: The effects of acute and chronic ethanol administration on the wild-type (WT), long-fin striped (LFS), and blue long-fin (BLF) strains of zebrafish were investigated. In the LFS strain, acute exposure to 0.25% (v/v) ethanol inhibited the startle reaction and increased both the area occupied by a group of subjects and the average distance between each fish and its nearest neighbor. Similar effects were found in the WT fish although higher concentrations of ethanol were required. No effects on the behavior of the BLF fish were observed with up to 1.0% (v/v) ethanol. Brain alcohol levels were comparable among the three strains precluding a pharmacokinetic explanation for the behavioral results. In LFS zebrafish, behavioral tolerance was observed after 1 week of continual exposure to ethanol. Conversely, chronic ethanol exposure of the WT fish for up to 2 weeks did not result in the development of tolerance, but rather appeared to increase the disruptive action of the drug. The present results suggest the observed strain differences in the effects of ethanol reflect genotypic differences in both the response of the central nervous system (CNS) to ethanol as well as the ability of the CNS to adapt to ethanol exposure. Although preliminary, the present study indicates that the zebrafish is an excellent model system to investigate the genetic determinants involved in regulating the responses to ethanol.
Descriptors: zebrafish, WT, LFS and BLF strains, behavior drug effects, central nervous system depressants pharmacology, ethanol pharmacology, brain metabolism, central nervous system depressants metabolism, drug dose response relationship, ethanol metabolism, genotype, species specificity, startle reaction drug effects, swimming physiology.

Gerlai, R., V. Lee, and R. Blaser (2006). **Effects of acute and chronic ethanol exposure on the behavior of adult zebrafish (*Danio rerio*).** *Pharmacology, Biochemistry, and Behavior* 85(4): 752-61. ISSN: 0091-3057.

NAL Call Number: 0091-3057

Abstract: The zebrafish has been a popular subject of embryology and genetic research for the past three decades. Recently, however, the interest in its neurobiology and behavior has also increased. Nevertheless, compared to other model organisms, e.g., rodents, zebrafish behavior is understudied and very few behavioral paradigms exist for mutation or drug screening purposes. Alcoholism is one of the biggest and costliest diseases whose mechanisms are not well understood. Model organisms such as the zebrafish may be utilized in this line of research. Previously, we investigated the effects of acute ethanol exposure on adult zebrafish using four behavioral paradigms and employing manual quantification methods. Here, we study the effects of chronic ethanol exposure and analyze how it modifies the effects of acute ethanol treatment. We employ a videotracking-based automated quantification method in a predator model paradigm and show that this method is capable of detecting an avoidance reaction that is ameliorated by higher doses of ethanol, a potential anxiolytic effect. Importantly, we also demonstrate that chronic, two week long, exposure to ethanol results in significant adaptation to this substance in adult zebrafish. Overall, our results suggest that zebrafish will be an appropriate subject for high throughput screening applications aimed at the analysis of the mechanisms and pharmacology of acute and chronic ethanol induced changes in the vertebrate brain.

Descriptors: zebrafish, alcoholism psychology and model, behavior drug effects, ethanol pharmacology, ethanol administration and dosage.

Goldsmith, P. (2004). **Zebrafish as a pharmacological tool: the how, why and when.** *Current Opinion in Pharmacology* 4(5): 504-12. ISSN: 1471 4892.

NAL Call Number: RM1

Abstract: Zebrafish combine the relevance of a vertebrate with the scalability of an invertebrate. They can live in 96-well plate format and readily absorb chemicals from the water. These features have stimulated the use of zebrafish by medical researchers to model human disease and then assess the action of compounds in a whole organism. Examples of the power of this system have been illustrated with the cloning of zebrafish human ether-a-go-go-related gene (HERG), which shows near 100% homology in key domains, and the associated ability to identify drugs that prolong the QT interval both rapidly and with tiny amounts (micrograms) of compound.

Descriptors: zebrafish, preclinical drug evaluation methods, pharmacology models, mammals physiology, species specificity.

Goldsmith, P., Z. Golder, J. Hunt, S. Berghmans, D. Jones, J.P. Stables, L. Murphree, D. Howden, P.E. Newton, and F.M. Richards (2007). **GBR12909 possesses anticonvulsant activity in zebrafish and rodent models of generalized epilepsy but cardiac ion channel effects limit its clinical utility.** *BioMed Central Pharmacology* 79(4): 250-8. ISSN: 1472-6904.

NAL Call Number: RM1

Abstract: BACKGROUND/AIMS: GBR12909 has been reported to possess anticonvulsant activity with focal brain perfusion to the hippocampus of pilocarpine, although an earlier publication suggested any anticonvulsant effects were only mild. Here we further explored the anticonvulsant potential of GBR12909 with a suite of anticonvulsant assays in both zebrafish and mammals and then explored whether it possessed any QT effects which might limit clinical utility. METHODS: We assessed the anticonvulsant effects of GBR12909 in zebrafish pentylenetetrazole (PTZ), mammalian maximal electroshock and PTZ models of generalized epilepsy and a rodent hippocampal kindling model. Cardiac effects were assessed in zebrafish and man.

RESULTS: GBR12909 possesses anticonvulsant activity in zebrafish and rodent models of generalized epilepsy. However, phase 1 human data indicated potential QT effects. Subsequent testing in a zebrafish QT assay confirmed marked arrhythmogenic potential. CONCLUSION: Further clinical development of GBR12909 in epilepsy was considered inappropriate because of insufficient window between the therapeutic effects and the cardiac arrhythmia problems identified in zebrafish assays. Any further development based on this mechanism of action should avoid the GBR12909 chemical scaffold, or involve structure-activity dissociation of its neurological and cardiac effects. 2007 S. Karger AG, Basel

Descriptors: zebrafish, anticonvulsants pharmacology, dopamine uptake inhibitors pharmacology, epilepsy drug therapy, ether a go go potassium channels metabolism , myocardium metabolism, piperazines pharmacology, anticonvulsants adverse effects, cardiac arrhythmias chemically induced, dogs , dopamine uptake inhibitors adverse effects, electrocardiography, mice, piperazines adverse effects, sprague dawley rats.

Gonzalez Nunez, V., G. Arsequell, E. Szemenyei, G. Toth, G. Valencia, and R.E. Rodriguez (2005). **Binding profile of the endogenous novel heptapeptide Met-enkephalin-Gly-tyr in zebrafish and rat brain.** *Journal of Pharmacology and Experimental Therapeutics, The* 314(2): 862-7. ISSN: 0022-3565.

Abstract: Zebrafish is considered a model organism, not only for the study of the biological functions of vertebrates but also as a tool to analyze the effects of some drugs or toxic agents. Five opioid precursor genes homologous to the mammalian opioid propeptide genes have recently been identified; one of these, the zebrafish proenkephalin, codes a novel heptapeptide, the Met-enkephalin-Gly-Tyr (MEGY). To analyze the pharmacological properties of this novel ligand, we have labeled it with tritium ([(3)H]MEGY). In addition, we have also synthesized two analogs: (d-Ala(2))-MEGY (Y-d-Ala-GFMGY) and (d-Ala(2), Val(5))-MEGY (Y-d-Ala-GFVGY). The binding profile of these three agents has been studied in zebrafish and rat brain membranes. [(3)H]MEGY presents one binding site in zebrafish, as well as in rat brain membranes, although it shows a slight higher affinity in zebrafish brain. The observed saturable binding is displaced by naloxone, thus confirming the opioid nature of the binding sites. Competition binding assays indicate that the methionine residue is essential for high-affinity binding of MEGY and probably of other peptidic agonists in zebrafish, whereas the change of a Gly for a d-Ala does not dramatically affect the ligand affinity. Our results show that the percentage of [(3)H]MEGY displaced by all the ligands studied is higher than 100%, thus inferring that naloxone (used to determine nonspecific binding) does not bind to all the sites labeled by [(3)H]MEGY. Therefore, we can deduct that some of the MEGY binding sites should not be considered classical opioid sites.

Descriptors: zebrafish, proenkephalin brain metabolism, methionine enkephalin analogs and derivatives, amino acid substitution, binding, competitive drug effects, diprenorphine pharmacokinetics, methionine enkephalin chemical synthesis and pharmacokinetics, membranes drug effects and metabolism, methionine metabolism, naloxone pharmacokinetics, narcotic antagonists pharmacokinetics, rats, species specificity.

Gonzalez Nunez, V., A. Barrallo, J.R. Traynor, and R.E. Rodriguez (2006). **Characterization of opioid-binding sites in zebrafish brain.** *Journal of Pharmacology and Experimental Therapeutics, The* 316(2): 900-4. ISSN: 0022-3565.

Abstract: The pharmacological profile of opioid-binding sites in zebrafish brain homogenates has been studied using radiolabeled binding techniques. The nonselective antagonist [(3)H]diprenorphine binds with high affinity (K(D) = 0.27 +/- 0.08 nM and a B(max) = 212 +/- 14.3 fmol/mg protein), displaying two different binding sites with affinities of K(D1) = 0.08 +/- 0.02 nM and K(D2) = 17.8 +/- 9.18 nM. The nonselective agonist [(3)H]bremazocine also binds with

high affinity to zebrafish brain membranes but only displays one single binding site with a K(D) = 1.1 +/- 0.09 nM and a B(max) = 705 +/- 19.3 fmol/mg protein. Competition binding assays using [(3)H]diprenorphine and several unlabeled ligands were performed. The synthetic selective agonists for mammalian opioid receptors DPDPE ([DPen(2),D-Pen(5)]-enkephalin), DAMGO ([D-Ala(2),NMe-Phe(4),Gly(5)-ol]-enkephalin), and U69,593 [(5alpha,7alpha,8beta)-(+)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide] failed to effectively displace [(3)H]diprenorphine binding, whereas nonselective ligands and the endogenous opioid peptides such as dynorphin A showed good affinities in the nanomolar range, although several of the endogenous peptides only displaced approximately 50% of the specifically bound [(3)H] diprenorphine. Our results provide evidence that, although the selective synthetic compounds for mammalian receptors do not fully recognize the opioid-binding sites in zebrafish brain, the activity of the endogenous zebrafish opioid system might not significantly differ from that displayed by the mammalian opioid system. Hence, the study of zebrafish opioid activity may contribute to an understanding of endogenous opioid systems in higher vertebrates.
Descriptors: zebrafish, benzomorphans pharmacology, brain metabolism and opiod binding sites, diprenorphine pharmacology, narcotic antagonists pharmacology, opioid receptors metabolism, brain drug effects, ligands, radioligand assay.

Gurvich, N., M.G. Berman, B.S. Wittner, R.C. Gentleman, P.S. Klein, and J.B.A. Green (2005). **Association of valproate-induced teratogenesis with histone deacetylase inhibition in vivo**. *Federation of American Societies for Experimental Biology Journal* 19(7) ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: Chemically induced birth defects are an important public health and human problem. Here we use *Xenopus* and zebrafish as models to investigate the mechanism of action of a well-known teratogen, valproic acid (VPA). VPA is a drug used in treatment of epilepsy and bipolar disorder but causes spina bifida if taken during pregnancy. VPA has several biochemical activities, including inhibition of histone deacetylases (HDACs). To investigate the mechanism of action of VPA, we compared its effects in *Xenopus* and zebrafish embryos with those of known HDAC inhibitors and noninhibitory VPA analogs. We found that VPA and other HDAC inhibitors cause very similar and characteristic developmental defects whereas VPA analogs with poor inhibitory activity in vivo have little teratogenic effect. Unbiased microarray analysis revealed that the effects of VPA and trichostatin A (TSA), a structurally unrelated HDAC inhibitor, are strikingly concordant. The concordance is apparent both by en masse correlation of fold-changes and by detailed similarity of dose-response profiles of individual genes. Together, the results demonstrate that the teratogenic effects of VPA are very likely mediated specifically by inhibition of HDACs. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, behavior, pharmacology, toxicology, nervous system, neural coordination, development, enzymology, epilepsy, nervous system disease, drug therapy, bipolar disorder, behavioral and mental disorders, drug therapy, spina bifida, nervous system disease, congenital disease, drug induced, etiology, teratogenesis, valproic acid, *Xenopus* embryos .

Hamad, A., M. Kluk, J. Fox, M. Park, and J.E. Turner (2007). **The effects of aromatase inhibitors and selective estrogen receptor modulators on eye development in the zebrafish *(Danio rerio*).** *Current Eye Research* 32(10): 819-27. ISSN: 0271 3683.
Abstract: PURPOSE: It has been shown that the steroid hormone estrogen plays an important role in the development of the central nervous system. The purpose of these studies was to determine the effect of estrogen on zebrafish eye maturation. METHODS: In these experiments, the estrogen receptor blockers tamoxifen and clomiphene, selective estrogen receptor modulators (SERMs), were used to determine the role of estrogen in the developing eye by morphometric

microscopic techniques. Also, in this study the synthesis of estrogen was inhibited with aminoglutethimide or 4-hydroxyandrostenedione treatments, both of which are aromatase inhibitors (AIs). RESULTS: After these various treatments, the zebrafish were analyzed for eye maturation and growth rates, which were significantly diminished with both AI and SERM treatments. In a competition assay, where tamoxifen and excess estrogen were added at the same time normal eye development and body growth took place, indicating that estrogen out-competed tamoxifen and that these phenomena were most likely exerted at the level of the estrogen receptor. Specifically, morphometric analysis revealed a significantly diminished thickness in most retinal layers, and, most particularly, in the two plexiform layers where synaptic contacts are established. CONCLUSIONS: These studies have demonstrated that the presence of estrogen in the early developing embryo is critical for retinal development and overall body growth.

Descriptors: zebrafish , aromatase inhibitors pharmacology, physiology, eye embryology competition assay, selective estrogen receptor modulators pharmacology, aminoglutethimide pharmacology, androstenedione analogs and derivatives and pharmacology, clomiphene pharmacology, embryonic development drug effects, estrogens pharmacology, retina embryology, tamoxifen pharmacology.

Holmberg, A., C. Olsson, and G.W. Hennig (2007). **TTX-sensitive and TTX-insensitive control of spontaneous gut motility in the developing zebrafish (*Danio rerio*) larvae.** *Journal of Experimental Biology* 210(Pt 6): 1084-91. ISSN: 0022-0949.

NAL Call Number: 442.8B77

Abstract: Spontaneous regular gut motility in zebrafish begins around 4 days post fertilisation (d.p.f.) and is modulated by release of acetylcholine and nitric oxide. The role of intrinsic or extrinsic innervation for initiating and propagating the spontaneous contractions, however, is not well understood. By creating spatiotemporal maps, we could examine spontaneous motility patterns in zebrafish larvae in vivo at 4 and 7 d.p.f. in more detail. Tetrodotoxin (TTX) was added to elucidate the importance of nervous control. Anterograde and retrograde contraction waves originated in the same region, just posterior to the intestinal bulb. This area correlates well with the distribution of Hu (human neuronal protein C/D)-immunoreactive nerve cell bodies. Whereas numerous immunoreactive nerve cells were present in the mid and distal intestine at both 4 and 7 d.p.f., fewer cells were seen anterior to the origin of contractions. The overall frequency of contractions (1.16+/-0.15 cycles min(-1), N=14 at 4 d.p.f.; 1.05+/-0.09 cycles min(-1), N=13 at 7 d.p.f.) and the interval between individual anterograde contraction waves (54.8+/-7.9 s at 4 d.p.f., N=14; 56.9+/-4.4 s, N=13 at 7 d.p.f.) did not differ between the two stages but the properties of the contractions were altered. The distance travelled by each wave increased from 591.0+/-43.8 microm at 4 d.p.f. (N=14) to 719.9+/-33.2 microm at 7 d.p.f. (N=13). By contrast, the velocity decreased from 4 d.p.f. (49.5+/-5.5 microm s(-1), N=12) to 7 d.p.f. (27.8+/-3.6 microm s(-1), N=13). At 4 d.p.f., TTX did not affect any of the parameters whereas at 7 d.p.f. anterograde frequency (control 1.07+/-0.12 cycles min(-1), N=8; TTX 0.55+/-0.13 cycles min(-1), N=8) and distance travelled (control 685.1+/-45.9 microm, N=8; TTX 318.7+/-88.7 microm, N=6) were decreased. In conclusion, enteric or extrinsic innervation does not seem to be necessary to initiate spontaneous contractions of the gut in zebrafish larvae. However, later in development, nerves have an increasingly important role as modulators of intestinal activity.

Descriptors: zebrafish larva, gastrointestinal motility drug effects, tetrodotoxin pharmacology, zebrafish growth and development , immunohistochemistry, larva drug effects and physiology, muscle contraction drug effects, time factors.

Holmberg, A., C. Olsson, and S. Holmgren (2006). **The effects of endogenous and exogenous nitric oxide on gut motility in zebrafish *Danio rerio* embryos and larvae.** *Journal of Experimental Biology* 209(Pt 13): 2472-9. ISSN: 0022-0949.

NAL Call Number: 442.8B77

Abstract: Using motion analysis, the ontogeny of the nitrergic control system in the gut was studied in vivo in zebrafish *Danio rerio* embryos and larvae. For the first time we show the presence of a nitrergic tonus, modulating both anterograde and retrograde contraction waves in the intestine of developing zebrafish. At 4 d.p.f. (days post fertilisation), the nitric oxide synthase (NOS) inhibitor L-NAME (three boluses of 50-100 nl, 10(-3) mol l(-1)) increased the anterograde contraction wave frequency by 0.50+/-0.10 cycles min(-1). Subsequent application of the NO donor sodium nitroprusside (SNP; three boluses of 50-100 nl, 10(-4) mol l(-1)) reduced the frequency of propagating anterograde waves (-0.71+/-0.20 cycles min(-1)). This coincided with the first appearance of an excitatory cholinergic tonus, observed in an earlier study. One day later, at 5 d.p.f., in addition to the effect on anterograde contraction waves, application of L-NAME increased (0.39+/-0.15 cycles min(-1)) and following SNP application reduced (-1.61+/-0.36 cycles min(-1)) the retrograde contraction wave frequency. In contrast, at 3 d.p.f., when no spontaneous motility is observed, application of L-NAME did not induce contraction waves in either part of the gut, indicating the lack of a functional inhibitory tonus at this early stage. Gut neurons expressing NOS-like immunoreactivity were present in the distal and middle intestine as early as 2 d.p.f., and at 1 day later in the proximal intestine. In conclusion, the present study suggests that a nitrergic inhibitory tonus develops shortly before or at the time for onset of exogenous feeding.

Descriptors: zebrafish embryos, gastrointestinal motility physiology, nitric oxide physiology and donors pharmacology, nitroprusside pharmacology, anatomy and histology, drug effects, physiology, gastrointestinal motility drug effects, immunohistochemistry, larva physiology, muscle contraction drug effects and physiology, muscle tonus drug effects and physiology, ng nitroarginine methyl ester pharmacology, nitrergic neurons drug effects and physiology, nitric oxide pharmacology.

Hsu, H.J., M.R. Liang, C.T. Chen, and B.C. Chung (2006). **Pregnenolone stabilizes microtubules and promotes zebrafish embryonic cell movement.** *Nature* 439(7075): 480-3. ISSN: 0028-0836.

NAL Call Number: 472 N21

Abstract: Embryonic cell movement is essential for morphogenesis and the establishment of body shapes, but little is known about its mechanism. Here we report that pregnenolone, which is produced from cholesterol by the steroidogenic enzyme Cyp11a1 (cholesterol side-chain cleavage enzyme, P450scc), functions in promoting cell migration during epiboly. Epiboly is a process in which embryonic cells spread from the animal pole to cover the underlying yolk. During epiboly, cyp11a1 is expressed in an extra-embryonic yolk syncytial layer. Reducing cyp11a1 expression in zebrafish using antisense morpholino oligonucleotides did not perturb cell fates, but caused epibolic delay. This epibolic defect was partially rescued by the injection of cyp11a1 RNA or the supplementation of pregnenolone. We show that the epibolic delay is accompanied by a decrease in the level of polymerized microtubules, and that pregnenolone can rescue this microtubule defect. Our results indicate that pregnenolone preserves microtubule abundance and promotes cell movement during epiboly.

Descriptors: zebrafish embryos, cell movement drug effects, cytology, drug effects, microtubules drug effects, pregnenolone pharmacology, 3 hydroxysteroid dehydrogenases genetics and dehydrogenases metabolism, blastomeres metabolism, cholesterol side chain cleavage enzyme genetics and metabolism, embryology, developmental gene expression regulation, microtubules metabolism, pregnenolone metabolism.

Iversen, P.L. and S. Newbry (2005). **Manipulation of zebrafish embryogenesis by phosphorodiamidate morpholino oligomers indicates minimal non-specific teratogenesis.** *Current Opinion in Molecular Therapeutics* 7(2): 104-8. ISSN: 1464 8431.

Abstract: The incidence of teratogenicity caused by therapeutic agents is a significant concern in drug development. Approaches to screening active agents in drug development tend to be limited by relevance to humans, cost from the large number of animals required for testing and complicated by barriers to drug entry into the test organism. The era post-human, -mouse and -zebrafish genome sequence determination should result in more precise experimental approaches to the evaluation of teratogenic potential. Peer-reviewed publications (204 papers) from independent investigators evaluating phosphorodiamidate morpholino oligomers (PMOs) in functional genomic studies are reviewed. These studies utilized over 47,000 embryos and resulted in 0.3% off-targeted or potential teratogenic effects, while at the same time producing the 74% phenotypic penetrance expected for the targeted gene. This represents a very compelling case for the minimal teratogenic potential of PMO chemistry.

Descriptors: zebrafish, drug induced abnormalities, morpholines pharmacology.

Jensen, F.B. (2007). **Nitric oxide formation from nitrite in zebrafish.** *Journal of Experimental Biology* 210(Pt 19): 3387-94. ISSN: 0022-0949.

NAL Call Number: 442.8B77

Abstract: Nitrite is a potential nitric oxide (NO) donor and may have important biological functions at low concentrations. The present study tests the hypothesis that nitrite accumulation across the gills in fish will cause a massive NO production from nitrite. Zebrafish were exposed to three different nitrite levels for variable time periods, and changes in blood nitrosylhemoglobin (HbNO), methemoglobin (metHb), oxygenated hemoglobin (oxyHb) and deoxygenated hemoglobin (deoxyHb) were evaluated by spectral deconvolution. Blood HbNO (a biomarker of internal NO production) was low in controls, increased to a stable level around 3.7% of total Hb in fish exposed to 0.6 mmol l(-1) nitrite, and to 12.1% (at day 2) in fish exposed to 2 mmol l(-1) nitrite. The very high HbNO levels testify to an extensive conversion of nitrite to NO. With deoxyHb-mediated reduction of nitrite being a major NO-producing mechanism, the data reveal the significance of this mechanism, when hemoglobin cycles between full and intermediate oxygen saturations in the arterial-venous circulation. Fish exposed to 0.6 mmol l(-1) nitrite for up to 5 days could be divided into responding (with elevated metHb) and non-responding individuals. Exposure to 2 mmol l(-1) nitrite caused a time-dependent increase in metHb to 59% of total Hb within 2 days. Taking HbNO into account, the functional (potential O2 carrying) Hb was reduced to 29% at this stage. Total blood [Hb] was also significantly decreased. In spite of the reduced blood O2 capacitance, and the possibility that excess NO may inhibit mitochondrial respiration, whole animal routine oxygen consumption was not depressed.

Descriptors: zebrafish gills, nitric oxide metabolism, sodium nitrite exposure and metabolism, drug dose response relationship, hemoglobins metabolism, oxygen consumption, sodium nitrite pharmacology, time factors, water chemistry, zebrafish blood.

Kamkar, M., M.A. Steggles, and C.C. Martin (2006). **Fish pharming: identifying gene function and therapeutic targets using pharmacologic treatment of zebrafish embryos**. *Current PharmacoGenomics* 4(2): 145-152. ISSN: 1570 1603.

Abstract: One of the remaining challenges for the post-genomic era researcher is the systematic assignment of gene function to a sequenced genome. The zebrafish is an effective model organism for conducting comparative analyses of the human genome. The ability to obtain large numbers of zebrafish embryos, grow them in a 96-well dish, and then expose them to molecules dissolved in their water, underline the valuable characteristics of this organism that is currently being

explored by academic and private researchers. This high throughput strategy is being applied to evaluate the specificity and the cellular toxicity of environmental toxins and new drug therapies. Similarly, small molecules, metabolic inhibitors, enzyme antagonist and agonist or receptor/ligand substitutes have been used to enhance our understanding of developmental and physiological pathways and gene cascades. These studies also introduce valuable systems that can be employed to investigate the pathologies that can occur when these pathways are disrupted. Pharmacologics act primarily at the post-translational level, the proteome. These results are further supported by comparative genomic studies using the numerous zebrafish mutants that are available or by targeted gene knockdowns using antisense morpholino oligonucleotides. The conservation of genetic mechanisms across a wide range of phyla ensures that the results obtained from fish can be directly transferred to humans. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, pharmacology model, molecular genetics, development, cardiac morphogenesis, sex differentiation, gene identification.

Kidd, K.R. and B.M. Weinstein (2003). **Fishing for novel angiogenic therapies.** *British Journal of Pharmacology* 140(4): 585-594. ISSN: 0007 1188.

Descriptors: zebrafish, cardiovascular system, transport and circulation, development, angiogenesis model, therapeutic, vascular embryogenesis.

Kohli, G., E. Clelland, and C. Peng (2005). **Potential targets of transforming growth factor-beta1 during inhibition of oocyte maturation in zebrafish.** *Reproductive Biology and Endocrinology* 3: 53. ISSN: 1477-7827.

NAL Call Number: QP1

Abstract: BACKGROUND: TGF-beta is a multifunctional growth factor involved in regulating a variety of cellular activities. Unlike mammals, the function of TGF-beta in the reproduction of lower vertebrates, such as fish, is not clear. Recently, we showed that TGF-beta1 inhibits gonadotropin- and 17alpha, 20beta-dihydroxyprogesterone (DHP)-induced maturation in zebrafish. The aim of the present study was to investigate the mechanisms underlying this action. METHOD: To determine if the effect of TGF-beta1 on oocyte maturation involves transcription and/or translation, ovarian follicles were pre-treated with actinomycin D, a blocker of transcription, and cyclohexamide, an inhibitor of translation, and incubated with hCG or DHP, either alone or in combination with TGF-beta1 and oocyte maturation scored. To determine the effect of TGF-beta1 on mRNA levels of several key effectors of oocyte maturation, three sets of experiments were performed. First, follicles were treated with control medium or TGF-beta1 for 2, 6, 12, and 24 h. Second, follicles were treated with different concentrations of TGF-beta1 (0 to 10 ng/ml) for 18 h. Third, follicles were incubated with hCG in the absence or presence of TGF-beta1 for 18 h. At the end of each experiment, total RNA was extracted and reverse transcribed. PCR using primers specific for 20beta-hydroxysteroid dehydrogenase (20beta-HSD) which is involved in DHP production, follicle stimulating hormone receptor (FSHR), luteinizing hormone receptor (LHR), the two forms of membrane progestin receptor: mPR-alpha and mPR-beta, as well as GAPDH (control), were performed. RESULTS: Treatment with actinomycin D, a blocker of transcription, reduced the inhibitory effect of TGF-beta1 on DHP-induced oocyte maturation, indicating that the inhibitory action of TGF-beta1 is in part due to regulation of gene transcription. Treatment with TGF-beta1 caused a dose and time-dependent decrease in mRNA levels of 20beta-HSD, LHR and mPR-beta in follicles. On the other hand, TGF-beta1 had no effect on mPR-alpha mRNA expression and increased FSHR mRNA levels. Furthermore, hCG upregulated 20beta-HSD, LHR and mPR-beta mRNA levels, but this stimulatory effect was blocked by TGF-beta1. CONCLUSION: These findings suggest that TGF-beta1 acts at multiple sites, including LHR, 20beta-HSD and mPR-beta, to inhibit zebrafish oocyte maturation.

Descriptors: zebrafish oocytes, physiology, transforming growth factor beta physiology, chorionic gonadotropin pharmacology, cortisone reductase biosynthesis, cortodoxone analogs and derivatives , cycloheximide pharmacology, dactinomycin pharmacology, down regulation, gene expression drug effects, hydroxyprogesterones antagonists and inhibitors, oocytes drug effects, ovarian follicle drug effects and physiology, fsh receptors biosynthesis, lh receptors biosynthesis and metabolism, progesterone receptors biosynthesis , transforming growth factor beta1.

Kopeika, J., T. Zhang, and D. Rawson (2006). **Zebrafish embryos (*Danio rerio*) using microinjection.** *Cryo Letters* 27(5): 319-28. ISSN: 0143 2044.
NAL Call Number: QC278.C72
Abstract: Low membrane permeability is one of the major obstacles to the successful cryopreservation of zebrafish embryos. The aim of the present study was to explore if this could be overcome by yolk modification with different cryoprotectants by micro-injection. Initial investigation of two cryoprotectants, methanol and sucrose, was undertaken to determine their suitability for micro-injection supplementation of the yolk mass. Intact zebrafish embryos at 50% epiboly stage were injected with Hanks' solution, 5.2 M methanol or 1.3 M sucrose yielding approximate final concentrations of 2.0 and 0.5 M of the cryoprotectants within the yolk sac respectively. After micro-manipulation, the embryos were cultured at 28 degree C for three days and their survival assessed at the hatching stage. All micro-manipulations performed in the present study resulted in a significant decrease in embryo survival ($P < 0.05$). Embryos micro-injected with methanol or sucrose were also subjected to a cooling procedure. They were placed in 3M methanol + 0.5 M sucrose at room temperature for 30 min and then cooled from 20 degree C to 0 degree C at 2 degree/min, from 0 degree C to -7.5 degree/min at 1 degree/min, seeded at -7.5 degree C and held for 10 min, before cooling at 0.3 degree/min to - 20 degree C or until full crystallization in all embryos. The processes of extra- and intracellular crystallization were studied by cryomicroscopy. The temperature of intracellular crystallization did not differ significantly between control and injected embryos. However, it was found that intracellular crystallization did not always happen instantly after extracellular crystallization.
Descriptors: zebrafish embryos, cryopreservation methods, cryoprotective agents administration, dosage and pharmacology, methanol administration and dosage, sucrose administration and dosage, cryoelectron microscopy, crystallization, embryo physiology and ultrastructure, embryonic development, heart embryology, intracellular fluid chemistry, methanol pharmacology, microinjections, sucrose pharmacology, survival, yolk sac.

Kuster, E. and R. Altenburger (2006). **Comparison of cholin- and carboxylesterase enzyme inhibition and visible effects in the zebra fish embryo bioassay under short-term paraoxon-methyl exposure.** *Biomarkers* 11(4): 341-54. ISSN: 1354 750X.
Abstract: The acute zebra fish embryo test (*Danio rerio* Hamilton-Buchanan, 1822) is an accepted bioassay to assess the toxicity of waste water that may be used for the replacement of testing with adult fish. It is also suggested for chemical hazard characterization and assessment, although only a few groups of substances have yet been studied. Specifically acting substances such as neurotoxic insecticides pose a potentially hazard for non-target fish. To establish whether the proposed zebra fish embryo test protocol and the inhibition of cholinesterases (acetylcholinesterase EC 3.1.1.7, propionylcholinesterase EC 3.1.1.8) and carboxylesterase (EC 3.1.1.1) enzymes can be used in a similar fashion for hazard characterization and risk assessment of chemicals and environmental samples, two types of experiments were conducted. Visual effects of exposure to the organophosphate metabolite paraoxon-methyl after 24 and 48 h in the zebra fish embryo test system were analysed with the use of an inverse microscope (rate of mortality, developmental disturbances, heart rate and others). The inhibition to cholinesterases and carboxy-

lesterase was also measured. Enzyme inhibition as a biomarker of exposure was about 70 times more sensitive than the effects in the zebra fish embryo test with an IC50 below 1.2 micromol compared with an EC50 of 91 micromol. The dose-response relationships showed different curve characteristics with a linear increase of enzyme inhibition compared with a sigmoidal curve for the overt effects. Significant overt effects could only be seen at concentrations at which already 80% of the activities of the different esterases were inhibited.

Descriptors: zebrafish embryos, carboxylic ester hydrolases antagonists and inhibitors, cholinesterase inhibitors pharmacology, metabolism, enzyme inhibitors pharmacology, paraoxon analogs and derivatives, biological assay, biological markers chemistry, drug dose response relationship and effects, inhibitory concentration 50, paraoxon pharmacology and toxicity, time factors.

Lassen, N., T. Estey, R.L. Tanguay, A. Pappa, M.J. Reimers, and V. Vasiliou (2005). **Molecular cloning, baculovirus expression, and tissue distribution of the zebrafish aldehyde dehydrogenase 2.** *Drug Metabolism and Disposition* 33(5): 649-56. ISSN: 0090 9556.

Abstract: Ethanol is metabolized to acetaldehyde mainly by the alcohol dehydrogenase pathway and, to a lesser extent, through microsomal oxidation (CYP2E1) and the catalase-H(2)O(2) system. Acetaldehyde, which is responsible for some of the deleterious effects of ethanol, is further oxidized to acetic acid by aldehyde dehydrogenases (ALDHs), of which mitochondrial ALDH2 is the most efficient. The aim of this study was to evaluate zebrafish (*Danio rerio*) as a model for ethanol metabolism by cloning, expressing, and characterizing the zebrafish ALDH2. The zebrafish ALDH2 cDNA was cloned and found to be 1892 bp in length and encoding a protein of 516 amino acids (M(r) = 56,562), approximately 75% identical to mammalian ALDH2 proteins. Recombinant zebrafish ALDH2 protein was expressed using the baculovirus expression system and purified to homogeneity by affinity chromatography. We found that zebrafish ALDH2 is catalytically active and efficiently oxidizes acetaldehyde (K(m) = 11.5 microM) and propionaldehyde (K(m) = 6.1 microM). Similar kinetic properties were observed with the recombinant human ALDH2 protein, which was expressed and purified using comparable experimental conditions. Western blot analysis revealed that ALDH2 is highly expressed in the heart, skeletal muscle, and brain with moderate expression in liver, eye, and swim bladder of the zebrafish. These results are the first reported on the cloning, expression, and characterization of a zebrafish ALDH, and indicate that zebrafish is a suitable model for studying ethanol metabolism and, therefore, toxicity.

Descriptors: zebrafish, aldehyde dehydrogenase genetics, metabolism and biosynthesis, baculoviridae metabolism, amino acid sequence, Western blotting, brain chemistry, central nervous system depressants metabolism and toxicity, chromosome mapping, molecular cloning, complementary DNA biosynthesis and genetics, polyacrylamide gel electrophoresis, ethanol metabolism and toxicity, kinetics, molecular sequence data, skeletal muscle metabolism, myocardium metabolism, tissue distribution.

Levin, E.D., Z. Bencan, and D.T. Cerutti (2007). **Anxiolytic effects of nicotine in zebrafish.** *Physiology and Behavior* 90(1): 54-8. ISSN: 0031-9384.

NAL Call Number: QP1.P4

Abstract: Anxiolytic effects of nicotine have been documented in studies with rodents and humans. Understanding the neural basis of nicotine-induced anxiolysis can help both with developing better aids for smoking cessation as well as with the potential development of novel nicotinic ligands for treating anxiety. Complementary non-mammalian models may be useful for determining the molecular bases of nicotine effects on neurobehavioral function. The current project examined whether a zebrafish model of anxiety would be sensitive to nicotine. When zebrafish are placed in a novel environment, they dive to the bottom of the tank. In the wild,

diving could help to escape predation. We tested the anxiolytic effect of nicotine on the novelty-elicited diving response and subsequent habituation. Zebrafish placed in a novel tank spent the majority of time at the bottom third of the tank during the first minute of a 5-min session and then show a gradual decrease in time spent at the tank bottom. Nicotine treatment at 100 mg/l for 3 min by immersion before testing caused a significant decrease in diving throughout the session, while 50 mg/l was effective during the first minute when the greatest bottom dwelling was seen in controls. Nicotine effects were reversed by the nicotinic antagonist mecamylamine given together with nicotine, but not when administered shortly before the test session after prior nicotine dosing. This implies that the effect of nicotine on diving was due to net stimulation at nicotinic receptors, an effect that is blocked by mecamylamine; and that once invoked, this effect is no longer dependent on continuing activation of nicotinic receptors. The effect of nicotine on diving did not seem to be the result of a general disorientation of the fish. The 100 mg/ml nicotine dose was shown in our earlier study to significantly improve spatial-discrimination learning in zebrafish. Nicotine-induced anxiolytic effects can be modeled in the zebrafish. This preparation will help in the investigation of the molecular bases of this effect.
Descriptors: zebrafish anxiety drug model, anti anxiety agents pharmacology, anxiety drug therapy, exploratory behavior drug effects, locomotion drug effects, nicotine pharmacology, preclinical drug evaluation methods, reproducibility of results, spatial behavior drug effects, swimming.

Levin, E.D., J. Limpuangthip, T. Rachakonda, and M. Peterson (2006). **Timing of nicotine effects on learning in zebrafish.** *Psychopharmacology* 184(3-4): 547-52. ISSN: 0069-8811.
Abstract: RATIONALE: Nicotine has been shown in many, but not all, studies to improve cognitive function in a number of species including rats, mice, monkeys, and humans. Recently, we have found that nicotine also improves memory in zebrafish. Nicotinic agonists are being developed as novel treatments for Alzheimer's disease and other cognitive impairments. OBJECTIVES: In screening the therapeutic potential of novel nicotinic agonists, it is important to have a rapid assay of cognitive improvement. Zebrafish can help with this effort. METHODS: We have developed a method of rapidly assessing spatial position discrimination learning in zebrafish in one session of seven trials. We used this method to determine the cognitive effects of nicotine. RESULTS: Nicotine (100 mg/l administered during 3 min of immersion) caused a significant improvement in percent correct performance. This dose was within the effective range we found to improve the choice accuracy performance of zebrafish using the more time-intensive delayed spatial alternation procedure. Interestingly, the positive effect of nicotine was seen at 20-40 min postadministration, but not earlier, and declined at 80 and 160 min posttreatment. At the 40-min postdosing interval, 200 mg/l nicotine was also found to significantly improve choice accuracy. Nicotine-induced accuracy improvement was reversed by the nicotinic antagonist mecamylamine given shortly before testing but not when given concurrently with nicotine. CONCLUSIONS: This position discrimination procedure in zebrafish effectively demonstrated the cognitive-enhancing effects of nicotine. This model may be useful in the early screening of novel nicotinic compounds for treatment of cognitive dysfunction.
Descriptors: zebrafish memory model, mental recall drug effects, nicotine pharmacology, orientation drug effects, choice behavior drug effects, discrimination learning drug effects, drug dose response relationship, mecamylamine pharmacology, nicotinic antagonists pharmacology, reaction time drug effects, retention psychology drug effects.

Li, Z.L., H.G. Chen, Y. Xu, and Z.M. Kong (2006). **Toxicological effects of three veterinary drugs and feed additives on fish**. *Journal of Ecology and Rural Environment* 22(1): 84-86. ISSN: 1673 4831.
Abstract: Olaquindox,arsanilic acid, and oxytetracycline are three common kinds of veterinary

drugs and feed additives. Their eco-toxicological effects on zebrafish (*Brachydanio rerio*) and carp (*Cyprinus carpio*) were studied through acute toxicity test,and embryo test of zebrafish, and single kidney cell gel electrophoresis test (SCGE) in vivo of carp. Results show that acute toxicities of the three substances were not significant at concentrations of the ecological sense; olaquindox and arsanilicacid showed a significant teratogenic effect on the embryo, olaquindox and oxytetracycline could bring about obvious great DNA damage to carp kidney cells in vivo and have potential genotoxicity to fish. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, pharmacology , toxicology, cell biology, gel electrophoresis, DNA damage, genotoxicity, toxicological effect.
Language of Text: Chinese.

Lindsay, S.M. and R.G. Vogt (2004). **Behavioral responses of newly hatched zebrafish (*Danio rerio*) to amino acid chemostimulants.** *Chemical Senses* 29(2): 93-100. ISSN: 0379 864X.
NAL Call Number: QP456.C5
Abstract: The zebrafish chemosensory systems of olfaction, taste and solitary chemosensory cells (SCCs) are established during the first week after fertilization (a.f.). These systems presumably support the early development of feeding behaviors required as yolk supplies diminish over the same period. Yet there is no previous data reporting early chemosensory responses in zebrafish. We therefore assayed the chemosensory behavior of newly hatched zebrafish on days 3, 4 and 5 a.f. Responses were compared between fish exposed to water alone versus water containing a mixture of 12 amino acids (100 microM each) flowing through a 50 ml test chamber at 4 ml/min; computer-assisted motion analysis was used to quantify responses. Behavioral responses were first observed at day 4 a.f.; the number of fish swimming, their swimming speeds, and their net-to-gross displacement (NGDR) all increased significantly in response to amino acid stimulation. Because taste buds first appear 4-5 days a.f. and the SCCs may not respond to amino acids, these initial chemosensory responses of day 4 fish may be mediated by already established olfactory neurons. The onset of chemosensitivity in day 4 fish corresponded with an easily recognizable developmental phenotype of inactive floating; day 3 fish were inactive and resting on the bottom while day 5 fish were active and moving through the water column. The ease of identifying responsive day 4 fish suggests these animals may be useful for characterizing odorant sensitivity or developmental plasticity or for screening for chemosensory mutations.
Descriptors: zebrafish, five day old fish, chemosensory responses, amino acids pharmacology, behavior physiology, chemoreceptors physiology and drug effects, motor activity physiology, mutation genetics, neuronal plasticity drug effects, odors, olfactory receptor neurons drug effects and physiology, phenotype, chemical stimulation, swimming physiology, water movements.

Lockwood, B., S. Bjerke, K. Kobayashi, and S. Guo (2004). **Acute effects of alcohol on larval zebrafish: a genetic system for large-scale screening.** *Pharmacology, Biochemistry, and Behavior* 77(3): 647-54. ISSN: 0091-3057.
NAL Call Number: 0091-3057
Abstract: Larval zebrafish are used extensively for developmental genetic studies due to their salient features, such as small size, external development, optical transparency, and accessibility in large numbers. However, their use for the study of drug and alcohol abuse has not been explored. Here we investigated the response of larval zebrafish to acute treatment of alcohol. Our analyses showed that like adults, the larval zebrafish exhibited a dose-dependent locomotor response to ethanol: intermediate doses led to hyperactivity, whereas high doses have a neurodepressive effect resulting in hypoactivity and sedation. Alcohol also induced morpho-

logical changes of melanocytes, providing a visible cellular measure of the biological effects of alcohol in vivo. In addition, alcohol induced thigmotaxis behavior (preference for the edge of a compartment). In the behaviors we analyzed, genetic background influenced the locomotor responses to alcohol. The present study demonstrates that larval zebrafish exert a response to the acute treatment of alcohol, which is genetically modifiable. Therefore, the larval zebrafish represent a tractable vertebrate model system for a large-scale genetic analysis of the biological effects of alcohol.

Descriptors: zebrafish larvae, ethanol administration and dosage, genetic screening methods, drug dose response relationship, locomotion drug effects, locomotion genetics.

McMenamin, S., S. Reinsch, and G. Conway (2003). **Direct comparison of common fixation methods for preservation of microtubules in zebrafish embryos.** *BioTechniques* 34(3): 468-70, 472. ISSN: 0736 6205.

NAL Call Number: RB37.1B56

Descriptors: zebrafish embryos, microtubules drug effects, physiology and pathology, tissue preservation methods, chorion cytology, drug effects and physiology, formaldehyde pharmacology, organ preservation solutions pharmacology, polymers pharmacology, quality control.

Michel, W.C., M.J. Sanderson, J.K. Olson, and D.L. Lipschitz (2003). **Evidence of a novel transduction pathway mediating detection of polyamines by the zebrafish olfactory system.** *Journal of Experimental Biology* 206(Pt 10): 1697-706. ISSN: 0022-0949.

NAL Call Number: 442.8B77

Abstract: To better understand the full extent of the odorant detection capabilities of fish, we investigated the olfactory sensitivity of zebrafish to a monoamine and several polyamines using electrophysiological and activity-dependent labeling techniques. Electro-olfactogram (EOG) recording methods established the relative stimulatory effectiveness of these odorants as: spermine >> spermidine approximately agmatine > glutamine > putrescine >or= cadaverine >or= histamine > artificial freshwater. The detection threshold for the potent polyamines was approximately 1 micromol l(-1). Cross-adaptation experiments suggested that multiple receptors are involved in polyamine detection. Three observations indicated that polyamine signaling may involve a transduction cascade distinct from those used by either amino acids or bile salts. Like bile salts and the adenylate cyclase activator forskolin, but unlike amino acid odorants, polyamines failed to stimulate activity-dependent labeling of olfactory sensory neurons with the cation channel permeant probe agmatine, suggesting a signaling pathway different from that used by amino acid stimuli. Also supporting distinct amino acid and polyamine signaling pathways is the finding that altering phospholipase C activity with the inhibitor U-73122 significantly reduced amino acid-evoked responses, but had little effect on polyamine- (or bile salt-) evoked responses. Altering cyclic nucleotide-mediated signaling by adenylate cyclase activation with forskolin, which significantly reduced responses to bile salts, failed to attenuate polyamine responses, suggesting that polyamines and bile salts do not share a common transduction cascade. Collectively, these findings suggest that polyamines are a new class of olfactory stimuli transduced by a receptor-mediated, second messenger signaling pathway that is distinct from those used by amino acids or bile salts.

Descriptors: zebrafish olfactory system, biogenic polyamines analysis, odors analysis, smell physiology, physiological adaptation, biogenic polyamines pharmacology, electrophysiology, forskolin pharmacology, olfactory receptor neurons drug effects and physiology, second messenger systems, signal transduction, smell drug effects.

Milan, D.J., T.A. Peterson, J.N. Ruskin, R.T. Peterson, and C.A. MacRae (2003). **Drugs that induce repolarization abnormalities cause bradycardia in zebrafish.** *Circulation* 107(10): 1355-8. ISSN: 0009 7322.

NAL Call Number: RC681.A1C8

Abstract: BACKGROUND: Drug-induced QT prolongation and torsades de pointes remain significant and often unpredictable clinical problems. Current in vitro preclinical assays are limited by biological simplicity, and in vivo models suffer from expense and low throughput. METHODS AND RESULTS: During a screen for the effects of 100 small molecules on the heart rate of the zebrafish, *Danio rerio*, we found that drugs that cause QT prolongation in humans consistently caused bradycardia and AV block in the zebrafish. Of 23 such drugs tested, 18 were positive in this initial screen. Poor absorption explained 4 of 5 false-negative results, as demonstrated by microinjection. Overall, 22 of 23 compounds that cause repolarization abnormalities were positive in this assay. Antisense "knockdown" of the zebrafish KCNH2 ortholog yielded bradycardia in a dose dependent manner confirming the effects of reduction of repolarizing potassium current in this model. Classical drug-drug interactions between erythromycin and cisapride, as well as cimetidine and terfenadine, were also reproduced. CONCLUSION: This simple high-throughput assay is a promising addition to the repertoire of preclinical tests for drug-induced repolarization abnormalities. The genetic tractability of the zebrafish will allow the exploration of heritable modifiers of such drug effects.

Descriptors: zebrafish hearts, chemically induced bradycardia , cation transport proteins, heart rate drug effects, potassium channels, voltage gated, arrhythmia chemically induced, bradycardia etiology, bradycardia physiopathology, drug interactions, electric conductivity, ether a go go potassium channels, heart block chemically induced, antisense oligonucleotides pharmacology, potassium channels genetics and physiology.

Morris, J.P.4., S. Berghmans, D. Zahrieh, D.S. Neuberg, J.P. Kanki, and A.T. Look (2003). **Zebrafish sperm cryopreservation with N,N-dimethylacetamide.** *BioTechniques* 35(5): 956-962 . ISSN: 0736 6205.

NAL Call Number: RB37.1B56

Abstract: High fecundity, rapid generation time, and external development of optically clear embryos make the zebrafish (*Danio rerio*) a convenient vertebrate model for genetic, developmental, and disease studies. Efficient sperm cryopreservation enhances the zebrafish model system by optimizing productive use of facility space, extending the reproductive lifetime of males, providing an alternative to live stocks for strain recovery, and ensuring the survival of valuable mutant lines. Here we identify a cryoprotective medium, 10% N,N-dimethylacetamide (DMA) (v/v) diluted in buffered sperm motility-inhibiting solution (BSMIS), as well as parameters for zebrafish sperm cryopreservation that enhance cryopreservation efficiency and significantly increase the yield of live embryos from archived stocks. Our experiments emphasize the effect of the ratio of sperm and medium volume and the use of large egg clutches to maximize the recovery of viable embryos.

Descriptors: zebrafish sperm cryopreservation, acetamides pharmacology, cryopreservation methods, cryoprotective agents pharmacology, semen preservation methods, sperm motility drug effects, spermatozoa drug effects and physiology, cell membrane permeability drug effects, fertility drug effects.

Mukhi, S., L. Torres, and R. Patino (2007). **Effects of larval-juvenile treatment with perchlorate and co-treatment with thyroxine on zebrafish sexratios.** *General and Comparative Endocrinology* 150(3): 486-94. ISSN: 0016-6480.

NAL Call Number: 444.8G28

Abstract: The objective of this study was to determine the effect of larval-juvenile exposure to perchlorate, a thyroid hormone synthesis inhibitor, on the establishment of gonadal sex ratios in zebrafish. Zebrafish were exposed to untreated water or water containing perchlorate at 100 or 250 ppm for a period of 30 days starting at 3 days postfertilization (dpf). Recovery treatments consisted of a combination of perchlorate and exogenous thyroxine (T4; 10 nM). Thyroid histology was assessed at the end of the treatment period (33 dpf), and gonadal histology and sex ratios were determined in fish that were allowed an additional 10-day period of growth in untreated water. As expected, exposure to perchlorate caused changes in thyroid histology consistent with hypothyroidism and these effects were reversed by co-treatment with exogenous T4. Perchlorate did not affect fish survival but co-treatment with T4 induced higher mortality. However, relative to the corresponding perchlorate concentration, co-treatment with T4 caused increased mortality only at a perchlorate concentration of 100 ppm. Perchlorate alone or in the presence of T4 suppressed body length at 43 dpf relative to control values. Perchlorate exposure skewed the sex ratio toward female in a concentration-dependent manner, and co-treatment with T4 not only blocked the feminizing effect of perchlorate but also overcompensated by skewing the sex ratio towards male. Moreover, co-treatment with T4 advanced the onset of spermatogenesis in males. There was no clear association between sex ratios and larval survival or growth. We conclude that endogenous thyroid hormone plays a role in the establishment of gonadal sex phenotype during early development in zebrafish.

Descriptors: zebrafish larvae, juvenile stages, sex ratio statistics, environmental pollutants pharmacology, gonads drug effects, perchloric acid pharmacology, sex differentiation drug effects, thyroxine metabolism, age factors, analysis of variance, drug dose response relationship, drug interactions, environmental pollutants administration and dosage, gonads growth and development, larva drug effects, larva growth and development, perchloric acid administration and dosage, nonparametric statistics, thyroid gland drug effects, growth, development and metabolism, thyroxine administration and dosage.

Murphey, R.D. and L.I. Zon (2006). **Small molecule screening in the zebrafish.** *Methods* 39(3): 255-61. ISSN: 0882-4010.

NAL Call Number: QP519.7.M47

Abstract: The zebrafish is an ideal organism for small molecule studies. The ability to use the whole organism allows complex in vivo phenotypes to be assayed and combines animal testing with screening. Embryos are easily treatable by waterborne exposure. The small size and abundance of embryos make zebrafish suitable for screening in a high-throughput manner in 96- or 48-well plates. Zebrafish embryos have successfully been used in chemical genetic screens to elucidate biological pathways and find chemical suppressors. Small molecules discovered by screening zebrafish disease models may also be useful as lead compounds for drug development as there appears to be a high level of conservation of drug activity between mammals and zebrafish. Here we provide the technical aspects of treating embryos with small molecules and performing chemical screens with zebrafish.

Descriptors: zebrafish embryology, preclinical drug evaluation methods and instrumentation, drug effects, genotype, toxicity tests instrumentation and methods, .

Ou, H.C., D.W. Raible, and E.W. Rubel (2007). **Cisplatin-induced hair cell loss in zebrafish (*Danio rerio*) lateral line.** *Hearing Research* 233(1-2): 46-53. ISSN: 0378-5955.

Abstract: We have used time-lapse imaging to study cisplatin-induced hair cell death in lateral line neuromasts of zebrafish larvae in vivo. We found that cisplatin-induced hair cell

death occurred much more slowly than had been shown to occur in aminoglycoside-induced hair cell death. By prelabeling hair cells with FM1-43FX, and assessing hair cell damage, it was established that cisplatin causes hair cell loss in the lateral line in a dose-dependent fashion. The kinetics of hair cell loss during exposure to different concentrations of cisplatin was also assessed and it was found that the onset of hair cell loss correlated with the accumulated dose of cisplatin. These data demonstrate the feasibility and repeatability of cisplatin damage protocols in the zebrafish lateral line and set the stage for future evaluations of modulation of cisplatin-induced hair cell death.

Descriptors: zebrafish growth and development, cisplatin pharmacology, hair cells, auditory drug effects, lateral line system drug effects, lateral line system pathology, cell death, cisplatin administration and dosage , drug dose response relationship, feasibility studies, kinetics, larva drug effects, lateral line system physiopathology, reproducibility of results, time factors.

Parker, B. and V.P. Connaughton (2007). **Effects of nicotine on growth and development in larval zebrafish.** *Zebrafish* 4(1): 59-68. ISSN: 1545-8547.

Abstract: In this study, larval zebrafish (*Danio rerio*) were chronically exposed to nicotine concentrations of 5, 10, or 20 mg/L (corresponding to 10, 20, or 40muM) beginning at 1 day postfertilization. Larval growth, morphology, and behavior were examined at day 10. Results indicate that nicotine exposure significantly reduces notochord length and eye diameter (growth), adversely affects the startle responses (behavior), and decreases survival. These findings parallel results on the deleterious effects of nicotine on development in other animals and indicate that zebrafish may be a good model to further study factors affecting vertebrate development.

Descriptors: zebrafish larva, nicotine, larval growth, morphology, behavior, notochord length, eye diameter, behavior.

Parng, C., N. Anderson, C. Ton, and P. McGrath (2004). **Zebrafish apoptosis assays for drug discovery.** *Methods in Cell Biology* 76: 75-85. ISSN: 0091-679X.

NAL Call Number: 442P92

Descriptors: zebrafish embryology, apoptosis drug effects, preclinical drug evaluation methods, acridine orange analysis and chemistry, apoptosis genetics and physiology, caspases antagonists and inhibitors and metabolism, cysteine proteinase inhibitors pharmacology, drug dose response relationship, fluorescent dyes metabolism, in situ nick end labeling methods, fluorescence microscopy, neomycin pharmacology, fluorescence spectrometry, sTaurosporine pharmacology.

Peitsaro, N., M. Sundvik, O.V. Anichtchik, J. Kaslin, and P. Panula (2007). **Identification of zebrafish histamine H1, H2 and H3 receptors and effects of histaminergic ligands on behavior.** *Biochemical Pharmacology* 73(8): 1205-14. ISSN: 0006 2952.

NAL Call Number: 396.8 B52

Abstract: Neuronal histamine regulates several functions in the vertebrate brain. The zebrafish brain contains a widespread histaminergic system and H(3) receptor ligand binding has been reported. In this study we provide evidence for the existence of histamine H(1), H(2) and H(3) receptor genes in zebrafish. Single copies of putative histamine H(1), H(2) and H(3) receptors were identified and cloned from the zebrafish brain. Expression analysis suggested that they are expressed in the brain and a few other tissues. Widespread distribution of zebrafish H(2) receptor binding sites was detected with [(125)I]iodoaminopotentidine in brain sections. Zebrafish larvae were exposed to 1, 10 or 100 microM of the H(1) ligand pyrilamine, the H(2) ligand cimetidine and the H(3) ligands thioperamide and

immepip for 5 days. Significant decreases in swimming distance were observed with the highest dose of all ligands, whereas cimetidine gave a significant decrease also with 1 and 10 microM doses. These results provide the first molecular biological evidence for the presence of histamine receptors in zebrafish. These histamine receptors resemble those of higher vertebrates and they provide a useful model for pharmacological and behavioral studies for characterizing the functions of histamine in more detail.
Descriptors: zebrafish histamines receptors, behavior drug effects, histamine agonists pharmacology, histamine antagonists pharmacology, histamine receptors H1, H2 and H3 isolation and purification, behavior physiology, cimetidine pharmacology, imidazoles pharmacology, piperidines pharmacology.

Peterson, R.T. and M.C. Fishman (2004). **Discovery and use of small molecules for probing biological processes in zebrafish.** *Methods in Cell Biology* 76: 569-91. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: preclinical drug evaluation methods, drug effects, organic chemicals pharmacology, chromatography, affinity methods, combinatorial chemistry techniques, gene expression profiling, developmental gene expression regulation, morphogenesis, mutation, organic chemicals metabolism, phenotype, PCR.

Pisharath, H. (2007). **Validation of nitroreductase, a prodrug-activating enzyme, mediated cell death in embryonic zebrafish (*Danio rerio*).** *Comparative Medicine* 57(3): 241-6. ISSN: 1532 0820.
NAL Call Number: SF77.C65
Abstract: 1Escherichia coli gene nfsB encodes a nitroreductase (NTR) enzyme that converts prodrugs like metronidazole (Met) and CB 1954 to cytotoxic metabolites. My coworkers and I have validated this prodrug-enzyme system in zebrafish (*Danio rerio*) by ubiquitously expressing NTR-EGFP fusion protein and exposing these embyos to CB 1954 and Met. These embryos showed extensive gross pathologic changes and death by 24 h of incubation in the prodrugs. They also exhibited widespread and marked apoptotic changes by 8 h of incubation in Met. Neither the prodrugs themselves nor the NTR-EGFP fusion protein were toxic to the developing zebrafish embryos. Thus the NTR-CB 1954 and NTR-Met systems can be used to ablate a wide variety of cells and tissues in zebrafish embryos.
Descriptors: zebrafish, pathology, enzymologic gene expression regulation drug effects, green fluorescent nitroreductases genetics, prodrugs pharmacology, aziridines pharmacology, cell death, molecular cloning, enzymology, gene transfer techniques, in situ nick end labeling, metronidazole pharmacology, confocal microscopy, genetically modified, PCR, recombinant fusion proteins genetics.

Prasch, A.L., R.L. Tanguay, V. Mehta, W. Heideman, and R.E. Peterson (2006). **Identification of zebrafish ARNT1 homologs: 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity in the developing zebrafish requires ARNT1.** *Molecular Pharmacology* 69(3): 776-87. ISSN: 0026-895X.
NAL Call Number: QP901.M6
Abstract: To use the zebrafish (*Danio rerio*) as a model to study 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) developmental toxicity, it is essential to know which proteins are involved in mediating toxicity. Previous work has identified zfAHR2 as the receptor that binds TCDD mediating downstream responses. Although zfARNT2b can form a functional heterodimer with zfAHR2 in vitro, zfarnt2 null mutants show no protection against endpoints of TCDD developmental toxicity, demonstrating that zfARNT2b cannot be the physiological dimeriza-

tion partner for zfAHR2 mediating responses to TCDD in zebrafish embryos. The purpose of the current study was to identify an alternate dimerization partner(s) for zfAHR2 that may function to mediate TCDD developmental toxicity. By searching zebrafish genomic sequence and using the polymerase chain reaction-based rapid amplification of cDNA ends technique, three forms of cDNA that seem to be alternate mRNA splice variants of a zebrafish homolog of ARNT1 were detected. Analysis of the zfARNT1 proteins in vitro demonstrates that the two longest forms of zfARNT1, zfARNT1b and zfARNT1c, can form functional heterodimers with zfAHR2. However, the shortest form, zfARNT1a, seems to be nonfunctional with zfAHR2 in vitro. To determine whether a zfARNT1 protein functions with zfAHR2 in vivo, a morpholino targeted against the 5' end of zfARNT1 (zfarnt1-MO) was used. Injection of the zfarnt1-MO before TCDD treatment significantly decreases the induction of zfCYP1A mRNA and protein. In addition, zfarnt1 morphants show complete protection against TCDD-induced pericardial edema and show partial protection against reduced blood flow and craniofacial malformations caused by TCDD, demonstrating the role of zfARNT1 proteins in mediating these responses.

Descriptors: zebrafish embryos, aryl hydrocarbon receptor nuclear translocator metabolism, classification and genetics, drug resistance genetics, tetrachlorodibenzodioxin toxicity, proteins metabolism, amino acid sequence, COS cells, *Cercopithecus aethiops*, complementary DNA genetics, drug effects, embryology, molecular sequence data, phylogeny, RNA splicing, aryl hydrocarbon receptors metabolism, trans activators classification, genetics and metabolism, proteins classification and genetics.

Ruuskanen, J.O., J. Laurila, H. Xhaard, V.V. Rantanen, K. Vuoriluoto, S. Wurster, A. Marjamaki, M. Vainio, M.S. Johnson, and M. Scheinin (2005). **Conserved structural, pharmacological and functional properties among the three human and five zebrafish alpha 2-adrenoceptors.** *British Journal of Pharmacology* 144(2): 165-77. ISSN: 0007 1188.

Abstract: 1. Zebrafish has five distinct alpha(2)-adrenoceptors. Two of these, alpha(2Da) and alpha(2Db), represent a duplicated, fourth alpha(2)-adrenoceptor subtype, while the others are orthologue of the human alpha(2A)-, alpha(2B)- and alpha(2C)-adrenoceptors. Here, we have compared the pharmacological properties of these receptors to infer structural determinants of ligand interactions. 2. The zebrafish alpha(2)-adrenoceptors were expressed in Chinese hamster ovary cells and tested in competitive ligand binding assays and in a functional assay (agonist-stimulated [(35)S]GTPgammaS binding). The affinity results were used to cluster the receptors and, separately, the ligands using both principal component analysis and binary trees. 3. The overall ligand binding characteristics, the order of potency and efficacy of the tested agonists and the G-protein coupling of the zebrafish and human alpha(2)-adrenoceptors, separated by approximately 350 million years of evolution, were found to be highly conserved. The binding affinities of the 20 tested ligands towards the zebrafish alpha(2)-adrenoceptors are generally comparable to those of their human counterparts, with a few compounds showing up to 40-fold affinity differences. 4. The alpha(2A) orthologues and the zebrafish alpha(2D) duplicates clustered as close pairs, but the relationships between the orthologues of alpha(2B) and alpha(2C) were not clearly defined. Applied to the ligands, our clustering methods segregated the ligands based on their chemical structures and functional properties. As the ligand binding pockets formed by the transmembrane helices show only minor differences among the alpha(2)-adrenoceptors, we suggest that the second extracellular loop--where significant sequence variability is located --might contribute significantly to the observed affinity differences.

Descriptors: zebrafish, conserved sequence, adrenoceptor alpha 2 chemistry, metabolism

and classification, cho cells, *Cricetinae*, protein binding physiology, secondary protein structure physiology.

Ruuskanen, J.O., N. Peitsaro, J.V. Kaslin, P. Panula, and M. Scheinin (2005). **Expression and function of alpha-adrenoceptors in zebrafish: drug effects, mRNA and receptor distributions.** *Journal of Neurochemistry* 94(6): 1559-69. ISSN: 0022-3042.
NAL Call Number: QP351.J6
Abstract: The alpha2-adrenoceptors are G-protein-coupled receptors that mediate many of the physiological effects of norepinephrine and epinephrine. Mammals have three subtypes of alpha2-adrenoceptors, alpha2A, alpha2B and alpha2C. Zebrafish, a teleost fish used widely as a model organism, has five distinct alpha2-adrenoceptor genes. The zebrafish has emerged as a powerful tool to study development and genetics, with many mutations causing diseases reminiscent of human diseases. Three of the zebrafish adra2 genes code for orthologues of the mammalian alpha2-adrenoceptors, while two genes code for alpha2Da- and alpha2Db- adrenoceptors, representing a duplicated, fourth alpha2-adrenoceptor subtype. The three different mammalian alpha2-adrenoceptor subtypes have distinct expression patterns in different organs and tissues, and mediate different physiological functions. The zebrafish alpha2-adrenergic system, with five different alpha2-adrenoceptors, appears more complicated. In order to deduce the physiological functions of the zebrafish alpha2-adrenoceptors, we localized the expression of the five different alpha2-adrenoceptor subtypes using RT-PCR, mRNA in situ hybridization, and receptor autoradiography using the radiolabelled alpha2-adrenoceptor antagonist [ethyl-3H]RS-79948-197. Localization of the alpha2A-, alpha2B- and alpha2C-adrenoceptors in zebrafish shows marked conservation when compared with mammals. The zebrafish alpha2A, alpha2Da, and alpha2Db each partially follow the distribution pattern of the mammalian alpha2A: a possible indication of subfunction partitioning between these subtypes. The alpha2-adrenergic system is functional in zebrafish also in vivo, as demonstrated by marked locomotor inhibition, similarly to mammals, and lightening of skin colour induced by the specific alpha2-adrenoceptor agonist, dexmedetomidine. Both effects were antagonized by the specific alpha2-adrenoceptor antagonist atipamezole.
Descriptors: zebrafish, brain metabolism , catecholamines metabolism, messenger RNA metabolism, adrenoceptor alpha 2 metabolism, adrenergic alpha agonists pharmacology, adrenergic alpha antagonists pharmacology, binding, competitive physiology, brain anatomy and histology, isoquinolines metabolism, mammals anatomy and histology , motor activity drug effects and physiology, naphthyridines metabolism, pigmentation drug effects and physiology, messenger RNAdrug effects, radioligand assay, adrenoceptor alpha 2 drug effects and genetics, viscera metabolism, zebrafish anatomy and histology.

Satora, L. and J. Morawska (2003). **Szczepionka, metadon i "dzieci slonca". [Vaccine, methadone and zebrafish]**. *Przeglad Lekarski* 60(4): 311-3. ISSN: 0033-2240.
Abstract: Substances of abuse have been known since antiquity till now. Current trends in neurobiological and genetical study related to drug dependency were presented in the study. The trial of preparation a cocaine vaccine and the new hope for dependent people coming from investigation of Zebrafish were also described.
Descriptors: zebrafish, cocaine related disorders prevention and control, methadone pharmacology, narcotics pharmacology, vaccines administration and dosage, disease models.
Language of Text: Polish.

Seng, W.L., K. Eng, J. Lee, and P. McGrath (2004). **Use of a monoclonal antibody specific for activated endothelial cells to quantitate angiogenesis in vivo in zebrafish after drug treatment.** *Angiogenesis* 7(3): 243-53. ISSN: 0969 6970.

NAL Call Number: QP106.6.A54

Abstract: We have recently generated a monoclonal antibody (mAb), Phy-V002, which specifically labels activated vascular endothelial cells (EC) in zebrafish. Here, we show that this mAb labels activated EC in newly formed vessels in vivo without staining mature vessels or other tissues. Using this mAb, drug effects on in vivo EC migration and vessel formation were visually assessed by whole-mount immunochemical staining in the transparent embryo. In addition, we have developed a quantitative microplate-based ELISA that measures EC proliferation in vivo after drug treatment. We have validated the quantitative in vivo ELISA using several antiangiogenic small molecules with different mechanisms of action which were added directly to the fish water. Some of these drugs, including: 2-methoxyestradiol, flavopiridol, paclitaxel, and genistein, are currently in clinical trials. We also injected large molecule drugs, including 3TSR and TSR2+KRFK, recombinant human antiangiogenic peptides of thrombospondin-1, a natural protein. To demonstrate that proangiogenic effects can also be assessed in zebrafish, we assessed effects of penicillamine and simvastatin, two proangiogenic compounds shown to stimulate vessel formation in rodents. Using whole-mount immunochemical staining with Phy-V002, inhibition of EC migration and inhibition or stimulation of vessel formation were visually observed for each compound. Next, using the quantitative in vivo angiogenesis ELISA, we generated dose-response curves for each compound. Compared to conventional assays, advantages of using zebrafish to assess drug effects on angiogenesis include: (1) a short assay time; (2) easy animal maintenance; (3) use of small quantities of drug; (4) single dosing; (5) a quantitative assay format; and (6) use of statistically significant number of animals per test.

Descriptors: zebrafish angiogenesis, angiogenesis inducing agents pharmacology and inhibitors pharmacology, monoclonal antibodies pharmacology, vascular endothelium drug effects, zebrafish immunology, monoclonal antibodies immunology, blood vessels drug effects, blood vessels immunology, cell movement drug effects, cell proliferation drug effects, drug dose response relationship, preclinical drug evaluation methods , immunology, endothelial cells drug effects and immunology, vascular endothelium cytology and immunology, ELISA, physiologic neovascularization drug effects, penicillamine pharmacology, simvastatin pharmacology, staining and labeling methods.

Snaar Jagalska, B.E., S.F. Krens, I. Robina, L.X. Wang, and H.P. Spaink (2003). **Specific activation of ERK pathways by chitin oligosaccharides in embryonic zebrafish cell lines.** *Glycobiology* 13(10): 725-32. ISSN: 0959-6658.

NAL Call Number: QP552.G59G593

Abstract: Chitin oligosaccharides (COs) play a role in plant development and are presumed to affect body plan formation during vertebrate embryogenesis. The mechanisms of COs recognition and cellular processes underlying embryonic development are still not understood. We analyze the possible link with the mitogen-activated protein kinase pathway that is conserved in evolution through the plant and animal kingdom and has been implicated in diverse cellular processes, including cell growth, proliferation, differentiation, survival, and vertebrate development. We show that in vivo stimulation of embryonic zebrafish cells ZF13 and ZF29 with chitin tetrasaccharides at 10-9 M concentration transiently induced activation/phosphorylation of extracellular regulated kinases (ERKs), with a maximum after 15 min. Furthermore the biological specificity of chitin tetrasaccharides and various derivatives was examined. The replacement of one or two GlcNAc residues of the chitin backbone

by glucose and fucosylation of chitin tetrasaccharides at the reducing terminus caused a complete loss of their activity. We also tested a chitin tetrasaccharide analogue in which the oxygen atoms in glycosidic linkages were replaced by sulfur atoms. This analog, which could not be enzymatically hydrolyzed, was as potent an inducer as chitin tetrasaccharide. These results suggest that the observed activation of ERKs is chitin tetrasaccharide-specific and does not require further enzymatic processing. We examined possible signaling pathways leading to ERK activation by COs by use of phosphospecific antibodies and inhibitors. We conclude that a high-affinity CO receptor system exists that links to the Raf, MEK, and ERK pathway in zebrafish cells.

Descriptors: zebrafish embryo cell lines, chitin pharmacology, MAP kinase signaling system drug effects, mitogen activated protein kinases metabolism, carbohydrate sequence, cell line, cytology, drug effects, enzymology, enzyme activation drug effects, molecular sequence data, molecular structure.

Sun, S.N., Y.H. Gui, H.Y. Song, T. Zhong, Y.X. Wang, and Q. Jiang (2007). **Folic acid antagonist methotrexate causes the development malformation of heart and down-regulates the BMP2b and HAS2 expressions in zebrafish**. *Chinese Journal of Contemporary Pediatrics* 9(2): 159-63. ISSN: 1008-8830.

Abstract: OBJECTIVE: To study the effect of methotrexate (MTX), a folic acid antagonist which can lead to folic acid deficient, on the cardiac development and on the expressions of BMP2b and HAS2 in zebrafish. METHODS: The zebrafish embryos at 6-48 hrs post fertilization (hpf) were treated with various concentrations of MTX (0.5 x 10(-3), 1.0 x 10(-3) and 2.0 x 10(-3) M). At 48 hpf, the percentage of cardiac malformation and heart rate were recorded. The zebrafish embryos at 6-10 hpf treated with 1.5 x 10(-3) M MTX were used as the MTX treatment group. At 24 and 48 hpf the cardiac morphology was observed under a microscope. The expressions of BMP2b and HAS2 in zebrafish were detected by in situ antisense RNA hybridization and real-time PCR. RESULTS: 6-12 hpf, the early embryonic developmental stage, was a sensitive period that MTX affected cardiac formation of zebrafish. The retardant cardiac development and the evidently abnormal cardiac morphology was found in the MTX treatment group. The results of in situ antisense RNA hybridization showed that the expressions of BMP2b and HAS2 in the zebrafish heart were reduced in the MTX treatment group at 36 and 48 hpf. The real-time PCR results demonstrated that the BMP2b expression decreased at 12, 24, 36 and 48 hpf, and that the HAS2 expression decreased at 24, 36 and 48 hpf in the treatment group compared with the control group without MTX treatment. CONCLUSIONS: The inhibition of folic acid function may affect cardiac development of early embryos, resulting in a retardant development and a morphological abnormality of the heart in zebrafish, possibly by down-regulating the expressions of BMP2b and HAS2.

Descriptors: zebrafish, drug induced abnormalities etiology, bone morphogenetic folic acid antagonists toxicity, gene expression regulation drug effects, glucuronosyltransferase genetics, chemically induced congenital heart defects , methotrexate toxicity , down regulation, PCR.

Language of Text: Chinese.

Tamaru, Y., T. Wakasa, S. Akiyama, and T. Tanaka (2005). **Transcriptome analysis on an antidepressant drug, imipramine by zebrafish DNA microarray**. *Journal of Pharmacological Sciences* 97(Suppl. 1): 76P. ISSN: 1347 8613.

Descriptors: zebrafish, pharmacology, development, molecular genetics, transcriptome analysis, genetic techniques, DNA microarray, genetic techniques, embryogenesis, imipramine, antidepressant drugs.

Teraoka, H., S. Urakawa, S. Nanba, Y. Nagai, W. Dong, T. Imagawa, R.L. Tanguay, K. Svoboda, H.M. Handley Goldstone, J.J. Stegeman, and T. Hiraga (2006). **Muscular contractions in the zebrafish embryo are necessary to reveal thiuram-induced notochord distortions.** *Toxicology and Applied Pharmacology* 212(1): 24-34. ISSN: 0041-008X.

NAL Call Number: 391.8T662

Abstract: Dithiocarbamates form a large group of chemicals that have numerous uses in agriculture and medicine. It has been reported that dithiocarbamates, including thiuram (tetramethylthiuram disulfide), cause wavy distortions of the notochord in zebrafish and other fish embryos. In the present study, we investigated the mechanism underlying the toxicity of thiuram in zebrafish embryos. When embryos were exposed to thiuram (2-1000 nM: 0.48-240 microg/L) from 3 h post fertilization (hpf) (30% epiboly) until 24 hpf (Prim-5), all embryos develop wavy notochords, disorganized somites, and have shortened yolk sac extensions. The thiuram response was specific and did not cause growth retardation or mortality at 24 hpf. The thiuram-dependent responses showed the same concentration dependence with a waterborne EC50 values of approximately 7 nM. Morphometric measurements revealed that thiuram does not affect the rate of notochord lengthening. However, the rate of overall body lengthening was significantly reduced in thiuram-exposed animals. Other dithiocarbamates, such as ziram, caused similar malformations to thiuram. While expression of genes involved in somitogenesis was not affected, the levels of notochord-specific transcripts were altered after the onset of malformations. Distortion of the notochord started precisely at 18 hpf, which is concomitant with onset of spontaneous rhythmic trunk contractions. Abolishment of spontaneous contractions using tricaine, alpha-bungarotoxin, and a paralytic mutant sofa potato, resulted in normal notochord morphology in the presence of thiuram. These results indicate that muscle activity is necessary to reveal the underlying functional deficit and suggest that the developmental target of dithiocarbamates impairs trunk plasticity through an unknown mechanism.

Descriptors: zebrafish, physiology, fungicides, industrial toxicity, skeletal muscle physiology, notochord abnormalities, thiram toxicity, cHeLating agents pharmacology, collagen metabolism, drug dose response relationship, drug effects, in situ hybridization, laminin metabolism, muscle contraction drug effects and physiology, skeletal muscle abnormalities, thiocarbamates toxicity.

Tokumoto, T., M. Tokumoto, and Y. Nagahama (2005). **Induction and inhibition of oocyte maturation by EDCs in zebrafish.** *Reproductive Biology and Endocrinology* 3: 69. ISSN: 1477-7827.

NAL Call Number: QP1

Abstract: BACKGROUND: Oocyte maturation in lower vertebrates is triggered by maturation-inducing hormone (MIH), which acts on unidentified receptors on the oocyte surface and induces the activation of maturation-promoting factor (MPF) in the oocyte cytoplasm. We previously described the induction of oocyte maturation in fish by an endocrine-disrupting chemical (EDC), diethylstilbestrol (DES), a nonsteroidal estrogen. METHODS: In this study, stimulatory and inhibitory effects of EDCs and natural steroids on oocyte maturation were examined in zebrafish. For effective agents, some details about the mechanism in induction or inhibition of maturation were examined. Possible groups of DES interacting with the MIH receptor are discussed based on relative potency of steroids to induce maturation. RESULTS: Among agents tested, tamoxifen (TAM) and its metabolite 4-hydroxytamoxifen (4-OHT) showed stimulatory activity similar to DES. The time courses of the change in germinal vesicle breakdown and an intracellular molecular event (the synthesis of cyclin B) induced by TAM were indistinguishable from those induced by MIH. In contrast, pen-

tachlorophenol (PCP) had a potent inhibitory effect on MIH-induced oocyte maturation. PCP inhibited not only MIH-induced maturation but also DES- and TAM-induced maturation. Methoxychlor also inhibited maturation when oocytes were pre-treated with this agent. CONCLUSION: These results suggest that EDCs act as agonists or antagonists in the induction of oocyte maturation in fish.

Descriptors: zebrafish, endocrine disruptors pharmacology and chemistry, oocytes drug effects, diethylstilbestrol chemistry and pharmacology, oocytes cytology, growth and development, pentachlorophenol chemistry and pharmacology, steroid receptors metabolism, steroids chemistry and pharmacology , tamoxifen chemistry and pharmacology.

Wang, N., L. Mackenzie, A.G. De Souza, H. Zhong, G. Goss, and L. Li (2007). **Proteome profile of cytosolic component of zebrafish liver generated by LC-ESI MS/MS combined with trypsin digestion and microwave-assisted acid hydrolysis.** *Journal of Proteome Research* 6(1): 263-72. ISSN: 1535-3893.

NAL Call Number: QD431.A1

Abstract: The zebrafish genome has recently been sequenced and annotated allowing for high-throughput proteomic analysis. Here, we report for the first time a proteomic subset of zebrafish liver, an important organ for metabolizing toxins. Using a newly developed analytical procedure, we have identified 1204 proteins from the cytosolic component of a zebrafish liver tissue sample. Our methods involve cell-compartment fractionation of liver tissue samples, four levels of protein digestion, and off-line two-dimensional liquid chromatography (2-D LC) separations of resultant peptides. Proteins are identified using an electrospray ionization quadrupole time-of-flight tandem mass spectrometer (ESI-QTOF MS/MS), which provides high-resolution and high-accuracy mass measurement of peptide ions and their fragment ions. We demonstrate that greater proteome coverage can be achieved by combining the results obtained from four methods of protein digestion: three tryptic digests (one in buffer, one in methanol, and another in SDS), and a microwave-assisted acid hydrolysate of the protein extracts. Identified proteins--which included several groups of established protein biomarkers--were functionally classified. We discuss the functions and implications of these biomarkers within the context of zebrafish toxicology.

Descriptors: zebrafish, cytosol metabolism, liver metabolism, microwaves, proteomics methods, trypsin pharmacology, ion exchange chromatography, hydrolysis, peptide mapping methods, proteome, electrospray ionization mass spectrometry, subcellular fractions, time factors.

Wang, W., X. Liu, D. Gelinas, B. Ciruna, and Y. Sun (2007). **A fully automated robotic system for microinjection of zebrafish embryos.** *Public Library of Science: One* 2(9): E862. ISSN: 0739 5175.

Abstract: As an important embodiment of biomanipulation, injection of foreign materials (e.g., DNA, RNAi, sperm, protein, and drug compounds) into individual cells has significant implications in genetics, transgenics, assisted reproduction, and drug discovery. This paper presents a microrobotic system for fully automated zebrafish embryo injection, which overcomes the problems inherent in manual operation, such as human fatigue and large variations in success rates due to poor reproducibility. Based on computer vision and motion control, the microrobotic system performs injection at a speed of 15 zebrafish embryos (chorion unremoved) per minute, with a survival rate of 98% (n = 350 embryos), a success rate of 99% (n = 350 embryos), and a phenotypic rate of 98.5% (n = 210 embryos). The sample immobilization technique and microrobotic control method are applicable to other biological injection applications such as the injection of mouse oocytes/embryos and *Droso-*

phila embryos to enable high-throughput biological and pharmaceutical research.
Descriptors: zebrafish embryos, robotic system, microinjection.

Wang, W.D., Y. Wang, H.J. Wen, D.R. Buhler, and C.H. Hu (2004). **Phenylthiourea as a weak activator of aryl hydrocarbon receptor inhibiting 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced CYP1A1 transcription in zebrafish embryo.** *Biochemical Pharmacology* 68(1): 63-71. ISSN: 0006 2952.
NAL Call Number: 396.8 B52
Abstract: The aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor that can be activated by a diverse synthetic and naturally-occurring chemicals, such as the halogenated aromatic hydrocarbons (HAHs) and the non-halogenated polycyclic aromatic hydrocarbons (PAHs). The liganded AHR modulates the genetic activity of a variety of xenobiotic-responsive genes, including cytochrome P4501A1 (CYP1A1). The tyrosinase inhibitor 1-phenyl-2-thiourea (PTU) is widely used in zebrafish research to suppress pigmentation in developing embryos/fry. Here we showed that 0.2 mM PTU induced a basal level of CYP1A1 transcription in zebrafish embryonic integument as early as 24 h postfertilization (hpf) stage. Subsequently, PTU induced CYP1A1 transcription in blood vessels at 36 hpf. During larval stage, the liver and all pharyngeal arch vessels of PTU-treated embryos exhibited CYP1A1 transcription as well. Comparing to TCDD, PTU induces CYP1A1 transcription with much lower efficacy in zebrafish embryos. Coincubating the embryos with PTU and TCDD led to repressing TCDD-induced CYP1A1 transcription. Mechanistic studies indicated that both of PTU- and TCDD-mediated CYP1A1 transcriptions are modulated by the same AHR-ARNT signaling pathway.
Descriptors: zebrafish, cytochrome P450 CYPA1A1 metabolism, phenylthiourea pharmacology, aryl hydrocarbon receptors metabolism, genetic transcription drug effects, cytochrome P450 CYPA1A1 genetics, drug interactions, embryo drug effects, enzymology and metabolism, gene expression regulation drug effects, messenger RNAdrug effects and metabolism, signal transduction drug effects, signal transduction physiology, tetrachlorodibenzodioxin pharmacology.

Wang, Y. and W. Ge (2003). **Gonadotropin regulation of follistatin expression in the cultured ovarian follicle cells of zebrafish, *Danio rerio.*** *General and Comparative Endocrinology* 134(3): 308-15. ISSN: 0016-6480.
NAL Call Number: 444.8G28
Abstract: Follistatin is a single-chain glycoprotein initially identified in the mammalian ovary. As a specific binding protein of activin, it effectively modifies the paracrine/autocrine roles of activin in a variety of tissues including the ovary. In the zebrafish, we have demonstrated that the human chorionic gonadotropin (hCG)-induced oocyte maturation and oocyte maturational competence can be blocked by follistatin, suggesting a role for ovarian activin in the signaling pathway of gonadotropin in the zebrafish ovary. The up-regulation of zebrafish ovarian activin betaA subunit by gonadotropin further supports this hypothesis. Since follistatin has extremely high affinity for activin, its expression level in various tissues is critical in fine-tuning local activin activities. In the present study, we investigated the regulation of follistatin expression by gonadotropin in a primary culture of zebrafish ovarian follicle cells using semi-quantitative RT-PCR. Both hCG and goldfish pituitary extract strongly increased the expression of follistatin in the cultured follicle cells in clear time- and dose-dependant manners. The effect of hCG (15IU/ml) reached the maximal level at 2h of treatment and longer treatment (4-8h) led to decreased response. The up-regulation of follistatin expression by hCG could be mimicked by all the drugs that increase the intracellular

cAMP level including 8-Br-cAMP, db-cAMP, forskolin, and 3-isobutyl-1-methylxanthine (IBMX). The hCG (15IU/ml)- and forskolin (10microM)-induced follistatin expression could be blocked by H89 (10microM), a specific protein kinase A (PKA) inhibitor. These results strongly suggest that the regulation of follistatin expression in the zebrafish ovary by gonadotropin is primarily mediated by cAMP-PKA signaling pathway. The up-regulation of follistatin mRNA by gonadotropin in cultured zebrafish ovarian follicle cells, together with our previous studies on gonadotropin regulation of activin beta subunits, suggests that the ovarian activin-follistatin system is tightly controlled by gonadotropin in fish ovary.
Descriptors: zebrafish ovarian follicle cells, follistatin biosynthesis, gene expression regulation, gonadotropins pharmacology, ovarian follicle cytology and physiology, activins pharmacology, cyclic AMP analysis and dependent protein kinases pharmacology, reverse transcriptase PCR, up regulation.

Wentworth, J.N., R. Buzzeo, and R.S. Pollenz (2004). **Functional characterization of aryl hydrocarbon receptor (zfAHR2) localization and degradation in zebrafish (*Danio rerio*).** *Biochemical Pharmacology* 67(7): 1363-72. ISSN: 0006 2952.
NAL Call Number: 396.8 B52
Abstract: The basic-helix-loop-helix/PAS (bHLH/PAS) family of proteins is a group of transcription factors that regulate key pathways during normal development and in the response to stress. The aryl hydrocarbon receptor (AHR) is a member of this family. Recently, *Danio rerio* (zebrafish) has become an important model system in the study of the signal transduction pathway and complements the results seen in mammalian models. However, studies of the AHR protein have been limited by the lack of antibody reagents and thus, little is known concerning the localization and degradation of the zebrafish AHR (zfAHR). In this report, we describe the production and characterization of specific polyclonal antibodies to the zfAHR2 protein and the analysis of AHR-mediated signal transduction in the zebrafish liver cell line (ZFL). The results show that the zfAHR2 is degraded via the 26S proteasome following exposure of cells to beta-naphthoflavone (BNF). Interestingly, the time course is slower and the magnitude of zfAHR2 degradation is not as great as seen for the mammalian AHR. Studies also show that the zfAHR2 is rapidly degraded in a ligand-independent manner by exposure of cells to geldanamycin (GA) to levels consistent with mammalian AHR. Finally, immunohistochemical staining of the ZFL cells suggest that the unliganded AHR resides in both the cytoplasm and nucleus and undergoes active nucleocytoplasmic shuttling in the absence of ligand. These results suggest that there is conservation of function between fish and mammals with respect to ligand-dependent and -independent degradation of the AHR and that the zfAHR2 is degraded via the 26S proteasome.
Descriptors: zebrafish, mice, peptide hydrolases metabolism, proteasome endopeptidase complex, aryl hydrocarbon receptors metabolism, antibodies immunology, benzoquinones, binding sites, cultured cells, lactams, macrocyclic, quinones pharmacology, aryl hydrocarbon receptorsimmunology, subcellular fractions, transfection.

Wickstrom, E., M. Choob, K.A. Urtishak, X. Tian, N. Sternheim, S. Talbot, J. Archdeacon, V.A. Efimov, and S.A. Farber (2004). **Sequence specificity of alternating hydroyprolyl/phosphono peptide nucleic acids against zebrafish embryo mRNAs.** *Journal of Drug Targeting* 12(6): 363-72. ISSN: 1061-186X.
Abstract: Morpholino phosphorodiamidate (MO) DNA mimics display excellent water solubility and hybridization properties toward DNA and RNA, and have been utilized in the model vertebrate zebrafish (*Danio rerio*) for genome-wide, sequence-based, reverse genetic screens during embryonic development. Peptide nucleic acids (PNAs) exhibit

excellent mismatch discrimination, nuclease resistance, and protease resistance, but low solubility. Negatively charged DNA mimics composed of alternating residues of trans-4-hydroxy-L-proline peptide nucleic acid monomers and phosphono peptide nucleic acid monomers (HypNA-pPNA) combine all of the positive features of both MOs and PNAs. Thus, we evaluated PNA oligomers and HypNA-pPNA oligomers as an alternative to MOs for oligonucleotide inhibition of gene expression in zebrafish embryos. We observed that HypNA-pPNA 18-mers displayed comparable potency to MO 25-mers as knockdown agents against chordin, notail and uroD, with greater mismatch stringency. Furthermore, we observed that a specific HypNA-pPNA 18-mer elicited the dharma (bozozok)(-/-) phenotype in zebrafish embryos, which MO 25-mers do not. These observations validate HypNA-pPNAs as an alternative to MO oligomers for reverse genetic studies. The stronger hybridization and greater specificity of HypNA-pPNAs enable knockdown of mRNAs unaffected by MO oligomers.

Descriptors: zebrafish, drug effects , hydroxyproline chemistry, antisense oligoribonucleotides pharmacology, peptide nucleic acids chemistry, phosphonic acids chemistry, messenger RNAantagonists and inhibitors, base sequence, molecular mimicry, antisense oligoribonucleotides chemistry, messenger RNAbiosynthesis.

Wu, J.Y., C.Y. Lin, T.W. Lin, C.F. Ken, and Y.D. Wen (2007). **Curcumin affects development of zebrafish embryo.** *Biological and Pharmaceutical Bulletin* 30(7): 1336-9. ISSN: 0918 6158.

NAL Call Number: QP501

Abstract: Embryotoxic and teratogenic effects of curcumin on the development of zebrafish embryo were investi-gated in this study. The LD(50) values of curcumin (24-h incubation) were estimated at 7.5 microM and 5 microM for embryos and larvae, respectively. The developmental defects caused by curcumin treatments include bent or hook-like tails, spinal column curving, edema in pericardial sac, retarded yolk sac resorption, and shorter body length. In curcumin-treated larvae, fluorescence signals of curcumin were found in edamae sac and some skin cells. Together, these results indicate that zebrafish are suitable model organisms to study the toxic effects of curcumin.

Descriptors: zebrafish larvae, drug induced abnormalities, curcumin toxicity, drug effects, curcumin pharmacokinetics, drug dose response relationship, metabolism, larva drug effects, larva metabolism, lethal dose 50.

Zhang, T., X.H. Liu, and D.M. Rawson (2003). **Effects of methanol and developmental arrest on chilling injury in zebrafish *(Danio rerio*) embryos.** *Theriogenology* 59(7): 1545-56 . ISSN: 0093-691X.

NAL Call Number: QP251.A1T5

Abstract: Stage-dependent chilling sensitivity has been reported for many species of fish embryos. Most of these studies reveal that developmental stages beyond 50% epiboly are less sensitive to chilling, but the chilling sensitivity accelerates rapidly at subzero temperatures. In this study, the effects of methanol and developmental arrest on chilling injury were studied using zebrafish *(Danio rerio*) embryos at 64-cell, 50% epiboly, 6-somite, prim-6 and long-bud stages. Embryos were exposed to methanol or anoxic conditions before they were cooled to 0 or -5 degrees C with slow (1 degrees C/min), medium (30 degrees C/min) or fast (approximately 300 degrees C/min) cooling rates and were held at these temperatures for different time periods. Embryo survival was evaluated in terms of the percentage of treated embryos with normal developmental appearance after 3-day culture. Experiments on the effect of methanol on chilling sensitivity of the embryos showed that the addition of methanol to embryo medium increased embryo survival significantly at all developmental stages

and under all cooling conditions. Higher concentration of methanol treatment generally improved embryo survival when embryos were cooled at a fast cooling rate of 300 degrees C/min. Experiments on the effect of developmental arrest on chilling sensitivity of embryos showed that embryos at 50% epiboly and prim-6 stages underwent developmental arrest almost immediately after 15 min oxygen deprivation. After 4h in anoxia, the survival rates of the embryos were not significantly different from their respective aerobic controls. Anoxia and developmental arrest had no effect on the chilling sensitivity of zebrafish embryos.
Descriptors: zebrafish embryo, cold, drug effects, methanol pharmacology, cryopreservation methods, cryopreservation veterinary, drug dose response relationship, embryonic development, nitrogen chemistry , oxygen chemistry, time factors, zebrafish growth and development.

Zon, L.I. and R.T. Peterson (2005). **In vivo drug discovery in the zebrafish.** *Nature Reviews. Drug Discovery* 4(1): 35-44. ISSN: 1474-1776.
NAL Call Number: RM301.25
Abstract: The zebrafish has become a widely used model organism because of its fecundity, its morphological and physiological similarity to mammals, the existence of many genomic tools and the ease with which large, phenotype-based screens can be performed. Because of these attributes, the zebrafish might also provide opportunities to accelerate the process of drug discovery. By combining the scale and throughput of in vitro screens with the physiological complexity of animal studies, the zebrafish promises to contribute to several aspects of the drug development process, including target identification, disease modelling, lead discovery and toxicology.
Descriptors: zebrafish, drug design, research models, preclinical drug evaluation methods, toxicity tests methods.

Physiology

Adams, S.L., T. Zhang, and D.M. Rawson (2005). **The effect of external medium composition on membrane water permeability of zebrafish *(Danio rerio*) embryos.** *Theriogenology* 64(7): 1591-602. ISSN: 0093-691X.

NAL Call Number: QP251.A1T5

Abstract: The effect of external medium composition on chorion and plasma membrane permeability of zebrafish (*Danio rerio*) embryos was investigated in this study. Initially, survival of embryos spawned into varying strengths (10-40%) of Hank's solution (HBSS) was assessed. Development and hatching rates for embryos spawned into 30% and 40% HBSS were significantly lower than those obtained with embryos spawned into system water. The effect of embryo survival in 30% HBSS with different calcium levels was then investigated. Embryo survival in calcium free 30% HBSS or 30% HBSS with 10x the standard calcium concentration was similar to survival in standard 30% HBSS. Membrane water permeability was determined by measuring the floatation time of embryos in test solutions made up with heavy water (D2O) instead of deionized water. Intact embryos at early developmental stages were less permeable than later stages irrespective of the external medium that they were spawned into. In system water, the floatation time of embryos at one-cell and two-cell stages were 1323+/-83 and 1189+/-55 s, respectively, compared to 432+/-6 and 353+/-10 s at the high and 50% epiboly stages. Change of external medium composition had no effect on membrane permeability of intact embryos at early developmental stages. However, at later stages embryos spawned into 30% HBSS were less permeable than embryos spawned into system water, irrespective of calcium concentration. The flotation time of embryos at the high stage increased from 432+/-6s in system water to 468+/-10s in 30% HBSS. The study on dechorionated embryos showed that change of external medium composition had no effect on plasma membrane permeability.

Descriptors: zebrafish embryos, physiology, calcium analysis, cell membrane permeability, deuterium, isotonic solutions, osmolar concentration, solutions, water metabolism.

Airaksinen, S., C.M. Rabergh, A. Lahti, A. Kaatrasalo, L. Sistonen, and M. Nikinmaa (2003). **Stressor-dependent regulation of the heat shock response in zebrafish, *Danio rerio*.** *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology* 134(4): 839-46. ISSN: 1095 6433.

NAL Call Number: QP1C6

Abstract: Heat shock transcription factors (HSFs) regulate expression of heat shock proteins (Hsps). We have previously shown that in zebrafish a unique isoform, zHSF1b, disappears concomitant with heat shock-induced Hsp70 expression. To characterize the role of zHSF1a and zHSF1b isoforms in the regulation of the stress response in vivo, we have carried out cadmium (10-100 microM) and copper (10-30 microM) exposures in order to specify whether the disappearance of HSF1b is specific for heat stress. After 4-h metal exposures we analyzed the expression of hsp70, zHSF1a, zHSF1b and metallothionein (MT) by reverse transcriptase polymerase chain reaction in zebrafish liver, gonads and gills. Although cadmium is a known inducer of Hsps, it did not affect hsp70 expression significantly in the studied tissues. Induction of hsp70 was observed upon copper exposure in liver and gonads, but not in gills. Neither metal affected the zHSF1a/b ratio. Both cadmium and copper exposure caused upregulation of MT, regulator of metal homeostasis and detoxification, confirming that the tissues were subjected to metal loads. Thus, hsp70 appears to be more weakly induced upon metal exposure than in response to heat shock and HSF1 isoforms may participate in stressor-specific regulation of hsp70.

Descriptors: heat shock response, DNA binding proteins genetics, gene expression regulation, HSP70 heat shock reverse transcriptase PCR, transcription factors.

Aizawa, H., I.H. Bianco, T. Hamaoka, T. Miyashita, O. Uemura, M.L. Concha, C. Russell, S.W. Wilson, and H. Okamoto (2005). **Laterotopic representation of left-right information onto the dorso-ventral axis of a zebrafish midbrain target nucleus.** *Current Biology* 15(3): 238-43. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: The habenulae are part of an evolutionarily highly conserved limbic-system conduction pathway that connects telencephalic nuclei to the interpeduncular nucleus (IPN) of the midbrain . In zebrafish, unilateral activation of the Nodal signaling pathway in the left brain specifies the laterality of the asymmetry of habenular size . We show "laterotopy" in the habenulo-interpeduncular projection in zebrafish, i.e., the stereotypic, topographic projection of left-sided habenular axons to the dorsal region of the IPN and of right-sided habenular axons to the ventral IPN. This asymmetric projection is accounted for by a prominent left-right (LR) difference in the size ratio of the medial and lateral habenular sub-nuclei, each of which specifically projects either to ventral or dorsal IPN targets. Asymmetric Nodal signaling directs the orientation of laterotopy but is dispensable for the establishment of laterotopy itself. Our results reveal a mechanism by which information distributed between left and right sides of the brain can be transmitted bilaterally without loss of LR coding, which may play a crucial role in functional lateralization of the vertebrate brain .

Descriptors: zebrafish embryos and larva, brain mapping, mesencephalon physiology, signal transduction physiology, base sequence, DNA binding proteins genetics, functional laterality, gene library , green fluorescent proteins, habenula physiology, anatomy and histology, in situ hybridization, larva anatomy and histology and physiology, mesencephalon anatomy and histology, molecular sequence data, DNA sequence analysis, transcription factor brn 3, transcription factors genetics, transforming growth factor beta metabolism, trans anatomy and histology.

Albert, J.T., H. Winter, T.J. Schaechinger, T. Weber, X. Wang, D.Z. He, O. Hendrich, H.S. Geisler, U. Zimmermann, K. Oelmann, M. Knipper, M.C. Gopfert, and D. Oliver (2007). **Voltage-sensitive prestin orthologue expressed in zebrafish hair cells.** *Journal of Physiology* 580(Pt. 2): 451-61. ISSN: 0022-3751.

NAL Call Number: 447.8J82

Abstract: Prestin, a member of the solute carrier (SLC) family SLC26A, is the molecular motor that drives the somatic electromotility of mammalian outer hair cells (OHCs). Its closest reported homologue, zebrafish prestin (zprestin), shares approximately 70% strong amino acid sequence similarity with mammalian prestin, predicting an almost identical protein structure. Immunohistochemical analysis now shows that zprestin is expressed in hair cells of the zebrafish ear. Similar to mammalian prestin, heterologously expressed zprestin is found to generate voltage-dependent charge movements, giving rise to a non-linear capacitance (NLC) of the cell membrane. Compared with mammalian prestin, charge movements mediated by zprestin display a weaker voltage dependence and slower kinetics; they occur at more positive membrane voltages, and are not associated with electromotile responses. Given this functional dissociation of NLC and electromotility and the structural similarity with mammalian prestin, we anticipate that zprestin provides a valuable tool for tracing the molecular and evolutionary bases of prestin motor function.

Descriptors: zebrafish ear hair cells, anion transport proteins metabolism, hair cells metabolism and physiology, anion transport proteins chemistry and genetics, cell membrane metabolism, electric capacitance, exons, gene expression, molecular structure, transfection, proteins chemistry and genetics.

Ason, B., D.K. Darnell, B. Wittbrodt, E. Berezikov, W.P. Kloosterman, J. Wittbrodt, P.B. Antin, and R.H. Plasterk (2006). **Differences in vertebrate microRNA expression**. *Proceedings of the National Academy of Sciences of the United States of America* 103(39): 14385-14389. ISSN: 0027 8424.
NAL Call Number: 500N21P
Abstract: attenuate gene expression by means of and . They are abundant, highly conserved, and predicted to regulate a large number of transcripts. Several hundred miRNA classes are known, and many are associated with cell proliferation and differentiation. Many exhibit tissue-specific expression, which aids in evaluating their functions, and it has been assumed that their high level of sequence conservation implies a high level of expression conservation. A limited amount of data supports this, although discrepancies do exist. By comparing the expression of approximate to 100 miRNAs in medaka and chicken with existing data for and mouse, we conclude that the timing and location of miRNA expression is not strictly conserved. In some instances, differences in expression are associated with changes in miRNA copy number, genomic context, or both between species. Variation in miRNA expression is more pronounced the greater the differences in physiology, and it is enticing to speculate that changes in miRNA expression may play a role in shaping the physiological differences produced during animal development. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, biochemistry, molecular biophysics, microRNAs, translational inhibition, mRNA degradation.

Bagatto, B. (2005). **Ontogeny of cardiovascular control in zebrafish (*Danio rerio*): effects of developmental environment.** *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology* 141(4): 391-400. ISSN: 1095 6433.
NAL Call Number: QP1C6
Abstract: The goal of this symposium paper was to identify and quantify developmental plasticity in the onset of cardiovascular responses in the zebrafish. Developmental plasticity was induced by altering the developmental environment in one of three ways: (1) by developing zebrafish in a constant current of 5 body lengths per second, (2) by developing zebrafish at a colder temperature (20 degrees C), and (3) by developing zebrafish in severe hypoxia (DO=0.8 mg/L). Early morphological development was significantly affected by each of the treatment environments with hypoxia slowing development the most and producing the highest variation in measurements. Development in constant water current did not significantly affect the timing onset of cardiovascular responses to the pharmacological agents applied. Development at 20 degrees C significantly delayed the onset of all cardiovascular responses measured by 2-3 days. Development in hypoxia, however, not only delayed onset of all cardiovascular responses, but also shifted the onset relative to the developmental program. Hypoxia clearly has a profound affect on the onset of cardiovascular regulation and it will take many more studies to elucidate the mechanisms by which hypoxia is having its effect. Furthermore, long term studies are also needed to assess whether the plasticity measured in this study is adaptive in the evolutionary sense.
Descriptors: zebrafish embryo development, cardiovascular system, developmental gene expression regulation, adrenergic agonists metabolism, anoxia, cell size, cholinergic agonists metabolism, physiology, environment, heart rate, temperature, time factors, proteins.

Bang, A. and P. Gronkjaer (2005). **Otolith size-at-hatch reveals embryonic oxygen consumption in the zebrafish, *Danio rerio*.** *Marine Biology Berlin* 147(6): 1419-1423. ISSN: 0025 3162.
NAL Call Number: QH91.A1M35
Abstract: Otoliths have frequently been used to reconstruct growth histories in larval, juvenile and adult fish. However, there is growing evidence that otolith growth is directly determined by metabolic intensity and, consequently, only indirectly related to somatic growth. By performing

measurements of oxygen consumption rate and other early life-history traits on individual eggs of zebrafish (*Danio rerio*), we found that oxygen consumption explained residual variance in otolith size that is not accounted for by egg size. Total oxygen consumption during the embryonic stage explained 34% of the variance in sagitta size at hatch, whereas larval size at hatch (as a proxy for growth during the embryonic period) was not significantly correlated with sagitta size. This strongly suggests that otolith growth is directly related to metabolic rate, and yields a mechanism that may explain recent observations of a link between otolith size at hatch and viability in larval fish.
Descriptors: zebrafish , *Danio rerio*, size, oxygen consumption, embryonic, indication from otolith size at hatch, statoreceptors, otolith.

Bang, A., P. Gronkjaer, and H. Malte (2004). **Individual variation in the rate of oxygen consumption by zebrafish embryos**. *Journal of Fish Biology* 64(5): 1285-1296. ISSN: 0022-1112.
NAL Call Number: QL614.J68
Descriptors: zebrafish, animal metabolism, embryonic development , embryos, freshwater fish, oxygen consumption , respiration, *Danio rerio*, intraspecific varitions.

Bardet, P.L., B. Horard, V. Laudet, and J.M. Vanacker (2005). **The ERR alpha orphan nuclear receptor controls morphogenetic movements during zebrafish gastrulation.** *Developmental Biology* 281(1): 102-11. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: Gastrulation is a process involving cellular commitment and movements whereby the three fundamental germ layers are established in vertebrates embryos. Estrogen Receptor-Related (ERR) alpha is a nuclear receptor displaying high sequence identity to the Estrogen Receptors (ERs). However, ERRalpha is unable to bind and to be regulated by estrogens or any natural ligand to date. Whereas recent studies have suggested roles for ERRalpha in bone and adipose tissue metabolism in the mouse, little is known about its roles during embryonic development. In zebrafish embryos, ERRalpha is expressed from the beginning of gastrulation at the margin of the blastoderm that represents the presumptive mesendoderm. Using loss of function (morpholinos or a dominant-negative version of the protein) and gain of function (mRNA injection) strategies, we show here that ERRalpha is involved in epiboly and convergent-extension (CE) processes in the zebrafish. Altogether, these results propose ERRalpha as a new regulator of morphogenetic movement during gastrulation, independently of cell fate determination.
Descriptors: zebrafish, mice, gastrula physiology, morphogenesis physiology, cytoplasmic receptors nuclear genetics and nuclear metabolism, estrogen receptors metabolism and genetics, proteins metabolism, in situ hybridization, antisense oligonucleotides genetics and metabolism, random allocation.

Barth, K.A., A. Miklosi, J. Watkins, I.H. Bianco, S.W. Wilson, and R.J. Andrew (2005). **Fsi zebrafish show concordant reversal of laterality of viscera, neuroanatomy, and a subset of behavioral responses.** *Current Biology* 15(9): 844-50. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Abstract: Asymmetries in CNS neuroanatomy are assumed to underlie the widespread cognitive and behavioral asymmetries in vertebrates. Studies in humans have shown that the laterality of some cognitive asymmetries is independent of the laterality of the viscera; discrete mechanisms may therefore regulate visceral and neural lateralization. However, through analysis of visceral, neuroanatomical, and behavioral asymmetries in the frequent-situs-inversus (fsi) line of zebrafish, we show that the principal left-right body asymmetries are coupled to certain brain asymmetries and lateralized behaviors. fsi fish with asymmetry defects show concordant reversal of heart, gut,

and neuroanatomical asymmetries in the diencephalon. Moreover, the neuroanatomical reversals in reversed fsi fish correlate with reversal of some behavioral responses in both fry and adult fsi fish. Surprisingly, two behavioral asymmetries do not reverse, suggesting that at least two separable mechanisms must influence functional lateralization in the CNS. Partial reversal of CNS asymmetries may generate new behavioral phenotypes; supporting this idea, reversed fsi fry differ markedly from their normally lateralized siblings in their behavioral response to a novel visual feature. Revealing a link between visceral and brain asymmetry and lateralized behavior, our studies help to explain the complexity of the relationship between the lateralities of visceral and neural asymmetries.

Descriptors: zebrafish embryos, behavior physiology, body patterning physiology, frequent-situs-inversus (fsi) line, central nervous system embryology, physiology, functional laterality physiology, viscera embryology, analysis of variance, cytology, green fluorescent proteins, habenula cytology, immunohistochemistry, in situ hybridization, mutation genetics, psychomotor performance physiology, trans video recording.

Becker, C.G., J. Schweitzer, J. Feldner, M. Schachner, and T. Becker (2004). **Tenascin-R as a repellent guidance molecule for newly growing and regenerating optic axons in adult zebrafish.** *Molecular and Cellular Neuroscience* 26(3): 376-89. ISSN: 1044-7431.

Descriptors: zebrafish, axons physiology, nerve regeneration physiology, optic nerve injuries metabolism, tenascin physiology, zebrafish, cultured cells, gene expression regulation physiology, goldfish, mice, optic nerve physiology and injuries genetics, tenascin biosynthesis and genetics.

Biga, P.R. and F.W. Goetz (2006). **Zebrafish and giant danio as models for muscle growth: determinate vs. indeterminate growth as determined by morphometric analysis**. *American Journal of Physiology* 291(5(2)): R1327-R1337. ISSN: 0002 9513.

NAL Call Number: 447.8Am3

Abstract: The zebrafish has become an important genetic model, but their small size makes them impractical for traditional physiological studies. In contrast, the closely related giant danio is larger and can be utilized for physiological studies that can also make use of the extensive zebrafish genomic resources. In addition, the giant danio and zebrafish appear to exhibit different growth types, indicating the potential for developing a comparative muscle growth model system. Therefore, the present study was conducted to compare and characterize the muscle growth pattern of zebrafish and giant danio. Morphometric analyses demonstrated that giant danio exhibit an increased growth rate compared with zebrafish, starting as early as 2 wk posthatch. Total myotome area, mean fiber area, and total fiber number all exhibited positive correlations with larvae length in giant danio but not in zebrafish. Morphometric analysis of giant danio and zebrafish larvae demonstrated faster, more efficient growth in giant danio larvae. Similar to larger teleosts, adult giant danio exhibited increased growth rates in response to growth hormone, suggesting that giant danio exhibit indeterminate growth. In contrast, adult zebrafish do not exhibit mosaic hyperplasia, nor do they respond to growth hormone, suggesting they exhibit determinate growth like mammals. These results demonstrate that giant danio and zebrafish can be utilized as a direct comparative model system for muscle growth studies, with zebrafish serving as a model organism for determinate growth and giant danio for indeterminate growth. Copyright © 2008 CABI

Descriptors: zebrafish, animal models, biological development, growth, laboratory animals, morphology, muscles, physiology, *Danio rerio.*

Blaser, R. and R. Gerlai (2006). **Behavioral phenotyping in zebrafish: comparison of three behavioral quantification methods.** *Behavior Research Methods* 38(3): 456-69. ISSN: 1554 351X.

Abstract: The zebrafish has been popular in developmental biology and genetics, but its brain function has rarely been studied. High-throughput screening of mutation or drug-induced changes in brain function requires simple and automatable behavioral tests. This article compares three behavioral quantification methods in four simple behavioral paradigms that test a range of characteristics of adult zebrafish, including novelty-induced responses, social behavior, aggression, and predator-model-induced responses. Two quantification methods, manual recording and computerized videotracking of location and activity, yielded very similar results, suggesting that automated videotracking reliably measures activity parameters and will allow high-throughput screening. However, observation-based event recording of posture patterns was found generally not to correlate with videotracking measures, suggesting that further refinement of automated behavior quantification may be considered.

Descriptors: zebrafish, aggression, behavior and exploratory behavior physiology, phenotype, social behavior, analysis of variance, behavioral sciences methods, escape reaction physiology, motor activity physiology, reference values, nonparametric statistics.

Boisen, A.M., J. Amstrup, I. Novak, and M. Grosell (2003). **Sodium and chloride transport in soft water and hard water acclimated zebrafish (*Danio rerio*).** *Biochimica Et Biophysica Acta* 1618(2): 207-18. ISSN: 0006 3002.

NAL Call Number: 381B522

Abstract: While the zebrafish is commonly used for studies of developmental biology and toxicology, very little is known about their osmoregulatory physiology. The present investigation of Na(+) and Cl(-) transport revealed that the zebrafish is able to tolerate extremely low ambient ion concentrations and that this is achieved at least in part by a greatly enhanced apparent uptake capacity and affinity for both ions. Zebrafish maintain plasma and whole body electrolyte concentrations similar to most other freshwater teleosts even in deionized water containing only 35 microM NaCl, i.e soft water. We recorded an extremely low transport affinity constant (K(m)) of 8+/-1 microM for the active uptake of Cl(-) in soft water acclimated fish, while other transport kinetic parameters were in agreement with reports for other freshwater organisms. While both Na(+) and Cl(-) uptake in soft water clearly depends on apical proton pump activity, changes in abundance and possibly localization of this protein did not appear to contribute to soft water acclimation. Active Cl(-) uptake was strongly dependent on branchial carbonic anhydrase (CA) activity regardless of water type, while the response of Na(+) transport to a CA inhibitor was more variable. Differential response of Na(+) uptake to amiloride depending on acclimation medium suggests that different Na(+) transport mechanisms are employed by zebrafish acclimated to soft and hard water.

Descriptors: zebrafish, amiloride analogs and derivatives, chlorides and sodium metabolism, biological transport, Western blotting, down regulation, ethoxzolamide, fresh water, gills metabolism, immunohistochemistry, kinetics, macrolides, proton pumps analysis and antagonists and inhibitors, proton pumps metabolism, zebrafish blood.

Brustein, E., L. Saint Amant, R.R. Buss, M. Chong, J.R. McDearmid, and P. Drapeau (2003). **Steps during the development of the zebrafish locomotor network.** *Journal of Physiology, Paris* 97(1): 77-86. ISSN: 0928-4257.

NAL Call Number: QP1

Abstract: This review summarizes recent data from our lab concerning the development of motor activities in the developing zebrafish. The zebrafish is a leading model for studies of verte-

brate development because one can obtain a large number of transparent, externally and rapidly developing embryos with motor behaviors that are easy to assess (e.g. for mutagenic screens). The emergence of embryonic motility was studied behaviorally and at the cellular level. The embryonic behaviors appear sequentially and include an early, transient period of spontaneous, alternating tail coilings, followed by responses to touch, and swimming. Patch clamp recording in vivo revealed that an electrically coupled network of a subset of spinal neurons generates spontaneous tail coiling, whereas a chemical (glutamatergic and glycinergic) synaptic drive underlies touch responses and swimming and requires input from the hindbrain. Swimming becomes sustained in larvae once serotonergic neuromodulatory effects are integrated. We end with a brief overview of the genetic tools available for the study of the molecular determinants implicated in locomotor network development in the zebrafish. Combining genetic, behavioral and cellular experimental approaches will advance our understanding of the general principles of locomotor network assembly and function.
Descriptors: zebrafish locomotor network model, motor activity physiology, nerve net and neurons physiology, genetically modified physiology, behavior larva, motor activity genetics, mutagenicity tests methods, nerve net embryology, neurons classification.
Notes: A review.

Bryson Richardson, R.J. and P.D. Currie (2004). **Optical projection tomography for spatio-temporal analysis in the zebrafish.** *Methods in Cell Biology* 76: 37-50. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, three dimensional imaging methods, tomography methods, computer assisted image processing methods, confocal microscopy methods, fluorescence microscopy, propidium chemistry, software, tissue fixation methods, tomography trends.

Buckingham, S.D. and D.W. Ali (2005). **Computer simulations of high-pass filtering in zebrafish larval muscle fibres.** *Journal of Experimental Biology* 208(Pt 16): 3055-63. ISSN: 0022-0949.
NAL Call Number: 442.8B77
Abstract: Larval somatic muscle of the zebrafish, *Danio rerio*, like that of some other organisms, responds to a sustained depolarization with one, and only one, action potential. Here, we report computer simulations, using the NEURON simulation programme, of sodium and potassium currents of somatic muscle of larval Danio rerio to investigate their possible contribution to once-only firing. Our computer model incorporated simulated sodium and potassium ion channels based on steady-state and kinetic parameters derived from a recent electrophysiological study. The model responded to sustained depolarizations with a single action potential at all levels of depolarization above threshold. By varying several parameters of the sodium and potassium currents systematically, the minimum changes necessary to produce repetitive firing were found to be a positive shift in the half-inactivation and a negative shift in the half-activation potentials for the sodium current, accompanied by a slowing of the rate of inactivation to half of the experimentally observed values. This suggests that once-only spiking can be attributed to the steady-state values of activation and inactivation of the sodium current, along with a slower rate of inactivation. Mapping of the resultant firing properties against steady-state and kinetic ion channel parameters revealed a high safety factor for once-only firing and showed that the time constant of inactivation of the sodium current was the key determinant of once-only or repetitive firing. The rapidly inactivating potassium current does not influence once-only firing or the maximum rate of firing in response to periodic excitation in these simulations. Although a contribution of other currents to produce once-only firing has not been excluded, this model suggests that the properties of the sodium current are sufficient to account for once-only firing.
Descriptors: zebrafish larval muscle computer simulation, ion channels metabolism, muscle

contraction physiology, muscle fibers physiology and metabolism, skeletal muscle physiology and metabolism, action potentials physiology, computer simulation, larva physiology, potassium and sodium metabolism.

Burgess, H.A. and M. Granato (2007). **Modulation of locomotor activity in larval zebrafish during light adaptation.** *Journal of Experimental Biology* 210(Pt 14): 2526-39. ISSN: 0022-0949.
NAL Call Number: 442.8B77
Abstract: The neural basis of behavioral choice in vertebrates remains largely unknown. Zebrafish larvae have a defined locomotor repertoire as well as a simple nervous system and are therefore an attractive vertebrate system in which to study this process. Here we describe a high-throughput system for quantifying the kinematics of motor events in zebrafish larvae in order to measure the initiation frequency of different maneuvers. We use this system to analyze responses to photic stimuli and find that larvae respond to changes in illumination with both acute responses and extended behavioral programs. Reductions in illumination elicit large angle turns, distinct from startle responses, which orient larvae toward the source of light. In continuing darkness, larvae are transiently hyperactive before adopting a quiescent state. Indeed, locomotor activity is controlled by the state of light or dark adaptation similar to masking phenomena in higher vertebrates where light directly regulates motor activity. We propose that regulation of motor activity by photic stimuli in zebrafish larvae serves a behavioral goal of maximizing exposure to well lit environments optimal for feeding.
Descriptors: zebrafish larva, acclimatization, behavior radiation effects, light, locomotion, zebrafish growth, development and physiology, biomechanics, larva physiology and radiation effects, photic stimulation.

Cahill, G.M. (2007). **Automated video image analysis of larval zebrafish locomotor rhythms.** *Methods in Molecular Biology* 362: 83-94. ISSN: 1064-3745.
Abstract: A method is described for measurement of the circadian activity rhythms of up to 150 larval zebrafish simultaneously with a single video image analysis system. Most of the required equipment and software are commercially available, although some components are custom-built.
Descriptors: zebrafish larva, circadian rhythm physiology, motor activity physiology, statistical data interpretation, larva physiology, physiologic monitoring methods, physiologic monitoring statistics and numerical data, software , swimming physiology, video recording methods.

Cardona Costa., J. and F. Garcia Ximenez (2007). **Vitrification of zebrafish embryo blastomeres in microvolumes.** *Cryo Letters* 28(4): 303-09. ISSN: 0143 2044.
NAL Call Number: QC278.C72
Abstract: Cryopreservation of fish embryos may play an important role in biodiversity preservation and in aquaculture, but it is very difficult. In addition, the cryopreservation of fish embryo blastomeres makes conservation strategies feasible when they are used in germ-line chimaerism, including interspecific chimaerism. Fish embryo blastomere cryopreservation has been achieved by equilibrium procedures, but to our knowledge, no data on vitrification procedures are available. In the present work, zebrafish embryo blastomeres were successfully vitrified in microvolumes: a number of 0.25 microl drops, sufficient to contain all the blastomeres of an embryo at blastula stage (from 1000-cell stage to oblong stage), were placed over a 2.5 cm loop of nylon filament. In this procedure, where intracellular cryoprotectant permeation is not required, blastomeres were exposed to cryoprotectants for a maximum of 25 sec prior vitrification. The assayed cryoprotectants (ethylene glycol, propylene glycol, dimethyl sulphoxide, glycerol and methanol) are all frequently used in fish embryo and blastomere cryopreservation. Methanol was finally rejected because of the excessive concentration required for the vitrification (15M). All other

cryoprotectants were prepared (individually) at 5 M in Hanks' buffered salt solution (sigma) plus 20% FBS (vitrification solutions: vs). After direct thawing in Hanks' buffered salt solution plus 20% FBS, acceptable survival rates were obtained with ethylene glycol: 82.8%, propylene glycol: 87.7%, dimethyl sulphoxide: 93.4%, and glycerol: 73.9% ($p < 0.05$). Dimethyl sulphoxide showed the highest blastomere survival rate and allowed the rescue of as much as 20% of the total blastomeres from each zebrafish blastula embryo.
Descriptors: zebrafish embryos, biodiversity preservation, blastomeres, dimethyl sulphoxide, cryoprotectants, chimaerism.

Chen, S., C. Li, G. Yuan, and F. Xie (2007). **Anatomical and histological observation on the pancreas in adult zebrafish.** *Pancreas* 34(1): 120-5. ISSN: 0885-3177.
Abstract: OBJECTIVES: To investigate the anatomic structure of the pancreas and the distribution of the islets in adult zebrafish. METHODS: In situ immunofluorescent staining, electron microscopy, and serial paraffin-embedded sectioning with hematoxylin/eosin staining were applied. RESULTS: The pancreas along the intestine included 4 relatively independent and concentrated lobes, in which 4 kinds of islets-principal islets, Brockmann bodies, diffusely existing islets, and single beta-cell-were observed. Some islets contained both alpha and beta cells, whereas some contained only beta cells. The islet number in each adult zebrafish averaged 84.53 +/- 43.77; and the lower quartile, median, and upper quartile were 55.25, 70.50, and 112.00, respectively ($n = 40$). The different islets were differently distributed in the 4 pancreatic lobes with statistical significance ($P < 0.05$). Meanwhile, 3 kinds of secretory granules were found in the cytoplasm of different islet cells. CONCLUSIONS: According to the distinct distribution, concentration of the pancreas, and different contents of the islets within the pancreas, 4 lobes of the pancreas along the intestine-the gallbladder-spleen lobe, the middle lobe, the left lobe, and the ventral lobe-were identified in adult zebrafish.
Descriptors: zebrafish pancreas anatomy and physiology, glucagon secreting cells cytology, insulin secreting cells cytology, exocrine pancreas anatomy and histology, age factors, fluorescent antibody technique, glucagon metabolism, secreting cells metabolism and secreting cells ultrastructure, insulin metabolism and secreting cells metabolism and ultrastructure, electron microscopy, exocrine pancreas ultrastructure.

Chen, X. and B.M. Gumbiner (2006). **Crosstalk between different adhesion molecules**. *Current Opinion in Cell Biology* 18(5): 572-578. ISSN: 0955 0674.
NAL Call Number: QH573.C87
Abstract: Cell adhesion molecules mediate cell-cell and cellextracellular matrix adhesions, and coordination between these molecules is essential for tissue formation and morphogenesis. Crosstalk between integrins and cadherins may result from a physical response to integrin-mediated adhesion, complex cell differentiation processes, or direct signaling pathways linking the two adhesion systems. Nectins have recently been shown to regulate the organization of cadherins into adherens junctions and the formation of tight junctions by several processes. Furthermore, protocadherins can interact with extracellular matrix proteins or function by regulating classical cadherins. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, cadherins, integrins, nectins, adherins, cell biology, cell differentiation, signaling pathway, morphogenesis, tissue formation, cell extracellular matrix adhesion.

Cheng, S.H. (2007). **Nature and nurture, the influence of genetic and xenobiotic factors in zebrafish embryonic development**. *Comparative Biochemistry and Physiology, Part a: Molecular & Integrative Physiology* 148(Suppl.1): S8. ISSN: 1095-6433.
NAL Call Number: QP1C6

Descriptors: zebrafish, embryonic development, environmental effects, freshwater fish, phylogenetics, *Danio rerio*, reproduction and development.

Cibrian Uhalte, E., A. Langenbacher, X. Shu, J.N. Chen, and S. Abdelilah Seyfried (2007). **Involvement of zebrafish Na+,K+ ATPase in myocardial cell junction maintenance.** *Journal of Cell Biology* 176(2): 223-30. ISSN: 0021-9525.
NAL Call Number: 442.8J828
Abstract: Na(+),K(+) ATPase is an essential ion pump involved in regulating ionic concentrations within epithelial cells. The zebrafish heart and mind (had) mutation, which disrupts the alpha1B1 subunit of Na(+),K(+) ATPase, causes heart tube elongation defects and other developmental abnormalities that are reminiscent of several epithelial cell polarity mutants, including nagie oko (nok). We demonstrate genetic interactions between had and nok in maintaining Zonula occludens-1 (ZO-1)-positive junction belts within myocardial cells. Functional tests and pharmacological inhibition experiments demonstrate that Na(+),K(+) ATPase activity is positively regulated via an N-terminal phosphorylation site that is necessary for correct heart morphogenesis to occur, and that maintenance of ZO-1 junction belts requires ion pump activity. These findings suggest that the correct ionic balance of myocardial cells is essential for the maintenance of epithelial integrity during heart morphogenesis.
Descriptors: zebrafish heart morphogenesis model, intercellular junctions physiology, myocardium enzymology, amino acid sequence, cell membrane metabolism, cell polarity physiology, cytoplasm metabolism, drug effects, embryology, metabolism, guanylate cyclase genetics and metabolism, heart embryology and physiology, intercellular junctions drug effects, isoenzymes genetics and metabolism, membrane proteins genetics and metabolism, molecular sequence data, morphogenesis physiology, mutation, myocardium cytology, Na+ K+ exchanging ATPase antagonists, inhibitors, metabolism and genetics, ouabain pharmacology, phosphoproteins genetics and metabolism, phosphorylation, protein kinase C genetics and metabolism, amino acid sequence homology, transfection, proteins antagonists, inhibitors, genetics and metabolism.

Cinquin, O. (2007). **Repressor dimerization in the zebrafish somitogenesis clock.** *Public Library of Science Computational Biology* 3(2): E32. ISSN: 1553-734X.
Abstract: The oscillations of the somitogenesis clock are linked to the fundamental process of vertebrate embryo segmentation, yet little is known about their generation. In zebrafish, it has been proposed that Her proteins repress the transcription of their own mRNA. However, in its simplest form, this model is incompatible with the fact that morpholino knockdown of Her proteins can impair expression of their mRNA. Simple self-repression models also do not account for the spatiotemporal pattern of gene expression, with waves of gene expression shrinking as they propagate. Here we study computationally the networks generated by the wealth of dimerization possibilities amongst transcriptional repressors in the zebrafish somitogenesis clock. These networks can reproduce knockdown phenotypes, and strongly suggest the existence of a Her1-Her7 heterodimer, so far untested experimentally. The networks are the first reported to reproduce the spatiotemporal pattern of the zebrafish somitogenesis clock; they shed new light on the role of Her13.2, the only known link between the somitogenesis clock and positional information in the paraxial mesoderm. The networks can also account for perturbations of the clock by manipulation of FGF signaling. Achieving an understanding of the interplay between clock oscillations and positional information is a crucial first step in the investigation of the segmentation mechanism.
Descriptors: zebrafish embryos, biological clocks physiology, body patterning physiology, repressor, signal transduction and, computer simulation, dimerization, gene expression physiology, proteins chemistry.

Clouthier, D.E. and T.F. Schilling (2004). **Understanding endothelin-1 function during craniofacial development in the mouse and zebrafish.** *Birth Defects Research* 72(2): 190-9. ISSN: 1542 0752.

NAL Call Number: QL991.T4

Descriptors: zebrafish, endothelin 1 physiology, developmental gene expression regulation, neural crest embryology, basic helix loop helix transcription factors, body patterning, branchial region metabolism, DNA binding proteins genetics, proteins metabolism, endothelin 1 metabolism, gene expression regulation, jaw embryology, mice, mutation, pharynx embryology, receptor, endothelin a metabolism, signal transduction, time factors, transcription factors metabolism, zebrafish, proteins.

Cohen, S.A., H. Hatt, J. Kubanek, and N.A. McCarty (2008). **Reconstitution of a chemical defense signaling pathway in a heterologous system**. *Journal of Experimental Biology* 211(4): 599-605. ISSN: 0022-0949.

NAL Call Number: 442.8B77

Descriptors: zebrafish, chemoreceptors, conductance, cystic fibrosis, deterrents, electrophysiology, oocytes, predators, signal transduction, *Danio rerio*, *Thalassoma bifasciatum*, *Xenopus laevis.*

Colwill, R.M., M.P. Raymond, L. Ferreira, and H. Escudero (2005). **Visual discrimination learning in zebrafish (*Danio rerio*).** *Behavioural Processes* 70(1): 19-31. ISSN: 0376 6357.

Abstract: Three experiments demonstrated visual discrimination learning in zebrafish (*Danio rerio*). In each experiment, zebrafish were given a choice between two visually distinct arms of a T-maze. Choice of one stimulus was always followed by a food reward, but choice of the other stimulus was not rewarded. Different colored sleeves fitted around the arms of the T-maze were used in Experiments 1 (green and purple) and 2 (red and blue). The stimuli used in Experiment 3 were white sleeves lined with horizontal or vertical black stripes. In all three experiments, zebrafish acquired a significant preference for the stimulus that led to a food reward. Experiments 1 and 2 also showed that zebrafish could learn a reversal of the discrimination. Finally, the effect of discontinuing food rewards was examined after reversal training in Experiment 2 and after initial discrimination training in Experiments 1 and 3. Non-reinforcement led to a decrease in correct responding in Experiments 2 and 3 independent of stimulus identity, but to an asymmetrical pattern of responding in Experiment 1. The median latency to make a choice response decreased over the course of acquisition in all three experiments; during extinction, median response times did not change at all in Experiment 1 and increased only very slightly in Experiment 2, but showed a substantial increase in Experiment 3. The implications of these results for the zebrafish as a model system for genetic studies of learning and memory are discussed.

Descriptors: zebrafish visual learning model, discrimination learning, visual perception, behaviors, choice behavior, psychological extinction, memory, reward.

Connaughton, V.P. (2003). **Zebrafish retinal slice preparation.** *Methods in Cell Science* 25(1-2): 49-58. ISSN: 1381-5741.

NAL Call Number: QH573.T52

Abstract: This paper describes the protocol for generating thin (approximately 100 microm) slices of the zebrafish retina. Retinal slices retain the cytoarchitecture and synaptic contacts found in vivo, allowing neurons to be identified prior to physiological recordings. These characteristics distinguish retinal slices from both isolated cell and eyecup preparations. Studies using the zebrafish retinal slice have classified different retinal cell types, documented voltage- and ligand-gated current responses in distal bipolar neurons, and correlated physiological responses with neuronal morphology. Data collected using this protocol have provided baseline information

about retinal circuitry that can be directly applied to behavioral studies examining visual function and/or mutants with visual system defects.

Descriptors: zebrafish retina protocol, eye anatomy and histology, ion channel gating physiology, retina anatomy and histology, electroretinography, eye cytology, retina physiology.

Costa, M.L., R. Escaleira, M. Manasfi, L.F. de Souza, and C.S. Mermelstein (2003). **Cytoskeletal and cellular adhesion proteins in zebrafish (*Danio rerio*) myogenesis.** *Brazilian Journal of Medical and Biological Research* 36(8): 1117-20. ISSN: 0100 897X.

NAL Call Number: R850.A1B72

Abstract: The current myogenesis and myofibrillogenesis model has been based mostly on in vitro cell culture studies, and, to a lesser extent, on in situ studies in avian and mammalian embryos. While the more isolated artificial conditions of cells in culture permitted careful structural analysis, the actual in situ cellular structures have not been described in detail because the embryos are more difficult to section and manipulate. To overcome these difficulties, we used the optically clear and easy to handle embryos of the zebrafish *Danio rerio*. We monitored the expression of cytoskeletal and cell-adhesion proteins (actin, myosin, desmin, alpha-actinin, troponin, titin, vimentin and vinculin) using immunofluorescence microscopy and video-enhanced, background-subtracted, differential interference contrast of 24- to 48-h zebrafish embryos. In the mature myotome, the mononucleated myoblasts displayed periodic striations for all sarcomeric proteins tested. The changes in desmin distribution from aggregates to perinuclear and striated forms, although following the same sequence, occurred much faster than in other models. All desmin-positive cells were also positive for myofibrillar proteins and striated, in contrast to that which occurs in cell cultures. Vimentin appeared to be striated in mature cells, while it is developmentally down-regulated in vitro. The whole connective tissue septum between the somites was positive for adhesion proteins such as vinculin, instead of the isolated adhesion plaques observed in cell cultures. The differences in the myogenesis of zebrafish in situ and in cell culture in vitro suggest that some of the previously observed structures and protein distributions in cultures could be methodological artifacts.

Descriptors: zebrafish embryos, myogenesis, cell adhesion physiology, cytoskeletal proteins metabolism, adhesion proteins, muscle development physiology.

Craig, P.M., C.M. Wood, and G.B. McClelland (2007). **Gill membrane remodeling with soft-water acclimation in zebrafish (*Danio rerio*).** *Physiological Genomics* 30(1): 53-60. ISSN: 1094-8341.

Abstract: Little is known regarding the ionoregulatory abilities of zebrafish exposed to soft water despite the popularity of this model organism for physiology and aquatic toxicology. We examined genomic and nongenomic changes to gills of zebrafish as they were progressively acclimated from moderately hard freshwater to typical soft water over 7 days and held in soft water for another 7 days. Gills were sampled daily and mRNA expression levels of gill Na(+)-K(+)-ATPase (NKA) alpha1a subunit, epithelium calcium channel (ECaC), carbonic anhydrase-1 and 2 (CA-1, CA-2), Na(+)/H(+) exchanger (NHE-2), V-type proton (H(+))-ATPase, and copper transport protein (CTR-1) were quantified by real-time PCR. Changes in enzyme activities of gill NKA were determined and protein levels of NKA and ECaC were quantified by Western blotting. Levels of mRNA for ECaC increased fourfold after day 6, with an associated increase in ECaC protein levels after 1 wk in soft water. CA-1 and CA-2 exhibited a 1.5- and 6-fold increase in gene expression on days 6 and 5, respectively. Likewise, there was a fivefold increase in NHE-2 expression after day 6. Surprisingly, CTR-1 mRNA showed a large transient increase (over threefold) on day 6, while H(+)-ATPase mRNA did not change. These data demonstrate a high degree of phenotypic plasticity in zebrafish gills exposed to an ion-poor environment. This not only enhances our understanding of ionoregulatory processes in fish but also highlights the need for

proper experimental design for studies involving preacclimation to soft water (e.g., metal toxicity).

Descriptors: zebrafish ionoregulatory processes, gills metabolism, ions pharmacology, water pharmacology, acclimatization drug effects and genetics, Western blotting, calcium channels genetics and metabolism, carbonic anhydrases genetics and metabolism, gene expression profiling and regulation drug effects, gills drug effects, hydrogen ion concentration, magnesium metabolism, membrane transport membrane transport proteins metabolism, Na+ K+ exchanging ATPase genetics and metabolism, potassium metabolism, messenger RNA genetics and metabolism, reverse transcriptase PCR, sodium metabolism, time factors, vacuolar proton translocating ATPases genetics and metabolism.

Cutler, C.P., A.-S. Martinez, and G. Cramb (2007). **The role of aquaporin 3 in teleost fish**. *Comparative Biochemistry and Physiology, Part a: Molecular & Integrative Physiology* 148(1): 82-91. ISSN: 1095-6433.

NAL Call Number: QP1C6

Descriptors: zebrafish, anadromous species, DNA, genomes, nucleotide sequence, esophagus, osmoregulation, population genetics, *Danio rerio*, *Oncorhynchus mykiss*, rainbow trout.

Dahm, R., H.B. Schonthaler, A.S. Soehn, J. van Marle, and G.F. Vrensen (2007). **Development and adult morphology of the eye lens in the zebrafish.** *Experimental Eye Research* 85(1): 74-89. ISSN: 0014 4835.

NAL Call Number: QP474

Abstract: The zebrafish has become an important vertebrate model organism to study the development of the visual system. Mutagenesis projects have resulted in the identification of hundreds of eye mutants. Analysis of the phenotypes of these mutants relies on in depth knowledge of the embryogenesis in wild-type animals. While the morphological events leading to the formation of the retina and its connections to the central nervous system have been described in great detail, the characterization of the development of the eye lens is still incomplete. In the present study, we provide a morphological description of embryonic and larval lens development as well as adult lens morphology in the zebrafish. Our analyses show that, in contrast to other vertebrate species, the zebrafish lens delaminates from the surface ectoderm as a solid cluster of cells. Detachment of the prospective lens from the surface ectoderm is facilitated by apoptosis. Primary fibre cell elongation occurs in a circular fashion resulting in an embryonic lens nucleus with concentric shells of fibres. After formation of a monolayer of lens epithelial cells, differentiation and elongation of secondary lens fibres result in a final lens morphology similar to that of other vertebrate species. As in other vertebrates, secondary fibre cell differentiation includes the programmed degradation of nuclei, the interconnection of adjacent fibres via protrusions at the fibre cells' edges and the establishment of gap junctions between lens fibre cells. The very close spacing of the nuclei of the differentiating secondary fibres in a narrow zone close to the equatorial epithelium, however, suggests that secondary fibre cell differentiation deviates from that described for mammalian or avian lenses. In summary, while there are similarities in the development and final morphology of the zebrafish lens with mammalian and avian lenses, there are also significant differences, suggesting caution when extrapolating findings on the zebrafish to, for example, human lens development or function.

Descriptors: zebrafish eye lens development and morphology model, crystalline lens cytology, anatomy and histology, apoptosis physiology, cell differentiation physiology, cell nucleus ultrastructure, anatomy and histology, cytology, ultrastructure, embryonic development physiology, epithelial cells cytology and ultrastructure, gap junctions ultrastructure, in situ nick end labeling methods, iris anatomy and histology, crystalline lens embryology and ultrastructure, electron

microscopy methods, scanning electron microscopy methods, interference microscopy methods, species comparison.

Danilova, N., V.E. Krupnik, D. Sugden, and I.V. Zhdanova (2004). **Melatonin stimulates cell proliferation in zebrafish embryo and accelerates its development.** *Federation of American Societies for Experimental Biology Journal* 18(6): 751-3. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: All vertebrates show a dramatic circadian rhythm in circulating melatonin with high levels at night and very low levels during daytime. In adults, melatonin is thought to synchronize other circadian rhythms and regulate seasonal rhythms in photoperiodic animals by acting on specific G-protein coupled receptors. The role of melatonin in development is unknown, even though melatonin receptors appear to be more highly expressed in developing embryos and neonates than in adults. In this study on zebrafish embryos, we describe a role for melatonin in increasing cell proliferation and accelerating development. We propose that melatonin has a role in extending the safe limit of proliferation rate at night to allow more rapid development when potentially damaging ultraviolet light is absent.
Descriptors: zebrafish, melatonin physiology, cell division, cytology, drug effects, kinetics, ligands, melatonin pharmacology, melatonin receptor, mt2 metabolism and physiology.

Danos, N. and G.V. Lauder (2007). **The ontogeny of fin function during routine turns in zebrafish *Danio rerio*.** *Journal of Experimental Biology* 210(Pt 19): 3374-86. ISSN: 0022-0949.
NAL Call Number: 442.8B77
Abstract: Zebrafish *Danio rerio* exhibit spontaneous, routine turns as part of their normal foraging behavior from the early free-swimming stage to adulthood. Given the importance of this behavior and its pervasiveness during zebrafish life history, the functional requirements of routine turning should play an important role in development. Conversely, the ontogeny of turning performance should reflect morphological development. In this paper we analyze the kinematics of routine turning during ontogeny in zebrafish and compare the scaling of turning kinematics to predictions from two existing models. Twenty-nine fish ranging in size from 0.38 to 1.97 cm in fork length (FL) were filmed at 1000 frames s(-1) while performing routine turns. Images were analyzed using image cross-correlation to calculate body and fin velocities. We performed piecewise linear regression to identify variables that do not have a constant rate of change across ontogeny and found that two variables, turn angle and angular velocity, have a transition in slope at a body size of approximately 1 cm. Other variables show a constant positive (pectoral and caudal fin velocity, turn duration), negative (body curvature) or zero (head velocity) rate of change across ontogeny. We interpret these trends in light of morphological changes over ontogeny as well as relevant hydrodynamic conditions. We also compare the slope of the log-transformed variables to predictions from two scaling models of change in function with increasing size. We find mixed support for both models with no single model being better at predicting a single type of variable such as linear velocities. We conclude that morphological development of the paired and median fins and of the skeleton, is an important factor in determining the performance of routine turning over ontogeny. Three-dimensional kinematics and ecological behavior information will further elucidate the ontogenetic patterns observed here.
Descriptors: zebrafish ontogene patterns, swimming physiology, fin velocity, turn angle of fins, body size, video recording.

Dekens, M.P., C. Santoriello, D. Vallone, G. Grassi, D. Whitmore, and N.S. Foulkes (2003). **Light regulates the cell cycle in zebrafish.** *Current Biology* 13(23): 2051-57. ISSN: 0960 9822.
NAL Call Number: QH301.C85

Abstract: The timing of cell proliferation is a key factor contributing to the regulation of normal growth. Daily rhythms of cell cycle progression have been documented in a wide range of organisms. However, little is known about how environmental, humoral, and cell-autonomous factors contribute to these rhythms. Here, we demonstrate that light plays a key role in cell cycle regulation in the zebrafish. Exposure of larvae to light-dark (LD) cycles causes a range of different cell types to enter S phase predominantly at the end of the day. When larvae are raised in constant darkness (DD), a low level of arrhythmic S phase is observed. In addition, light-entrained cell cycle rhythms persist for several days after transfer to DD, both observations pointing to the involvement of the circadian clock. We show that the number of LD cycles experienced is essential for establishing this rhythm during larval development. Furthermore, we reveal that the same phenomenon exists in a zebrafish cell line. This represents the first example of a vertebrate cell culture system where circadian rhythms of the cell cycle are observed. Thus, we implicate the cell-autonomous circadian clock in the regulation of the vertebrate cell cycle by light.
Descriptors: zebrafish, cell cycle physiology, circadian rhythm physiology, bromodeoxyuridine, cultured cells, ELISA, larva physiology, light, myocardium cytology, photoperiod, staining and labeling.

Desplantez, T., I. Marics, T. Jarry Guichard, R. Veteikis, J.P. Briand, R. Weingart, and D. Gros (2003). **Characterization of zebrafish Cx43.4 connexin and its channels.** *Experimental Physiology* 88(6): 681-90. ISSN: 0928 0670.

Abstract: Connexins (Cx) form intercellular junctional channels which are responsible for metabolic and electrical coupling. We report here on the biochemical and immunohistochemical characterization of zebrafish connexin zfCx43.4, an orthologue of mammalian and avian Cx45, and the electrophysiological properties of junctional channels formed by this protein. The investigations were performed on transfected COS-7 cells or HeLa cells. Using site-directed antibodies, zfCx43.4 cDNA (GenBank accession no. X96712) was demonstrated to code for a protein with a M(r) of 45 000. In transfected cells, zfCx43.4 was localized in cell-cell contact areas as expected for a gap junction protein. zfCx43.4 channels were shown to transfer Lucifer Yellow. The multichannel currents were sensitive to the transjunctional voltage (V(j)). Their properties were consistent with a two-state model and yielded the following Boltzmann parameters for negative/positive V(j): V(j,0) = -38.4/41.9 mV; g(j,min) = 0.19/0.18; z = 2.6/2.3. These parameters deviate somewhat from those of zfCx43.4 channels expressed in *Xenopus* oocytes and from those of Cx45, an orthologue of zfCx43.4, expressed in mammalian cells or *Xenopus* oocytes. Conceivably, the subtle differences may reflect differences in experimental methods and/or in the expression system. The single channel currents yielded two prominent levels attributable to a main conductance state (gamma(j,main) = 33.2 +/- 1.5 pS) and a residual conductance state (gamma(j,residual) = 11.9 +/- 0.6 pS).
Descriptors: zebrafish, cell communication physiology, gap junctions physiology, membrane potentials physiology, membrane, COS cells, *Cercopithecus aethiops*, molecular cloning, electric conductivity, HeLa cells, membrane proteins chemistry and genetics, molecular weight, recombinant proteins metabolism, tissue distribution, proteins chemistry and genetics.

Dezfuli, B.S., S. Capuano, E. Simoni, M. Previati, and L. Giari (2007). **Rodlet cells and the sensory systems in zebrafish (*Danio rerio*).** *Anatomical Record* 290(4): 367-74. ISSN: 0003 276X.

Abstract: This preliminary work was designed to study, using routine procedures for light and transmission electron microscopy, the presence of rodlet cells (RCs) in or near the sensory systems of 12 adult specimens (4.0 +/- 1.2 cm, LT +/- SD) of zebrafish, *Danio rerio* Hamilton, 1822. Rodlet cells, characterized by a distinctive cell cortex (range, 0.4-1.5 microm in thickness) and conspicuous inclusions named "rodlets," have a round to ovoid nucleus with irregular outline.

Mature RCs are 11.5 +/- 1.2 microm (mean +/- SD) long and 7.8 +/- 1.1 microm (mean +/- SD) wide. These cells are more numerous near neuromasts enclosed by an epithelial roof and/or ossified canal wall. In contrast, very few RCs were noticed near superficial neuromasts. Based on the presence of RCs around the two cranial neuromasts of each fish, a variable number from 1 to 15 rodlet cells was found (10.4 +/- 3.6, mean +/- SD). The RCs were located 1.5 microm (minimal) to 73.3 microm (maximal) from the neuromast (27.9 +/- 17.2, mean +/- SD). Moreover, RCs were found in olfactory epithelium and in proximity to some taste buds. Interestingly, RCs were absent in the inner ear, eye, and brain.
Descriptors: zebrafish rodlet cells , mechanoreceptors ultrastructure, mechanoreceptors physiology, light and transmission electron microscopy.

Dmochowski, I. (2004). **New molecular imaging reagents for biological investigations.** *Abstracts of Papers American Chemical Society* 228(Part 1): U834. ISSN: 0065 7727.
NAL Call Number: 381Am33Pa
Descriptors: zebrafish, cancer, neoplastic disease, pathology, magnetic resonance imaging, diagnostic techniques, imaging and microscopy techniques, fluorescence imaging, trojan horse protein, drug delivery device.

Dong, Q., K. Svoboda, T.R. Tiersch, and W.T. Monroe (2007). **Photobiological effects of UVA and UVB light in zebrafish embryos: evidence for a competent photorepair system.** *Journal of Photochemistry and Photobiology. B, Biology* 88(2-3): 137-46. ISSN: 1011-1344.
NAL Call Number: QH515.J68
Abstract: The consequences of UVB and UVA irradiation on hatch rate, mortality, and malformation were studied in embryonic zebrafish (Danio rerio). The use of zebrafish embryos has expanded from traditional developmental models to diverse studies, including many techniques utilizing light exposure. To characterize useful indicators of photodamage, the responses and threshold limits of UV radiation as a function of embryonic stage and fish source were evaluated. Significant differences in UVB susceptibility were observed in embryos at 3, 6-7, 12, and 24h post-fertilization (hpf), with the 1000-cell stage (3 hpf) having greatest tolerance to UVB. Embryos derived from zebrafish raised in outdoor ponds were more tolerant to UVB than were embryos from laboratory-raised fish. Combinations of UVB and UVA exposure were used to confirm the presence of a competent photorepair system in zebrafish that could return otherwise malformed embryos to a normal phenotype. Overall, embryonic zebrafish had large tolerances (LD(50) of 850 J/cm(2)) to UVA, confirming their suitability for photoactivation and photorepair studies.
Descriptors: zebrafish embryo, radiation effects, embryonic development radiation effects, developmental gene expression regulation radiation effects, ultraviolet rays adverse effects, radiation dose response relationship, embryo metabolism and ultrastructure, embryonic development physiology, developmental gene expression regulation physiology, time factors, .

Drummond, I. (2003). **Making a zebrafish kidney: a tale of two tubes.** *Trends in Cell Biology* 13(7): 357-65. ISSN: 0962-8924.
NAL Call Number: QH573.T73
Abstract: The kidney can be thought of as the pairing of two tubes: an epithelial tube (the nephron), carrying filtered blood and engaged in ion and water transport; and endothelial tubes (the blood vessels), delivering blood and carrying away recovered solute. The development of the nephron presents several interesting questions. How does an epithelial tube form and how is it patterned into functionally distinct components and segments? What guides the interaction between the vasculature and kidney epithelia? How are epithelial cell shape and lumen diameter

maintained, and what goes wrong when kidney tubules balloon into cysts? Here, I outline the progress that has been made in answering these questions using the zebrafish pronephros as a simple, accessible model of nephron development.

Descriptors: zebrafish nephron development model, cell differentiation physiology, epithelial cells metabolism, kidney tubules blood supply and embryology, renal artery embryology, cell communication physiology, cell size physiology, epithelial cells ultrastructure, cystic kidney diseases etiology, pathology and physiopathology, kidney tubules ultrastructure, renal artery ultrastructure, renal circulation physiology.

Eimon, P.M., E. Kratz, E. Varfolomeev, S.G. Hymowitz, H. Stern, J. Zha, and A. Ashkenazi (2006). **Delineation of the cell-extrinsic apoptosis pathway in the zebrafish.** *Cell Death and Differentiation* 13(10): 1619-30. ISSN: 1350 9047.

NAL Call Number: QH671

Abstract: The mammalian extrinsic apoptosis pathway is triggered by Fas ligand (FasL) and Apo2 ligand/tumor necrosis factor (TNF)-related apoptosis-inducing ligand (Apo2L/TRAIL). Ligand binding to cognate receptors activates initiator caspases directly in a death-inducing signaling complex. In *Drosophila*, TNF ligand binding activates initiator caspases indirectly, through JNK. We characterized the extrinsic pathway in zebrafish to determine how it operates in a nonmammalian vertebrate. We identified homologs of FasL and Apo2L/TRAIL, their receptors, and other components of the cell death machinery. Studies with three Apo2L/TRAIL homologs demonstrated that they bind the receptors zHDR (previously linked to hematopoiesis) and ovarian TNFR (zOTR). Ectopic expression of these ligands during embryogenesis induced apoptosis in erythroblasts and notochord cells. Inhibition of zHDR, zOTR, the adaptor zFADD, or caspase-8-like proteases blocked ligand-induced apoptosis, as did antiapoptotic Bcl-2 family members. Thus, the extrinsic apoptosis pathway in zebrafish closely resembles its mammalian counterpart and cooperates with the intrinsic pathway to trigger tissue-specific apoptosis during embryogenesis in response to ectopic Apo2L/TRAIL expression.

Descriptors: zebrafish , apoptosis physiology, apoptosis genetics, developmental gene expression regulation, hematopoiesis, ligands, notochord cytology, signal transduction, metabolism, zebrafish proteins genetics and metabolism.

Engeszer, R.E., M.J. Ryan, and D.M. Parichy (2004). **Learned social preference in zebrafish.** *Current Biology* 14(10): 881-4. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: How social aggregations arise and persist is central to our understanding of evolution, behavior, and psychology. When social groups arise within a species, evolutionary divergence and speciation can result. To understand this diversifying role of social behavior, we must examine the internal and external influences that lead to nonrandom assortment of phenotypes. Many fishes form aggregations called shoals that reduce predation risk while enhancing foraging and reproductive success. Thus, shoaling is adaptive, and signals that maintain shoals are likely to evolve under selection. Given the diversity of pigment patterns among *Danio* fishes, visual signals might be especially important in mediating social behaviors in this group. Our understanding of pigment pattern development in the zebrafish *D. rerio* allows integrative analyses of how molecular variation leads to morphological variation among individuals and how morphological variation influences social interactions. Here, we use the zebrafish pigment mutant nacre/mitfa to test roles for genetic and environmental determinants in the development of shoaling preference. We demonstrate that individuals discriminate between shoals having different pigment pattern phenotypes and that early

experience determines shoaling preference. These results suggest a role for social learning in pigment pattern diversification in danios.
Descriptors: zebrafish pigment pattern descrimination, association learning physiology, DNA binding proteins genetics, pigmentation physiology, social behavior, transcription factors genetics, fish communication, DNA binding, genotype, microphthalmia associated transcription factor, mutation genetics, transcription factors physiology.

Esaki, M., K. Hoshijima, S. Kobayashi, H. Fukuda, K. Kawakami, and S. Hirose (2007). **Visualization in zebrafish larvae of Na(+) uptake in mitochondria-rich cells whose differentiation is dependent on foxi3a.** *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 292(1): R470-80. ISSN: 0363 6119.
Abstract: Uptake of Na(+) from the environment is an indispensable strategy for the survival of freshwater fish, as they easily lose Na(+) from the plasma to a diluted environment. Nevertheless, the location of and molecules involved in Na(+) uptake remain poorly understood. In this study, we utilized Sodium Green, a Na(+)-dependent fluorescent reagent, to provide direct evidence that Na(+) absorption takes place in a subset of the mitochondria-rich (MR) cells on the yolk sac surface of zebrafish larvae. Combined with immunohistochemistry, we revealed that the Na(+)-absorbing MR cells were exceptionally rich in vacuolar-type H(+)-ATPase (H(+)-ATPase) but moderately rich in Na(+)-K(+)-ATPase. We also addressed the function of foxi3a, a transcription factor that is specifically expressed in the H(+)-ATPase-rich MR cells. When foxi3a was depleted from zebrafish embryos by antisense morpholino oligonucleotide injection, differentiation of the MR cells was completely blocked and Na(+) influx was severely reduced, indicating that MR cells are the primary sites for Na(+) absorption. Additionally, foxi3a expression is initiated at the gastrula stage in the presumptive ectoderm; thus, we propose that foxi3a is a key gene in the control of MR cell differentiation. We also utilized a set of ion transport inhibitors to assess the molecules involved in the process and discuss the observations.
Descriptors: zebrafish larva, hepatocyte nuclear factor 3 gamma genetics, larva physiology, mitochondria metabolism, sodium metabolism, calcium physiology, cation transport proteins antagonists and inhibitors, cell differentiation drug effects, computer assisted image processing, fluorescent reagent, immunohistochemistry, in situ hybridization, morpholines pharmacology, antisense oligonucleotides pharmacology, organic chemicals, proton translocating ATPases metabolism, sodium radioisotopes diagnostic use, sodium hydrogen antiporter metabolism, yolk sac cytology and metabolism.

Evans, T.G., Z. Belak, N. Ovsenek, and P.H. Krone (2007). **Heat shock factor 1 is required for constitutive Hsp70 expression and normal lens development in embryonic zebrafish.** *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology* 146(1): 131-40. ISSN: 1095 6433.
NAL Call Number: QP1C6
Abstract: Heat shock factors (HSFs) are the major transcription factors responsible for heat-induced upregulation of heat shock protein (Hsp) genes. All three mammalian HSFs (HSF1, HSF2, HSF4) have also been shown to be required for normal mammalian development. It is currently unknown if HSFs play similarly important roles during normal development of non-mammalian vertebrates. In the present study, a morpholino modified antisense oligonucleotide (MO) approach targeted against hsf1 mRNA (hsf1-MO) was used to examine the requirement of HSF1 in zebrafish development. Embryos depleted of HSF1 displayed a reproducible small eye phenotype characterized by an immature lens and a disorganized retinal structure. These defects were strikingly similar to those observed when constitutive,

lens specific Hsp70 expression was reduced through the microinjection of MO targeting hsp70. The data suggest that HSF1 is involved in regulating constitutive lens specific expression of hsp70 in the embryonic zebrafish. This conclusion is supported by a marked reduction in Hsp70 protein in hsf1-MO injected embryos. Microinjection of MO targeted to hsf2 mRNA (hsf2-MO) did not result in a small eye phenotype in a significant number of embryos. These data also suggest that HSF1 and HSF2 play distinct roles in non-mammalian vertebrates, similarly to what has been demonstrated previously in mouse.
Descriptors: zebrafish embryo, physiology, HSP70 heat shock proteins biosynthesis, heat shock, crystalline lens embryology, electrophoretic mobility shift assay, developmental gene expression regulation, morpholines pharmacology, oligodeoxyribonucleotides, antisense pharmacology, phenotype, .

Fan, L. and P. Collodi (2006). **Zebrafish embryonic stem cells.** *Methods in Enzymology* 418: 64-77. ISSN: 0076-6879.
NAL Call Number: QP601M49
Abstract: Methods are presented for the derivation of zebrafish embryonic stem (ES) cell cultures that are initiated from blastula and gastrula stage embryos. To maintain pluripotency, the ES cells are cocultured with rainbow trout spleen cells from the RTS34st cell line. ES cells maintained for multiple passages on a feeder layer of growth-arrested RTS34st exhibit in vitro characteristics of pluripotency and produce viable germ cells following transplantation into a host embryo. The ES cells are able to undergo targeted plasmid insertion by homologous recombination, and methods are described for the introduction of a targeting vector by electroporation. Two strategies are described for the efficient isolation of homologous recombinants using a visual marker screen and positive-negative selection.
Descriptors: zebrafish embryos, embryonic stem cells cytology, blastula cytology and physiology, cell culture techniques methods, culture media embryonic development physiology, gastrula cytology and physiology.

Fekete, D.M. (2003). **Developmental biology. Rocks that roll zebrafish.** *Science* 302(5643): 241-2. ISSN: 0036-8075.
NAL Call Number: 470Sci2
Abstract: The vestibular organs of the inner ear of higher vertebrates control balance, and their counterparts in fish control both balance and hearing. Essential to the operation of these sensory organs are the biomineralized structures--otoconia in higher vertebrates or otoliths in fish--that deflect the sensory hair bundles situated beneath them. In her Perspective, Fekete explores the fascinating world of otolith biomineralization in zebrafish; revealing the importance of a protein called Starmaker for coordinating the shape and type of crystal in fish otoliths (Sollner et al.).
Descriptors: zebrafish, otolithic membrane physiology, physiologic calcification, calcium carbonate chemistry, crystallization, morphogenesis, musculoskeletal equilibrium, otolithic membrane chemistry, growth, development and ultrastructure, protein precursors chemistry, amino acid sequence homology, swimming, zebrafish anatomy, histology, proteins genetics.

Feng, J., S.H. Cheng, P.K. Chan, and H.H. Ip (2005). **Reconstruction and representation of caudal vasculature of zebrafish embryo from confocal scanning laser fluorescence microscopic images.** *Computers in Biology and Medicine* 35(10): 915-31. ISSN: 0010 4825.
NAL Call Number: RA409.5.C64
Abstract: Three-dimensional (3D) reconstruction from a series of sections is an important technique in medical imaging, particularly for visualization of blood vessels from angiogra-

phy. Here, we present a framework for automatic segmentation and registration of different kind of blood vessels from 2-day-old zebrafish embryos. Series of optical sections were acquired from confocal microscopy with the blood vessels labeled by fluorescent microbeads (0.02 microm) injected into blood stream of 2-day-old zebrafish embryos. Blood vessels were extracted and their morphological parameters, including length and diameter, were calculated. At the same time, individual blood vessels were registered automatically. Vasculature was represented by attributed vessel represent graph (AVRG), which contained morphological data and connectivity of every blood vessel. Using AVRG to represent a vasculature made the comparison between vasculatures of different embryos more easy. Visualization, as well as quantification, of reconstructed 3D model of AVRG was presented in an interactive interface. The framework was implemented by Visual C++ as Windows-based program.
Descriptors: zebrafish, blood vessels ultrastructure, cardiovascular system embryology, computer assisted image processing, confocal microscopy, fluorescence microscopy.

Gilland, E., R. Baker, and W. Denk (2003). **Long duration three-dimensional imaging of calcium waves in zebrafish using multiphoton fluorescence microscopy.** *Biological Bulletin, The* 205(2): 176-7. ISSN: 0006 3185.
NAL Call Number: 442.8B52
Descriptors: zebrafish embryos, calcium signaling physiology, metabolism, multiphoton fluorescence microscopy, .

Graham, C., P. Nalbant, B. Scholermann, H. Hentschel, R.K. Kinne, and A. Werner (2003). **Characterization of a type IIb sodium-phosphate cotransporter from zebrafish *(Danio rerio)* kidney.** *American Journal of Physiology. Renal Physiology* 284(4): F727-36. ISSN: 0363 6127.
NAL Call Number: 447.8Am3
Abstract: Zebrafish (*Danio rerio*) express two isoforms of the type IIb Na-dependent P(i) cotransporter (NaPi). Type NaPi-IIb1 has previously been cloned and characterized. Here, we report the cloning of the NaPi-IIb2 transcript from zebrafish kidney, its localization, and its functional characterization. RT-PCR with renal RNA and degenerate NaPi-IIb-specific primers resulted in a specific fragment. 3'-Rapid amplification of cDNA ends yielded a product that contained typical NaPi-IIb characteristics such as a cysteine-rich COOH terminus and a PDZ (PSD95- Dlg-zona occludens-1) binding motif. Several approaches were unsuccessful at cloning the 5' end of the transcript; products lacked an in-frame start codon. The missing information was obtained from an EST (GenBank accession number). The combined clone displayed a high degree of homology with published type IIb cotransporter sequences. Specific antibodies were raised against a COOH-terminal epitope of both NaPi-IIb1 and NaPi-IIb2 isoforms. Immunohistochemical mapping revealed apical expression of both isoforms in zebrafish renal and intestinal epithelia, as well as in bile ducts. The novel clone was expressed in oocytes, and function was assayed by the two-electrode voltage-clamp technique. The function of the new NaPi-IIb2 clone was found to be significantly different from NaPi-IIb1 despite strong structural similarities. NaPi-IIb2 was found to be strongly voltage sensitive, with higher affinities for both sodium and phosphate than NaPi-IIb1. Also, NaPi-IIb2 was significantly less sensitive to external pH than NaPi-IIb1. The strong structural similarity but divergent function makes these zebrafish transporters ideal models for the molecular mapping of functionally important regions in the type II NaPi-cotransporter family.
Descriptors: zebrafish kidney metabolism, symporters chemistry and genetics, amino acid sequence, molecular cloning, flounder, hydrogen ion concentration, immunohistochemistry, membrane potentials physiology, mice, microinjections, molecular sequence data, oocytes

metabolism, organ specificity, patch clamp techniques, phosphates chemistry, rats, sequence alignment, sodium chemistry, sodium phosphate cotransporter proteins, Type II sodium phosphate cotransporter proteins, Type IIb sodium phosphate cotransporter proteins, symporters biosynthesis, Xenopus laevis, zebrafish proteins biosynthesis, chemistry and genetics.

Grillitsch, S., N. Medgyesy, T. Schwerte, and B. Pelster (2005). **The influence of environmental P(O(2)) on hemoglobin oxygen saturation in developing zebrafish *Danio rerio*.** *Journal of Experimental Biology* 208(Pt 2): 309-16. ISSN: 0022-0949.
NAL Call Number: 442.8B77
Abstract: Several studies suggest that during early larval development of lower vertebrates convective blood flow is not essential to supply oxygen to the tissues, but information about the oxygenation status of larvae during the time of cutaneous respiration is still missing. If convective oxygen transport contributes to the oxygen supply to tissues, venous blood in the central circulatory system should be partly deoxygenated, and hyperoxia should increase the oxygen saturation of the hemoglobin. To analyze the changes in hemoglobin oxygen saturation induced by hyperoxic incubation, zebrafish larvae were incubated in a tiny chamber between polytetrafluoroethylene membranes (Teflon), so that the oxygen supply could be rapidly modified. Hemoglobin oxygen saturation was measured in vivo by combining video imaging techniques with a spectrophotometrical analysis of hemoglobin light absorption at specific wavelengths for maximal absorption of oxygenated and deoxygenated blood (413 nm and 431 nm, respectively) under normoxic conditions and after a 10 min period of hyperoxia (P(O(2))=100 kPa), assuming that at a P(O(2)) of 100 kPa the hemoglobin is fully saturated. The results demonstrated that red blood cell oxygenation of zebrafish larvae at 4 days post fertilization (d.p.f.), 5 d.p.f. and 12 d.p.f. could be increased by hyperoxia. The data suggest that at the time of yolk sac degradation (i.e. 4 d.p.f. and 5 d.p.f.), when the total surface area of the animal is reduced, bulk diffusion of oxygen may not be sufficient to prevent a partial deoxygenation of the hemoglobin. The decrease in hemoglobin oxygenation observed at 12 d.p.f. confirms earlier studies indicating that at 12-14 d.p.f., convective oxygen transport becomes necessary to ensure oxygen supply to the growing tissues.
Descriptors: zebrafish embryos, hemoglobins metabolism, oxygen blood, zebrafish blood, analysis of variance, diffusion, heart rate, larva metabolism, oxygen consumption physiology, partial pressure, spectrophotometry, video recording.

Guan, M., T. Zhang, and D.M. Rawson (2006). **Effects of improved controlled slow cooling protocols on the survival of zebrafish (*Danio rerio*) oocytes**. *Cryobiology* 53(3): 377-378 . ISSN: 0011-2240.
NAL Call Number: QH324.C7
Descriptors: zebrafish, cryobiology, cryopreservation, freshwater fish, oocytes, survival, *Danio rerio.*

Hagedorn, M., A. Peterson, P. Mazur, and F.W. Kleinhans (2004). **High ice nucleation temperature of zebrafish embryos: slow-freezing is not an option.** *Cryobiology* 49(2): 181-9. ISSN: 0011 2240.
NAL Call Number: QH324.C7
Abstract: Although fish embryos have been used in a number of slow-freezing cryopreservation experiments, they have never been successfully cryopreserved. In part this is because little is known about whether ice forms within the embryo during the slow-freezing dehydration process. Therefore, we examined the temperature of intraembryonic ice formation (T(IIF)) and the temperature of extraembryonic ice formation (T(EIF)), using a cryomicroscope. We

used both unmodified zebrafish embryos and those with water channels (aquaporin-3 or AQP3) inserted into their membranes to increase permeability to water and cryoprotectants, examined at 100% epiboly to the 6-somite stage. In these experiments we examined: (1) the spontaneous freezing of (external) solutions; (2) the spontaneous freezing of solutions containing embryos; (3) the effect of preloading the embryos with cryoprotectants on T(IIF); (4) whether preloading the embryos with cryoprotectant helps in survival after nucleating events in the solution; and (5) the damaging effects of extracellular nucleation events versus solution toxicity on the embryos. The solutes alone (embryo medium--EM, sucrose culture medium, 1 M propylene glycol in EM, and 1 M propylene glycol in a sucrose culture medium) froze at -14.9 +/- 1.1, -17.0 +/- 0.3, -17.8 +/- 1.0, and -17.7 +/- 1.4, respectively. There was no difference amongst these means (P > 0.05), thus adding cryoprotectant did not significantly lower the nucleation point. Adding embryos (preloaded with cryoprotectant or not) did not change the basic freezing characteristics of these solutes. In all these experiments, (T(EIF)) equaled (T(IIF)), and there was no difference in the freezing point of the solutions with or without the embryos (P > 0.05). Additionally, there was no difference in the freezing characteristics of embryos with and without aquaporins (P > 0.05). The formation of intra-embryonic ice was lethal to the zebrafish embryos in all cases. But this lethal outcome was not related to solution injury effects, because 88-98% of embryos survived when exposed to a higher solute concentration with no ice present. Taken together, these data suggest that slow-freezing is not a suitable option for zebrafish embryos. The mechanism of this high temperature nucleation event in zebrafish embryos is still unknown.
Descriptors: zebrafish embryology, cryopreservation methods, cryoprotective agents, ice.

Hammer, J.A. and X.S. Wu (2007). **Organelle motility: running on unleadened**. *Current Biology* 17(23): R1017-R1019. ISSN: 0960-9822.
NAL Call Number: QH301.C85
Descriptors: zebrafish, melanocytes, Motility, organelles, running, *Danio rerio.*

Harris, J.A., A.G. Cheng, L.L. Cunningham, G. MacDonald, D.W. Raible, and E.W. Rubel (2003). **Neomycin-induced hair cell death and rapid regeneration in the lateral line of zebrafish (*Danio rerio*).** *Journal of the Association for Research in Otolaryngology* 4(2): 219-34. ISSN: 1525-3961.
Abstract: Mechanoreceptive hair cells are extremely sensitive to aminoglycoside antibiotics, including neomycin. Hair cell survival was assessed in larval wild-type zebrafish lateral line neuromasts 4 h after initial exposure to a range of neomycin concentrations for 1 h. Each of the lateral line neuromasts was scored in live fish for the presence or absence of hair cells using the fluorescent vital dye DASPEI to selectively label hair cells. All neuromasts were devoid of DASPEI-labeled hair cells 4 h after 500 microM neomycin exposure. Vital DASPEI staining was proportional to the number of hair cells per neuromast identified in fixed larvae using immunocytochemistry for acetylated tubulin and phalloidin labeling. The time course of hair cell regeneration in the lateral line neuromasts was also analyzed following neomycin-induced damage. Regenerated hair cells were first observed using live DASPEI staining 12 and 24 h following neomycin treatment. The potential role of proliferation in regenerating hair cells was analyzed. A 1 h pulse-fix protocol using bromodeoxyuridine (BrdU) incorporation was used to identify S-phase cells in neuromasts. BrdU incorporation in neomycin-damaged neuromasts did not differ from control neuromasts 4 h after drug exposure but was dramatically upregulated after 12 h. The proliferative cells identified during a 1 h period at 12 h after neomycin treatment were able to give rise to new hair cells by 24-48 h after drug treatment. The results presented here provide a standardized prepara-

tion for studying and identifying genes that influence vertebrate hair cell death, survival, and regeneration following ototoxic insults.

Descriptors: zebrafish hair cell model, hair cells drug effects and physiology, neomycin poisoning, regeneration, acetylation, actins metabolism, bromodeoxyuridine, cell death, coloring agents, drug dose response relationship, hair cells ultrastructure, larva, scanning electron microscopy, neomycin administration and dosage, phalloidine, pyridinium compounds, staining and labeling, tubulin metabolism, .

Hentschel, D.M., M. Mengel, L. Boehme, F. Liebsch, C. Albertin, J.V. Bonventre, H. Haller, and M. Schiffer (2007). **Rapid screening of glomerular slit diaphragm integrity in larval zebrafish.** *American Journal of Physiology. Renal Physiology* 293(5): F1746-50. ISSN: 0363 6127.

NAL Call Number: 447.8Am3

Abstract: Gene array-type experiments have identified large numbers of genes thought to be important for the integrity of the glomerular slit diaphragm. Confirmation of individual proteins has been limited by the expenses and time involved in generating transgenic or knockout mice for each candidate. We present a functional screening assay based on the clearance of a 70-kDa fluorescent dextran in another vertebrate system that is rapid and low in cost. In the pronephric glomerulus of larval zebrafish, we have demonstrated quantifiable loss of slit diaphragm integrity in a zebrafish model of puromycin aminonucleoside (PA) toxicity. In addition, after knockdown of CD2-associated protein (CD2AP) and podocin, two well-characterized genetic contributors to podocyte differentiation in mammals, we observed glomerular loss of serum macromolecules similar to that seen in mammalian kidneys with inborn mutations in these genes. Increased filtration of 70-kDa FITC-labeled dextran correlates with effacement of podocyte foot processes in ultrastructural analysis. These findings document the value of the zebrafish model in genomics and pharmacological screening applications.

Descriptors: zebrafish gene array-type experiments, diagnostic techniques, urological, kidney diseases diagnosis, kidney glomerulus drug effects, metabolism and pathology, signal transducing adaptor cytoskeletal dextrans pharmacokinetics, chemically induced edema, fluorescein 5 isothiocyanate analogs, derivatives and pharmacokinetics, gene deletion, injections, intracellular signaling peptides and kidney diseases genetics, metabolism and pathology, larva anatomy and histology, macromolecular substances metabolism, membrane electron microscopy, podocytes pathology, puromycin aminonucleoside administration and dosage and poisoning, time factors.

Hentschel, D.M., Park Kwon Moo, L. Cilenti, A.S. Zervos, I. Drummond, and J.V. Bonventre (2005). **Acute renal failure in zebrafish: a novel system to study a complex disease**. *American Journal of Physiology* 288(5(2)): F923-F929. ISSN: 0002 9513.

NAL Call Number: 447.8Am3

Abstract: Acute renal failure (ARF) is characterized by a very high mortality essentially unchanged over the past 40 years. Simple vertebrate models are needed to improve our understanding of ARF and facilitate the development of novel therapies for this clinical syndrome. Here, we demonstrate that gentamicin, a commonly used nephrotoxic antibiotic, causes larval zebrafish to develop ARF characterized by histological and functional changes that mirror aminoglycoside toxicity in higher vertebrates and inability of zebrafish to maintain fluid homeostasis. We developed a novel method to quantitate renal function in larval zebrafish and demonstrate a decline in glomerular filtration rate after gentamicin exposure. The antineoplastic drug cisplatin, whose use in humans is limited by kidney toxic-

ity, also causes typical histological changes and a decline in renal function in larval zebrafish. A specific inhibitor of Omi/HtrA2, a serine protease implicated in cisplatin-induced apoptosis, prevented renal failure and increased survival. This protective effect was confirmed in a mouse model of cisplatin-induced nephrotoxicity. Therefore, zebrafish provides a unique model system, amenable to genetic manipulation and drug screening, to explore the pathophysiology of ARF and establish novel therapies with potential use in mammals. Copyright © 2008 CABI

Descriptors: zebrafish acute renal failure model, aminoglycoside antibiotics, animal models, animal testing alternatives, antibacterial agents, antibiotics, apoptosis, drug development, gentamicin, glomerular filtration rate, kidneys, laboratory animals, larvae, nephrotoxicity, renal failure, renal function, serine proteinases, toxicity, zebrafish.

Herbomel, P. and J.P. Levraud (2005). **Imaging early macrophage differentiation, migration, and behaviors in live zebrafish embryos.** *Methods in Molecular Medicine* 105: 199-214. ISSN: 1543-1894.

Abstract: Because zebrafish embryos are transparent, cell behaviors and interactions can be directly imaged noninvasively in live embryos using differential interference contrast-Nomarski light microscopy. We found that the imaging quality can be much improved by coupling differential interference contrast-Nomarski to true (analogical) color video so as to visualize the image in real time on a high-resolution colour video monitor. We explain here how to apply this approach to the in vivo imaging of embryonic macrophages, which constitute a distinct early macrophage lineage, that originates from the rostral-most lateral mesoderm--adjacent to the cardiac field, differentiate in the yolk sac, and rapidly spread in embryonic tissues, although still retaining proliferative capacity.

Descriptors: zebrafish embryos, cell differentiation physiology, cell movement physiology, macrophages physiology, confocal and video microscopy methods.

Higgs, D.M., A.K. Rollo, M.J. Souza, and A.N. Popper (2003). **Development of form and function in peripheral auditory structures of the zebrafish (*Danio rerio*).** *Journal of the Acoustical Society of America* 113(2): 1145-54 . ISSN: 0001-4966.

Abstract: Investigations of the development of auditory form and function have, with a few exceptions, thus far been largely restricted to birds and mammals, making it difficult to postulate evolutionary hypotheses. Teleost fishes represent useful models for developmental investigations of the auditory system due to their often extensive period of posthatching development and the diversity of auditory specializations in this group. Using the auditory brainstem response and morphological techniques we investigated the development of auditory form and function in zebrafish (*Danio rerio)* ranging in size from 10 to 45 mm total length. We found no difference in auditory sensitivity, response latency, or response amplitude with development, but we did find an expansion of maximum detectable frequency from 200 Hz at 10 mm to 4000 Hz at 45 mm TL. The expansion of frequency range coincided with the development of Weberian ossicles in zebrafish, suggesting that changes in hearing ability in this species are driven more by development of auxiliary specializations than by the ear itself. We propose a model for the development of zebrafish hearing wherein the Weberian ossicles gradually increase the range of frequencies available to the inner ear, much as middle ear development increases frequency range in mammals.

Descriptors: zebrafish auditory form and function, evoked potentials, auditory, brain stem physiology, evolution, hearing physiology, growth and development, age factors, auditory threshold physiology, biometry, brain stem growth and development, ear ossicles growth and

development, larva growth and development, pitch discrimination physiology, species specificity.

Hirata, H. (2007). **Locomotion research with zebrafish**. *Japanese Journal of Psychopharmacology* 27(3): 127-34. ISSN: 0285-5313.

Abstract: Recent genetic analysis of behavior was successfully performed in *C. elegans* and *Drosophila*. However, since the organization and morphology of their nervous system is different from those of vertebrates, it is unclear how applicable these findings are for vertebrates. Additionally, electrophysiological analysis is difficult in these invertebrates. On the other hand, electrophysiology is well established in vertebrate animals, such as mice and *Xenopus*, but large-scale mutagenesis is costly and difficult with these vertebrates. I started a genetic analysis of behavior in zebrafish to overcome these difficulties. First, zebrafish can be studied using large-scale mutagenesis. Second, electrophysiological methods are available to analyze the CNS, NMJ and muscles in zebrafish. Third, the early behaviors are simple and stereotyped in zebrafish embryos. Fourth, zebrafish embryos are transparent making it possible to visualize dynamic events in vivo such as Ca transients and axon outgrowth. These advantages make the zebrafish an ideal research tool for functional development of neurons and muscles. I characterized accordion and bandoneon mutants, which were defective in calcium pump in muscle and glycine receptor in the CNS, respectively. Interestingly, both of these mutants exhibited similar simultaneous bilateral muscle contraction and shortening of the tail in response to touch.

Descriptors: zebrafish disease model, locomotion, movement disorders genetics, skeletal muscle physiology and physiopathology, calcium metabolism and transporting ATPases genetics, central nervous system, disease models, electrophysiology, motor neurons physiology, muscle contraction, mutation, glycine receptors genetics.

Language of Text: Japanese.

Hirayama, J., M. Kaneko, L. Cardone, G. Cahill, and P. Sassone Corsi (2005). **Analysis of circadian rhythms in zebrafish.** *Methods in Enzymology* 393: 186-204. ISSN: 0076-6879.

NAL Call Number: QP601M49

Abstract: The zebrafish probably constitutes the best animal system to study the complexity of the circadian clock machinery and the influence that light has on it. The possibilities of producing transgenic fishes, to establish light-responsive cultured cells, and to directly explore light phototransduction on single clock cells are all remarkable features of this circadian system. This article describes some of the most useful methodologies to analyze the behavioral, cellular, and molecular aspects of the zebrafish circadian clock system.

Descriptors: zebrafish, circadian rhythm, clock, light, cells, molecular changes.

Hodel, C., S.C. Neuhauss, and O. Biehlmaier (2006). **Time course and development of light adaptation processes in the outer zebrafish retina.** *Anatomical Record. Part A, Discoveries in Molecular, Cellular, and Evolutionary Biology* 288(6): 653-62. ISSN: 1552 4884.

Abstract: Retinomotor movements are morphological changes in the outer retina in response to changing light conditions. They can be separated into two components: Migration of pigment granules within the microvilli of the retinal pigment epithelium (RPE) and positional changes in photoreceptor cells. These positional changes optimize exposure of the cone and rod photoreceptors to light. The aim of this study was to analyze both the time course of retinomotor movements in the adult zebrafish and the maturation of these processes in the developing fish. We show that retinomotor movements are used as a dark/light adaptation mechanism in zebrafish. In adult zebrafish, melanin granules of the RPE migrate

with constant speed and reach the fully light adapted (LA) state approximately after 1 h. In contrast, about two thirds of double cone outer segment movements are finished in 5 min, and are fully completed in 10 to 20 min. During development there are three crucial stages leading to mature retinomotor movements in response to light: at 5 dpf (days post fertilization) the migration of pigment granules begins, at 20 dpf the pigment granules condense in the apical part of the RPE microvilli, and at 28 dpf, concomitant with the functional maturation of rods, the double cones contract as in adult retinas.
Descriptors: zebrafish, adaptation, ocular physiology, retina cones physiology, retina physiology, cell movement, retina cones cytology, cytoplasmic granules physiology, dark adaptation, pigment epithelium of eye cytology and physiology, retina cytology, retinal pigments physiology, time factors, zebrafish anatomy and histology.

Horng, J.L., L.Y. Lin, C.J. Huang, F. Katoh, T. Kaneko, and P.P. Hwang (2007). **Knockdown of V-ATPase subunit A (atp6v1a) impairs acid secretion and ion balance in zebrafish (*Danio rerio*).** *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 292(5): R2068-76. ISSN: 0363 6119.
Abstract: In the skin of zebrafish embryo, the vacuolar H(+)-ATPase (V-ATPase, H(+) pump) distributed mainly in the apical membrane of H(+)-pump-rich cells, which pump internal acid out of the embryo and function similarly to acid-secreting intercalated cells in mammalian kidney. In addition to acid excretion, the electrogenic H(+) efflux via the H(+)-ATPases in the gill apical membrane of freshwater fish was proposed to act as a driving force for Na(+) entry through the apical Na(+) channels. However, convincing molecular physiological evidence in vivo for this model is still lacking. In this study, we used morpholino-modified antisense oligonucleotides to knockdown the gene product of H(+)-ATPase subunit A (atp6v1a) and examined the phenotype of the mutants. The H(+)-ATPase knockdown embryos revealed several abnormalities, including suppression of acid-secretion from skin, growth retardation, trunk deformation, and loss of internal Ca(2+) and Na(+). This finding reveals the critical role of H(+)-ATPase in embryonic acid -secretion and ion balance, as well.
Descriptors: zebrafish, acids metabolism, proton translocating ATPases deficiency, vacuolar proton translocating ATPases genetics and metabolism, water electrolyte balance , metabolism, fresh water, enzymologic gene expression regulation, mutation, protein subunits deficiency, protein subunits genetics, protein subunits metabolism, proton translocating ATPases chemistry, genetics and metabolism, protons, vacuolar proton translocating ATPases deficiency, yolk sac metabolism.

Hove, J.R. (2004). **In vivo biofluid dynamic imaging in the developing zebrafish.** *Birth Defects Research* 72(3): 277-89. ISSN: 1542 0752.
NAL Call Number: QL991.T4
Descriptors: zebrafish biofluid flow in embryos, cardiovascular physiology, cardiovascular system embryology, three dimensional imaging, regional blood flow physiology, models, .
Notes: A review.

Hsu, E. and M.F. Criscitiello (2006). **Diverse immunoglobulin light chain organizations in fish retain potential to revise B cell receptor specificities**. *Journal of Immunology* 177(4): 2452-2462. ISSN: 0022 1767.
NAL Call Number: 448.8J8232
Abstract: We have characterized the genomic organization of the three zebrafish L chain isotypes and found they all differed from those reported in other teleost fishes. Two of the

zebrafish L chain isotypes are encoded by two loci, each carrying multiple V gene segments. To understand the derivation of these L chain genes and their organizations, we performed phylogenetic analyses and show that IgL organization can diverge considerably among closely related species. Except in zebrafish, the teleost fish IgL each contain only two to four recombinogenic components (one to three V, one J) and exist in multiple copies. BCR heterogeneity can be generated, but this arrangement apparently provides neither combinatorial diversification nor an opportunity for the secondary rearrangements that, in mammals, take place during receptor editing, a process crucial to the promotion of tolerance in developing lymphocytes. Examination of the zebrafish IgL recombination possibilities gave insight into how the suppression of self-reactivity by receptor editing might be managed, including in miniloci. We suggest that, despite the diverse IgL organizations in early and higher vertebrates, two elements essential to generating the Ab repertoire are retained: the numerous genes/loci for ligand-binding diversification and the potential for correcting unwanted specificities that arise. Copyright © 2008 CABI

Descriptors: zebrafish, B lymphocytes, genes, heterogeneity, immunoglobulins, isotypes, loci, phylogenetics, *Osteichthyes*.

Hu, G., J. Tang, B. Zhang, Y. Lin, J. Hanai, J. Galloway, V. Bedell, N. Bahary, Z. Han, R. Ramchandran, B. Thisse, C. Thisse, L.I. Zon , and V.P. Sukhatme (2006). **A novel endothelial-specific heat shock protein HspA12B is required in both zebrafish development and endothelial functions in vitro.** *Journal of Cell Science* 119(Pt 19): 4117-26. ISSN: 0021-9533.

NAL Call Number: QH301.J6

Abstract: A zebrafish transcript dubbed GA2692 was initially identified via a whole-mount in situ hybridization screen for vessel specific transcripts. Its mRNA expression during embryonic development was detected in ventral hematopoietic and vasculogenic mesoderm and later throughout the vasculature up to 48 hours post fertilization. Morpholino-mediated knockdown of GA2692 in embryos resulted in multiple defects in vasculature, particularly, at sites undergoing active capillary sprouting: the intersegmental vessels, sub-intestinal vessels and the capillary sprouts of the pectoral fin vessel. During the course of these studies, a homology search indicated that GA2692 is the zebrafish orthologue of mammalian HspA12B, a distant member of the heat shock protein 70 (Hsp70) family. By a combination of northern blot and real-time PCR analysis, we showed that HspA12B is highly expressed in human endothelial cells in vitro. Knockdown of HspA12B by small interfering RNAs (siRNAs) in human umbilical vein endothelial cells blocked wound healing, migration and tube formation, whereas overexpression of HspA12B enhanced migration and accelerated wound healing - data that are consistent with the in vivo fish phenotype obtained in the morpholino-knockdown studies. Phosphorylation of Akt was consistently reduced by siRNAs against HspA12B. Overexpression of a constitutively active form of Akt rescued the inhibitory effects of knockdown of HspA12B on migration of human umbilical vein endothelial cells. Collectively, our data suggests that HspA12B is a highly endothelial-cell-specific distant member of the Hsp70 family and plays a significant role in endothelial cells during development and angiogenesis in vitro, partially attributable to modulation of Akt phosphorylation.

Descriptors: zebrafish embryo in vitro studies, vascular endothelium physiology, HSP70 heat shock heat shock, alkaline phosphatase metabolism, cell movement drug effects and genetics, cultured cells, coronary angiography methods embryonic development genetics, endothelial cells metabolism, developmental gene expression regulation, HSP70 heat shock and genetics, physiologic neovascularization genetics, antisense oligonucleotides pharma-

cology, oncogene protein v akt metabolism, phosphorylation, RNA interference, small interfering RNA adverse effects, transfection.

Huang, H., B. Ju, K.Y. Lee, and S. Lin (2003). **Protocol for nuclear transfer in zebrafish.** *Cloning and Stem Cells* 5(4): 333-7. ISSN: 1536 2302.
NAL Call Number: QH442.2.C57
Descriptors: zebrafish, cloning, organism methods, nuclear transfer techniques, cell cycle, cultured cells, electroporation methods, embryo transfer, fibroblasts cytology and physiology , microinjections methods, oocytes physiology.

Huang, L., H. Maaswinkel, and L. Li (2005). **Olfactoretinal centrifugal input modulates zebrafish retinal ganglion cell activity: a possible role for dopamine-mediated Ca2+ signalling pathways.** *Journal of Physiology* 569(Pt 3): 939-48. ISSN: 0022-3751.
NAL Call Number: 447.8J82
Abstract: The vertebrate retina receives centrifugal input from the brain. In zebrafish, the major centrifugal input originates in the terminal nerve (TN). TN cell bodies are located in the olfactory bulb and ventral telencephalon. The TN projects axons to the retina where they branch in the inner plexiform layer (IPL) and synapse onto several inner retinal cell types, including dopaminergic interplexiform cells (DA-IPCs). This olfactoretinal centrifugal input plays a role in modulating retinal ganglion cell (RGC) activity, probably via dopamine-mediated Ca2+ signalling pathways. Normally, dopamine inhibits RGC firing by decreasing the inward Ca2+ current. Olfactory stimulation with amino acids decreases dopamine release in the retina, thereby reducing dopaminergic inhibition of RGCs. This model of olfacto-visual integration was directly tested by recording single-unit RGC activity in response to olfactory stimulation in the presence or absence of dopamine receptor blockers. Stimulation of the olfactory neurones increased RGC activity. However, this effect diminished when the dopamine D1 receptors were pharmacologically blocked. In isolated RGCs, the application of dopamine or a dopamine D1 receptor agonist decreased voltage-activated Ca2+ current and lowered Ca2+ influx. Together, the data suggest that olfactory input has a modulatory effect on RGC firing, and that this effect is mediated by dopamine D1 receptor-coupled Ca2+ signalling pathways.
Descriptors: zebrafish, calcium signaling physiology, dopamine metabolism, retinal ganglion cells metabolism, R 2,3,4,5 tetrahydro 8 chloro 3 methyl 5 phenyl 1H 3 benzazepin 7 ol pharmacology, amino acids pharmacology, calcium signaling drug effects, dopamine pharmacology, dopamine antagonists pharmacology, evoked potentials drug effects, methionine pharmacology, models, neural inhibition, olfactory pathways drug effects and metabolism, photic stimulation, dopamine D1 receptors drug effects and metabolism, retinal ganglion cells drug effects, sensory thresholds drug effects, synaptic transmission drug effects and physiology.

Huang, Y.Y. and S.C. Neuhauss (2008). **The optokinetic response in zebrafish and its applications.** *Frontiers in Bioscience: a Journal and Virtual Library* 13: 1899-916. ISSN: 1093-9946.
NAL Call Number: QH301.F76
Abstract: The optokinetic response (OKR) is a stereotyped eye movement in response to movement in he surround. The OKR serves to stabilize the visual image on the retina, and allows for high resolution vision. Due to its high selection value, all vertebrates display this basic behavior. Here, we review the properties of the OKR with a focus on the zebrafish, including methodological aspects of measuring eye movements in small larvae. The genetic amenabilities of the zebrafish model permit the use of this reflexive behavior in genetic

screens. Such approaches have led to the isolation of mutant strains with specific defects in the visual pathway. In addition to the use of the OKR as a screening assay, mutations with characteristic abnormalities in the execution of this behavior will enable the analysis of sensory-motor control in great detail. A case in point is the belladonna mutation, where an axonal misrouting effect at the optic chiasm leads to a reversed OKR with a number of interesting properties.

Descriptors: zebrafish optokinetic response, eye metabolism and movements, ocular physiology, kinetics, mutation, saccades physiology, vision, visual pathways, .

Isayeva, A., T. Zhang, and D.M. Rawson (2004). **Studies on chilling sensitivity of zebrafish (*Danio rerio*) oocytes.** *Cryobiology* 49(2): 114-22. ISSN: 0011 2240.

NAL Call Number: QH324.C7

Abstract: Human activity in the last few decades has had a devastating effect on the diversity of fresh water and marine fish. Further decline of fish population may have serious economic and ecological consequences. One of the most promising techniques to preserve fish population is to cryopreserve their germ cells. Cryopreservation has been successfully applied to fish sperm of many species, but there has been no success with fish embryo cryopreservation and fish oocyte cryopreservation has never been studied systematically. The aim of this study is to investigate the chilling sensitivity of fish oocytes. Experiments were conducted with zebrafish stage III (vitellogenic) and stage V (mature) oocytes, which were chilled at 10, 5, 0, -5 or -10 degrees C for 15 or 60 min using a low temperature bath. Control oocytes were kept at room temperature at 22 degrees C. Oocyte viability was assessed using three different methods: trypan blue staining (TB), thiazolyl blue tetrazolium bromide (MTT) staining and observation of germinal vesicle breakdown (GVBD). The results showed that zebrafish oocyte are very sensitive to chilling and their survival decreased with decreasing temperature and increasing exposure time periods. Normalised survivals assessed with TB staining after exposure to 0, -5 or -10 degrees C for 15 or 60 min were 90.1+/-6.0, 77.8+/-7.6, and 71.2+/-9.3%, and 60.2+/-3.8, 49.6+/-6.7, and 30.4+/-3.0%, respectively. The study found that the sensitivity of viability assessment methods increase in the order of MTT < TB < GVBD. It was found that stage III oocytes were more susceptible to chilling than stage V oocytes, and that individual female had a significant influence ($p < 0.0001$) on oocyte chilling sensitivity. Zebrafish oocyte chilling sensitivity may also be one of the limiting factors for development of protocol of their cryopreservation.

Descriptors: zebrafish oocytes, cryopreservation methods , oocytes cytology, growth and development , cell survival, temperature, time factors.

Jonz, M.G., I.M. Fearon, and C.A. Nurse (2004). **Neuroepithelial oxygen chemoreceptors of the zebrafish gill.** *Journal of Physiology* 560(Pt 3): 737-52. ISSN: 0022-3751.

NAL Call Number: 447.8J82

Abstract: In aquatic vertebrates, hypoxia induces physiological changes that arise principally from O(2) chemoreceptors of the gill. Neuroepithelial cells (NECs) of the zebrafish gill are morphologically similar to mammalian O(2) chemoreceptors (e.g. carotid body), suggesting that they may play a role in initiating the hypoxia response in fish. We describe morphological changes of zebrafish gill NECs following in vivo exposure to chronic hypoxia, and characterize the cellular mechanisms of O(2) sensing in isolated NECs using patch-clamp electrophysiology. Confocal immunofluorescence studies indicated that chronic hypoxia (P(O(2)) = 35 mmHg, 60 days) induced hypertrophy, proliferation and process extension in NECs immunoreactive for serotonin or synaptic vesicle protein (SV2). Under voltage clamp, NECs responded to hypoxia (P(O(2)) = 25-140 mmHg) with a dose-dependent decrease

in K(+) current. The current-voltage relationship of the O(2)-sensitive current (I(KO(2))) reversed near E(K) and displayed open rectification. Pharmacological characterization indicated that I(KO(2)) was resistant to 20 mM tetraethylammonium (TEA) and 5 mM 4-aminopyridine (4-AP), but was sensitive to 1 mm quinidine. In current-clamp recordings, hypoxia produced membrane depolarization associated with a conductance decrease; this depolarization was blocked by quinidine, but was insensitive to TEA and 4-AP. These biophysical and pharmacological characteristics suggest that hypoxia sensing in zebrafish gill NECs is mediated by inhibition of a background K(+) conductance, which generates a receptor potential necessary for neurosecretion and activation of sensory pathways in the gill. This appears to be a fundamental mechanism of O(2) sensing that arose early in vertebrate evolution, and was adopted later in mammalian O(2) chemoreceptors.
Descriptors: zebrafish , chemoreceptors physiology, gills physiology, oxygen physiology, gills drug effects and pathology, hypoxia, brain physiopathology, membrane potentials drug effects and physiology, neuroepithelial cells drug effects, physiology and pathology, quinidine pharmacology.

Jonz, M.G., I.M. Fearon, and C.A. Nurse (2003). **Potential oxygen sensing pathways in the zebrafish gill.** *Advances in Experimental Medicine and Biology* 536: 217-23. ISSN: 0065 2598.
NAL Call Number: QP901.A33
Descriptors: zebrafish, gills metabolism, oxygen metabolism, chemoreceptors metabolism, epithelial cells metabolism, fluorescent antibody technique, gills innervation, afferent neurons metabolism, patch clamp techniques, serotonin metabolism.

Jonz, M.G. and C.A. Nurse (2005). **Development of oxygen sensing in the gills of zebrafish.** *Journal of Experimental Biology* 208(Pt 8): 1537-49. ISSN: 0022-0949.
NAL Call Number: 442.8B77
Abstract: Previous studies have described the morphology, innervation and O(2)-chemoreceptive properties of neuroepithelial cells (NECs) of the zebrafish gill filaments. The present work describes the ontogenesis of these cells, and the formation of functional O(2)-sensing pathways in developing zebrafish. Confocal immunofluorescence was performed on whole-mount gill preparations using antibodies against serotonin (5-HT) and a zebrafish-derived neuronal marker (zn-12) to identify the appearance and innervation of gill NECs during larval stages. NECs were first expressed in gill filament primordia of larvae at 5 days postfertilization (d.p.f.) and were fully innervated by 7 d.p.f. In vivo ventilation frequency analysis revealed that a behavioural response to hypoxia (11.2+/-2.8 min(-1)) developed in embryos as early as 2 d.p.f., and a significant increase (P<0.05) in the ventilatory response to hypoxia (200.8+/-23.0 min(-1)) coincided with innervation of NECs of the filaments. In addition, exogenous application of quinidine, a blocker of O(2)-sensitive background K(+) channels in NECs, induced hyperventilation in adults in a dose-dependent manner and revealed the development of a quinidine-sensitive ventilatory response in 7 d.p.f. larvae. This study shows that NEC innervation in the gill filaments may account for the development of a functional O(2)-sensing pathway and the hyperventilatory response to hypoxia in zebrafish larvae. At earlier stages, however, O(2)-sensing must occur through another pathway. The possibility that a new type of 5-HT-positive NEC of the gill arches may account for this earlier hypoxic response is discussed.
Descriptors: zebrafish, chemoreceptors physiology, oxygen physiology, analysis of variance, chemoreceptors embryology, drug dose response relationship, gills innervation, embryol-

ogy and physiology, fluorescence microscopy, neuroepithelial cells physiology and cytology, oxygen consumption drug effects, quinidine pharmacology.

Jonz, M.G. and C.A. Nurse (2003). **Neuroepithelial cells and associated innervation of the zebrafish gill: a confocal immunofluorescence study.** *Journal of Comparative Neurology* 461(1): 1-17. ISSN: 0021-9967.
Descriptors: zebrafish, chemoreceptors chemistry, epithelial cells chemistry, gills cytology and innervation, neurons chemistry, biological markers analysis, epithelium anatomy, histology, chemistry and innervation, fluorescent antibody technique, membrane glycoproteins analysis, confocal microscopy, nerve degeneration, nerve tissue proteins analysis, organ culture techniques, oxygen blood, purinergic P2 receptor analysis, serotonin analysis, tissue distribution.

Jung, D.W., D. Williams, S.M. Khersonsky, T.W. Kang, N. Heidary, Y.T. Chang, and S.J. Orlow (2005). **Identification of the F1F0 mitochondrial ATPase as a target for modulating skin pigmentation by screening a tagged triazine library in zebrafish.** *Molecular BioSystems* 1(1): 85-92. ISSN: 1742-206X.
NAL Call Number: QH506.M66426
Abstract: A triazine-based combinatorial library of small molecules was screened in zebrafish to identify compounds that produced interesting phenotypes. One compound (of 1536 screened) induced a dramatic increase in the pigmentation of early stage zebrafish embryos. This compound, PPA, was also found to increase pigmentation in cultured mammalian melanocytes. The cellular target was identified as the mitochondrial F1F0-ATP synthase (ATPase) by affinity chromatography. Oligomycin, a small molecule known to inhibit the mitochondrial ATPase, competed with PPA for its cellular target in melanocytes. In addition, PPA was shown to alter the membrane potential of mitochondria, consistent with inhibition of the mitochondrial ATPase. Thus, PPA has been successfully used as a chemical probe in a forward chemical genetic approach to establish a link between the phenotype and the protein. The results attest to the power of screening small molecule libraries in zebrafish as a means of identifying mammalian targets and suggest the mitochondrial ATPase as a target for modulating pigmentation in both melanocytes and melanoma cells.
Descriptors: zebrafish, mitochondrial, skin pigmentation physiology, amino acid sequence, cultured cells melanins metabolism, melanocytes physiology, membrane potentials, inbred C57Bl mice, mitochondrial membranes physiology, mitochondrial proteins antagonists and inhibitors, mitochondrial proton translocating ATPases antagonists, inhibitors and physiology , molecular sequence data, oligomycins pharmacology, triazines pharmacology, proteins antagonists and inhibitors.

Kaneko, M., N. Hernandez Borsetti, and G.M. Cahill (2006). **Diversity of zebrafish peripheral oscillators revealed by luciferase reporting.** *Proceedings of the National Academy of Sciences of the United States of America* 103(39): 14614-9. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: In various multicellular organisms, circadian clocks are present not only in the central nervous system, but also in peripheral organs and tissues. In mammals peripheral oscillators are not directly responsive to light, but are entrained by the central oscillator in the suprachiasmatic nucleus. These individual oscillators are diverse in their free-running periods and phases. In contrast, cultured peripheral tissues and cell lines from zebrafish are not only rhythmic, but can also be directly entrained by light. Because of the convenience of studying rhythms in cultured cells, however, little has been known about properties of individual oscil-

lators in intact zebrafish. Here, we show the remarkable diversity and consistency of oscillator properties in various peripheral organs and tissues from the period3-luciferase (per3-luc) transgenic zebrafish. Tissue-dependent differences were found in free-running period, phase, response to light, and temperature compensation. Furthermore, cycling amplitudes were reduced at lower temperatures in some, but not all, of the organs tested. Finally, we found that per3-luc rhythms can free run in both constant dark and constant light with remarkably similar amplitudes, phases, and periods, despite the fact that the mRNA of per2 and per1 has been shown not to oscillate in constant light.

Descriptors: transgenic zebrafish, biological clocks physiology, reporter luciferases metabolism, genetically modified, biological clocks radiation effects, circadian rhythm physiology and radiation effects light and darkness, heart physiology, luciferases genetics, luminescent proteins metabolism, nuclear proteins metabolism, organ specificity, spleen physiology, temperature, transcription factors metabolism.

Kanungo, J., Z. Kozmik, S.K. Swamynathan, and J. Piatigorsky (2003). **Gelsolin is a dorsalizing factor in zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 100(6): 3287-92. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: The gene for gelsolin (an actin-binding, cytoskeletal regulatory protein) was shown earlier to be specialized for high corneal expression in adult zebrafish. We show here that zebrafish gelsolin is required for proper dorsalization during embryogenesis. Inhibition of gelsolin expression by injecting fertilized eggs with a specific morpholino oligonucleotide resulted in a range of concentration-dependent ventralized phenotypes, including those lacking a brain and eyes. These were rescued by coinjection of zebrafish gelsolin or chordin (a known dorsalizing agent) mRNAs, or human gelsolin protein. Moreover, injection of gelsolin mRNA or human gelsolin protein by itself dorsalized the developing embryos, often resulting in axis duplication. Injection of the gelsolin-specific morpholino oligonucleotide enhanced the expression of Vent mRNA, a ventral marker downstream of bone morphogenetic proteins, whereas injection of gelsolin mRNA enhanced the expression of chordin and goosecoid mRNAs, both dorsal markers. Our results indicate that gelsolin also modulates embryonic dorsalventral pattern formation in zebrafish.

Descriptors: zebrafish embryos, injecting fertilized eggs, gelsolin physiology, intercellular signaling peptides and proteins, base sequence, body patterning genetics and physiology, bone morphogenetic proteins metabolism, cornea embryology and metabolism, gelsolin antagonists and inhibitors and genetics, developmental gene expression regulation, glycohomeodomain in situ hybridization, oligodeoxyribonucleotides, antisense genetics and pharmacology, messenger RNA genetics and metabolism, repressor signal transduction.

Kawakami, A., Y. Nojima, A. Toyoda, M. Takahoko, M. Satoh, H. Tanaka, H. Wada, I. Masai, H. Terasaki, Y. Sakaki, H. Takeda, and H. Okamoto (2005). **The zebrafish-secreted matrix protein you/scube2 is implicated in long-range regulation of Hedgehog signaling.** *Current Biology* 15(5): 480-8. ISSN: 0960 9822.

NAL Call Number: QH301.C85

Abstract: The Hedgehog (Hh) signal plays a pivotal role in induction of ventral neuronal and muscle cell types around the midline during vertebrate development [1]. We report that the gene disrupted in zebrafish you mutants, in which Hh signaling is impaired, encodes the secreted matrix protein Scube2. Consistently, epistasis analyses suggested that Scube2 functions upstream of Hh ligands or through a parallel pathway. In addition, overexpression analyses suggested that Scube2 is an essential, but a permissive, mediator of Hh signaling in

zebrafish embryos. Surprisingly, the you gene is expressed in the dorsal neural tube, raising the possibility that Scube2 could indirectly act via a long-range regulator of Hh signaling. The dorsal Bmps have a long-range and opposing influence on Hh signaling [2-5]. We show that neural plate patterning is affected in you mutants in a way that is consistent with the aberrant long-range action of a Bmp-dependent signal. We further show that Bmp activity can be attenuated by the coexpression of Scube2. Our data support the idea that Scube2 can modulate the long-range action of Bmp-dependent signaling in the neural tube and somites.
Descriptors: zebrafish, body patterning physiology, central nervous system embryology, extracellular matrix proteins metabolism, phenotype, signal transduction physiology, trans activators metabolism, amino acid and base sequence, Western blotting, bone morphogenetic proteins metabolism, chromosome mapping, DNA primers, epistasis, genetic, extracellular matrix gene components, genotype, Hedgehog proteins, in situ hybridization, molecular sequence data, mutation genetics, sequence alignment, DNA sequence analysis.

Kawakami, Y., A. Raya, R.M. Raya, C. Rodriguez Esteban, and J.C. Belmonte (2005). **Retinoic acid signalling links left-right asymmetric patterning and bilaterally symmetric somitogenesis in the zebrafish embryo.** *Nature* 435(7039): 165-71. ISSN: 0028-0836.
NAL Call Number: 472 N21
Abstract: During embryogenesis, cells are spatially patterned as a result of highly coordinated and stereotyped morphogenetic events. In the vertebrate embryo, information on laterality is conveyed to the node, and subsequently to the lateral plate mesoderm, by a complex cascade of epigenetic and genetic events, eventually leading to a left-right asymmetric body plan. At the same time, the paraxial mesoderm is patterned along the anterior-posterior axis in metameric units, or somites, in a bilaterally symmetric fashion. Here we characterize a cascade of laterality information in the zebrafish embryo and show that blocking the early steps of this cascade (before it reaches the lateral plate mesoderm) results in random left-right asymmetric somitogenesis. We also uncover a mechanism mediated by retinoic acid signalling that is crucial in buffering the influence of the flow of laterality information on the left-right progression of somite formation, and thus in ensuring bilaterally symmetric somitogenesis.
Descriptors: zebrafish, body patterning physiology, metabolism, signal transduction, somites and tretinoin metabolism, cilia physiology, embryology, molecular sequence data, somites cytology.

Ken, C.F., C.T. Lin, J.F. Shaw, and J.L. Wu (2003). **Characterization of fish Cu/Zn-superoxide dismutase and its protection from oxidative stress**. *Marine Biotechnology* 5(2): 167-173. ISSN: 1436-2228.
NAL Call Number: TP248.27.M37M37
Descriptors: zebrafish larva, biological resistance, biological stress, copper, defence mechanisms, detergents, enzymes, fish larvae, injuries, oxidation, stability, superoxide dismutase, temperature effects, zinc, pH effects, *Danio rerio*, copper-zinc superoxide dismutase.

Kim, D.H., C.N. Hwang, Y. Sun, S.H. Lee, B. Kim, and B.J. Nelson (2006). **Mechanical analysis of chorion softening in prehatching stages of zebrafish embryos.** *Institute of Electrical and Electronics Engineers: Transactions on Nanobioscience* 5(2): 89-94. ISSN: 1536-1241 .
Abstract: During early development, the chorion envelope of the zebrafish embryo undergoes a thinning process called "chorion softening," which has so far only been characterized chemically. In this study, a micromechanical force sensing system was used to characterize and quantitate mechanical modifications of the zebrafish embryo chorion during early

development. Quantitative relationships between applied forces and chorion structural deformations were established at various embryonic stages. The measured penetration force into the chorion at the blastula stage was 1.3-fold greater than those at the prehatching stage. Furthermore, chorion elastic modulus values were determined by using a biomembrane elastic model. The elastic modulus of the chorion at the blastula stage was 1.66-fold greater than that at the prehatching stage, thus indicating that the chorion envelope become mechanically "softened" at the prehatching stage. The experimental results quantitatively describe "chorion softening," which is most likely due to proteolytic activities at the prehatching stage. Gradual chorion softening during embryonic development was also artificially achieved by treating blastula chorion with pronase, a proteolytic enzyme. The forces required to penetrate the pronase-treated chorion were similar to those at the prehatching stage. This similarity suggests that "chorion softening" may be induced by the release of protease from the embryos, and the chemical nature of the process involves proteolytic fragmentation of the ZP2 protein.
Descriptors: zebrafish, chorion embryology and physiology, egG, embryo physiology, hardness tests instrumentation, membrane glyco, cell surface receptors physiology, biomechanics instrumentation and methods, computer simulation, elasticity, hardness tests methods, morphogenesis physiology, physical stimulation instrumentation and methods, mechanical stress.

Kim, D.H., Y. Sun, S. Yun, S.H. Lee, and B. Kim (2005). **Investigating chorion softening of zebrafish embryos with a microrobotic force sensing system.** *Journal of Biomechanics* 38(6): 1359-63. ISSN: 0021-9290.
NAL Call Number: TA166.J6
Abstract: The zebrafish is a model organism for addressing questions of vertebrate embryo development. In this paper, the softening phenomenon of the chorion envelope of zebrafish embryos at different developmental stages was mechanically quantitated by using a microrobotic force sensing system. The microrobotic system integrates a piezoelectric cellular force sensor to measure the required forces for penetrating the chorion envelope. Magnitude of penetration forces was found to decrease as an embryo develops. The results mechanically quantitate "chorion softening" in zebrafish embryos due to protease activities subtly modifying the chorion structure, providing an understanding of zebrafish embryo development.
Descriptors: zebrafish embryo development, chorion physiology, hardness tests instrumentation, physical stimulation instrumentation, robotics instrumentation, transducers , elasticity, equipment design, equipment failure analysis, gestational age, hardness tests methods, miniaturization, physical stimulation methods, reproducibility of results, robotics methods, sensitivity and specificity, mechanical stress.

Kim, J., E. Lee, B.S. Chang, C.S. Oh, G.H. Mun, Y.H. Chung, and D.H. Shin (2005). **The presence of megamitochondria in the ellipsoid of photoreceptor inner segment of the zebrafish retina.** *Anatomia Histologia Embryologia* 34(6): 339-42. ISSN: 0340 2096.
NAL Call Number: SF761.Z4
Abstract: Although the megamitochondria (MM) were localized in various pathological conditions, normal retina of some mammalian species was reported to include MM for various physiological roles. However, it was not clearly confirmed whether the MM is present in the retina of lower vertebrate as well. In this study, we tried to show the presence of the MM in the zebrafish retina using electron microscopic technique. In all the photoreceptors including rods, cones and double cones of the zebrafish retina, MM were observed in the ellipsoid of inner segment. In the photoreceptor epllipsoid of the zebrafish retina, the mitochondria located in the central portion of the ellipsoid had a highly electron-dense matrix, which were accompanied by the mitochondria with electron-lucent matrix in the

apical portion of the ellipsoid. The presence of MM was more clearly discernable in the rods, which were localized under the double cones. This finding is somewhat different from those observed in the previous studies because MM were localized in the inner segment of cones, but were not in those of rods in the case of mammalian retina. Although the exact physiological meaning for the presence of MM in some vertebrate species should be further studied, the present study could show that the MM in the ellipsoid of the retinal photoreceptors was not only restricted in some mammalian species.
Descriptors: zebrafish, mitochondria ultrastructure, vertebrate photoreceptors ultrastructure, retina ultrastructure, zebrafish anatomy, histology and physiology, electron transmission microscopy.

Kondo, M. (2007). **Bone morphogenetic proteins in the early development of zebrafish.** *Federation of European Biochemical Societies Journal* 274(12): 2960-7. ISSN: 1742 464X.
NAL Call Number: QP501.E8
Abstract: Bone morphogenetic proteins (BMPs) are known to be widely involved in various biological processes. Many of the members of the BMP family, as well as related factors, receptors and molecules in the BMP signaling pathway, have been isolated, but their precise functions are still unclear. In addition to the 'classical' model organism *Xenopus*, zebrafish, *Danio rerio*, is now considered to be a suitable model organism to study the roles of the BMP signaling pathway during embryogenesis. Mutagenesis screens have identified a number of mutants in the pathway. Although they do not cover the entire members of the BMP signaling cascade that are currently known, they serve as a powerful tool to broaden our understanding of BMP functions, in combination with other experimental techniques.
Descriptors: zebrafish, bone morphogenetic protein receptors, genetics, metabolism and physiology, body patterning, physiology, developmental gene expression regulation, mutation, signal transduction.

Kopp, R., B. Pelster, and T. Schwerte (2007). **How does blood cell concentration modulate cardiovascular parameters in developing zebrafish (*Danio rerio*)?** *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology* 146(3): 400-7. ISSN: 1095 6433.
NAL Call Number: QP1C6
Abstract: Blood flow and shear forces are considered to be important parameters possibly stimulating angiogenesis or cardiovascular remodeling. The main objective of this study was to test the hypothesis that a significant reduction in shear forces as a consequence of a significant isovolemic anemia induced by microsurgical techniques during early larval development of the zebrafish might induce a compensatory stimulation of erythropoiesis and/or induce a modification of cardiac activity or even the formation of the heart and may influence the shaping of the vascular bed. Blood from 2 day old zebrafish larvae was withdrawn and replaced by zebrafish Ringer's solution, so that the blood cell concentration was reduced by at least 75%. At 5 days post fertilization (dpf) a partial recovery in blood cell concentration was observed and reached a value of 814.55+/-85.42 cells/nL, while in control animals blood cell concentration amounted to 1856.00+/-131.59 cells/nL. At 7 dpf the value of blood cell concentration was 1023.89+/-95.75 cells/nL versus 1701.54+/-146.03 cells/nL in control animals. Compared to control animals, heart rate and cardiac output were significantly reduced in anemic animals and alterations in the formation of the vascular bed were also observed. A significant decrease in the end-diastolic volume suggested that ventricular volume was reduced. Thus, within a few days zebrafish larvae were nearly able to compensate for an isovolemic anemia by an enhanced erythropoiesis. However, several changes in cardiovascular system indicated that phenotypic plasticity is established even at an early

developmental stage.
Descriptors: zebrafish, blood cell count, zebrafish blood, anemia blood, blood vessels growth and development, blood viscosity, erythropoiesis, heart growth and development.

Krauss, A. and C. Neumeyer (2003). **Wavelength dependence of the optomotor response in zebrafish (*Danio rerio*).** *Vision Research* 43(11): 1273-82. ISSN: 0042-6989.
Abstract: The action spectrum of motion detection in zebrafish (*Danio rerio*) was measured using the optomotor response in the light adapted state. The function has a single maximum at 550-600 nm, and is similar to the spectral sensitivity function of the L-cone type in the mid and long wavelength range. At shorter wavelengths the values of three of the five fish tested are lower. As in goldfish [Vis. Res. 36 (1996) 4025], the result indicates a dominance of the L-cone type with an inhibitory influence of M- or S-cones. Experiments with a red/green striped cylinder showed that the optomotor response was at minimum whenever the L-cone type was not modulated by the moving pattern. This demonstrates that motion vision in zebrafish is "color blind", using mainly one of the four cone types probably involved in color vision.
Descriptors: zebrafish, motion perception physiology, color perception physiology, color vision defects, optometry methods, visual pattern recognition physiology.

Kubota, Y., Y. Oike, S. Satoh, Y. Tabata, Y. Niikura, T. Morisada, M. Akao, T. Urano, Y. Ito, T. Miyamoto, N. Nagai, G.Y. Koh, S. Watanabe, and T. Suda (2005). **Cooperative interaction of angiopoietin-like proteins 1 and 2 in zebrafish vascular development.** *Proceedings of the National Academy of Sciences of the United States of America* 102(38): 13502-7. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: Angiopoietin-like protein (Angptl) 1 and Angptl2, which are considered orphan ligands, are highly homologous, particularly in the fibrinogen-like domain containing the putative receptor binding site. This similarity suggests potentially cooperative functions between the two proteins. In this report, the function of Angptl1 and Angptl2 is analyzed by using morpholino antisense technology in zebrafish. Knockdown of both Angptl1 and Angptl2 produced severe vascular defects due to increased apoptosis of endothelial cells at the sprouting stage. In vitro studies showed that Angptl1 and Angptl2 have antiapoptotic activities through the phosphatidylinositol 3-kinase/Akt pathway, and coinjection of constitutively active Akt/protein kinase B mRNA rescued impaired vascular development seen in double knockdown embryos. These results provide a physiological demonstration of the cooperative interaction of Angptl1 and Angptl2 in endothelial cells through phosphatidylinositol 3-kinase/Akt mediated antiapoptotic activities.
Descriptors: zebrafish embryo, angiopoietins metabolism, apoptosis physiology, blood vessels embryology, endothelial cells metabolism, intercellular signaling peptides and proteins metabolism, proteins metabolism, 1 phosphatidylinositol 3 kinase metabolism, angiopoietins genetics, apoptosis drug effects, blood vessels cytology, endothelial cells cytology, gene silencing, oligodeoxyribonucleotides, antisense administration and dosage, oligodeoxyribonucleotides, antisense genetics, protein serine threonine kinases metabolism, proto oncogene proteins metabolism, proto oncogene proteins C Akt, signal transduction drug effects, signal transduction physiology, proteins genetics.

Kurita, K. and N. Sakai (2004). **Functionally distinctive testicular cell lines of zebrafish to support male germ cell development.** *Molecular Reproduction and Development* 67(4): 430-8. ISSN: 1040-452X.

NAL Call Number: QP251.M64
Descriptors: testicular zebrafish cell lines, cell communication physiology, spermatogenesis physiology, spermatozoa cytology, testis cytology, coculture techniques, high mobility group proteins genetics and metabolism, messenger RNA genetics and metabolism, spermatogenesis genetics, spermatozoa physiology, testis physiology, transcription factors genetics and metabolism, Wt1 proteins genetics and metabolism, zebrafish anatomy, histology, proteins genetics and proteins metabolism.

Lai, Y.S., L.F. Chiue, and T. Hsu (2006). **Low-molecular-weight vitellogenin 1-like proteins are components of a UV-damaged-DNA binding activity highly expressed in zebrafish (*Danio rerio*) embryos.** *Journal of Experimental Zoology. Part A, Comparative Experimental Biology* 305(3): 215-24. ISSN: 1548-8969.
NAL Call Number: QL1.J854
Descriptors: zebrafish embryos, DNA metabolism, DNA damage physiology, DNA binding proteins genetics, proteins metabolism, pyrimidine dimers metabolism, vitellogenins metabolism, proteins metabolism, Western blotting, chelating agents pharmacology, DNA radiation effects, electrophoretic mobility shift assay, peptide mapping, phenanthrolines pharmacology, spectrometry, mass, matrix assisted laser desorption ionization, vitellogenins genetics.

Lam, S.H., C.L. Winata, Y. Tong, S. Korzh, W.S. Lim, V. Korzh, J. Spitsbergen, S. Mathavan, L.D. Miller, E.T. Liu, and Z. Gong (2006). **Transcriptome kinetics of arsenic-induced adaptive response in zebrafish liver.** *Physiological Genomics* 27(3): 351-61. ISSN: 1094-8341.
Abstract: Arsenic is a prominent environmental toxicant and carcinogen; however, its molecular mechanism of toxicity and carcinogenicity remains poorly understood. In this study, we performed microarray-based expression profiling on liver of zebrafish exposed to 15 parts/million (ppm) arsenic [As(V)] for 8-96 h to identify global transcriptional changes and biological networks involved in arsenic-induced adaptive responses in vivo. We found that there was an increase of transcriptional activity associated with metabolism, especially for biosyntheses, membrane transporter activities, cytoplasm, and endoplasmic reticulum in the 96 h of arsenic treatment, while transcriptional programs for proteins in catabolism, energy derivation, and stress response remained active throughout the arsenic treatment. Many differentially expressed genes encoding proteins involved in heat shock proteins, DNA damage/repair, antioxidant activity, hypoxia induction, iron homeostasis, arsenic metabolism, and ubiquitin-dependent protein degradation were identified, suggesting strongly that DNA and protein damage as a result of arsenic metabolism and oxidative stress caused major cellular injury. These findings were comparable with those reported in mammalian systems, suggesting that the zebrafish liver coupled with the available microarray technology present an excellent in vivo toxicogenomic model for investigating arsenic toxicity. We proposed an in vivo, acute arsenic-induced adaptive response model of the zebrafish liver illustrating the relevance of many transcriptional activities that provide both global and specific information of a coordinated adaptive response to arsenic in the liver.
Descriptors: zebrafish, arsenic toxicity, gene expression regulation, liver metabolism, physiological adaptation, arsenic metabolism, down regulation, gene expression profiling, genomics, liver drug effects and pathology, metabolic networks and pathways drug effects, oligonucleotide array sequence analysis, genetic transcription , up regulation.

Lee, J.W., D.S. Na, S.K. Chae, C. Kim, J.Y. Kang, B.K. Ju, H. Lee, S.U. Kim, C.N. Hwang, and S.H. Lee (2005). **Using the chorions of fertilized zebrafish eggs as a biomaterial for the attachment and differentiation of mouse stem cells.** *Langmuir* 21(17): 7615-20. ISSN:

0743-7463.
NAL Call Number: QD506.A1L34
Abstract: The development of proper biomaterials is critical for the success of cell therapy and modern tissue engineering. Here, we extruded the yolk and remaining inner mass from fertilized zebrafish eggs and used the resulting chorions as a biomaterial for the differentiation and attachment of mouse P19 embryonic carcinoma (EC) cells. Cells inserted into the chorion showed the spontaneous formation of embryoid body due to the repulsive cell adhesion of the chorion and differentiated specifically into neural cells and cardiomyocytes. In contrast, dissolved chorion extracellular matrix (ECM) conferred enhanced cell attachment on it, suggesting that a unique property of the zebrafish chorion with nanoporous structure appears to be responsible for the simple and controllable embryoid formation for stem cell differentiation. These results indicate that chorions from fertilized zebrafish eggs may be used as an extracellular matrix alternative and applied for stem cell differentiation to specific cell lineages.
Descriptors: zebrafish, mice, biocompatible materials chemistry and pharmacology, cell differentiation drug effects, chorion chemistry, tumor stem cells cytology and drug effects, biocompatible materials isolation and purification, cell adhesion drug effects, cell culture techniques methods, tumor cell line.

Lessman, C.A. (2004). **Computer-aided screening for zebrafish embryonic motility mutants.** *Methods in Cell Biology* 76: 285-313. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, computer assisted image processing methods and instrumentation, motor activity genetics, mutation, automatic data processing methods, physiology, glycoheparan sulfate proteoglycans genetics, intercellular signaling peptides and larva genetics and physiology, motor activity physiology, phenotype, software, statistical distributions, proteins genetics.

Leung, Y.T., G.L. Fain, and H.R. Matthews (2007). **Simultaneous measurement of current and calcium in the ultraviolet-sensitive cones of zebrafish.** *Journal of Physiology* 579(Pt 1): 15-27. ISSN: 0022-3751.
NAL Call Number: 447.8J82
Abstract: In rods and visible cone photoreceptors, multiple measurements cannot be made of intracellular Ca2+ concentration from the same cell using fluorescent dyes, because a single exposure of the measuring light bleaches too large a fraction of the rod or cone photopigment. We have therefore identified and characterized UV-sensitive cones of the zebrafish, whose wavelength of maximum sensitivity is at 360 nm which is far enough from the wavelength of our measuring light (514.5 nm) so that it has been possible to make multiple determinations of photocurrent and Ca2+ concentration from the same cells. We show that for a limited number of measurements, for which the bleaching of the cone photopigment is too small to affect flash kinetics, the outer segment Ca2+ concentration closely follows the wave form of the flash response convolved with the dominant time constant for Ca2+ removal by Na+-Ca2+-K+ exchange. For a larger number of measurements, significant acceleration of the response kinetics by pigment bleaching inevitably occurs, but the Ca2+ concentration nevertheless rises and falls in approximate agreement with the flash wave form. During exposure to steady background light, the Ca2+ concentration falls in proportion to the steady-state current for dim backgrounds at all times and for bright backgrounds at steady state. At early times following the onset of bright backgrounds, however, the Ca2+ concentration is markedly higher than expected from the current of the cone. We show this

to be the result of light-dependent Ca2+ release by bright background light, which can be abolished by pre-exposure of the cone to the membrane-permeant acetoxymethyl ester of the Ca2+ chelator BAPTA. Our results therefore demonstrate that the cone outer segment Ca2+ concentration is predominantly a function of the rate of influx and efflux of Ca2+ across the plasma membrane, but that a release of Ca2+ in bright light most probably from buffer sites within the cell can transiently elevate the Ca2+ concentration above the level expected from the open probability of the light-dependent channels.
Descriptors: zebrafish UV sensitive eye cones, calcium metabolism, calcium channels physiology, retina cones physiology, phototransduction physiology, ultraviolet rays, aniline compounds, color perception physiology, fluorescent dyes, homeostasis physiology, photic stimulation, xanthenes.

Levraud, J.P., P. Boudinot, I. Colin, A. Benmansour, N. Peyrieras, P. Herbomel, and G. Lutfalla (2007). **Identification of the zebrafish IFN receptor: implications for the origin of the vertebrate IFN system.** *Journal of Immunology* 178(7): 4385-94. ISSN: 0022-1767.
NAL Call Number: 448.8J8232
Abstract: The recent description of virus-induced fish IFNs has raised questions about the evolution of this complex antiviral system. Identification of the receptor of the zebrafish virus-induced IFN (zIFN) was sought to help resolve these questions. We set up an experimental system to study the zIFN system in the course of a viral infection of zebrafish embryos. In this setting, zIFN was induced by viral infection, and we identified zIFN-dependent induced transcripts. Embryos quickly died from the infection, but zIFN overexpression increased their survival. We took advantage of this experimental system to perform in vivo loss and gain of function analysis of candidate receptors of the class II helical receptor family and identified zCRFB1 and zCRFB5 as the two subunits of the zebrafish IFN receptor. Based on the organization of the zIFN gene and the protein structure of the identified receptor components, the virus-induced fish IFNs appear as orthologs of mammalian IFN-lambda, specifying type III IFN as the ancestral antiviral system of vertebrates.
Descriptors: zebrafish, interferons metabolism, protein subunits physiology, interferon receptors physiology, immunology, alternative splicing, amino acid sequence, embryo chemistry, immunology and virology, interferons genetics, molecular sequence data, promoter regions genetics, protein subunits analysis and genetics, interferon receptors analysis and genetics, genetic transcription.

Liang, P., C.A. Jones, B.W. Bisgrove, L. Song, S.T. Glenn, H.J. Yost, and K.W. Gross (2004). **Genomic characterization and expression analysis of the first nonmammalian renin genes from zebrafish and pufferfish.** *Physiological Genomics* 16(3): 314-22. ISSN: 1094-8341.
Abstract: Renin is a key enzyme in the renin-angiotensin system (RAS), a pathway which plays an important physiological role in blood pressure and electrolyte homeostasis. The origin of the RAS is believed to have accompanied early evolution of vertebrates. However, renin genes have so far only been unequivocally identified in mammals. Whether or not a bona fide renin gene exists in nonmammalian vertebrates has been an intriguing question of physiological and evolutionary interest. Using a genomic analytical approach, we identified renin genes in two nonmammalian vertebrates, zebrafish *(Danio rerio*) and pufferfish (*Takifugu rubripes*). Phylogenetic analysis demonstrates that the predicted fish renins cluster together with mammalian renins to form a distinct subclass of vertebrate aspartyl proteases. RT-PCR results confirm generation of the predicted zebrafish mRNA and its expression in association with the opisthonephric kidney of adult zebrafish. Comparative in situ hybrid-

ization analysis of wild-type and developmental mutants indicates that renin expression is first detected bilaterally in cells of the interrenal primordia at 24 h postfertilization, which subsequently migrate to lie adjacent to, but distinct from, the glomerulus of the developing pronephric kidney. Our report provides the first molecular evidence for the existence of renin genes in lower vertebrates. The observation that the earliest renin-expressing cells, arising during ontogeny of this teleost vertebrate, are of adrenocortical lineage raises an interesting hypothesis as regards the origin of renin-expressing cells in the metanephric kidney of higher vertebrates.

Descriptors: zebrafish, gene expression profiling, genomics, renin genetics, tetraodontiformes genetics, adrenal glands embryology and metabolism, amino acid sequence, computational biology, molecular evolution, gene expression regulation, genome, kidney metabolism, molecular sequence data, phylogeny, messenger RNA genetics and metabolism, renin chemistry, sequence alignment, DNA sequence analysis, transcription initiation site.

Lin, L.Y., J.L. Horng, J.G. Kunkel, and P.P. Hwang (2006). **Proton pump-rich cell secretes acid in skin of zebrafish larvae.** *American Journal of Physiology. Cell Physiology* 290(2): C371-8. ISSN: 0363-6143.

Abstract: The mammalian kidney excretes its metabolic acid load through the proton-transporting cells, intercalated cells, in the distal nephron and collecting duct. Fish excrete acid through external organs, gill, or skin; however, the cellular function is still controversial. In this study, molecular and electrophysiological approaches were used to identify a novel cell type secreting acid in skin of zebrafish *(Danio rerio*) larvae. Among keratinocytes covering the larval surface, novel proton-secreting ionocytes, proton pump (H(+)-ATPase)-rich cells, were identified to generate strong outward H(+) flux. The present work demonstrates for the first time, with a noninvasive technique, H(+)-secreting cells in an intact animal model, the zebrafish, showing it to be a suitable model in which to study the functions of vertebrate transporting epithelia in vivo.

Descriptors: zebrafish acid secretion system, larva anatomy and histology and metabolism, proton pumps metabolism, skin cytology, concanavalin a metabolism , electrophysiology methods, enzyme inhibitors metabolism, in situ hybridization, macrolides metabolism, Na+ K+ exchanging ATPase genetics and metabolism, proton translocating ATPases genetics and metabolism, skin metabolism.

Link, V., L. Carvalho, I. Castanon, P. Stockinger, A. Shevchenko, and C.P. Heisenberg (2006). **Identification of regulators of germ layer morphogenesis using proteomics in zebrafish.** *Journal of Cell Science* 119(Pt 10): 2073-83. ISSN: 0021-9533.

NAL Call Number: QH301.J6

Abstract: During vertebrate gastrulation, a well-orchestrated series of morphogenetic changes leads to the formation of the three germ layers: the ectoderm, mesoderm and endoderm. The analysis of gene expression patterns during gastrulation has been central to the identification of genes involved in germ layer formation. However, many proteins are regulated on a translational or post-translational level and are thus undetectable by gene expression analysis. Therefore, we developed a 2D-gel-based comparative proteomic approach to target proteins involved in germ layer morphogenesis during zebrafish gastrulation. Proteomes of ectodermal and mesendodermal progenitor cells were compared and 35 significantly regulated proteins were identified by mass spectrometry, including several proteins with predicted functions in cytoskeletal organization. A comparison of our proteomic results with data obtained in an accompanying microarray-based gene expression analysis revealed no significant overlap, confirming the complementary nature of proteomics and transcrip-

tomics. The regulation of ezrin2, which was identified based on a reduction in spot intensity in mesendodermal cells, was independently validated. Furthermore, we show that ezrin2 is activated by phosphorylation in mesendodermal cells and is required for proper germ layer morphogenesis. We demonstrate the feasibility of proteomics in zebrafish, concluding that proteomics is a valuable tool for analysis of early development.
Descriptors: zebrafish, germ layers physiology , proteomics methods, cytoskeletal proteins biosynthesis and genetics and physiology , gene expression profiling, developmental gene expression regulation, genomics methods, germ layers cytology and metabolism, mass spectrometry methods, microarray analysis, morphogenesis, antisense oligonucleotides genetics.

Liu, Q., R.A. Frey, S.G. Babb Clendenon, B. Liu, J. Francl, A.L. Wilson, J.A. Marrs, and D.L. Stenkamp (2007). **Differential expression of photoreceptor-specific genes in the retina of a zebrafish cadherin2 mutant glass onion and zebrafish cadherin4 morphants.** *Experimental Eye Research* 84(1): 163-75. ISSN: 0014 4835.
NAL Call Number: QP474
Abstract: Cadherins are Ca2+ -dependent transmembrane molecules that mediate cell-cell adhesion through homophilic interactions. Cadherin2 (also called N-cadherin) and cadherin4 (also called R-cadherin), members of the classic cadherin subfamily, have been shown to be involved in development of a variety of tissues and organs including the visual system. To gain insight into cadherin2 and cadherin4 function in differentiation of zebrafish photoreceptors, we have analyzed expression patterns of several photoreceptor-specific genes (crx, gnat1, gnat2, irbp, otx5, rod opsin, rx1, and uv opsin) and/or a cone photoreceptor marker (zpr-1) in the retina of a zebrafish cadherin2 mutant, glass onion (glo) and in zebrafish embryos injected with a cadherin4 specific antisense morpholino oligonucleotide (cdh4MO). We find that expression of all these genes, and of zpr-1, is greatly reduced in the retina of both the glo and cadherin4 morphants. Moreover, in these embryos, expression of some genes (e.g. gnat1, gnat2 and irbp) is more affected than others (e.g. rod opsin and uv opsin). In embryos with both cadherins functions blocked (glo embryos injected with the cdh4MO), the eye initially formed, but became severely and progressively disintegrated and expressed little or no crx and otx5 as development proceeded. Our results suggest that cadherin2 and cadherin4 play important roles in the differentiation of zebrafish retinal photoreceptors.
Descriptors: glass onion zebrafish embryo mutant, cadherins physiology, eye, vertebrate photoreceptors metabolism, cadherins genetics, cell differentiation genetics and physiology, eye embryology and developmental gene expression regulation, mutation, retina embryology and metabolism.

Logan, D.W., S.F. Burn, and I.J. Jackson (2006). **Regulation of pigmentation in zebrafish melanophores.** *Pigment Cell Research* 19(3): 206-13. ISSN: 0893-5785.
Abstract: In comparison with the molecular genetics of melanogenesis in mammals, the regulation of pigmentation in poikilothermic vertebrates is poorly understood. Mammals undergo morphological colour change under hormonal control, but strikingly, many lower vertebrates display a rapid physiological colour change in response to the same hormones. The recent provision of extensive genome sequencing data from teleost zebrafish, *Danio rerio*, provides the opportunity to define the genes and proteins mediating this physiological pigment response and characterise their function biologically. Here, we illustrate the background adaptation process in adults and larvae and describe a novel assay to visualize and directly quantify the rate of zebrafish melanophore pigment translocation in unprecedented detail. We demonstrate the resolution of this assay system; quantifying the zebrafish melanophore response to melanin-concentrating and melanocyte-stimulating hormones.

Furthermore, we investigate the intracellular signalling downstream of hormone stimulation and the biomechanical processes involved in zebrafish pigment translocation, confirming the importance of cyclic adenosine monophosphate (cAMP) as a mediator of pigment translocation and finding intact microtubules are essential for both melanin dispersion and aggregation in zebrafish, but that microfilament disruption affects aggregation only. In conclusion, we propose these data establish the zebrafish as an experimental model for studying both physiological colour change and the molecular basis of pigment translocation.
Descriptors: zebrafish pigment translocation, hypothalamic hormones physiology, melanins physiology, melanocyte stimulating hormones physiology, melanophores physiology , pituitary hormones physiology, skin pigmentation physiology, physiological adaptation, biological assay, cyclic AMP, intracellular signaling peptides and proteins , microfilaments physiology, microtubules physiology, transport vesicles physiology.

Lopez Olmeda, J.F., J.A. Madrid, and F.J. Sanchez Vazquez (2006). **Light and temperature cycles as zeitgebers of zebrafish (*Danio rerio*) circadian activity rhythms.** *Chronobiology International* 23(3): 537-50. ISSN: 0742 0528.
NAL Call Number: QP84.6.C47
Abstract: Light and temperature cycles are the most important synchronizers of biological rhythms in nature. However, the relative importance of each, especially when they are not in phase, has been poorly studied. The aim of this study was to analyze the entrainment of daily locomotor activity to light and/or temperature cycles in zebrafish. Under two constant temperatures (20 degrees C and 26 degrees C) and 12:12 light-dark (LD) cycles, zebrafish were most active during the day (light) time and showed higher total activity at the warmer temperature, while diurnalism was higher at 20 degrees C than at 26 degrees C (87% and 77%, respectively). Under thermocycles (12:12 LD, 26:20 degrees C thermophase:chryophase or TC), zebrafish daily activity synchronized to the light phase, both when the thermophase and light phase were in phase (LD/TC) or in antiphase (LD/CT). Under constant dim light (3 lux), nearly all zebrafish synchronized to thermocycles (tau=24 h), although activity rhythms (60% to 67% of activity occurred during the thermophase) were not as marked as those observed under the LD cycle. Under constant dim light of 3 lux and constant temperature (22.5 degrees C), 4 of 6 groups of zebrafish previously entrained to thermocycles displayed free-running rhythms (tau=22.9 to 23.6 h). These results indicate that temperature cycles alone can also entrain zebrafish locomotor activity.
Descriptors: zebrafish, circadian rhythm physiology, activity cycles physiology, motor activity physiology, photoperiod, light and temperature cycles.

Lopez-Schier, H. and A.J. Hudspeth (2006). **A two-step mechanism underlies the planar polarization of regenerating sensory hair cells**. *Proceedings of the National Academy of Sciences, USA* 103(49): 18615-18620. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish sensory hairs, epithelia, freshwater fish, hair cells, lateral line, maintenance, mutants, physiology, planning, polarity, polarization, restoration, sensory neurons, stem cells, regeneration, *Danio rerio.*

Luna, V.M. and P. Brehm (2006). **An electrically coupled network of skeletal muscle in zebrafish distributes synaptic current.** *Journal of General Physiology* 128(1): 89-102. ISSN: 0022-1295.
NAL Call Number: 447.8 J822
Abstract: Fast and slow skeletal muscle types are readily distinguished in larval zebrafish

on the basis of differences in location and orientation. Additionally, both muscle types are compact, rendering them amenable to in vivo patch clamp study of synaptic function. Slow muscle mediates rhythmic swimming, but it does so purely through synaptic drive, as these cells are unable to generate action potentials. Our patch clamp recordings from muscle pairs of zebrafish reveal a network of electrical coupling in slow muscle that allows sharing of synaptic current within and between segmental boundaries of the tail. The synaptic current exhibits slow kinetics (tau(decay) approximately 4 ms), which further facilitates passage through the low pass filter, a consequence of the electrically coupled network. In contrast to slow muscle, fast skeletal muscle generates action potentials to mediate the initial rapid component of the escape response. The combination of very weak electrical coupling and synaptic kinetics (tau(decay) <1 ms) too fast for the network low pass filter minimizes intercellular sharing of synaptic current in fast muscle. These differences between muscle types provide insights into the physiological role(s) of electrical coupling in skeletal muscle. First, intrasegmental coupling among slow muscle cells allows effective transfer of synaptic currents within tail segments, thereby minimizing differences in synaptic depolarization. Second, a fixed intersegmental delay in synaptic current transit, resulting from the low pass filter properties of the slow muscle network, helps coordinate the rostral-caudal wave of contraction.
Descriptors: zebrafish, cell communication physiology, skeletal muscle physiology, synaptic transmission physiology, action potentials and cell communication drug effects, electrophysiology, fluorescent dyes metabolism, gap junctions drug effects and physiology, glycyrrhetinic acid analogs, derivatives and pharmacology, kinetics, muscle contraction physiology, fast twitch muscle fibers physiology, slow twitch muscle fibers physiology, skeletal muscle drug effects, neuromuscular junction, patch clamp techniques, tetrodotoxin pharmacology.

Maaswinkel, H. and L. Li (2003). **Olfactory input increases visual sensitivity in zebrafish: a possible function for the terminal nerve and dopaminergic interplexiform cells.** *Journal of Experimental Biology* 206(Pt 13): 2201-9. ISSN: 0022-0949.
NAL Call Number: 442.8B77
Abstract: Centrifugal innervation of the neural retina has been documented in many species. In zebrafish *Danio rerio*, the only so-far described centrifugal pathway originates from terminal nerve (TN) cell bodies that are located in the olfactory bulb. Most of the TN axons terminate in the forebrain and midbrain, but some project via the optic nerve to the neural retina, where they synapse onto dopaminergic interplexiform cells (DA-IPCs). While the anatomical pathway between the olfactory and visual organs has been described, it is unknown if and how olfactory signals influence visual system functions. We demonstrate here that olfactory input is involved in the modulation of visual sensitivity in zebrafish. As determined by a behavioral assay and by electroretinographic (ERG) recording, zebrafish visual sensitivity was increased upon presentation of amino acids as olfactory stimuli. This effect, however, was observed only in the early morning hours when zebrafish are least sensitive to light. The effect of olfactory input on vision was eliminated after lesion of the olfactory bulbs or after the destruction of DA-IPCs. Intraocular injections of a dopamine D(2) but not a D(1) receptor antagonist blocked the effect of olfactory input on visual sensitivity. Although we cannot exclude the involvement of other anatomical pathways, our data suggest that the TN and DA-IPCs are the prime candidates for olfactory modulation of visual sensitivity.
Descriptors: zebrafish visual sensitivity, olfactory pathways physiology, visual perception drug effects, visual perception physiology, amino acids pharmacology, dopamine pharmacology, electroretinography, immunohistochemistry, photic stimulation , chemical stimulation.

Maaswinkel, H. and L. Li (2003). **Spatio-temporal frequency characteristics of the optomotor response in zebrafish.** *Vision Research* 43(1): 21-30. ISSN: 0042-6989.
Descriptors: zebrafish visual systems function, motion perception physiology, motor activity physiology, psychomotor performance physiology, photic stimulation methods, rotation, time factors.

MacCormack, T., N. Wang, A. Desouza, L. MacKenzie, L. Li, and G. Goss (2007). **Proteomic profiles of the zebrafish (*Danio rerio*) gill and liver**. *Comparative Biochemistry and Physiology, Part a: Molecular & Integrative Physiology* 148(Suppl.1): S11. ISSN: 1095-6433.
NAL Call Number: QP1C6
Descriptors: zebrafish proteomics, gills, *Danio rerio*, physiology, biochemistry, biophysics.

Mann, K.D., E.R. Turnell, J. Atema, and G. Gerlach (2003). **Kin recognition in juvenile zebrafish (*Danio rerio*) based on olfactory cues.** *Biological Bulletin, The* 205(2): 224-5. ISSN: 0006 3185.
NAL Call Number: 442.8B52
Descriptors: juvenile zebrafish, kin recognition psychology physiology, smell physiology.

Margaritis, M. and D. Higgs (2005). **The effects of stimulus parameters on the auditory brainstem response of zebrafish (*Danio rerio*)**. *Journal of the Acoustical Society of America* 117(4): 2440. ISSN: 0001-4966.
Descriptors: zebrafish, amplitude, auditory stimuli, brain, freshwater fish, sound waves, *Danio rerio.*

Marshall, C.R., J.A. Fox, S.L. Butland, B.F.F. Ouellette, F.S.L. Brinkman, and G.F. Tibbits (2005). **Phytogeny of Na super(+)/Ca super(2+) exchanger (NCX) genes from genomic data identifies new gene duplications and a new family member in fish species**. *Physiological Genomics* 21(2): 161-173. ISSN: 1094-8341.
Descriptors: zebrafish, calcium homeostasis, evolutionary genetics, NCX1 protein, Na super(+), Ca super(2+)-exchanging ATPase, phylogeny, genomics, molecular evolution, *Danio rerio.*

McClelland, G.B., P.M. Craig, K. Dhekney, and S. Dipardo (2006). **Temperature- and exercise-induced gene expression and metabolic enzyme changes in skeletal muscle of adult zebrafish (*Danio rerio*).** *Journal of Physiology* 577(Pt 2): 739-51. ISSN: 0022-3751.
NAL Call Number: 447.8J82
Abstract: Both exercise training and cold acclimatization induce muscle remodelling in vertebrates, producing a more aerobic phenotype. In ectothermic species exercise training and cold-acclimatization represent distinct stimuli. It is currently unclear if these stimuli act through a common mechanism or if different mechanisms lead to a common phenotype. The goal of this study was to survey responses that represent potential mechanisms responsible for contraction- and temperature-induced muscle remodelling, using an ectothermic vertebrate. Separate groups of adult zebrafish (*Danio rerio*) were either swim trained or cold acclimatized for 4 weeks. We found that the mitochondrial marker enzyme citrate synthase (CS) was increased by 1.5x in cold and by 1.3x with exercise ($P<0.05$). Cytochrome c oxidase (COx) was increased by 1.2x following exercise training ($P<0.05$) and 1.2x ($P=0.07$) with cold acclimatization. However, only cold acclimatization increased beta-hydroxyacyl-CoA dehydrogenase (HOAD) compared to exercise-trained (by 1.3x) and pyruvate kinase (PK) relative to control zebrafish. We assessed the whole-animal performance outcomes of these

treatments. Maximum absolute sustained swimming speed (Ucrit) was increased in the exercise trained group but not in the cold acclimatized group. Real-time PCR analysis indicated that increases in CS are primarily transcriptionally regulated with exercise but not with cold treatments. Both treatments showed increases in nuclear respiratory factor (NRF)-1 mRNA which was increased by 2.3x in cold-acclimatized and 4x in exercise-trained zebrafish above controls. In contrast, peroxisome proliferator-activated receptor (PPAR)-alpha mRNA levels were decreased in both experimental groups while PPAR-beta1 declined in exercise training only. Moreover, PPAR-gamma coactivator (PGC)-1alpha mRNA was not changed by either treatment. In zebrafish, both temperature and exercise produce a more aerobic phenotype, but there are stimulus-dependent responses (i.e. HOAD and PK activities). While similar changes in NRF-1 mRNA suggest that common responses might underlie aerobic muscle remodelling there are distinct changes (i.e. CS and PPAR-beta1 mRNA) that contribute to specific temperature- and exercise-induced phenotypes.
Descriptors: zebrafish, acclimatization physiology, cold, enzymologic gene expression regulation, skeletal muscle enzymology, physical conditioning physiology, metabolism, 3 hydroxyacyl Co A dehydrogenases metabolism, citrate si synthase metabolism, electron transport complex IV metabolism, eye proteins metabolism, muscle fatigue, skeletal muscle metabolism, nuclear respiratory factor 1 metabolism, peroxisome proliferator activated receptors metabolism, phenotype, PCR, pyruvate kinase metabolism, messenger RNA metabolism, time factors.

McElligott, M.B. and D.M. O'Malley (2005). **Prey tracking by larval zebrafish: axial kinematics and visual control.** *Brain Behavior and Evolution* 66(3): 177-196. ISSN: 0006 8977.
Abstract: High- speed imaging was used to record the prey-tracking behavior of larval zebrafish as they fed upon paramecium. Prey tracking is comprised of a variable set of discrete locomotor movements that together align the larva with the paramecium and bring it into close proximity, usually within one body length. These tracking behaviors are followed by a brief capture swim bout that was previously described (Borla et al., 2002). Tracking movements were classified as either swimming or turning bouts. The swimming bouts were similar to a previously characterized larval slow swim (Budick and O'Malley, 2000), but the turning movements consisted of unique J-shaped bends which appear to minimize forward hydrodynamic disturbance when approaching the paramecium. Such J-turn tracking bouts consisted of multiple unilateral contractions to one side of the body. J-turns slowly and moderately alter the orientation of the larva-this is in contrast to previously described escape and routine turns. Tracking behaviors appear to be entirely visually guided. Infra- red (IR) imaging of locomotor behaviors in a dark environment revealed a complete absence of tracking behaviors, even though the normal repertoire of other locomotive behaviors was recorded. Concomitantly, such larvae were greatly impaired in consuming paramecia. The tracking behavior is of interest because it indicates the presence of sophisticated locomotor control circuitry in this relatively simple model organism. Such locomotor strategies may be conserved and elaborated upon by other larval and adult fishes.
Descriptors: zebrafish, *Danio rerio*, biomechanics, axial kinematics, photoreception , visual control, protozoan prey, paramecium , axial kinematics and visual control of prey tracking, foraging, swimming.

McHenry, M.J. and S.M. van Netten (2007). **The flexural stiffness of superficial neuromasts in the zebrafish (*Danio rerio*) lateral line.** *Journal of Experimental Biology* 210(Pt 23): 4244-53. ISSN: 0022-0949.
NAL Call Number: 442.8B77

Abstract: Superficial neuromasts are structures that detect water flow on the surface of the body of fish and amphibians. As a component of the lateral line system, these receptors are distributed along the body, where they sense flow patterns that mediate a wide variety of behaviors. Their ability to detect flow is governed by their structural properties, yet the micromechanics of superficial neuromasts are not well understood. The aim of this study was to examine these mechanics in zebrafish (*Danio rerio*) larvae by measuring the flexural stiffness of individual neuromasts. Each neuromast possesses a gelatinous cupula that is anchored to hair cells by kinocilia. Using quasi-static bending tests of the proximal region of the cupula, we found that flexural stiffness is proportional to the number of hair cells, and consequently the number of kinocilia, within a neuromast. From this relationship, the flexural stiffness of an individual kinocilium was found to be 2.4x10(-20) N m(2). Using this value, we estimate that the 11 kinocilia in an average cupula generate more than four-fifths of the total flexural stiffness in the proximal region. The relatively minor contribution of the cupular matrix may be attributed to its highly compliant material composition (Young's modulus of approximately 21 Pa). The distal tip of the cupula is entirely composed of this material and is consequently predicted to be at least an order of magnitude more flexible than the proximal region. These findings suggest that the transduction of flow by a superficial neuromast depends on structural dynamics that are dominated by the number and height of kinocilia.
Descriptors: zebrafish larva hair cells capula, flexure, stiffness, superficial neuromasts, lateral line.

McKenzie, P., R. Lehtinen, and D. Fraga (2004). **Neurotrophin-3 improves recovery time following noise-induced sensorineural hearing loss and causes hair cell growth in the colorless mutant of zebrafish**. *Ohio Journal of Science* 104(1): A14-A15. ISSN: 0030-0950.
Descriptors: zebrafish, defects, freshwater fish, growth, hair, mutations, recovery, symptoms, therapy, *Danio rerio.*

Melani, C., M. Campana, B. Lombardot, B. Rizzi, F. Veronesi, C. Zanella, P. Bourgine, K. Mikula, N. Peyrieras, and A. Sarti (2007). **Cells tracking in a live zebrafish embryo.** *Engineering in Medicine and Biology Magazine* 1: 1631-34. ISSN: 0739 5175.
Abstract: We designed a set of procedures for achieving the tracking of cell nuclei and the identification of cell divisions in live zebrafish embryos using 3D+time images acquired by confocal laser scanning microscopy (CLSM). Our strategy includes image signal enhancement with feature preserving denoising algorithm, automated identification of the nuclei position, extraction of the optical flow from 3D images sequences and tracking of nuclei.
Descriptors: zebrafish embryo, cells, nucluei, microscopy, 3D imaging, live embryotracking cell nuclei and identifying cell divisions.

Milan, D.J., I.L. Jones, P.T. Ellinor, and C.A. MacRae (2006). **In vivo recording of adult zebrafish electrocardiogram and assessment of drug-induced QT prolongation.** *American Journal of Physiology. Heart and Circulatory Physiology* 291(1): H269-73. ISSN: 0363 6135.
Abstract: In the last decade the zebrafish has become a major model organism for the study of development and organogenesis. To maximize the experimental utility of this organism, it will be important to establish methods for adult phenotyping. We previously proposed that the embryonic zebrafish may be useful in high-throughput screening for drug-induced cardiotoxicity. We now describe a method for the reproducible recording of the adult zebrafish ECG and illustrate its application in the investigation of QT-prolonging drugs. Zebrafish ECGs were obtained by inserting two needle electrodes through the ventral epidermis. Fish

were perfused orally, and motion artifacts were eliminated with a paralytic dose of mu-conotoxin GIIIB. Test compounds were delivered via the perfusion system. Without a means of hydration and oxygenation, the fish succumb rapidly. The use of a perfusion system allowed stable recording for > 6 h. Baseline conduction intervals were as follows: PR, 66 ms (SD 14); QRS, 34 ms (SD 11); QT, 242 ms (SD 54); and R-R, 398 ms (SD 77). The known QT-prolonging agents astemizole, haloperidol, pimozide, and terfenadine caused corrected QT increases of 18% (SD 9), 16% (SD 11), 17% (SD 9), and 11% (SD 6), respectively. The control drugs clonidine, penicillin and propranolol did not prolong the corrected QT interval. In conclusion, perfusion and muscular paralysis allows stable, low-noise recording of zebrafish ECGs. Agents known to cause QT prolongation in humans caused QT prolongation in fish in each case. The development of rigorous tools for the phenotyping of adult zebrafish will complement the high-throughput assays currently under development for embryonic and larval fish.
Descriptors: zebrafish, disease models, electrocardiography methods, long QT syndrome diagnosis, long QT syndrome physiopathology, aging, chemically induced long QT syndrome , species specificity.

Mothersill, C., R.W. Smith, N. Agnihotri, and C.B. Seymour (2007). **Characterization of a radiation-induced stress response communicated in vivo between zebrafish.** *Environmental Science and Technology* 41(9): 3382-7. ISSN: 0013 936X.
NAL Call Number: TD420.A1E5
Abstract: Radiation-induced communication of stress signals between rainbow trout (*Oncorhynchus mykiss* W) have recently been described by this group and linked to the bystander effect. This paper addresses the question of whether another totally unrelated fish species (*Danio rerio* L) can demonstrate the effect and also looks at attenuation of both the bystander signal, from irradiated fish, and the bystander effect, in naive fish. The data show that zebrafish produce bystander signals, and that, as with rainbow trout these can affect naive (i.e., non-irradiated) fish placed in water with X-rayed fish or in water previously occupied by X-rayed fish. Skin explants from directly X-rayed fish still reduce HPV-G reporter cell growth 6 h after X-ray, but the bystander signal to naive fish is lost. Twelve h after X-ray the signal is lost in X-rayed fish. The bystander effect is also attenuated if induction was by placing naive fish in water which previously held the X-rayed fish. However, the effect is retained if induction was by placing X-rayed and naive fish together. This suggests the signal is not retained by water for long periods of time. Individual fish data reveal unique responses by bystander fish which could indicate varying levels of sensitivity to signal strength among individuals.
Descriptors: zebrafish larva, bystander effect radiation effects, X rays, bystander effect physiology, genes, BCL 2 radiation effects, genes, myc radiation effects, gills metabolism, gills radiation effects, skin metabolism, skin radiation effects, stress.

Mueller, U.K., J.G.M. Van den Boogaart, and J.L. van Leeuwen (2008). **Flow patterns of larval fish: undulatory swimming in the intermediate flow regime**. *Journal of Experimental Biology* 211(2): 196-205. ISSN: 0022-0949.
NAL Call Number: 442.8B77
Descriptors: zebrafish, body size, boundary layers, head, stroke, swimming, tails, travel, waves, *Danio rerio*, orientation, migration, locomotion.

Nakada, T., K. Hoshijima, M. Esaki, S. Nagayoshi, K. Kawakami, and S. Hirose (2007). **Localization of ammonia transporter Rhcg1 in mitochondrion-rich cells of yolk sac, gill, and kidney of zebrafish and its ionic strength-dependent expression.** *American Journal of*

Physiology. Regulatory, Integrative and Comparative Physiology 293(4): R1743-53. ISSN: 0363 6119.

Abstract: Members of the Rh glycoprotein family have been shown to be involved in ammonia transport in a variety of species. Here we show that zebrafish Rhcg1, a member of the Rh glycoprotein family, is highly expressed in the yolk sac, gill, and renal tubules. Molecular cloning and characterization indicate that zebrafish Rhcg1 shares 82% sequence identity with the pufferfish ortholog fRhcg1. RT-PCR, combined with in situ hybridization, revealed that Rhcg1 is first expressed in vacuolar-type H(+)-ATPase/mitochondrion-rich cells (vH-MRC) on the yolk sac of larvae at 3 days postfertilization (dpf) and later in vH-MRC-like cells in the gill at 4-5 dpf. Ammonia excretion from zebrafish larvae increased in parallel with the expression of Rhcg1. At larval stages, Rhcg1 mRNA was detected only on the yolk sac and gill; however, the kidney, as well as the gill, becomes a major site of Rhcg1 expression in adults. Using a zebrafish Tol2 transgenic line whose vH-MRC are labeled with green fluorescent protein (GFP) and an antibody against zebrafish Rhcg1, we demonstrate that Rhcg1 is located in the apical regions of 1) vH-MRC on the yolk sac and vH-MRC-like cells (cell population with the expression of Rhcg1 and GFP) in the gill and 2) cells in the renal distal tubule and intercalated cell-like cells in the collecting duct of the kidney. Remarkably, expression of Rhcg1 mRNA at the larval stage was changed by environmental ionic strength. These results suggest that roles of zebrafish Rhcg1 are not solely ammonia secretion to eliminate nitrogen from the gill.

Descriptors: Tol2 transgenic zebrafish, cation transport proteins genetics and metabolism, gills cytology, kidney cytology, mitochondria metabolism, yolk sac cytology, and metabolism, amino acid sequence, genetically modified, base sequence, cation transport proteins chemistry, gene expression regulation, green fluorescent proteins, osmolar concentration, phylogeny, protein transport, messenger RNA genetics and metabolism, water chemistry.

Nelson, R., A.M. Bender, and V.P. Connaughton (2003). **Stimulation of sodium pump restores membrane potential to neurons excited by glutamate in zebrafish distal retina.** *Journal of Physiology* 549(Pt 3): 787-800. ISSN: 0022-3751.

NAL Call Number: 447.8J82

Abstract: Glutamate either depolarizes or hyperpolarizes retinal neurons. Those are the initial and primary effects. Using a voltage probe (oxonol, DiBaC4 (5)) to study dissociated zebrafish retinal neurons, we find a secondary, longer-term effect: a post-excitatory restoration of membrane potential, termed after-hyperpolarization (AHP). AHP occurs only in neurons that are depolarized by glutamate and typically peaks about 5 min after glutamate application. AHP is seen in dissociated horizontal cells (HCs) and hyperpolarizing, or OFF type, bipolar cells (HBCs). These cells commonly respond with only an AHP component. AHP never occurs in depolarizing, or ON type, bipolar cells (DBCs), which are cell types hyperpolarized by glutamate. AHP is blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). It is evoked by kainate, AMPA and the AMPA-selective agonist (S)-5-fluorowillardiine, but not by NMDA, D-aspartate, the kainate-selective agonist SYM 2081 or by DL-2-amino-4-phosphonobutyric acid (DL-AP4). Cells with exclusively AHP responses are tonically depolarized. Resting potentials can be restored by nifedipine, suggesting a tonic, depolarizing action of L-type Ca2+ channels. However AHP is not blocked by nifedipine and is insensitive to [Cl-]o. AHP is blocked by Li+o substitution for Na+o and by ouabain. A mechanism is proposed in which Na+ entering through ionotropic AMPA channels stimulates Na+,K+-ATPase, which, by electrogenic action, restores membrane potential, generating the AHP response. Patterns of ATPase immunoreactivity support localization in the outer plexiform layer (OPL) as cone pedicles, HCs and BCs were positively labelled. Labelling

was weaker in the inner plexiform layer (IPL) than in nuclear layers, though two IPL bands of immunoreactive BC terminals could be discerned, one in sublamina a and the other in sublamina b. Persistent stimulation of distal retina by photoreceptor glutamate may induce increased expression and activity of Na+,K+-ATPase, with a consequent impact on distal glutamate responses.

Descriptors: zebrafish, excitatory amino acid agonists pharmacology, glutamic acid pharmacology, membrane potentials drug effects, Na+ K+ exchanging ATPase metabolism, neurons metabolism, retina metabolism, 6 cyano 7 nitroquinoxaline 2,3 dione pharmacology, calcium channels, l type metabolism, calcium channels, l type physiology, calibration, chlorides metabolism, electric stimulation, enzyme inhibitors pharmacology, excitatory amino acid antagonists pharmacology, immunohistochemistry, interneurons drug effects, interneurons metabolism, ion channel gating drug effects, Na+ K+ exchanging ATPase antagonists and inhibitors, neurons drug effects, ouabain pharmacology, potassium channels drug effects and metabolism, glutamate receptors drug effects, retina cytology, retina drug effects, sodium channels drug effects and metabolism, chemical stimulation.

Nguyen, N., M. Sugimoto, and Y. Zhu (2006). **Production and purification of recombinant somatolactin beta and its effects on melanosome aggregation in zebrafish.** *General and Comparative Endocrinology* 145(2): 182-7. ISSN: 0016-6480.

NAL Call Number: 444.8G28

Abstract: A second form of somatolactin, somatolactin beta (SLbeta), was recently discovered in zebrafish (*Danio rerio*). This novel subtype of somatolactin is distantly related to somatolactin alpha (SLalpha) found in teleost species and is produced in a different region of the pituitary. To date, no physiological study of SLbeta has been reported. In order to study the physiological functions of SLbeta, recombinant SLbeta protein has been produced and purified. The cDNA of zebrafish SLbeta was cloned into a pET100 bacteria expression vector and His-tagged fusion proteins were produced in BL21 (DE3) *Escherichia coli* cells. The majority of recombinant somatolactins produced by *E. coli* were isolated in inclusion bodies although a small percentage of recombinant proteins (<1%) were also found in soluble form. Fusion proteins were solubilized from inclusion bodies using 6M guanidine hydrochloride. Pure recombinant somatolactins were obtained by affinity purification. The estimated molecular weight of 28 kDa by sodium dodecyl sulfate-polyacrylamide gel electrophoresis correlates with the molecular mass calculated from the deduced amino acid sequence of SLbeta. Thereafter, specific polyclonal antibodies against the recombinant SLbeta were developed. These antibodies recognized specifically a group of cells located in the anterior pars intermedia of the pituitary. The antibodies did not react with SLalpha, growth hormone or prolactin cells in the zebrafish pituitary glands. Furthermore, recombinant SLbeta induced melanosome aggregation in a concentration-dependent manner in skin of zebrafish scales. Significant melanosome aggregation was observed in zebrafish melanophores at a concentration of 1 microg/ml. These results, combined with previous reports demonstrate that the recombinant SLbeta proteins produced here are bioactive. The function of inducing melanosome aggregation is conserved among the somatolactin functions.

Descriptors: zebrafish, fish proteins biosynthesis, glycoproteins biosynthesis, melanosomes drug effects, pituitary hormones biosynthesis, recombinant proteins biosynthesis, proteins biosynthesis, molecular cloning, complementary DNA metabolism, polyacrylamide gel electrophoresis, fish proteins genetics and pharmacology, glycoproteins genetics and pharmacology, melanocytes physiology and ultrastructure, melanosomes physiology, pituitary gland cytology and metabolism, pituitary hormones genetics and pharmacology, recombinant pro-

teins isolation and purification and pharmacology, skin drug effects, skin ultrastructure and pharmacology.

Novak, A.E. and A.B. Ribera (2005). **The zebrafish as an integrative physiology model**. *Integrative Physiology in the Proteomics and Post-Genomics Age,* TOTOWA, NJ 07512-1165 USA, p. 215-237. ISBN: 1588293157.
Descriptors: zebrafish, development, nervous system, neural coordination.

O'Malley, D.M., N.S. Sankrithi, M.A. Borla, S. Parker, S. Banden, E. Gahtan, and H.W.3. Detrich (2004). **Optical physiology and locomotor behaviors of wild-type and nacre zebrafish.** *Methods in Cell Biology* 76: 261-84. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, behavior physiology, locomotion physiology, genetically modified, axotomy methods, brain stem and calcium signaling physiology, calmodulin genetics and metabolism, cell survival drug effects, central nervous system physiology, DNA binding proteins genetics, dextrans chemistry and pharmacology, diagnostic imaging methods, escape reaction physiology, fluorescent dyes chemistry, immediate genes early genetics and physiology, FOS and physiology, genotype, green fluorescent proteins genetics and metabolism, larva physiology, locomotion genetics, microphthalmia associated transcription factor, confocal microscopy methods, fluorescence microscopy methods, mutation, myosin light chain kinase genetics, myosin light chain kinase metabolism, peptide fragments genetics and metabolism, phenylthiourea pharmacology, photic stimulation, photosensitizing agents chemistry and pharmacology, physical stimulation, pigmentation drug effects, spinal cord physiology, swimming physiology, transcription factors genetics.

Obara, T., S. Mangos, Y. Liu, J. Zhao, S. Wiessner, A.G. Kramer Zucker, F. Olale, A.F. Schier, and I.A. Drummond (2006). **Polycystin-2 immunolocalization and function in zebrafish.** *Journal of the American Society of Nephrology* 17(10): 2706-18. ISSN: 1555-9041.
Abstract: Polycystin-2 functions as a cation-permeable transient receptor potential ion channel in kidney epithelial cells and when mutated results in human autosomal dominant polycystic kidney disease. For further exploration of the in vivo functions of Polycystin-2, this study examined its expression and function during zebrafish embryogenesis. pkd2 mRNA is ubiquitously expressed, and its presence in the larval kidney could be confirmed by reverse transcription-PCR on isolated pronephroi. Immunostaining with anti-zebrafish Polycystin-2 antibody revealed protein expression in motile kidney epithelial cell cilia and intracellular cell membranes. Intracellular localization was segment specific; in the proximal nephron segment, Polycystin-2 was localized to basolateral cell membranes, whereas in the caudal pronephric segment, Polycystin-2 was concentrated in subapical cytoplasmic vesicles. Polycystin-2 also was expressed in muscle cells and in a variety of sensory cells that are associated with mechanotransduction, including cells of the ear, the lateral line organ, and the olfactory placodes. Disruption of Polycystin-2 mRNA expression resulted in pronephric kidney cysts, body axis curvature, organ laterality defects, and hydrocephalus-defects that could be rescued by expression of a human PKD2 mRNA. In-frame deletions in the first extracellular loop and C-terminal phosphofurin acidic cluster sorting protein-1 (PACS-1) binding sites in the cytoplasmic tail caused Polycystin-2 mislocalization to the apical cell surface. Unlike zebrafish intraflagellar transport protein (IFT) mutants, cyst formation was not associated with cilia defects and instead correlated with reduced kidney fluid output, expansion of caudal duct apical cell membranes, and occlusion of the caudal pronephric nephron segment.
Descriptors: zebrafish , trpp cation channels metabolism, amino acid sequence, cell mem-

brane metabolism, embryonic development, developmental gene expression regulation, kidney embryology and metabolism, microinjections, fluorescence microscopy, molecular sequence data, antisense oligoribonucleotides pharmacology, messenger RNA metabolism, subcellular fractions.

Okamoto, H., Y. Hirate, and H. Ando (2004). **Systematic identification of factors in zebrafish regulating the early midbrain and cerebellar development by odered differential display and caged mRNA technology.** *Frontiers in Bioscience: a Journal and Virtual Library* 9: 93-9. ISSN: 1093-9946.
NAL Call Number: QH301.F76
Abstract: During brain development, various transcription factors are activated in the regional specific manner and define the identities characteristic to individual regions, and many of such factors have been identified in the vertebrate brain, by taking advantage of the structural and functional conservation of them with the invertebrate counterparts. However, it is still largely unknown why individual transcription factors can define the final morphology and function of the tissues expressing these factors because of the lack of knowledge on which genes are actually up- or down-regulated as downstream targets of individual transcription factors. In this review, we introduce novel technologies which we have invented or improved as a part of our endeavor to identify and functionally analyze the downstream target genes of Isle-3 which are involved in development of the midbrain and the midbrain/hindbrain boundary region in zebrafish embryos. Our strategy and technologies can be applied to analyzing the downstream genes of any other transcription factors.
Descriptors: zebrafish developing brain, biotechnology methods, cerebellum physiology, eye, homeodomain, mesencephalon physiology, nerve tissue, cerebellum embryology, eye homeodomain mesencephalon embryology, nerve tissue messenger RNA analysis.

Orger, M.B., E. Gahtan, A. Muto, P. Page McCaw, M.C. Smear, and H. Baier (2004). **Behavioral screening assays in zebrafish.** *Methods in Cell Biology* 77: 53-68. ISSN: 0091-679X.
NAL Call Number: 442P92
Descriptors: zebrafish, behavior physiology, screening assays, methods, mutation, genetics.

Pan, T.C., B.K. Liao, C.J. Huang, L.Y. Lin, and P.P. Hwang (2005). **Epithelial Ca(2+) channel expression and Ca(2+) uptake in developing zebrafish.** *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 289(4): R1202-11. ISSN: 0363 6119.
Abstract: The purpose of the present work was to study the possible role of the epithelial Ca(2+) channel (ECaC) in the Ca(2+) uptake mechanism in developing zebrafish (*Danio rerio*). With rapid amplification of cDNA ends, full-length cDNA encoding the ECaC of zebrafish (zECaC) was cloned and sequenced. The cloned zECaC was 2,578 bp in length and encoded a protein of 709 amino acids that showed up to 73% identity with previously described vertebrate ECaCs. The zECaC was found to be expressed in all tissues examined and began to be expressed in the skin covering the yolk sac of embryos at 24 h postfertilization (hpf). zECaC-expressing cells expanded to cover the skin of the entire yolk sac after embryonic development and began to occur in the gill filaments at 96 hpf, and thereafter zECaC-expressing cells rapidly increased in both gills and yolk sac skin. Corresponding to ECaC expression profile, the Ca(2+) influx and content began to increase at 36-72 hpf. Incubating zebrafish embryos in low-Ca(2+) (0.02 mM) freshwater caused upregulation of the whole body Ca(2+) influx and zECaC expression in both gills and skin. Colocalization of zECaC mRNA and the Na(+)-K(+)-ATPase alpha-subunit (a marker for mitochondria-rich cells) indicated that only a portion of the mitochondria-rich cells expressed zECaC mRNA.

These results suggest that the zECaC plays a key role in Ca(2+) absorption in developing zebrafish.
Descriptors: zebrafish embryology, calcium metabolism, calcium channels chemistry and metabolism, developmental gene expression regulation physiology, metabolism, amino acid sequence, calcium signaling physiology, molecular sequence data, organ specificity, amino acid sequence homology, tissue distribution, proteins chemistry.

Pan, X., H. Yu, X. Shi, V. Korzh, and T. Wohland (2007). **Characterization of flow direction in microchannels and zebrafish blood vessels by scanning fluorescence correlation spectroscopy.** *Journal of Biomedical Optics* 12(1): 014034. ISSN: 1083-3668.
Abstract: The investigation of flow profiles in microstructures and tissues by fluorescence correlation spectroscopy (FCS) has been a challenging topic in the past decade. Due to its inherent optical configuration, a circular focused laser beam, FCS is unable to resolve microfluidic flow directions. Earlier schemes reported the use of two laser beams or the use of nonsymmetrical laser foci to break the symmetry of the measurement system. This, however, is difficult to combine with confocal systems since it would require modifications that interfere with the imaging capabilities. We propose a method called line-scan FCS to measure different flow angles in microchannels and tissues. This method is implemented on a combined laser scanning confocal microscopy (LSCM) and FCS system that enables uncompromised imaging and spectroscopy measurements. We demonstrate that by scanning the laser beam with a defined speed and direction we can measure flow direction with the current system at an optimal resolution of at least 3 microm. The combination system is assessed by measuring flow profiles in a microchannel with and without obstruction. To extend the technique to live tissue measurements we demonstrate that line-scan FCS can determine the flow direction in zebrafish small blood vessels in a label-free approach.
Descriptors: zebrafish, blood flow velocity physiology, microcirculation physiology, microfluidics instrumentation, confocal microscopy instrumentation, fluorescence microscopy instrumentation, fluorescence spectrometry instrumentation, equipment design, equipment failure analysis, microfluidics methods, confocal microscopy methods, fluorescence microscopy methods, reproducibility of results, sensitivity and specificity, fluorescence spectrometry methods.

Papasani, M.R., B.D. Robison, R.W. Hardy, and R.A. Hill (2006). **Early developmental expression of two insulins in zebrafish (*Danio rerio*).** *Physiological Genomics* 27(1): 79-85. ISSN: 1094-8341.
Abstract: We have cloned a second insulin gene in zebrafish and studied temporal and spatial expression of two zebrafish insulin genes. Zebrafish insulin-a (insa) and -b (insb) mRNAs are derived from two different DNA contigs on chromosomes 5 and 14, respectively, representing two different insulin genes. Real-time PCR studies suggest that insa is a maternal and also a postzygotic transcript. insa was observed at 1 h postfertilization (hpf) and was rapidly degraded by 6 and 12 hpf but induced at 24 hpf (i.e., after pancreas formation). Expression levels at 24 hpf were approximately 220-fold higher than at 6 hpf and were significantly different from earlier time points. At 72 hpf (at time of hatching), zebrafish insa mRNA levels tended to be higher than at 24 hpf and were approximately 727-fold higher compared with 6 hpf. This further increase in insa expression may be one of the many rapid physiological changes associated with hatching. insb expression was observed from 1 hpf and was significantly decreased from 12 hpf onward. Its expression levels at 12 and 24 hpf were approximately twofold and sixfold lower, respectively, compared with expression at 6 hpf. insb expression levels at 48 hpf were significantly lower than at 24 hpf but not different from

72 hpf. Expression levels at 72 hpf were approximately 61-fold lower than at 6 hpf. In situ hybridization studies showed insb expression in proliferating blastomeres at 3 and 4 hpf. At later time points, insb expression was restricted to the brain and pancreas (24 and 48 hpf). insa expression was observed in the pancreas at 24 and 48 hpf. Expression of insb in blastomeres and head suggests that insb could be acting as a pro-growth, survival, and neurotrophic factor during development. Pancreatic insa and insb may both be involved in regulation of glucose homeostasis as in mammals.

Descriptors: zebrafish embryos, insulin genetics, amino acid and base sequence, chromosome mapping, molecular cloning, gene expression, in situ hybridization, insulin metabolism, molecular sequence data, messenger RNA analysis, sequence alignment.

Patten, S.A. and D.W. Ali (2007). **AMPA receptors associated with zebrafish Mauthner cells switch subunits during development.** *Journal of Physiology* 581(Pt 3): 1043-56. ISSN: 0022-3751.

NAL Call Number: 447.8J82

Abstract: Glutamate AMPA receptors (AMPARs) are major excitatory receptors in the vertebrate CNS. In many biological systems there is a developmental speeding in AMPAR kinetics, which occurs either because of a switch in AMPAR subunits or a change in synaptic morphology. We studied the development of AMPAR-mediated miniature excitatory postsynaptic currents (AMPAR-mEPSCs) in zebrafish Mauthner cells (M-cells) to determine the reasons underlying the speeding of AMPA mEPSCs in this preparation. We recorded AMPAR-mEPSCs in zebrafish ranging in age from 33 h postfertilization (hpf) to 72 hpf. We found that the glutamate waveform in the synaptic cleft did not change during development, suggesting that synaptic morphology played little role in shaping the mEPSC. The current-voltage (I-V) relationship was linear at 33 hpf and outwardly rectified in older animals, while AMPAR decay kinetics were slower at positive potentials, compared with negative potentials. The relative change in tau with depolarization was found to be greater at 48 hpf than at 33 hpf. AMPARs in 33 hpf fish had a conductance of approximately 9 pS, and in older fish approximately 15 pS. Finally, the desensitization blocker, cyclothiazide, increased tau by approximately 4-fold in 48 hpf preparations, but only 1.5-fold in 33 hpf fish. These results are consistent with the hypothesis that the major mechanism underlying the developmental speeding in AMPAR kinetics in zebrafish CNS is a switch in receptor subunits. To our knowledge this is the first study to suggest that AMPARs change subunits during development in fish.

Descriptors: zebrafish Mauthner cells, glutamic acid metabolism, neurons metabolism, AMPA receptors metabolism, rhombencephalon metabolism, synaptic transmission drug effects, metabolism, benzothiadiazines pharmacology, excitatory postsynaptic potentials, ion channel gating, kinetics, neurons drug effects, protein subunits metabolism, AMPA receptors drug effects, rhombencephalon drug effects, embryology, growth and development.

Patterson, L.J. and R. Patient (2006). **The "Ets" factor: vessel formation in zebrafish--the missing link?** *Public Library of Science Biology* 4(1): E24. ISSN: 1544-9173.

NAL Call Number: QH301.P56

Descriptors: zebrafish embryos, blood vessels embryology, vascular endothelium embryology, signal transduction physiology, vascular endothelial growth factor A physiology.

Pelster, B., S. Grillitsch, and T. Schwerte (2005). **NO as a mediator during the early development of the cardiovascular system in the zebrafish.** *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology* 142(2): 215-20. ISSN: 1095 6433.

NAL Call Number: QP1C6

Abstract: As a general pattern innervation of the cardiovascular system appears late during development in vertebrate embryos, and cardiovascular control may be achieved by hormonal activity in early stages. However, very little is known about the onset of NO-responsiveness during development, which in adult vertebrates is known to play a key function in many physiological processes such as control of vascular tone, neurotransmission, macrophage activity, and angiogenesis. Analysis of the effect of NO on the cardiovascular system in zebrafish (*Danio rerio*) embryos and larvae revealed almost no effect on cardiac activity during chronic exposure to NO-producing chemicals, whereas vascular reactivity was observed in veins and arteries of the zebrafish in early developmental stages (5-6 days post fertilization). Chronic exposure also modified the development of the vascular system. The presence of an NO donor (sodium nitroprusside) did not change the patterning of the vascular bed, but it induced an earlier appearance of some blood vessels in the trunk region of the zebrafish larvae. The data reveal that NO plays an important role in the development of the cardiovascular system and in the ontogeny of the cardiovascular control system in fish.

Descriptors: zebrafishembryos, cardiovascular system, nitric oxide physiology, fertilization, guanylate cyclase metabolism, heart rate, macrophages metabolism, pathologic neovascularization, neurons metabolism, neurotransmitter agents, nitric oxide metabolism, nitric oxide donors pharmacology, nitroprusside pharmacology, protein isoforms, temperature, time factors.

Pelster, B., A.M. Sanger, M. Siegele, and T. Schwerte (2003). **Influence of swim training on cardiac activity, tissue capillarization, and mitochondrial density in muscle tissue of zebrafish larvae.** *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 285(2): R339-47. ISSN: 0363 6119.

Abstract: Larval zebrafish (*Danio rerio*) of two different age classes ("swim-up" larvae, 9 days old; "free-swimming" larvae, 21 days old) were exposed to either an endurance/continuous training or interval training. Control animals were kept in stagnant water. A comparison of cardiac activity of trained (either endurance or interval) and untrained animals at the end of the training regime revealed no differences in heart rate, end-diastolic and end-systolic ventricular volume, and cardiac output. Training also had no influence on the concentration of erythrocytes in the blood. Thus, at the level of total oxygen transport in the blood, training did not provoke any improvement during the first 32 days of development. Significant changes, however, were observed at the tissue level. In free-swimming larvae [i.e., between 21 and 32 days postfertilization (dpf)] endurance training increased the capillarization of both axial muscle caudal to the anus and the tail fin. In addition, mitochondrial density of red and intermediate muscle fibers increased significantly. In contrast to capillarization, even swim-up larvae, trained between 9 and 15 dpf, were affected. The observed increase in mitochondrial content indicates a high demand for oxygen and energy-rich metabolites for oxidative phosphorylation. In older larvae, this is met by the increase in capillarization that improves the blood supply and with it the required oxygen and metabolite supply of muscle tissue. Both of these adaptational changes result in a reduction of diffusion distances (between capillary and muscle fiber as well as mitochondria) and may contribute to a higher resistance toward oxygen deficiency. Furthermore, this study indicates that plasticity of muscle tissue is already established in early stages of development at both the tissue and cellular levels.

Descriptors: zebrafish larva, capillaries physiology, heart physiology, mitochondria physiology, skeletal muscle blood supply, physiologic neovascularization, swimming physiology, erythrocytes cytology, exertion physiology, larva cytology, physiology and ultrastructure,

mitochondria ultrastructure, skeletal muscle cytology and ultrastructure, zebrafish growth and development.

Puech, P.H., A. Taubenberger, F. Ulrich, M. Krieg, D.J. Muller, and C.P. Heisenberg (2005). **Measuring cell adhesion forces of primary gastrulating cells from zebrafish using atomic force microscopy.** *Journal of Cell Science* 118(Pt 18): 4199-206. ISSN: 0021-9533.
NAL Call Number: QH301.J6
Abstract: During vertebrate gastrulation, progenitor cells of different germ layers acquire specific adhesive properties that contribute to germ layer formation and separation. Wnt signals have been suggested to function in this process by modulating the different levels of adhesion between the germ layers, however, direct evidence for this is still lacking. Here we show that Wnt11, a key signal regulating gastrulation movements, is needed for the adhesion of zebrafish mesendodermal progenitor cells to fibronectin, an abundant extracellular matrix component during gastrulation. To measure this effect, we developed an assay to quantify the adhesion of single zebrafish primary mesendodermal progenitors using atomic-force microscopy (AFM). We observed significant differences in detachment force and work between cultured mesendodermal progenitors from wild-type embryos and from slb/wnt11 mutant embryos, which carry a loss-of-function mutation in the wnt11 gene, when tested on fibronectin-coated substrates. These differences were probably due to reduced adhesion to the fibronectin substrate as neither the overall cell morphology nor the cell elasticity grossly differed between wild-type and mutant cells. Furthermore, in the presence of inhibitors of fibronectin-integrin binding, such as RGD peptides, the adhesion force and work were strongly decreased, indicating that integrins are involved in the binding of mesendodermal progenitors in our assay. These findings demonstrate that AFM can be used to quantitatively determine the substrate-adhesion of cultured primary gastrulating cells and provide insight into the role of Wnt11 signalling in modulating cell adhesion at the single cell scale.
Descriptors: zebrafish, cell adhesion physiology, gastrula cytology, mesenchymal stem cells cytology, cultured cells, fibronectins metabolism , atomic force microscopy methods, Wnt proteins genetics and physiology.

Pullela, P.K., T. Chiku, M.J.I. Carvan, and D.S. Sem (2006). **Fluorescent probes for in vivo redox potential measurement and thiol rich protein function**. *Federation of American Societies for Experimental Biology Journal* 20(4, Part 1): A74. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: Fluorescence study of thiol rich proteins like metalothioneins and intracellular thiols have met with poor success. We synthesized a set of DSSA probes to study intracellular thiols and surface rich proteins. These reagents are shown to cross bacterial cell wall and are monitored by increase in fluorescence due to reaction of GSH with DSSA probe. When uptake studies are done with *E.coli* Origami cells, which are deficient in glutathione synthesis, much less fluorescence was observed. This suggests the utility of the DSSA probes for studying changes in intracellular GSH and changes in redox potential. The DSSA probes have redox potentials of around - 0.6 V, suggesting that their use in vivo will not disrupt intracellular redox state by oxidizing the cell. As a complement to these bacterial uptake studies, the DSSA probes are used to monitor thiol levels in a eukaryotic system: developing zebrafish embryos. Embryos were either incubated with the probe, or probe was microinjected past the chorion layer. In both cases, significant accumulation of probe was observed in the chorion suggesting the presence of thiol-rich proteins. We propose that, zebrafish have a mechanism by which the surface cysteine rich proteins serve as protective layer. Considering these results, these DSSA probes should have utility for monitoring thiol rich protein

function, dynamics and importantly, measuring redox potentials of intracellular components. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, methods and techniques, thiol rich proteins, cysteine rich proteins, redox potentials, fluorescence detection, spectrum analysis techniques.

Rawashdeh, O., N.H. de Borsetti, G. Roman, and G.M. Cahill (2007). **Melatonin suppresses nighttime memory formation in zebrafish.** *Science* 318(5853): 1144-6. ISSN: 0036-8075.
NAL Call Number: 470Sci2
Abstract: Memory processes are modulated by the biological clock, although the mechanisms are unknown. Here, we report that in the diurnal zebrafish both learning and memory formation of an operant conditioning paradigm occur better during the day than during the night. Melatonin treatment during the day mimics the nighttime suppression of memory formation. Training in constant light improves nighttime memory formation while reducing endogenous melatonin concentrations. Treatment with melatonin receptor antagonists at night dramatically improves memory. Pinealectomy also significantly improves nighttime memory formation. We adduce that melatonin is both sufficient and necessary for poor memory formation during the night.
Descriptors: zebrafish, circadian rhythm, melatonin physiology, memory physiology, darkness.

Redd, M.J., G. Kelly, G. Dunn, M. Way, and P. Martin (2006). **Imaging macrophage chemotaxis in vivo: studies of microtubule function in zebrafish wound inflammation.** *Cell Motility and the Cytoskeleton* 63(7): 415-22. ISSN: 0886 1544.
NAL Call Number: QH647.C45
Abstract: The inflammatory response is one of the most dramatic examples of directed cell movement in nature. Inflammation is triggered at the site of injury and results in the migration of immune cells to the site to protect the host from infection. We have devised an in vivo inflammation assay using translucent zebrafish embryos, which allow live imaging and pharmacological manipulation of macrophage chemotaxis to wounds inflicted with a laser. Using this assay, we test the role of the microtubule cytoskeleton in macrophage chemotaxis in vivo using nocodazole to disrupt microtubule polymerization. We find that de-stabilisation of microtubules with nocodazole impairs macrophage recruitment to wounds, but that addition of the Rho kinase inhibitor Y-27632 suppresses these effects and restores the recruitment of macrophages to wounds. Taken together, these results suggest that destabilizing microtubules activates Rho kinase and that this increase in Rho kinase activity interferes with leukocyte recruitment in vivo.
Descriptors: zebrafish embryos, chemotaxis physiology, macrophages physiology, microtubules metabolism, yolk sac cytology and injuries, amides pharmacology, chemotaxis drug effects enzyme inhibitors pharmacology, green fluorescent proteins metabolism, inflammation physiopathology, intracellular signaling peptides and proteins, macrophages drug effects, video microscopy, microtubules drug effects, nocodazole pharmacology, protein serine threonine kinases antagonists and inhibitors, pyridines pharmacology , wounds and injuries etiology and physiopathology.

Redd, M.J., L. Cooper, W. Wood, B. Stramer, and P. Martin (2004). **Wound healing and inflammation: embryos reveal the way to perfect repair**. *Philosophical Transactions of the Royal Society of London Biological Sciences* 359(1445): 777-784. ISSN: 0962 8436.
NAL Call Number: 501L84Pb
Abstract: Tissue repair in embryos is rapid, efficient and perfect and does not leave a scar,

an ability that is lost as development proceeds. Whereas adult wound keratinocytes crawl forwards over the exposed substratum to close the gap, a wound in the embryonic epidermis is closed by contraction of a rapidly assembled actin purse string. Blocking assembly of this cable in chick and mouse embryos, by drugs or by inactivation of the small GTPase Rho, severely hinders the re-epithelialization process. Live studies of epithelial repair in GFP-actin-expressing *Drosophila* embryos reveal actin-rich filopodia associated with the cable, and although these protrusions from leading edge cells appear to play little role in epithelial migration, they are essential for final zippering of the wound edges together-inactivation of Cdc42 prevents their assembly and blocks the final adhesion step. This wound re-epithelialization machinery appears to recapitulate that used during naturally occurring morphogenetic episodes as typified by *Drosophila* dorsal closure. One key difference between embryonic and adult repair, which may explain why one heals perfectly and the other scars, is the presence of an inflammatory response at sites of adult repair where there is none in the embryo. Our studies of repair in the PU.1 null mouse, which is genetically incapable of raising an inflammatory response, show that inflammation may indeed be partly responsible for scarring, and our genetic studies of inflammation in zebrafish *(Danio rerio*) larvae suggest routes to identifying gene targets for therapeutically modulating the recruitment of inflammatory cells and thus improving adult healing. Copyright©Thomson Reuters 2009
Descriptors: zebrafish emryos wound healing model, development, inflammation, wound healing, scarring.

Ren, J.Q. and L. Li (2004). **A circadian clock regulates the process of ERG b- and d-wave dominance transition in dark-adapted zebrafish.** *Vision Research* 44(18): 2147-52. ISSN: 0042-6989.
Abstract: In zebrafish, during dark adaptation following bright light adaptation, the dominance of electroretinogram (ERG) b- and d-waves switches. In the early dark adaptation, when visual sensitivity is cone-dominant, both the b- and d-waves are readily recorded. In the late dark adaptation, along with the increase of rod sensitivity, the b-wave becomes dominant whereas the d-wave is gradually lost. The time for the ERG b- and d-wave dominance transition varies between the day and night. The transition requires a longer amount of time in the night and early morning than in the afternoon. This pattern of timing for ERG b- and d-wave dominance transition persists in constant light and can be reversed after exposure to a reversed light-dark cycle. The data suggest that the transition of the dominance of ERG b- and d-waves is regulated by an endogenous circadian clock.
Descriptors: zebrafish, circadian rhythm physiology, dark adaptation physiology, electroretinography, retina cones physiology, retinal rodsphysiology.

Rigo, J.M., C.I. Badiu, and P. Legendre (2003). **Heterogeneity of postsynaptic receptor occupancy fluctuations among glycinergic inhibitory synapses in the zebrafish hindbrain.** *Journal of Physiology* 553(Pt 3): 819-32. ISSN: 0022-3751.
NAL Call Number: 447.8J82
Abstract: The amplitude of glycinergic miniature inhibitory postsynaptic currents (mIPSCs) varies considerably in neurons recorded in the isolated hindbrain of 50-h-old zebrafish larvae. At this age, glycinergic synapses are functionally mature. In order to measure the occupancy level of postsynaptic glycine receptors (GlyRs) and to determine the pre- and/or postsynaptic origin of its variability, we analysed mIPSCs within bursts evoked by alpha-latrotoxin (0.1-1 nM). Two types of burst were observed according to their mIPSC frequencies: 'slow' bursts with clearly spaced mIPSCs and 'fast' bursts characterised by superimposed events. Non-stationary noise analysis of mIPSCs in some 'slow' bursts recorded in the presence or in the

absence of Ca2+ denoted that mIPSC amplitude variance did not depend on the quantity of neurotransmitters released (presynaptic origin), but rather on intrinsic stochastic behaviour of the same group of GlyRs (postsynaptic origin). In these bursts, the open probability measured at the peak of the mIPSCs was close to 0.5 while the maximum open probability is close to 0.9 for the synaptic isoform of GlyRs (heteromeric alpha1/beta GlyRs). In 'fast' bursts with superimposed events, a correlation was found between the amplitude of mIPSCs and the basal current level measured at their onset, which could suggest that the same group of GlyRs is activated during such bursts. Altogether, our results indicate that glycine synapses can display different release modes in the presence of alpha-latrotoxin. They also indicate that, in our model, postsynaptic GlyRs cannot be saturated by the release of a single vesicle.
Descriptors: zebrafish hindbrain, evoked potentials drug effects, neurons physiology, glycine receptors physiology, rhombencephalon physiology, synapses physiology, electric stimulation, electrophysiology methods, kinetics, neurons drug effects, patch clamp techniques, spider venoms pharmacology, synapses drug effects.

Rinner, O., J.M. Rick, and S.C. Neuhauss (2005). **Contrast sensitivity, spatial and temporal tuning of the larval zebrafish optokinetic response.** *Investigative Ophthalmology and Visual Science* 46(1): 137-42. ISSN: 0146-0404.
Abstract: PURPOSE: To characterize the quantitative properties of the optokinetic response (OKR) in zebrafish larvae as a tool to test visual performance in genetically modified larvae. METHODS: Horizontal OKR was triggered in 5-day-old zebrafish larvae by stimulation with projected computer-generated gratings of varying contrast, angular velocity, temporal and spatial frequency, and brightness. Eye movements were analyzed by a custom-made eye tracker based on image analysis. RESULTS: The gain of the OKR slow phase was dependent on angular velocity, spatial frequency, and contrast of a moving grating, but largely independent on brightness. Eye velocity was a logarithmically linear function of grating contrast with a slope of approximately 0.8 per log unit contrast. CONCLUSIONS: The OKR of the larval zebrafish is not scaled for stimulus contrast and spatial frequency. These properties make the OKR a valuable tool to quantify behavioral visual performance such as visual acuity, contrast sensitivity, and light adaptation. This behavioral paradigm will be useful for analyzing visual performance in mutant and gene-knockdown larval zebrafish.
Descriptors: zebrafish larva optokinetic response, contrast sensitivity physiology, optokinetic nystagmus physiology, space perception physiology, behavior physiology, larva physiology, light.

Risner, M.L., E. Lemerise, E.V. Vukmanic, and A. Moore (2006). **Behavioral spectral sensitivity of the zebrafish (*Danio rerio*).** *Vision Research* 46(17): 2625-35. ISSN: 0042-6989.
Abstract: While the zebrafish *(Danio rerio*) continues to become an important animal model for the investigation of the genetic and physiological bases of visual processing of the vertebrate retina, its visual behavior, particularly regarding color processing, has received little attention. The purpose of this study was to obtain behavioral spectral sensitivity functions from adult zebrafish using an appetitive instrumental conditioning procedure. A three-chamber maze was implemented to train light-adapted adult zebrafish to swim into the chamber that contained a suprathreshold monochromatic stimulus for a food reward. Visual threshold was determined by varying the stimulus irradiance using a 'two-down one-up' staircase procedure. Threshold values were obtained for wavelengths from 340 to 640 nm. Spectral sensitivity functions obtained show contributions from two nonopponent cone mechanisms (UV and S) and two opponent mechanisms (M-S and L-M). These cone mechanisms are qualitatively similar to those obtained via physiological measures from the On-responses of

the zebrafish retina and optic tectum. However, the functions are not quantitatively similar suggesting that further visual processing takes place beyond the processing of the retinal circuitry and processing of the initial stages of the optic tectum. These results demonstrate that the zebrafish is an excellent model to examine and compare the relationship between physiological and behavioral color processing.
Descriptors: zebrafish retinal optic tectum, behavior physiology, color perception physiology, conditioning, operant physiology, retina cones physiology, discrimination learning, electroretinography, sensory thresholds physiology.

Rohde, L.A. and C.P. Heisenberg (2007). **Zebrafish gastrulation: cell movements, signals, and mechanisms.** *International Review of Cytology* 261: 159-92. ISSN: 0074-7696.
NAL Call Number: 442.8IN82
Abstract: Gastrulation is a morphogenetic process that results in the formation of the embryonic germ layers. Here we detail the major cell movements that occur during zebrafish gastrulation: epiboly, internalization, and convergent extension. Although gastrulation is known to be regulated by signaling pathways such as the Wnt/planar cell polarity pathway, many questions remain about the underlying molecular and cellular mechanisms. Key factors that may play a role in gastrulation cell movements are cell adhesion and cytoskeletal rearrangement. In addition, some of the driving force for gastrulation may derive from tissue interactions such as those described between the enveloping layer and the yolk syncytial layer. Future exploration of gastrulation mechanisms relies on the development of sensitive and quantitative techniques to characterize embryonic germ-layer properties.
Descriptors: zebrafish gastrulation, cell movement physiology, signal transduction, cell adhesion, physiology, extracellular matrix metabolism, gastrula physiology, Wnt proteins metabolism.

Romano, A., G. Kottra, A. Barca, N. Tiso, M. Maffia, F. Argenton, H. Daniel, C. Storelli, and T. Verri (2006). **High-affinity peptide transporter PEPT2 (SLC15A2) of the zebrafish *Danio rerio*: functional properties, genomic organization, and expression analysis.** *Physiological Genomics* 24(3): 207-17. ISSN: 1094-8341.
Abstract: Solute carrier 15 (SLC15) membrane proteins PEPT1 (SLC15A1) and PEPT2 (SLC15A2) have been described in great detail in mammals. In contrast, information in lower vertebrates is limited. We characterized the functional properties of a novel zebrafish peptide transporter orthologous to mammalian and avian PEPT2, described its gene (pept2) structure, and determined mRNA tissue distribution. An expressed sequence tag (EST) cDNA (Integrated Molecular Analysis of Gene Expression; IMAGE) corresponding to zebrafish pept2 was completed by inserting a stretch of 75 missing nucleotides in the coding sequence to obtain a 3,238-bp functional clone. The complete open reading frame (ORF) was 2,160 bp and encoded a 719-amino acid protein. Electrophysiological analysis after cRNA injection in Xenopus laevis oocytes suggested that zebrafish PEPT2 is a high-affinity/low-capacity transporter (K(0.5) for glycyl-L-glutamine approximately 18 microM at -120 mV and pH 7.5). Zebrafish pept2 gene was 19,435 kb, thus being the shortest vertebrate pept2 fully characterized so far. Also, zebrafish pept2 exhibited 23 exons and 22 introns, whereas human and rodent pept2 genes contain 22 exons and 21 introns only. Zebrafish pept2 mRNA was mainly detected in brain, kidney, gut, and, interestingly, otic vesicle, the embryonic structure that develops into the auditory/vestibular organ, homolog to the higher vertebrate inner ear, of the adult fish. Characterization of zebrafish pept2 will contribute to the investigation of peptide transporters using a well-established genetic model and will allow the elucidation of the evolutionary and functional relationships among vertebrate peptide

transporters. Moreover, it can represent a useful marker to screen mutations that affect choroid plexus and inner ear development.
Descriptors: zebrafish embryos, membrane transport symporters genetics, amino acid and base sequence, inner ear embryology and metabolism , electrophysiology in situ hybridization, larva genetics and metabolism, membrane transport proteins metabolism, genetic models, molecular sequence data, oocytes metabolism, phylogeny, messenger RNA metabolism, sequence analysis, symporters metabolism and pharmacokinetics, tissue distribution, *Xenopus* metabolism.

Rombough, P.J. (2007). **Ontogenetic changes in the toxicity and efficacy of the anaesthetic MS222 (tricaine methanesulfonate) in zebrafish (*Danio rerio*) larvae.** *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology* 148(2): 463-9. ISSN: 1095 6433.
NAL Call Number: QP1C6
Abstract: Median lethal (LC(50)) and effective (EC(50)) concentrations for 1-h and 24-h exposures to the anaesthetic MS222 (tricaine methanesulfonate) were determined for zebrafish *Danio rerio* larvae ranging in age from 3 days postfertilization (dpf) to 9 dpf. Cessation of heart beat was used as the indicator of death (LC(50)) while failure to respond to direct mechanical stimulation of the head region was taken as an indication of deep anaesthesia (EC(50)). 1-h LC(50)s, 1-h EC(50)s and 24-h EC(50)s all decreased gradually but significantly (all P<0.01) with age. Mean values for 1-h LC(50)s were 1633 mg L(-1) and 730 mg L(-1), respectively, for 3 dpf and 9 dpf larvae. Mean value for 1-h and 24-h EC(50) s were 106 mg L(-1) and 100 mg L(-1), respectively, at 3 dpf and 65 mg L(-1) and 31 mg L(-1), respectively, at 9 dpf. The gradual increase with age in sensitivity to the anaesthetic implied by these indicators is probably a reflection of ontogenetic changes in the activity of detoxification pathways. Mean values for the 24-h LC(50) also decreased significantly (P<0.001) with age, from 566 mg L(-1) at 3 dpf to 64 mg L(-1) at 9 dpf. However, unlike the other indicators, the decrease was not gradual but occurred in a step-like fashion with virtually all of the change occurring between 4 dpf and 7 dpf. This sharp increase in sensitivity coincides with the shift in the major site of systemic ionoregulatory activity from the skin to the gills. The implications of these ontogenetic changes in lethal and effective levels for researchers or others intending to use the anaesthetic with fish larvae are discussed.
Descriptors: zebrafish larva, aminobenzoic acids toxicity, anesthetics toxicity, consciousness drug effects, heart rate drug effects, drug dose response relationship, larva drug effects and growth and development, lethal dose 50, time factors.

Saint Amant, L. and P. Drapeau (2003). **Whole-cell patch-clamp recordings from identified spinal neurons in the zebrafish embryo.** *Methods in Cell Science* 25(1-2): 59-64. ISSN: 1381-5741.
NAL Call Number: QH573.T52
Abstract: We describe a preparation for obtaining patch-clamp recordings from identified embryonic spinal cord interneurons, motoneurons and sensory neurons in an in vivo zebrafish preparation. This preparation is used to study the spatial and temporal patterns of spontaneous and touch-evoked electrical activity during the initial development of circuitry in the spinal cord. The combination of these physiological techniques with the powerful genetic and molecular tools available in the zebrafish has the potential to increase our understanding of the complex interactions between genes and electrical activity during the development of the vertebrate nervous system.

Descriptors: zebrafish embryo, neurons physiology, patch clamp techniques, spinal cord physiology, electrophysiology.

Santos, E.M., V.L. Workman, G.C. Paull, A.L. Filby, K.J. Van Look, P. Kille, and C.R. Tyler (2007). **Molecular basis of sexand reproductive status in breeding zebrafish.** *Physiological Genomics* 30(2): 111-22. ISSN: 1094-8341.

Abstract: The zebrafish (*Danio rerio*) is used extensively as a model species for studies on vertebrate development and for assessing chemical effects on reproduction. Despite this, the molecular mechanisms controlling zebrafish reproduction are poorly understood. We analyzed the transcriptomic profiles of the gonads of individual zebrafish, using a 17k oligonucleotide microarray, to define the molecular basis of sex and reproductive status in sexually mature fish. The gonadal transcriptome differed substantially between sexes. Among the genes overexpressed in females, 11 biological processes were overrepresented including mitochondrion organization and biogenesis, and cell growth and/or maintenance. Among the genes overexpressed in males, six biological processes were overrepresented including protein biosynthesis and protein metabolism. Analysis of the expression of gene families known to be involved in reproduction identified a number of genes differentially expressed between ovaries and testes including a number of sox genes and genes belonging to the insulin-like growth factor and the activin-inhibin pathways. Real-time quantitative PCR confirmed the expression profiles for nine of the most differentially expressed genes and indicated that many transcripts are likely to be switched off in one of the sexes in the gonads of adult fish. Significant differences were seen between the gonad transcriptomes of individual reproductively active females reflecting their stage of maturation, whereas the testis transcriptomes were remarkably similar between individuals. In summary, we have identified molecular processes associated with (gonadal) sex specificity in breeding zebrafish and established a strong relationship between individual ovarian transcriptomes and reproductive status in females.

Descriptors: zebrafish, reproduction, sex factors, gene expression profiling, nucleic acid hybridization, oligonucleotide array sequence analysis, ovary metabolism, PCR, messenger RNA genetics, testis metabolism.

Schwerte, T. and R. Fritsche (2003). **Understanding cardiovascular physiology in zebrafish and Xenopus larvae: the use of microtechniques.** *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology* 135(1): 131-45. ISSN: 1095 6433.

NAL Call Number: QP1C6

Abstract: Zebrafish and *Xenopus*, genetically accessible vertebrates with an externally developing, optically clear embryo, are ideally suited for in vivo functional dissection of the embryonic development of the circulatory system. Physiological characterizations of the cardiovascular system are still imperative for a more complete understanding of the connections between genetic/epigenetic factors and cardiovascular development. Here, we review experimental tools and methods that have been developed to measure numerous cardiovascular parameters in these millimetre-sized animals.

Descriptors: zebrafish larva, *Xenopus larva*, cardiovascular physiology, physiologic monitoring methods, *Xenopus* physiology, biosensing techniques, blood cell count methods, blood vessels anatomy and histology and physiology, heart anatomy and histology and physiology, larva, miniaturization, physiologic monitoring trends, *Xenopus* and zebrafish anatomy and histology.

Schwerte, T., D. Uberbacher, and B. Pelster (2003). **Non-invasive imaging of blood cell concentration and blood distribution in zebrafish *Danio rerio* incubated in hypoxic conditions in vivo.** *Journal of Experimental Biology* 206(Pt 8): 1299-307. ISSN: 0022-0949.
NAL Call Number: 442.8B77
Abstract: This is the first study to use a combination of digital imaging techniques and vital video microscopy to study hypoxia-induced changes in blood cell concentration, angiogenesis and blood redistribution in entire animals. Zebrafish *Danio rerio*, which are known to be independent of convective oxygen transport until about 2 weeks post-fertilization, were raised under chronic hypoxia (P(O(2))=8.7 kPa) starting at 1 day after fertilization (d.p.f.) until 15 d.p.f. In control animals, the concentration of red cells (i.e. the number of red cells per nl blood) remained constant until 7 d.p.f., and than decreased by approximately 70% until 15 d.p.f. In hypoxic animals, however, the concentration of red cells remained significantly elevated compared to control animals at 12 and 15 d.p.f. Assuming that the hemoglobin content of the red cells is similar, hypoxic animals have a higher oxygen carrying capacity in their blood. Red cell distribution within the various parts of the circulatory system, taken as an indicator for blood distribution, revealed a significant modification in the number of blood cells perfusing the organs in hypoxic animals. At 12 d.p.f., gut perfusion was reduced by almost 50% in hypoxic animals, while perfusion of the segmental muscle tissue was increased to 350% of control values. No significant changes in brain perfusion were observed under these conditions. At 15 d.p.f., the reduction in gut perfusion was abolished, although muscle perfusion was still significantly elevated. At this time, growth of hypoxic animals was less compared to control animals, revealing that hypoxia had become deleterious for further development. The vascular bed of various organs was not obviously different in hypoxic animals compared to normoxic animals.
Descriptors: zebrafish, anoxia blood, hemodynamic processes physiology, zebrafish blood, analysis of variance, blood cell count, erythropoiesis, video recording.

Schwerte, T., S. Voigt, and B. Pelster (2005). **Epigenetic variations in early cardiovascular performance and hematopoiesis can be explained by maternal and clutch effects in developing zebrafish (*Danio rerio*).** *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology* 141(2): 200-9. ISSN: 1095 6433.
NAL Call Number: QP1C6
Abstract: This is the first study to show maternal and clutch effects on early developmental parameters like blood cell concentration and cardiac performance (heart rate, stroke volume and cardiac output) in developing zebrafish larvae (2-8 days post fertilization, dpf). Ten individuals per parental pair and developmental stage were analyzed. A pronounced interclutch variation of heart rate has been found in younger stages (2-4 dpf), while interclutch variation of heart rate was small in later stages (8 dpf). This effect was more pronounced in offspring from parental fish nourished with living food. The opposite effect was observed in interclutch variation of blood cell concentration. Here only older stages showed significant interclutch variations. Stroke volume and cardiac output had very small interclutch variations throughout all stages. Heart rate was strongly dependent with developmental stage in all groups. Nutritional maternal effects on heart performance and also in blood cell concentration could be detected in the offspring of parent animals either fed with flake food or with living food. Red blood cell count, calculated as a product from red blood cell concentration, was not significantly different in both feeding groups. The number of spawned eggs was not different. In summary, these data indicate that "clutch effects" caused by maternal and/or genetic influences can affect the developmental pattern of cardiac performance and blood cell concentration.

Descriptors: zebrafish larvae, cardiovascular physiology, epigenesis, genetic physiology, hematopoiesis physiology, cardiovascular system embryology, diet, egg yolk, erythrocyte count, heart rate, maternal exposure, paternal exposure.

Sedmera, D., M. Reckova, A. deAlmeida, M. Sedmerova, M. Biermann, J. Volejnik, A. Sarre, E. Raddatz, R.A. McCarthy, R.G. Gourdie, and R.P. Thompson (2003). **Functional and morphological evidence for a ventricular conduction system in zebrafish and *Xenopus* hearts.** *American Journal of Physiology. Heart and Circulatory Physiology* 284(4): H1152-60. ISSN: 0363 6135.

Abstract: Zebrafish and *Xenopus* have become popular model organisms for studying vertebrate development of many organ systems, including the heart. However, it is not clear whether the single ventricular hearts of these species possess any equivalent of the specialized ventricular conduction system found in higher vertebrates. Isolated hearts of adult zebrafish (*Danio rerio*) and African toads (*Xenopus laevis*) were stained with voltage-sensitive dye and optically mapped in spontaneous and paced rhythms followed by histological examination focusing on myocardial continuity between the atrium and the ventricle. Spread of the excitation wave through the atria was uniform with average activation times of 20 +/- 2 and 50 +/- 2 ms for zebrafish and *Xenopus* toads, respectively. After a delay of 47 +/- 8 and 414 +/- 16 ms, the ventricle became activated first in the apical region. Ectopic ventricular activation was propagated significantly more slowly (total ventricular activation times: 24 +/- 3 vs. 14 +/- 2 ms in zebrafish and 74 +/- 14 vs. 35 +/- 9 ms in *Xenopus*). Although we did not observe any histologically defined tracts of specialized conduction cells within the ventricle, there were trabecular bands with prominent polysialic acid-neural cell adhesion molecule staining forming direct myocardial continuity between the atrioventricular canal and the apex of the ventricle; i.e., the site of the epicardial breakthrough. We thus conclude that these hearts are able to achieve the apex-to-base ventricular activation pattern observed in higher vertebrates in the apparent absence of differentiated conduction fascicles, suggesting that the ventricular trabeculae serve as a functional equivalent of the His-Purkinje system.

Descriptors: zebrafish, *Xenopus*, heart anatomy and histology, heart physiology, heart conduction system anatomy and histology, heart conduction system physiology, *Xenopus laevis* anatomy, histology and physiology, atrial function, electrocardiography, heart atria anatomy and histology, heart rate, heart ventricles anatomy and histology and physiology, immunohistochemistry, myocardium chemistry, myosins analysis.

Seki, S., T. Kouya, D.M.J. Valdez, B. Jin, T. Hara, N. Saida, M. Kasai, and K. Edashige (2007). **The permeability to water and cryoprotectants of immature and mature oocytes in the zebrafish (*Danio rerio*).** *Cryobiology* 54(1): 121-4. ISSN: 0011 2240.

NAL Call Number: QH324.C7

Abstract: To identify a stage feasible for the cryopreservation of zebrafish oocytes, we investigated the permeability to water and cryoprotectants of immature (stage III) and mature (stage V) oocytes. The permeability to water (microm/min/atm) of immature oocytes at 25 degrees C (0.37) was significantly higher than that of mature oocytes (0.10). The permeability (x10(-3)cm/min) of immature oocytes to ethylene glycol, propylene glycol, and Me(2) SO (1.49-3.03) at 25 degrees C was substantially higher than that of mature oocytes approximately 0. The permeability of immature oocytes to glycerol was also high (1.75), although the permeability could not be measured in mature oocytes. Immature oocytes would be more suitable than mature oocytes for conservation of the zebrafish.

Descriptors: zebrafish oocytes, cell membrane permeability drug effects, cryopreservation methods, cryoprotective agents metabolism, oocytes metabolism, water metabolism, dim-

ethyl sulfoxide pharmacology, ethylene glycol pharmacology, propylene glycol pharmacology, time factors.

Seki, S., T. Kouya, R. Tsuchiya, D.MJ.r. Valdez, B.o. Jin, T. Hara, N. Saida, M. Kasai, and K. Edashige (2008). **development of a reliable in vitro maturation system for zebrafish oocytes**. *Reproduction* 135(3): 285-292. ISSN: 1470-1626.
NAL Call Number: QP251.J75
Descriptors: zebrafish oocytes, method, in vitro, maturation system.

Sepich, D.S. and L. Solnica Krezel (2005). **Analysis of cell movements in zebrafish embryos: morphometrics and measuring movement of labeled cell populations in vivo.** *Methods in Molecular Biology* 294: 211-33. ISSN: 1064 3745.
Abstract: Cell movements occur in all phases of animal life from embryogenesis, to maintaining adult organs, to comprising a critical component of pathology. During gastrulation, cells demonstrate a repertoire of morphogenetic movements coordinated with fate inductions to sculpt the embryonic body. The morphogenetic behaviors, underlying mechanisms, and their control, are the subject of much current study. External development of the transparent zebrafish embryo, the abundance of mutations influencing cell movements, as well as a range of observation and manipulation methods, make the zebrafish valuable for cell movement studies. This chapter offers a conceptual background for analysis of gastrulation cell movements by reviewing how region specific cell movements shape the wild-type zebrafish embryo, and how defective morphogenetic movements alone or in combination with altered cell fate specification distort the body plans of known zebrafish mutants. We furnish methods for the morphometric analysis of embryonic shape and organ rudiments in live and fixed embryos, and present data collected from live wild-type, dorsoventral patterning (somitabun and chordino) and convergence and extension (knypek and trilobite) classes of mutants. We provide a method for quantitative assessment of the movements of cell populations in vivo, and a method for determining whether cell fate and/or movement are disturbed.
Descriptors: zebrafish embryos, cell movement physiology , physiology, morphogenesis physiology, embryology methods, endoderm cytology and physiology, gastrula cytology and physiology, mesoderm cytology and physiology, research models, mutation.

Sharma, A., K.I. Anderson, and D.J. Muller (2005). **Actin microridges characterized by laser scanning confocal and atomic force microscopy**. *Federation of European Biochemical Societies Letters* 579(9): 2001-2008. ISSN: 0014-5793.
NAL Call Number: QD415.F41
Descriptors: zebrafish, actin, cells, lasers, microscopy, *Danio rerio*, physiology, biochemistry, biophysics.

Shcherbakov, D., M. Winklhofer, N. Petersen, J. Steidle, R. Hilbig, and M. Blum (2005). **Magnetosensation in zebrafish.** *Current Biology* 15(5): R161-2. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Descriptors: zebrafish, magnetics, sensation physiology, tilapia and zebrafish physiology, avoidance learning physiology, conditioning psychology physiology, electric stimulation, motor activity physiology, reinforcement psychology.

Sheets, L., D.G. Ransom, E.M. Mellgren, S.L. Johnson, and B.J. Schnapp (2007). **Zebrafish melanophilin facilitates melanosome dispersion by regulating dynein.** *Current Biology* 17(20): 1721-34. ISSN: 0960 9822.

NAL Call Number: QH301.C85
Abstract: BACKGROUND: Fish melanocytes aggregate or disperse their melanosomes in response to the level of intracellular cAMP. The role of cAMP is to regulate both melanosome travel along microtubules and their transfer between microtubules and actin. The factors that are downstream of cAMP and that directly modulate the motors responsible for melanosome transport are not known. To identify these factors, we are characterizing melanosome transport mutants in zebrafish. RESULTS: We report that a mutation (allele j120) in the gene encoding zebrafish melanophilin (Mlpha) interferes with melanosome dispersion downstream of cAMP. Based on mouse genetics, the current model of melanophilin function is that melanophilin links myosin V to melanosomes. The residues responsible for this function are conserved in the zebrafish ortholog. However, if linking myosin V to melanosomes was Mlpha's sole function, elevated cAMP would cause mlpha(j120) mutant melanocytes to hyperdisperse their melanosomes. Yet this is not what we observe. Instead, mutant melanocytes disperse their melanosomes much more slowly than normal and less than halfway to the cell margin. This defect is caused by a failure to suppress minus-end (dynein) motility along microtubules, as shown by tracking individual melanosomes. Disrupting the actin cytoskeleton, which causes wild-type melanocytes to hyperdisperse their melanosomes, does not affect dispersion in mutant melanocytes. Therefore, Mlpha regulates dynein independently of its putative linkage to myosin V. CONCLUSIONS: We propose that cAMP-induced melanosome dispersion depends on the actin-independent suppression of dynein by Mlpha and that Mlpha coordinates the early outward movement of melanosomes along microtubules and their later transfer to actin filaments.
Descriptors: zebrafish, carrier, dynein ATPase physiology, melanosomes physiology, amino acid and base sequence, biological transport, active drug effects, carrier DNA primers genetics, hypothalamic hormones pharmacology, melanins pharmacology, melanocyte stimulating hormones pharmacology, drug effects, physiology and ultrastructure, melanosomes drug effects, microtubules drug effects and physiology, molecular sequence data, mutation, phylogeny, pigmentation drug effects, genetics and physiology, pituitary hormones pharmacology, amino acid sequence homology.

Shepard, J.L., H.M. Stern, K.L. Pfaff, and J.F. Amatruda (2004). **Analysis of the cell cycle in zebrafish embryos.** *Methods in Cell Biology* 76: 109-25. ISSN: 0091 679X.
NAL Call Number: 442P92
Descriptors: zebrafish embryos, cell cycle physiology, physiology, acridine orange chemistry, apoptosis, bromodeoxyuridine analysis and metabolism, cell cycle genetics, centrosome chemistry, DNA analysis, drug screening assays, antitumor methods, flow cytometry, histones metabolism , immunohistochemistry methods, in situ hybridization methods, in situ nick end labeling methods, mitotic spindle apparatus chemistry, mutation, phosphorylation, propidium chemistry.

Sherwood, N.M., J.A. Tello, and G.J. Roch (2006). **Neuroendocrinology of protochordates: insights from Ciona genomics**. *Comparative Biochemistry and Physiology, Part a: Molecular & Integrative Physiology* 144(3): 254-271. ISSN: 1095-6433.
NAL Call Number: QP1C6
Descriptors: zebrafish, bibliographic information, cloning, endocrinology, evolution, gastrin, genomes, gonadotropin-releasing hormone, hormones, insulin, insulin-like growth factors, ligands, neuroendocrine system, neurophysiology, neurotransmitters, phylogenetics, phylogeny, receptor mechanisms, receptors, reviews, signal transduction, transcription, transcription factors, genomics, anura, chordata, *Ciona intestinalis*, *Ciona savignyi*, *Danio rerio*,

Drosophila, Nematoda.
Notes: A review.

Soellner, C., M. Burghammer, E. Busch-Nentwich, J. Berger, H. Schwarz, C. Riekel, and T. Nicolson (2003). **Control of crystal size and lattice formation by starmaker in otolith biomineralization**. *Science* 302(5643): 282-286. ISSN: 0036-8075.
NAL Call Number: 470Sci2
Descriptors: zebrafish, calcium carbonates, crystallization, crystals, mineralization, molecular structure, morphogenesis, otoliths, *Danio rerio*, biomineralization, starmaker gene, starmarker gene, physiology, biochemistry, biophysics.

Stock, D.W. (2007). **Zebrafish dentition in comparative context.** *Journal of Experimental Zoology. Part B. Molecular and Developmental Evolution* 308(5): 523-49. ISSN: 1552-5007.
NAL Call Number: QL1.J87
Descriptors: zebrafish tooth development, comparative anatomy, dentition, phylogeny, Cypriniformes classification and physiology, mammals growth and development, tooth anatomy and histology, tooth growth and development and loss genetics, variation genetics, vertebrates growth and development.

Strange, K. (2005). **The end of "naive reductionism": rise of systems biology or renaissance of physiology?** *American Journal of Physiology* 288(5(1)): C968-C974. ISSN: 0002 9513.
NAL Call Number: 447.8Am3
Abstract: Systems biology is an emerging discipline focused on tackling the enormous intellectual and technical challenges associated with translating genome sequence into a comprehensive understanding of how organisms are built and run. Physiology and systems biology share the goal of understanding the integrated function of complex, multicomponent biological systems ranging from interacting proteins that carry out specific tasks to whole organisms. Despite this common ground, physiology as an academic discipline runs the real risk of fading into the background and being superseded organizationally and administratively by systems biology. My goal in this article is to discuss briefly the cornerstones of modern systems biology, specifically functional genomics, nonmammalian model organisms and computational biology, and to emphasize the need to embrace them as essential components of 21st-century physiology departments and research and teaching programs. Copyright © 2008 CABI
Descriptors: zebrafish, animal physiology, genome analysis, genomes, molecular biology, teaching methods, *Danio rerio*, *Drosophila*, *Escherichia coli.*
Notes: Review article.

Sugimoto, M., M. Yuki, T. Miyakoshi, and K. Maruko (2005). **The influence of long-term chromatic adaptation on pigment cells and striped pigment patterns in the skin of the zebrafish, *Danio rerio*.** *Journal of Experimental Zoology. Part A, Comparative Experimental Biology* 303(6): 430-40. ISSN: 1548-8969.
NAL Call Number: QL1.J854
Descriptors: zebrafish chromatophore, physiological adaptation, chromatophores physiology, pigmentation physiology, skin physiology, annexin A5 metabolism, apoptosis physiology, fluorescence microscopy, time factors.

Tamai, T.K., A.J. Carr, and D. Whitmore (2005). **Zebrafish circadian clocks: cells that see light.** *Biochemical Society Transactions* 33(Pt 5): 962-6 . ISSN: 0300 5127.

NAL Call Number: QD415.A1B58

Abstract: In the classical view of circadian clock organization, the daily rhythms of most organisms were thought to be regulated by a central, 'master' pacemaker, usually located within neural structures of the animal. However, with the results of experiments performed in zebrafish, mammalian cell lines and, more recently, mammalian tissues, this view has changed to one where clock organization is now seen as being highly decentralized. It is clear that clocks exist in the peripheral tissues of animals as diverse as *Drosophila*, zebrafish and mammals. In the case of *Drosophila* and zebrafish, these tissues are also directly light-responsive. This light sensitivity and direct clock entrainability is also true for zebrafish cell lines and early-stage embryos. Using luminescent reporter cell lines containing clock gene promoters driving the expression of luciferase and single-cell imaging techniques, we have been able to show how each cell responds rapidly to a single light pulse by being shifted to a common phase, equivalent to the early day. This direct light sensitivity might be related to the requirement for light in these cells to activate the transcription of genes involved in DNA repair. It is also clear that the circadian clock in zebrafish regulates the timing of the cell cycle, demonstrating the wide impact that this light sensitivity and daily rhythmicity has on the biology of zebrafish.

Descriptors: zebrafish, luminescent reporter cell lines, biological clocks physiology and radiation effects, circadian rhythm physiology and radiation effects , darkness, *Drosophila*, light, luciferases metabolism, luminescence.

Tamai, T.K., L.C. Young, and D. Whitmore (2007). **Light signaling to the zebrafish circadian clock by Cryptochrome 1a.** *Proceedings of the National Academy of Sciences of the United States of America* 104(37): 14712-7. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: Zebrafish tissues and cells have the unusual feature of not only containing a circadian clock, but also being directly light-responsive. Several zebrafish genes are induced by light, but little is known about their role in clock resetting or the mechanism by which this might occur. Here we show that Cryptochrome 1a (Cry1a) plays a key role in light entrainment of the zebrafish clock. Intensity and phase response curves reveal a strong correlation between light induction of Cry1a and clock resetting. Overexpression studies show that Cry1a acts as a potent repressor of clock function and mimics the effect of constant light to "stop" the circadian oscillator. Yeast two-hybrid analysis demonstrates that the Cry1a protein interacts directly with specific regions of core clock components, CLOCK and BMAL, blocking their ability to fully dimerize and transactivate downstream targets, providing a likely mechanism for clock resetting. A comparison of entrainment of zebrafish cells to complete versus skeleton photoperiods reveals that clock phase is identical under these two conditions. However, the amplitude of the core clock oscillation is much higher on a complete photoperiod, as are the levels of light-induced Cry1a. We believe that Cry1a acts on the core clock machinery in both a continuous and discrete fashion, leading not only to entrainment, but also to the establishment of a high-amplitude rhythm and even stopping of the clock under long photoperiods.

Descriptors: zebrafish Cry1a, biological clocks physiology, circadian rhythm physiology, flavoproteins metabolism, light, signal transduction, cell line, immunohistochemistry, luciferases metabolism, luminescent measurements, oscillometry, retroviridae genetics, transfection, two hybrid system techniques.

Tan, Y. and Y.Y. Li (2006). **Application of fish in the study of endocrine disruption**. *Fisheries Science/Shuichan Kexue* 25(11): 583-587. ISSN: 1003-1111.
Descriptors: zebrafish, endocrine disruptors, fish assay, animal physiology, endocrinology, environmental assessment, hormones, screening, stock assessment, toxicity, toxicity tests, *Danio rerio*, *Oryzias latipes*, *medaka.*

Ton, C., D. Stamatiou, and C.C. Liew (2003). **Gene expression profile of zebrafish exposed to hypoxia during development.** *Physiological Genomics* 13(2): 97-106. ISSN: 1094-8341.
Abstract: Understanding how vertebrates respond to hypoxia can have important clinical implications. Fish have evolved the ability to survive long exposure to low oxygen levels. However, little is known about the specific changes in gene expression that result from hypoxia. In this study we used a zebrafish cDNA microarray to examine the expression of >4,500 genes in zebrafish embryos exposed to 24 h of hypoxia during development. We tested the hypotheses that hypoxia changes gene expression profile of the zebrafish embryos and that these changes can be reverted by reexposure to a normoxic (20.8% O(2)) environment. Our data were consistent with both of these hypotheses: indicating that zebrafish embryos undergo adaptive changes in gene expression in response to hypoxia. Our study provides a striking genetic portrait of the zebrafish embryos' adaptive responses to hypoxic stress and demonstrates the utility of the microarray technology as a tool for analyzing complex developmental processes in the zebrafish.
Descriptors: zebrafish, anoxia genetics, gene expression profiling methods, developmental gene expression regulation genetics, computer systems, complementary DNA genetics, oligonucleotide array sequence analysis methods, messenger RNA biosynthesis, reverse transcriptase PCR, proteins biosynthesis.

Traver, D., B.H. Paw, K.D. Poss, W.T. Penberthy, S. Lin, and L.I. Zon (2003). **Transplantation and in vivo imaging of multilineage engraftment in zebrafish bloodless mutants.** *Nature Immunology* 4(12): 1238-46. ISSN: 1529-2908.
NAL Call Number: QR180
Abstract: The zebrafish is firmly established as a genetic model for the study of vertebrate blood development. Here we have characterized the blood-forming system of adult zebrafish. Each major blood lineage can be isolated by flow cytometry, and with these lineal profiles, defects in zebrafish blood mutants can be quantified. We developed hematopoietic cell transplantation to study cell autonomy of mutant gene function and to establish a hematopoietic stem cell assay. Hematopoietic cell transplantation can rescue multilineage hematopoiesis in embryonic lethal gata1-/- mutants for over 6 months. Direct visualization of fluorescent donor cells in embryonic recipients allows engraftment and homing events to be imaged in real time. These results provide a cellular context in which to study the genetics of hematopoiesis.
Descriptors: zebrafish, cell lineage, bloodless mutants, blood development, hematopoietic stem cells physiology, system embryology and system growth and development, genetically modified, blood cells cytology, blood cells physiology, blood physiology, flow cytometry, hematopoietic stem cell transplantation, kidney cytology and physiology, mutation, spleen cytology and physiology.

Tseng, Y.C. and P.P. Hwang (2005). **Molecular evidence of glucose transporters in zebrafish**. *Journal of the Fisheries Society of Taiwan* 32(1): 105. ISSN: 0379-4180.
NAL Call Number: SH135.T3

Descriptors: zebrafish physiology, biological membranes, brain, embryonic development, glucose, neurons, neurophysiology, nucleotide sequence, *Danio rerio* .

Turesson, J., T. Schwerte, and L. Sundin (2006). **Late onset of NMDA receptor-mediated ventilatory control during early development in zebrafish (*Danio rerio*).** *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology* 143(3): 332-9. ISSN: 1095 6433.
NAL Call Number: QP1C6
Abstract: Increased ventilation frequency (fV) in response to hypoxia in adult fish depends on ionotropic N-methyl-D-aspartate (NMDA) receptors. Nonetheless, the ontogeny of central control mechanisms mediating hypoxic ventilatory chemoreflexes in lower vertebrates has not been studied. Therefore, the aim of this study was to determine when the hypoxic ventilatory response during zebrafish (*Danio rerio*) development is mediated via NMDA receptors, by performing physiological experiments and western blot analysis of NMDA receptor subunits. Zebrafish larvae at stages 4-16 days post-fertilisation (dpf) were exposed to an hypoxic pulse in control groups and in groups treated with MK801 (NMDA receptor antagonist). The hypoxic increase in fV was present at all larval stages, and it matured during development. The reflex became MK801 sensitive at 8 dpf, but did not completely rely on a glutamatergic transmission until 13 dpf. This, together with changing subunit composition during the different stages (increasing amounts of NMDAR1 subunits and appearance of NMDAR2A subunits in adults), suggests that the amount of functional NMDA receptors needed to achieve a fully developed reflex is not attained until later stages. Furthermore, our results suggest that other non-NMDA receptor mechanisms are responsible for the hypoxia-induced increase in fV during the earlier developmental stages.
Descriptors: zebrafish larva hypoxia, pulmonary ventilation physiology, N methyl D aspartate (NMDA) receptors physiology, anoxia physiopathology, dizocilpine maleate pharmacology, pulmonary ventilation drug effects, N methyl D aspartate receptors drug effects, respiratory mechanics.

Turnell, E.R., K.D. Mann, G.G. Rosenthal, and G. Gerlach (2003). **Mate choice in zebrafish (*Danio rerio*) analyzed with video-stimulus techniques.** *Biological Bulletin, The* 205(2): 225-6. ISSN: 0006 3185.
NAL Call Number: 442.8B52
Descriptors: zebrafish imaging, three dimensional imaging, mate selection, visual pattern recognition physiology, sexual behavior physiology.

Uchida, D., M. Yamashita, T. Kitano, and T. Iguchi (2004). **An aromatase inhibitor or high water temperature induce oocyte apoptosis and depletion of P450 aromatase activity in the gonads of genetic female zebrafish during sex-reversal**. *Comparative Biochemistry and Physiology A, Molecular and Integrative Physiology* 137(1): 11-20. ISSN: 1095 6433.
NAL Call Number: QP1C6
Abstract: Dietary administration of a cytochrome P450 aromatase (P450 arom) inhibitor (fadrozole) in genetic female juveniles of zebrafish (*Danio rerio*) was performed at 15-40 days post-hatching. The percentage of gonadal masculinization in the genetic all-females at 40 days post-hatching, treated with 0, 10, 100 and 1000 micro g fadrozole g-1 diet-1 were 0, 62.5, 100 and 100%, respectively. Rearing at high water temperature in genetic all-females was performed at 15-25 days post-hatching. The percentage of gonadal masculinization in the genetic all-females at 40 days post-hatching, at water temperatures of 28.5, 35 and 37 degrees C were 0, 68.8 and 100%, respectively. Terminal deoxynucleotidyl transferase-medi-

ated dUTP nick-end labeling (TUNEL)-positive oocytes of early diplotene and perinucleolar stages in fadrozole-treated genetic females (1000 micro g g-1 diet-1) were observed at 15-40 days post-hatching during sex-reversal. In contrast, apoptotic oocytes of early diplotene stage in high temperature-treated genetic females (at 35 and 37 degrees C) during sex-reversal and presumptive males of wild-type fish during sex differentiation were found at 15-27 days post-hatching. Our findings indicate that oocyte apoptosis, depletion of P450arom activity and differentiation of spermatogonia during gonadal sex-reversal are caused by treatments of aromatase inhibitor or high water temperature. Copyright © 2008 CABI
Descriptors: zebrafish juvenile oocytes, apoptosis, cytochrome P450, genetic effects, gonads, masculinizing effect, sex reversal, sexual maturity, water temperature, *Danio rerio.*

Valiunas, V., R. Mui, E. McLachlan, G. Valdimarsson, P.R. Brink, and T.W. White (2004). **Biophysical characterization of zebrafish connexin35 hemichannels.** *American Journal of Physiology. Cell Physiology* 287(6): C1596-604. ISSN: 0363 6143.
Abstract: A subset of connexins can form unopposed hemichannels in expression systems, providing an opportunity for comparison of hemichannel gating properties with those of intact gap junction channels. Zebrafish connexin35 (Cx35) is a member of the Cx35/Cx36 subgroup of connexins highly expressed in the retina and brain. In the present study, we have shown that Cx35 expression in *Xenopus* oocytes and N2A cells produced large outward whole cell currents on cell depolarization. Using whole cell, cell-attached, and excised patch configurations, we obtained multichannel and single-channel current recordings attributable to the Cx35 hemichannels (I(hc)) that were activated and increased by stepwise depolarization of membrane potential (V(m)) and deactivated by hyperpolarization. The currents were not detected in untransfected N2A cells or in control oocytes injected with antisense Cx38. However, water-injected oocytes that were not treated with antisense showed activities attributable to Cx38 hemichannels that were easily distinguishable from Cx35 hemichannels by a significantly larger unitary conductance (gamma(hc): 250-320 pS). The gamma(hc) of Cx35 hemichannels exhibited a pronounced V(m) dependence; i.e., gamma(hc) increased/decreased with relative hyperpolarization/depolarization (gamma(hc) was 72 pS at V(m) = -100 mV and 35 pS at V(m) = 100 mV). Extrapolation to V(m) = 0 mV predicted a gamma(hc) of 48 pS, suggesting a unitary conductance of intact Cx35 gap junction channels of approximately 24 pS. Channel gating was also V(m) dependent: open time declined with negative V(m) and increased with positive V(m). The ability to break down the complex gating of intact intercellular channels into component hemichannels in vitro will help to evaluate putative physiological roles for hemichannels in vivo.
Descriptors: zebrafish, *Xenopus*, connexins 35 genetics and metabolism, eye proteins genetics,chemistry and metabolism, ion channel gating physiology, connexins chemistry, gap junctions physiology, kinetics, membrane potentials physiology, antisense oligonucleotides, oocytes physiology, patch clamp techniques, quaternary protein structure, complementary RNA, transfection.

Vallone, D., K. Lahiri, T. Dickmeis, and N.S. Foulkes (2005). **Zebrafish cell clocks feel the heat and see the light!** *Zebrafish* 2(3): 171-187. ISSN: 1545 8547.
Abstract: The zebrafish has rapidly become established as one of the most valuable vertebrate models for studying circadian clock function. A major initial attraction was its utility in large-scale genetic screens. It subsequently emerged that most zebrafish cells possess circadian clocks that can be entrained directly by exposure to temperature or light dark cycles, a property shared by several zebrafish cell lines. This is not the case for mammals, where the retina is the primary source of light input to the clock. Furthermore, mammalian cell culture clocks

can only be entrained by acute culture treatments such as serum shocks. Thus, the zebrafish is proving invaluable to study light and temperature input to the vertebrate clock. In addition, the accessibility of its early developmental stages has placed the zebrafish at the forefront of studies aimed at understanding how the circadian clock is established during embryogenesis.
Descriptors: zebrafish cell lines, *Danio rerio,* biological clocks, circadian clock, function in relation to temperature and light dark cycles, photoperiod , temperature.

Van der Meulen, T., H. Schipper, J.G. van den Boogaart, M.O. Huising, S. Kranenbarg, and J.L. van Leeuwen (2006). **Endurance exercise differentially stimulates heart and axial muscle development in zebrafish (*Danio rerio*).** *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 291(4): R1040-8. ISSN: 0363 6119.
Abstract: Mechanical load is an important factor in the differentiation of cells and tissues. To investigate the effects of increased mechanical load on development of muscle and bone, zebrafish were subjected to endurance swim training for 6 h/day for 10 wk starting at 14 days after fertilization. During the first 3 wk of training, trained fish showed transiently increased growth compared with untrained (control) fish. Increased expression of proliferating cell nuclear antigen suggests that this growth is realized in part through increased cell proliferation. Red and white axial muscle fiber diameter was not affected. Total cross-sectional area of red fibers, however, was increased. An improvement in aerobic muscle performance was supported by an increase in myoglobin expression. At the end of 10 wk of training, heart and axial muscle showed increased expression of the muscle growth factor myogenin and proliferating cell nuclear antigen, but there were major differences between cardiac and axial muscle. In axial muscle, expression of the "slow" types of myosin and troponin C was increased, together with expression of erythropoietin and myoglobin, which enhance oxygen transport, indicating a shift toward a slow aerobic phenotype. In contrast, the heart muscle shifts to a faster phenotype but does not become more aerobic. This suggests that endurance training differentially affects heart and axial muscle.
Descriptors: zebrafish, heart growth, development and physiology, skeletal muscle growth and development and physiology, physical endurance physiology, behavior physiology, body size physiology, cell division genetics, conditioning psychology physiology, energy metabolism genetics, erythropoietin genetics , gene expression profiling and regulation , skeletal muscle cytology, myocardium cytology, myoglobin genetics, oxygen metabolism, physical conditioning physiology, swimming physiology, weight bearing physiology.

Vihtelic, T.S., J.E. Soverly, S.C. Kassen, and D.R. Hyde (2006). **Retinal regional differences in photoreceptor cell death and regeneration in light-lesioned albino zebrafish.** *Experimental Eye Research* 82(4): 558-75. ISSN: 0014 4835.
NAL Call Number: QP474
Abstract: Teleost fish regenerate retinal cells from a population of inner nuclear layer (INL) stem cells. To characterize photoreceptor regeneration in zebrafish (*Danio rerio*), adult albino fish were subjected to constant intense light to cause photoreceptor cell death. Retinal morphometry was performed on histological sections of control and light-lesioned albino retinas to compare the extent of light damage in the ventral, central and dorsal retinal regions. In addition, opsin immunohistochemistry and TUNEL were used to compare photoreceptor cell death in these different retinal areas, while PCNA immunolabeling quantified the cell proliferation that precedes the photoreceptor regeneration. Transgenic albino; Tg(alpha1-tubulin:egfp) zebrafish were also exposed to the intense light in order to examine regeneration-related gene expression changes. The light-lesioned retinas are characterized by extensive rod and cone photoreceptor cell death in the central and dorsal regions. In con-

trast, many of the rods and cones survive in the ventral retina. The highest levels of INL cell proliferation, which occurs subsequent to photoreceptor death, correspond to the retinal regions that suffer the greatest levels of photoreceptor damage. In the ventral retina, where photoreceptor cell death is minimal, cell proliferation is confined to the ONL. In addition, EGFP expression from the alpha1-tubulin promoter is increased in Muller glial cells in the light-damaged central and dorsal retina, while transgene expression in the ventral retina is restricted to small, round INL cells. Furthermore, expression of the HuC/D neuronal antigen is detected in a subpopulation of the Muller cells in the light-damaged superior retinal region. These data demonstrate that adult albino zebrafish display retinal regional differences in photoreceptor cell death and in the regeneration-related INL cell proliferation response. The high levels of INL cell proliferation and alpha1-tubulin:egfp transgene expression in the Muller cells may be graded in response to the degree of photoreceptor cell death. This suggests that the levels of photoreceptor damage may directly influence cell responses in the underlying retinal layers.

Descriptors: zebrafish albino, apoptosis physiology, vertebrate photoreceptors cytology, cell division physiology, retina cones cytology, gene expression genetics, immunohistochemistry methods, in situ nick end labeling methods, neuroglia cytology, opsin immunology, photic stimulation methods, retina cytology, rhodopsin immunology, rod outer segments cytology, retinal rodscytology, trans tubulin analysis.

Vijayan, M. and D. Alsop (2007). **Developmental plasticity of the stress axis in zebrafish**. *Comparative Biochemistry and Physiology, Part a: Molecular & Integrative Physiology* 148(1): S7. ISSN: 1095-6433.

NAL Call Number: QP1C6

Descriptors: zebrafish embryology, developmental stages, freshwater fish, *Danio rerio*, reproduction and development.

Vuilleumier, R., L. Besseau, G. Boeuf, A. Piparelli, Y. Gothilf, W.G. Gehring, D.C. Klein, and J. Falcon (2006). **Starting the zebrafish pineal circadian clock with a single photic transition.** *Endocrinology* 147(5): 2273-9. ISSN: 0013 7227.

NAL Call Number: 448.8EN2

Abstract: The issue of what starts the circadian clock ticking was addressed by studying the developmental appearance of the daily rhythm in the expression of two genes in the zebrafish pineal gland that are part of the circadian clock system. One encodes the photopigment exorhodopsin and the other the melatonin synthesizing enzyme arylalkylamine N-acetyltransferase (AANAT2). Significant daily rhythms in AANAT2 mRNA abundance were detectable for several days after fertilization in animals maintained in a normal or reversed lighting cycle providing 12 h of light and 12 h of dark. In contrast, these rhythms do not develop if animals are maintained in constant lighting or constant darkness from fertilization. In contrast to exorhodopsin, rhythmicity of AANAT2 can be initiated by a pulse of light against a background of constant darkness, by a pulse of darkness against a background of constant lighting, or by single light-to-dark or dark-to-light transitions. Accordingly, these studies indicate that circadian clock function in the zebrafish pineal gland can be initiated by minimal photic cues, and that single photic transitions can be used as an experimental tool to dissect the mechanism that starts the circadian clock in the pineal gland.

Descriptors: zebrafish, arylalkylamine N-acetyltransferase physiology, developmental gene expression regulation, pineal gland physiology, arylalkylamine N- acetyltransferase genetics, circadian rhythm, darkness, fertilization, in situ hybridization, light, electron microscopy,

photoperiod, vertebrate photoreceptors metabolism, pineal gland anatomy and histology and cytology, messenger RNA metabolism, retina metabolism, temperature, time factors.

Vulesevic, B., B. McNeill, and S.F. Perry (2006). **Chemoreceptor plasticity and respiratory acclimation in the zebrafish *Danio rerio*.** *Journal of Experimental Biology* 209(Pt 7): 1261-73. ISSN: 0022-0949.

NAL Call Number: 442.8B77

Abstract: The goals of this study were to assess the respiratory consequences of exposing adult zebrafish *Danio rerio* to chronic changes in water gas composition (hypoxia, hyperoxia or hypercapnia) and to determine if any ensuing effects could be related to morphological changes in branchial chemoreceptors. To accomplish these goals, we first modified and validated an established non-invasive technique for continuous monitoring of breathing frequency and relative breathing amplitude in adult fish. Under normal conditions 20% of zebrafish exhibited an episodic breathing pattern that was composed of breathing and non-breathing (pausing/apneic) periods. The pausing frequency was reduced by acute hypoxia (Pw(O)2<130 mmHg) and increased by acute hyperoxia (Pw(O)2>300 mmHg), but was unaltered by acute hypercapnia. Fish were exposed for 28 days to hyperoxia (Pw(O)2>350 mmHg), or hypoxia (Pw(O)2=30 mmHg) or hypercapnia (Pw(CO)2=9 mmHg). Their responses to acute hypoxia or hypercapnia were then compared to the response of control fish kept for 28 days in normoxic and normocapnic water. In control fish, the ventilatory response to acute hypoxia consisted of an increase in breathing frequency while the response to acute hypercapnia was an increase in relative breathing amplitude. The stimulus promoting the hyperventilation during hypercapnia was increased Pw(CO)2 rather than decreased pH. Exposure to prolonged hyperoxia decreased the capacity of fish to increase breathing frequency during hypoxia and prevented the usual increase in breathing amplitude during acute hypercapnia. In fish previously exposed to hyperoxia, episodic breathing continued during acute hypoxia until Pw(O)2 had fallen below 70 mmHg. In fish chronically exposed to hypoxia, resting breathing frequency was significantly reduced (from 191+/-12 to 165+/-16 min(-1)); however, the ventilatory responses to hypoxia and hypercapnia were unaffected. Long-term exposure of fish to hypercapnic water did not markedly modify the breathing response to acute hypoxia and modestly blunted the response to hypercapnia. To determine whether branchial chemoreceptors were being influenced by long-term acclimation, all four groups of fish were acutely exposed to increasing doses of the O(2) chemoreceptor stimulant, sodium cyanide, dissolved in inspired water. Consistent with the blunting of the ventilatory response to hypoxia, the fish pre-exposed to hyperoxia also exhibited a blunted response to NaCN. Pre-exposure to hypoxia was without effect whereas prior exposure to hypercapnia increased the ventilatory responses to cyanide. To assess the impact of acclimation to varying gas levels on branchial O(2) chemoreceptors, the numbers of neuroepithelial cells (NECs) of the gill filament were quantified using confocal immunofluorescence microscopy. Consistent with the blunting of reflex ventilatory responses, fish exposed to chronic hyperoxia exhibited a significant decrease in the density of NECs from 36.8+/-2.8 to 22.7+/-2.3 filament(-1).

Descriptors: zebrafish, physiological adaptation physiology, chemoreceptors physiology, oxygen analysis, water chemistry, carbon dioxide administration and dosage, cell count, fluorescent antibody technique, gills chemistry, cytology and physiology, confocal microscopy, neuroepithelial cells cytology, neuronal plasticity, oxygen and sodium cyanide administration and dosage.

Vulesevic, B. and S.F. Perry (2006). **Developmental plasticity of ventilatory control in zebrafish, *Danio rerio*.** *Respiratory Physiology and Neurobiology* 154(3): 396-405. ISSN: 1569-9048.
NAL Call Number: QP121.A1R4
Abstract: To determine whether development of ventilatory control in zebrafish (*Danio rerio*) exhibits plasticity, embryos were exposed to hypoxia, hyperoxia or hypercapnia for the first 7 days post-fertilization. Their acute reflex breathing responses to ventilatory stimuli (hypoxia, hypercapnia and external cyanide) were assessed when they had reached maturity (3 months or older). Zebrafish reared under hyperoxic conditions exhibited significantly higher breathing frequencies at rest (283+/-27min(-1) versus 212+/-16min(-1) in control fish); breathing frequency was unaffected in adult fish subjected to hyperoxia for 7 days. The respiratory responses of fish reared in hyperoxic water to acute hypoxia, hypercapnia or external cyanide were blunted (hypoxia, cyanide) or eliminated (hypercapnia). Adult fish exposed for 7 days to hyperoxia showed no change in acute responses to these stimuli. The respiratory responses to acute hypoxia, hypercapnia or external cyanide of fish reared under hypoxic or hypercapnic conditions were similar to those in fish reared under normal conditions. A subset of all fish examined exhibited episodic breathing; an analysis of breathing patterns demonstrated that fish reared under hypercapnic conditions had an increased tendency to display episodic breathing. The results of this study reveal that there is flexibility in the design and functioning of the embryonic or larval respiratory system in zebrafish.
Descriptors: zebrafish embryos, aging physiology, respiratory physiology, anoxia physiopathology, cyanides pharmacology, embryonic development, hypercapnia physiopathology, reflex physiology, respiration drug effects, respiratory mechanics.

Walker, M.B. and C.B. Kimmel (2007). **A two-color acid-free cartilage and bone stain for zebrafish larvae.** *Biotechnic and Histochemistry* 82(1): 23-8. ISSN: 1052 0295.
NAL Call Number: QH613.B56
Abstract: Traditionally, cartilage is stained by alcian blue using acidic conditions to differentiate tissue staining. The acidic conditions are problematic when one wishes to stain the same specimen for mineralized bone with alizarin red, because acid demineralizes bone, which negatively affects bone staining. We have developed an acid-free method to stain cartilage and bone simultaneously in zebrafish larvae. This method has the additional advantage that PCR genotyping of stained specimens is possible.
Descriptors: zebrafish larva, bone and bones anatomy and histology, cartilage anatomy and histology, coloring agents, histocytochemistry methods, larva anatomy and histology, magnesium chloride, acid free staining method, PCR.

Wang, R.Y., T. Zhang, Q. Bao, and D.M. Rawson (2006). **Study on fish embryo responses to the treatment of cryoprotective chemicals using impedance spectroscopy**. *European Biophysics Journal* 35(3): 224-230. ISSN: 0175 7571.
NAL Call Number: QH505 .A1B53
Abstract: Investigations using electrical impedance spectroscopy to measure the responses of fish embryos to the cryoprotective chemicals, methanol and dimethyl sulphoxide (DMSO), were carried out. Zebrafish (*Danio rerio*) embryos were used as a model to study the newly proposed technique. The normalised permittivity and conductivity changes of the embryos were measured continuously over a 20-min period in a customised embryo-holding chamber. The normalised permittivity and conductivity spectra were obtained during embryo exposure to different concentrations of methanol (1.0, 2.0 and 3.0 M) and DMSO (0.5, 1.0 and 2.0 M) solutions. The results showed significant permittivity and conductivity changes after embryo exposure to methanol and DMSO at the optimum embryo loading level (six

embryos). Embryos in different concentrations of methanol and DMSO also resulted in quantitative responses shown in the normalised permittivity and conductivity spectra. The results demonstrated that fish embryo membrane permeability to cryoprotective chemicals could be monitored in real-time. The measurement of permittivity at a lower frequency range (10-10(3)Hz) and conductivity at a higher frequency range (10(4)-10(6) Hz) during fish embryo exposure to cryoprotective chemicals using impedance spectroscopy can be used as a new tool for the fast screening of most effective cryoprotective chemicals. The results from the present study also demonstrated the possibility of quantifying the level of cryoprotective chemicals penetrating the fish embryos. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, methods and techniques, cell biology, electrical impedance spectroscopy, cryoprotective chemical screening, spectrum analysis techniques, embryo holding chamber, conductivity spectra.

Wang, X.M., F.Z. Cui, J. Ge, and Y. Wang (2004). **Hierarchical structural comparisons of bones from wild-type and liliput(dtc232) gene-mutated zebrafish.** *Journal of Structural Biology* 145(3): 236-45. ISSN: 1047-8477.
NAL Call Number: QH573.J68
Abstract: The alterations of hierarchical structures of bone by gene mutation in the zebrafish, which is associated with abnormal bone mineralization and bone disease, were reported for the first time in this paper. Bone samples from the liliput(dtc232) (lil) mutants as well as normal controls were studied by polarized light microscope, scanning electron microscope (SEM), transmission electron microscope (TEM), and atomic force microscope (AFM). Light microscopy examinations reveal that the lil bone has asymmetric mineralization and much thinner bone wall. The SEM studies show a lot of microcracks in lil bone wall. And the plywood-like structure of the normal bone does not exist in the lil bone, which is confirmed by the measurements of polarized light microscope. Furthermore, the TEM investigations display the collagen fibrils with two typical diameters. For the thinner collagen fibrils, the diameter of lil bone is about twice larger than that of the wild-type bone. And for the thicker one, there is a small increase in diameter after mutation and the band periodicity of the lil bone is similar with that of wild-type bone, which is consistent with the result of AFM. The morphologies of the minerals revealed that the mutated mineral was in bigger size and the shape was irregular but not plate-shaped.
Descriptors: zebrafish gene mutation, bone and bones physiology, mutation, bone and bones embryology, abnormal bone mineralization, bone and bones ultrastructure, collagen chemistry, collagen Type I chemistry, atomic force microscopy, scanning electron microscopy, electron transmission microscopy, polarization microscopy.

Webb, S.E. and A.L. Miller (2007). **Ca2+ signalling and early embryonic patterning during zebrafish development.** *Clinical and Experimental Pharmacology and Physiology* 34(9): 897-904. ISSN: 0305 1870.
Abstract: 1. It has been proposed that Ca2+ signalling, in the form of pulses, waves and steady gradients, may play a crucial role in key pattern-forming events during early vertebrate development. 2. With reference to the embryo of the zebrafish *(Danio rerio*), herein we review the Ca2+ transients reported from the cleavage to segmentation periods. This time-window includes most of the major pattern-forming events of early development, which transform a single-cell zygote into a complex multicellular embryo with established primary germ layers and body axes. 3. Data are presented to support our proposal that intracellular Ca2+ waves are an essential feature of embryonic cytokinesis and that propagating intercellular Ca2+ waves (both long and short range) may play a crucial role in: (i) the establishment

of the embryonic periderm and the coordination of cell movements during epiboly, convergence and extension; (ii) the establishment of the basic embryonic axes and germ layers; and (iii) definition of the morphological boundaries of specific tissue domains and embryonic structures, including future organ anlagen. 4. The potential downstream targets of these Ca2+ transients are also discussed, as well as how they may integrate with other pattern-forming signalling pathways known to modulate early developmental events.
Descriptors: zebrafish embryos, body patterning, calcium signaling, embryonic stem cells metabolism, blastula metabolism, cell movement, cleavage stage, ovum metabolism, cytokinesis, cytology, metabolism, gastrula metabolism, time factors.

Whitaker, M. (2006). **Calcium at fertilization and in early development**. *Physiological Reviews* 86(1): 25-88. ISSN: 0031 9333.
Abstract: Fertilization calcium waves are introduced, and the evidence from which we can infer general mechanisms of these waves is presented. The two main classes of hypotheses put forward to explain the generation of the fertilization calcium wave are set out, and it is concluded that initiation of the fertilization calcium wave can be most generally explained in invertebrates by a mechanism in which an activating substance enters the egg from the sperm on sperm-egg fusion, activating the egg by stimulating phospholipase C activation through a src family kinase pathway and in mammals by the diffusion of a sperm-specific phospholipase C from sperm to egg on sperm-egg fusion. The fertilization calcium wave is then set into the context of cell cycle control, and the mechanism of repetitive calcium spiking in mammalian eggs is investigated. Evidence that calcium signals control cell division in early embryos is reviewed, and it is concluded that calcium signals are essential at all three stages of cell division in early embryos. Evidence that phosphoinositide signaling pathways control the resumption of meiosis during oocyte maturation is considered. It is concluded on balance that the evidence points to a need for phosphoinositide/calcium signaling during resumption of meiosis. Changes to the calcium signaling machinery occur during meiosis to enable the production of a calcium wave in the mature oocyte when it is fertilized; evidence that the shape and structure of the endoplasmic reticulum alters dynamically during maturation and after fertilization is reviewed, and the link between ER dynamics and the cytoskeleton is discussed. There is evidence that calcium signaling plays a key part in the development of patterning in early embryos. Morphogenesis in ascidian, frog, and zebrafish embryos is briefly described to provide the developmental context in which calcium signals act. Intracellular calcium waves that may play a role in axis formation in ascidian are discussed. Evidence that the Wingless/ calcium signaling pathway is a strong ventralizing signal in *Xenopus*, mediated by phosphoinositide signaling, is adumbrated. The central role that calcium channels play in morphogenetic movements during gastrulation and in ectodermal and mesodermal gene expression during late gastrulation is demonstrated. Experiments in zebrafish provide a strong indication that calcium signals are essential for pattern formation and organogenesis. Copyright©Thomson Reuters 2009
Descriptors: zebrafish oocytes and embryos, calcium signals, fertilization organogenesis, development, gastrulation, ectodermal and mesodermal gene expression, pattern formation .

Widmer, S., F.B.G. Moore, and B. Bagatto (2006). **The effects of chronic developmental hypoxia on swimming performance in zebrafish**. *Journal of Fish Biology* 69(6): 1885-1891. ISSN: 0022 1112.
NAL Call Number: QL614.J68
Abstract: Relative to normoxia-reared fish, hypoxia-reared zebrafish Danio rerio achieved 24.9 and 21.4% slower maximal swim velocities in both normoxic and hypoxic waters,

respectively. Hypoxia-reared fish also produced 26.1 and 63.9% less lactate during resting conditions across both normoxic and hypoxic waters, respectively. During exercise, this trend continued as hypoxia-reared fish produced 68.2 and 55.1% less lactate across both normoxic and hypoxic waters, respectively. This reduction in performance, rather than representing a purely pathological (maladaptive) response to hypoxia, appears to represent a fundamental shift in the metabolic response to hypoxia. Copyright © 2008 CABI
Descriptors: zebrafish, dissolved oxygen, exercise, hypoxia versus normoxia rearing, lactic acid, metabolism, oxygen requirement, physical activity, *Danio rerio.*

Wilm, T.P. and L. Solnica Krezel (2003). **Radar breaks the fog: insights into dorsoventral patterning in zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 100(8): 4363-65. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish embryo growth, body patterning physiology, bone morphogenetic, transforming growth factor beta, body patterning genetics, bone morphogenetic developmental gene expression regulation, ligands, mutation, signal transduction.

Wilson Leedy, J.G. and R.L. Ingermann (2007). **Development of a novel CASA system based on open source software for characterization of zebrafish sperm motility parameters**. *Theriogenology* 67(3): 661-672. ISSN: 0093 691X.
NAL Call Number: QP251.A1T5
Abstract: Although computer-assisted sperm analysis (CASA) outperforms manual techniques, many investigators rely on non-automated analysis due to the high cost of commercial options. In this study, we have written and validated a free CASA software primarily for analysis of fish sperm. This software is a plugin for the free National Institutes of Health software ImageJ and is available with documentation at http://rsb.info.nih.gov/ij/plugins/casa.html. That it is open source makes possible external validation, should improve quality control and enhance the comparative value of data obtained among laboratories. In addition, we have improved upon the traditional velocity straight line (VSL) algorithm, eliminating inaccurate characterization of highly curved fish sperm paths. Using this system, the motion of zebrafish (*Danio rerio*) sperm was characterized relative to time post-activation and the impact of acquisition conditions upon data analysis determined. There were decreases in velocity and path straightness (STR), but not linearity (LIN), relative to time. From 30 to 300 frames/s, frame rate significantly affected curvilinear velocity (VCL) and STR measurements. Sperm density in the field of view did not affect any measured parameter. There was significant inter-male variation for VCL, VSL, velocity average path (VAP), percent motility, path character (STR, LIN), and duration of motility. Furthermore, relative sperm output (a measure reflecting both semen volume and concentration) was positively correlated to percent motility. For all motion parameters measured (except duration), the average CV was <=10%, comparable to values obtained using commercial systems. Copyright © 2008 CABI
Descriptors: zebrafish, automation, computer software, spermatozoa, techniques, computer-assisted sperm analysis (CASA), *Danio rerio.*

Wolff, C., S. Roy, and P.W. Ingham (2003). **Multiple muscle cell identities induced by distinct levels and timing of Hedgehog activity in the zebrafish embryo.** *Current Biology* 13(14): 1169-81. ISSN: 0960 9822.
NAL Call Number: QH301.C85
Abstract: BACKGROUND: In the zebrafish embryo, the differentiation of distinct muscle fiber types has been shown to require the activity of members of the Hedgehog (Hh) family

of secreted proteins. Evidence from other systems suggests that Hh behaves as a morphogen, inducing cell fates in a concentration-dependent manner. Exactly how Hh signaling contributes to the generation of the correct pattern of cells within the zebrafish myotome, however, has remained obscure. RESULTS: Here, we distinguish four distinct myotomal cell identities in the zebrafish embryo on the basis of their position, morphology, and gene expression patterns. Using morpholino oligonucleotides (MOs) to diminish the activities of the Hh pathway components Patched (Ptc), Fused (Fu), and Suppressor of Fused (Su(fu)), and the teratogen cyclopamine to inhibit the Hh transducer Smoothened (Smo), we show that the appropriate differentiation of each cell type depends upon the levels and range of Hh signaling within the myotome. In addition, by transiently modulating Hh activity by using cyclopamine and a heat-inducible transgene, we demonstrate that the competence of myotomal cells to respond to Hh changes with time. Finally, we show that the Gli1 and Gli2 transcription factors mediate most of the response of myotomal cells to Hh. CONCLUSIONS: Hh signaling acts in a dosage-dependent manner to specify cell fate in the zebrafish myotome. Allocation of the correct number of cells to a specific fate depends upon the range of Hh activity. The timing of exposure to Hh determines the response of cells to the signal.
Descriptors: zebrafish embryos, cell differentiation physiology, embryonic induction physiology, developmental gene expression regulation physiology, muscle cells cytology, trans activators physiology, DNA primers, Hedgehog proteins, immunohistochemistry, in situ hybridization, microinjections, muscle cells physiology, oncogene, reverse transcriptase PCR, signal transduction physiology, time factors, transcription factors physiology, veratrum alkaloids.

Wu, S., L. Page, and N.M. Sherwood (2006). **A role for GnRH in early brain regionalization and eye development in zebrafish.** *Molecular and Cellular Endocrinology* 257-258: 47-64. ISSN: 0303-7207.
NAL Call Number: QP187.A1M6
Abstract: Gonadotropin-releasing hormone (GnRH) is a highly conserved peptide that is expressed early in brain development in vertebrates. In zebrafish, we detected GnRH mRNA within 2h post fertilization by RT-PCR. To determine if GnRH is involved in development, we used gene knockdown techniques to block translation of gnrh2 or gnrh3 mRNA after which the expression patterns for gene markers were examined at 24h post fertilization with in situ hybridization. First, loss of either GnRH2 or GnRH3 affected regionalization of the brain as shown by a change in expression of fgf8 or pax2.1 genes in the midbrain-hindbrain boundary or diencephalon-midbrain boundary. Second, lack of GnRH2 and/or GnRH3 altered gene markers expressed in the formation of the eye cup (pax2.1, pax6.1, mab21l2 and meis1.1) or eye stalk (fgf8 and pax2.1). Third, knockdown of GnRH2 affected the size and shape of the midbrain and expression of gene markers therein. Results from assays with the TUNEL method and caspase-3 and -9 activity showed the brain and eye changes were unlikely to result from secondary apoptotic cell death before 24h post fertilization. These experiments suggest that GnRH loss-of-function affects early brain and eye formation during development.
Descriptors: zebrafish embryos, brain and eye embryology, gonadotropin releasing hormone physiology, amino acid sequence, apoptosis genetics, base sequence, body patterning, brain metabolism, diencephalon embryology and metabolism, eye metabolism and proteins metabolism, fibroblast growth factor 8 metabolism, gonadotropin releasing hormone analogs, derivatives, genetics and metabolism, homeodomain proteins metabolism, immunohistochemistry, mesencephalon embryology and metabolism, molecular sequence data, antisense oligonucleotides pharmacology, oligopeptides genetics, metabolism and physiology , organo-

genesis physiology, pax2 transcription factor metabolism, paired box transcription factors metabolism, pyrrolidonecarboxylic acid analogs and derivatives, pyrrolidonecarboxylic acid metabolism, RNA interference, epha4 metabolism receptor, repressor proteins metabolism, rhombencephalon embryology and metabolism, transcription factors metabolism.

Yamaguchi, M., E. Yoshimoto, and S. Kondo (2007). **Pattern regulation in the stripe of zebrafish suggests an underlying dynamic and autonomous mechanism.** *Proceedings of the National Academy of Sciences of the United States of America* 104(12): 4790-3. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: The mechanism by which animal markings are formed is an intriguing problem that has remained unsolved for a long time. One of the most important questions is whether the positional information for the pattern formation is derived from a covert prepattern or an autonomous mechanism. In this study, using the zebrafish as the model system, we attempted to answer this classic question. We ablated the pigment cells in limited areas of zebrafish skin by using laser irradiation, and we observed the regeneration of the pigmentation pattern. Depending on the area ablated, different patterns regenerated in a specific time course. The regenerated patterns and the transition of the stripes during the regeneration process suggest that pattern formation is independent of the prepattern; furthermore, pattern formation occurs by an autonomous mechanism that satisfies the condition of "local self-enhancement and long-range inhibition." Because the zebrafish is the only striped animal for which detailed molecular genetic studies have been conducted, our finding will facilitate the identification of the molecular and cellular mechanisms that underlie skin pattern formation.
Descriptors: zebrafish, body stripe patterning physiology, pigmentation physiology, computer simulation, regeneration, skin physiology.

Yang, C.T., R.D. Sengelmann, and S.L. Johnson (2004). **Larval melanocyte regeneration following laser ablation in zebrafish.** *Journal of Investigative Dermatology* 123(5): 924-9. ISSN: 0022-202X.
Abstract: A method to specifically ablate melanocytes in a genetically tractable organism would facilitate the analysis of melanocyte regeneration and regulation. We have demonstrated that a Q-switched neodymium:yttrium-aluminum-garnet dermatology laser kills larval melanocytes in zebrafish. Following melanocyte ablation, new melanocytes regenerate from unpigmented precursors. We show that melanocyte regeneration following laser ablation requires kit receptor tyrosine kinase.
Descriptors: zebrafish larva, melanocytes cytology and physiology, regeneration physiology, skin physiology, cell division physiology, lasers, proto oncogene proteins C kit physiology, skin cytology, skin growth and development, skin pigmentation physiology.

Yokogawa, T., W. Marin, J. Faraco, G. Pezeron, L. Appelbaum, J. Zhang, F. Rosa, P. Mourrain, and E. Mignot (2007). **Characterization of sleep in zebrafish and insomnia in hypocretin receptor mutants.** *Public Library of Science: Biology* 5(10): E277. ISSN: 1544-9173.
NAL Call Number: QH301.P56
Abstract: Sleep is a fundamental biological process conserved across the animal kingdom. The study of how sleep regulatory networks are conserved is needed to better understand sleep across evolution. We present a detailed description of a sleep state in adult zebrafish characterized by reversible periods of immobility, increased arousal threshold, and place preference. Rest deprivation using gentle electrical stimulation is followed by a sleep rebound, indicating homeostatic regulation. In contrast to mammals and similarly to birds, light suppresses sleep in zebrafish, with no evidence for a sleep rebound. We also identify a null

mutation in the sole receptor for the wake-promoting neuropeptide hypocretin (orexin) in zebrafish. Fish lacking this receptor demonstrate short and fragmented sleep in the dark, in striking contrast to the excessive sleepiness and cataplexy of narcolepsy in mammals. Consistent with this observation, we find that the hypocretin receptor does not colocalize with known major wake-promoting monoaminergic and cholinergic cell groups in the zebrafish. Instead, it colocalizes with large populations of GABAergic neurons, including a subpopulation of Adra2a-positive GABAergic cells in the anterior hypothalamic area, neurons that could assume a sleep modulatory role. Our study validates the use of zebrafish for the study of sleep and indicates molecular diversity in sleep regulatory networks across vertebrates.
Descriptors: zebrafish, characterization, sleep, insomnia, hypocretin, receptor, mutants.

Young, M.W. (2005). ***Methods in Enzymology***, Vol. 393, Elsevier Academic Press, Inc.: San Diego, CA 92101-4495 USA, 861 p. ISBN: 0121827984.
NAL Call Number: QP601.M49 v.393
Abstract: This 861-page book is volume 393 in the series Methods in Enzymology and the major focus of this volume is circadian rhythms. The book is structured into 12 sections and contains a total of 45 individually-authored chapters written in English. Section I focuses on genetic approaches to circadian clocks and contains 10 chapters which outline assays and genetic and molecular biological manipulative methods for the analysis of circadian rhythms in Neurospora, *Arabidopsis thaliana*, clock mutants of *Drosophila*, biological rhythms in *Drosophila*, circadian rhythm in zebrafish, *Xenopus*, mutant mice, methods to record wheel running activity in mice, genetic approaches to study human behavior and enhanced phenotyping of complex traits with a circadian clock model. Section II contains 6 chapters and this section focuses on methods of tracking circadian control of gene activity including real-time reporting of circadian-regulated gene expression by luciferase imaging in plants and mammalian cells, real-time luminescence reporting of circadian gene expression in mammals, transgenic cAMP response element reporter flies for monitoring circadian rhythms, analysis of circadian output rhythms of gene expression in Neurospora and mammalian cells in culture, molecular and statistical tools for circadian transcript profiling, and RNA profiling in circadian biology. The third section, which discusses molecular cycles and clock protein rhythms, contains 4 chapters and these chapters describe the analysis of posttranslational regulations of the Neurospora circadian clock, analysis of the degradation of PERIOD protein by ubiquitin-proteasome pathway in cultured *Drosophila* cells, casein kinase I in the mammalian circadian clock, and nucleocytoplasmic shuttling of clock proteins. Sections IV and V contain just 2 chapters each, which discuss the anatomical representation of neural clocks and mosaic circadian systems, respectively. Section VI contains 3 chapters on peripheral circadian clocks, in particular circadian rhythms in olfaction, the circadian effects of timed meals, and peripheral clocks in the regulation of cardiovascular and metabolic functions. Section VII contains 6 chapters on cell and tissue culture models of circadian rhythm and the eighth section contains 3 chapters on intercellular signaling. The ninth section focuses on photoresponsive clocks and contains 3 chapters, which discuss mammalian photoentrainment, cryptochromes and circadian photoreception in animals and nonvisual ocular photoreception in the mammal. The final 3 sections each contain 2 chapters and these sections focus on sleep cycles, circadian biology of populations, and circadian clocks affecting noncircadian biology. The book is indexed by author and by subject and highlights a list of contributors and their respective institutions. This book will be a very useful resource for biochemists, molecular biologists, physiologists and anyone with an interest in circadian rhythms. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, behavior, methods and techniques, molecular genetics, enzymology, biosynchronization, phenotyping , *Drosophila*, *Xenopus.*

Zeddies, D.G. and R.R. Fay (2005). **Development of the acoustically evoked behavioral response in zebrafish to pure tones.** *Journal of Experimental Biology* 208(Pt 7): 1363-72. ISSN: 0022-0949.

NAL Call Number: 442.8B77

Abstract: Zebrafish (*Danio rerio*) were placed in small wells that could be driven vertically with a series of calibrated sinusoids. Video images of the fish were obtained and analyzed to determine the levels and frequencies at which the fish responded to the stimulus tones. It was found that fish 4 days post fertilization (dpf) did not respond to the stimulus tones, whereas fish 5 dpf to adult did respond. It was further found that the stimulus thresholds and frequency bandwidth to which the fish responded did not change from 5 dpf to adult; indicating that the otolithic organ adaptations for high-frequency hearing are already present in larval fish. Deflating the swimbladders in adult fish eliminated their response, which is consistent with sensing sound pressure. Deflating the swimbladder in larval fish did not affect their thresholds, which is consistent with sensing the particle motion of the fluid directly. Because adult fish with Weberian ossicles have a greater input to the inner ear for a given sound pressure level (SPL), the finding that the adult and larval fish respond at the same SPL with intact swimbladders suggests that the acoustic startle response threshold is adjusted as the fish develop in order to maintain appropriate reactions to relevant stimuli.

Descriptors: zebrafishlarva and adults, auditory threshold physiology, hearing physiology, motor activity physiology, sound, acoustic stimulation, air sacs physiology, larva physiology, zebrafish growth and development.

Zhang, C., T. Miki, T. Shibasaki, M. Yokokura, A. Saraya, and S. Seino (2006). **Identification and characterization of a novel member of the ATP-sensitive K+ channel subunit family, Kir6.3, in zebrafish.** *Physiological Genomics* 24(3): 290-7. ISSN: 1094-8341.

Abstract: ATP-sensitive K+ (KATP) channels play a crucial role in coupling cellular metabolism to membrane potential. In addition to the orthologs corresponding to Kir6.1 and Kir6.2 of mammals, we have identified a novel member, designated Kir6.3 (zKir6.3), of the inward rectifier K+ channel subfamily Kir6.x in zebrafish. zKir6.3 is a protein of 432 amino acids that shares 66% identity with mammalian Kir6.2 but differs considerably from mammalian Kir6.1 and Kir6.2 in the COOH terminus, which contain an Arg-Lys-Arg (RKR) motif, an endoplasmic reticulum (ER) retention signal. Single-channel recordings of reconstituted channels show that zKir6.3 requires the sulfonylurea receptor 1 (SUR1) subunit to produce KATP channel currents with single-channel conductance of 57.5 pS. Confocal microscopic analysis shows that zebrafish Kir6.3 requires the SUR1 subunit for its trafficking to the plasma membrane. Analyses of chimeric protein between human Kir6.2 and zKir6.3 and a COOH-terminal deletion of zKir6.3 indicate that interaction between the COOH terminus of zKir6.3 and SUR1 is critical for both channel activity and trafficking to the plasma membrane. We also identified zebrafish orthologs corresponding to mammalian SUR1 (zSUR1) and SUR2 (zSUR2) by the genomic database. Both Kir6.3 and SUR1 are expressed in embryonic brain of zebrafish, as assessed by whole mount in situ hybridization. These data indicate that Kir6.3 and SUR1 form functional KATP channels at the plasma membrane in zebrafish through a mechanism independent from ER retention by the RKR motif.

Descriptors: zebrafish, adenosine triphosphate metabolism, potassium channels genetics, potassium channels, inwardly rectifying genetics, ATP binding cassette transporters genetics and metabolism, adenosine triphosphate genetics, amino acid sequence, cell membrane

metabolism, cultured cells, electrophysiology, gene library, confocal microscopy, genetic models, molecular sequence data, potassium channels metabolism, potassium channels, inwardly rectifying metabolism, protein subunits genetics , protein subunits metabolism, messenger RNA metabolism, receptors, drug genetics, receptors, drug metabolism, sequence alignment, structure activity relationship, transfection.

Zhang, T., A. Isayeva, S.L. Adams, and D.M. Rawson (2005). **Studies on membrane permeability of zebrafish (*Danio rerio*) oocytes in the presence of different cryoprotectants.** *Cryobiology* 50(3): 285-93. ISSN: 0011 2240.
NAL Call Number: QH324.C7
Abstract: Investigation into fish oocyte membrane permeability is essential for developing successful protocols for their cryopreservation. The aim of the present work was to study the permeability of the zebrafish *(Danio rerio*) oocyte membrane to water and cryoprotectants before cryopreservation protocol design. The study was conducted on stage III and stage V zebrafish oocytes. Volumetric changes of stage III oocytes in different concentrations of sucrose were measured after 20 min exposure at 22 degrees C and the osmotically inactive volume of the oocytes (Vb) was determined using the Boyle-van't Hoff relationship. Volumetric changes of oocytes during exposure to different cryoprotectant solutions were also measured. Oocytes were exposed to 2 M dimethyl sulphoxide (DMSO), propylene glycol (PG), and methanol for 40 min at 22 degrees C. Stage III oocytes were also exposed to 2 M DMSO at 0 degrees C. Oocyte images were captured on an Olympus BX51 cryomicroscope using Linkham software for image recording. Scion Image was used for image analysis and diameter measurement. The experimental data were fitted to a two-parameter model using Berkeley Madonna 8.0.1 software. Hydraulic conductivity (L(p)) and solute (cryoprotectant) permeability (Ps) were estimated using the model. The osmotically inactive volume of stage III zebrafish oocytes was found to be 69.5%. The mean values+/-SE of Lp were found to be 0.169+/-0.02 and 0.196+/-0.01 microm/min/atm in the presence of DMSO and PG, respectively, at 22 degrees C, assuming an internal isosmotic value for the oocyte of 272 mOsm. The Ps values were 0.000948+/-0.00015 and 0.000933+/-0.00005 cm/min for DMSO and PG, respectively. It was also shown that the membrane permeability of stage III oocytes decreased significantly with temperature. No significant changes in cell volume during methanol treatment were observed. Fish oocyte membrane permeability parameters are reported here for the first time. The Lp and Ps values obtained for stage III zebrafish oocytes are generally lower than those obtained from successfully cryopreserved mammalian oocytes and higher than those obtained with fish embryos and sea urchin eggs. It was not possible to estimate membrane permeability parameters for stage V oocytes using the methods employed in this study because stage V oocytes experienced the separation of outer oolemma membrane from inner vitelline during exposure to cryoprotectants.
Descriptors: zebrafish oocytes, membrane permeability physiology, cryopreservation veterinary, oocytes physiology, cell size, cryoprotective agents, dimethyl sulfoxide, computer assisted image processing, methanol, theoretical models, propylene glycol.

Zhang, T., R.Y. Wang, Q.Y. Bao, and D.M. Rawson (2006). **Development of a new rapid measurement technique for fish embryo membrane permeability studies using impedance spectroscopy**. *Theriogenology* 66(4): 982-988. ISSN: 0093 691X.
NAL Call Number: QP251.A1T5
Abstract: Information on fish embryo membrane permeability is vital in their cryopreservation. Whilst conventional volumetric measurement based assessment methods have been widely used in fish embryo membrane permeability studies, they are lengthy and reduce the

capacity for multi-embryo measurement during an experimental run. A new rapid 'real-time' measurement technique is required to determine membrane permeability during cryoprotectant treatment. In this study, zebrafish (*Danio rerio*) embryo membrane permeability to cryoprotectants was investigated using impedance spectroscopy. An embryo holding cell, capable of holding up to 10 zebrafish embryos was built incorporating the original system electrods for measuring the impedance spectra. The holding cell was tested with deionised water and a series of KCl solutions with known conductance values to confirm the performance of the modified system. Untreated intact embryos were then tested to optimise the loading capacity and sensitivity of the system. To study the impedance changes of zebrafish embryos during cryoprotectant exposure, three, six or nine embryos at 50% epiboly stage were loaded into the holding cell in egg water, which was then removed and replaced by 0.5, 1.0, 2.0 or 3 M methanol or dimethyl sulfoxide (DMSO). The impedance changes of the loaded embryos in different cryoprotectant solutions were monitored over 30 min at 22 degrees C, immediately following embryo exposure to cryoprotectants, at the frequency range of 10-10(6) Hz. The impedance changes of the embryos in egg water were used as controls. Results from this study showed that the optimum embryo loading level was six embryos per cell for each experimental run. The optimum frequency was identified at 10(3.14) or 1380 Hz which provided good sensitivity and reproducibility. Significant impedance changes were detected after embryos were exposed to different concentrations of cryoprotectants. The results agreed well with those obtained from conventional volumetric based studies. (c) 2006 Elsevier Inc. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, methods and techniques, membranes, cell biology, impedance spectroscopy , spectrum analysis techniques, cell membrane permeability.

Zhao, C.T., K.H. Shi, Y. Su, L.Y. Liang, Y. Yan, J. Postlethwait, and A.M. Meng (2003). **Two variants of zebrafish p100 are expressed during embryogenesis and regulated by Nodal signaling.** *Federation of European Biochemical Societies Journal* 543(1-3): 190-5. ISSN: 1742 464X.
NAL Call Number: QP501.E8
Abstract: Human p100 protein was first identified as a transcriptional coactivator of Epstein-Barr virus nuclear antigen 2, and has been shown to be a coactivator of other cellular transactivators. Its roles in development of vertebrate embryos, however, have not been reported. We have identified a zebrafish ortholog of the human p100 coactivator. The zebrafish p100 transcript is processed to two alternative variants, long and short forms, referred to as p100L and p100S, respectively. Both GFP-p100L and GFP-p100S fusion proteins are located in the cytoplasm of transfected culture cells and microinjected embryonic cells. Analysis of transcripts with Northern blots revealed the presence of p100L and lower amounts of p100S mRNAs from the one-cell stage throughout the life cycle. Whole-mount in situ hybridization shows that p100L and p100S share the same spatiotemporal expression pattern. Their zygotic expression is initially restricted to axial mesoderm precursors during gastrulation, and then spreads over other tissues during segmentation, and finally is constrained to some internal organs at day 5. We also find that Nodal signaling is essential for the zygotic expression of p100. These studies pave the way to understanding in depth the role of p100 during vertebrate embryogenesis.
Descriptors: zebrafish, alternative splicing, developmental gene expression regulation, transforming growth factor beta physiology, Xenopus embryology, Xenopus proteins, cytoplasm chemistry, embryo anatomy and histology, embryo chemistry, embryo metabolism, HeLa cells, in situ hybridization, protein biosynthesis, messenger RNA analysis, messenger RNA

biosynthesis, recombinant fusion proteins analysis, signal transduction, Xenopus genetics, Xenopus metabolism.

Zilberman Peled, B., L. Appelbaum, D. Vallone, N.S. Foulkes, S. Anava, A. Anzulovich, S.L. Coon, D.C. Klein, J. Falcon, B. Ron, and Y. Gothilf (2007). **Transcriptional regulation of arylalkylamine-N-acetyltransferase-2 gene in the pineal gland of the gilthead seabream**. *Journal of Neuroendocrinology* 19(1): 46-53. ISSN: 0953 8194.

Abstract: Pineal serotonin-N-acetyltransferase (arylalkylamine-N-acetyltransferase; AANAT) is considered the key enzyme in the generation of circulating melatonin rhythms; the rate of melatonin production is determined by AANAT activity. In all the examined species, AANAT activity is regulated at the post-translational level and, to a variable degree, also at the transcriptional level. Here, the transcriptional regulation of pineal aanat (aanat2) of the gilthead seabream (*Sparus aurata*) was investigated. Real-time polymerase chain reaction quantification of aanat2 mRNA levels in the pineal gland collected throughout the 24-h cycle revealed a rhythmic expression pattern. In cultured pineal glands, the amplitude was reduced, but the daily rhythmic expression pattern was maintained under constant illumination, indicating a circadian clock-controlled regulation of seabream aanat2. DNA constructs were prepared in which green fluorescent protein was driven by the aanat2 promoters of seabream and Northern pike. In vivo transient expression analyses in zebrafish embryos indicated that these promoters contain the necessary elements to drive enhanced expression in the pineal gland. In the light-entrainable clock-containing PAC-2 zebrafish cell line, a stably transfected seabream aanat2 promoter-luciferase DNA construct exhibited a clock-controlled circadian rhythm of luciferase activity, characteristic for an E-box-driven expression. In NIH-3T3 cells, the seabream aanat2 promoter was activated by a synergistic action of BMAL/CLOCK and orthodenticle homeobox 5 (OTX5). Promoter sequence analyses revealed the presence of the photoreceptor conserved element and an extended E-box (i.e. the binding sites for BMAL/CLOCK and OTX5 that have been previously associated with pineal-specific and rhythmic gene expression). These results suggest that seabream aanat2 is a clock-controlled gene that is regulated by conserved mechanisms. Copyright © 2008 CABI

Descriptors: zebrafish embryos, cell lines, circadian rhythm, embryos, enzyme activity, gene expression, genes, luciferases, melatonin, pineal body, promoters, synergism, transcription, sea bream, *Sparus aurata*.

Research Models

Anonymous (2007). **Recent papers on zebrafish and other aquarium fish models.** *Zebrafish* 4(1): 79-90. ISSN: 1545-8547.
Descriptors: zebrafish, models, aquarium fish.

Ackermann, G.E.P.B.H. (2003). **Zebrafish: a genetic model for vertebrate organogenesis and human disorders.** *Frontiers in Bioscience: a Journal and Virtual Library* 8: D1227-53. ISSN: 1093-9946.
NAL Call Number: QH301.F76
Abstract: Mutations may be tolerated without noticeable effect or may present with a specific phenotype that reveals information about the function of the mutated gene. This information is an inexhaustible source for understanding biology and let us ask particular questions about the molecular mechanisms of development, degeneration and disease. The zebrafish (*Danio rerio*) has been proven to be instrumental in the genetic analysis of spontaneous and induced mutations and has provided invaluable clues to the elucidation of complex molecular processes in vertebrate biology. Since completion of the two large-scale mutagenesis screens carried out at the Max-Planck Institute in Tuebingen and at the Massachusetts General Hospital in Boston, many of the recovered mutations have been cloned and the function of the mutated genes studied. Special interest laid in the analysis of mutations affecting structures and organ systems characteristic for vertebrates such as the notochord, neural crest, heart, vasculature, blood and kidney. This review updates our knowledge of heart, vessel, blood and kidney organogenesis in zebrafish and extrapolates our insights to human disorders by assessing common genetic pathways.
Descriptors: zebrafish, disease models, animal, genetic diseases, inborn genetics, organogenesis genetics.

Alestrom, P. (2005). **Sebrafisk som ny forskningsmodell innen veterinaer biomedisin [Norges Veterinaerhogskole] 1935-2005. [Research at the Norwegian Veterinary School 1935-2005]**. *Norsk Veterinaertidsskrift* 117(8): 632-634 . ISSN: 0332 5741.
Abstract: To mark the 70th anniversary of the founding of the School, a series of articles on work done in the School is being presented; two examples of current work are given in this issue of the journal. They deal with the use of zebrafish as experimental models in veterinary biomedicine (pp 632-634), and scrapie in sheep (pp 636-637). On pp 639-642 are short articles and notes on other work in progress, details of clinical summer courses, and doctorates of the NVH undertaken at other institutions. Copyright © 2008 CABI
Descriptors: zebrafish, research, veterinary education, Norwegian veterinary school, zebrafish as a model, biomedicine.
Language of Text: Norwegian.

Amali, A.A., R.D. Rekha, C.J. Lin, W.L. Wang, H.Y. Gong, G.M. Her, and J.L. Wu (2006). **Thioacetamide induced liver damage in zebrafish embryo as a disease model for steatohepatitis.** *Journal of Biomedical Science* 13(2): 225-32 . ISSN: 1021-7770.
NAL Call Number: R850.A1
Abstract: Steatohepatitis has recently been increasing as a cofactor influencing the progression of fibrosis, cirrhosis, adenoma and carcinoma in liver; however, the mechanisms by which it contributes to liver injury remain uncertain. We induced steatohepatitis in zebrafish embryos using thioacetamide (TAA). TUNEL assay revealed significant increasing of apoptosis in liver after 5 days post fertilization and the increasing of apoptosis was observed to be associated with the up-regulation of apoptotic genes such as, bad, bax, P-38a, caspase-3 and 8, and JNK-1. Histological sections by oil red O stain showed the accumulation of fatty droplets which causes the pushing of

the nucleus towards one side. Up-regulation of steatosis markers such as, ACC, adiponectin, PTL, CEBP- alpha and beta, SREBP-1 was also observed. Furthermore, the elevation of glutathione peroxidase in TAA treated embryos indicated that TAA induces lipid peroxidation which leads to causes liver damage. Zebrafish has already been considered as a good human disease model and in this context; TAA-treated zebrafish may serve as a good animal model to study the molecular pathogenesis of steatohepatitis. Moreover, non-availability of specific drugs to prevent steatohepatitis, this animal model may serve as a powerful preclinical platform to study the therapeutic strategies and for evaluating chemoprevention strategies for this disease.
Descriptors: zebrafish embryos, disease models, fat necrosis pathology, hepatitis pathology, thioacetamide adverse effects, apoptosis, apoptosis regulatory proteins genetics fat necrosis etiology and genetics, hepatitis etiology and genetics, liver diseases chemically induced, up regulation genetics.

Arnaout, R., T. Ferrer, J. Huisken, K. Spitzer, D.Y. Stainier, M. Tristani Firouzi, and N.C. Chi (2007). **Zebrafish model for human long QT syndrome.** *Proceedings of the National Academy of Sciences of the United States of America* 104(27): 11316-21. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: Long QT syndrome (LQTS) is a disorder of ventricular repolarization that predisposes affected individuals to lethal cardiac arrhythmias. To date, an appropriate animal model of inherited LQTS does not exist. The zebrafish is a powerful vertebrate model used to dissect molecular pathways of cardiovascular development and disease. Because fundamental electrical properties of the zebrafish heart are remarkably similar to those of the human heart, the zebrafish may be an appropriate model for studying human inherited arrhythmias. Here we describe the molecular, cellular, and electrophysiological basis of a zebrafish mutant characterized by ventricular asystole. Genetic mapping and direct sequencing identify the affected gene as kcnh2, which encodes the channel responsible for the rapidly activating delayed rectifier K(+) current (I(Kr)). We show that complete loss of functional I(Kr) in embryonic hearts leads to ventricular cell membrane depolarization, inability to generate action potentials (APs), and disrupted calcium release. A small hyperpolarizing current restores spontaneous APs, implying wild-type function of other ionic currents critical for AP generation. Heterozygous fish manifest overt cellular and electrocardiographic evidence for delayed ventricular repolarization. Our findings provide insight into the pathogenesis of homozygous kcnh2 mutations and expand the use of zebrafish mutants as a model system to study human arrhythmias.
Descriptors: zebrafish, disease models, long QT syndrome genetics, action potentials genetics, amino acid substitution genetics, electrocardiography, heart arrest genetics and physiopathology, long QT syndrome embryology and physiopathology, phenotype, potassium channels, voltage gated genetics, *Xenopus*, physiology.

Baden, K.N., J. Murray, R.A. Capaldi, and K. Guillemin (2007). **Early developmental pathology due to cytochrome C oxidase deficiency is revealed by a new zebrafish model.** *Journal of Biological Chemistry* 282(48): 34839-49. ISSN: 0021-9258.
NAL Call Number: 381J824
Abstract: Deficiency of cytochrome C oxidase (COX) is associated with significant pathology in humans. However, the consequences for organogenesis and early development are not well understood. We have investigated these issues using a zebrafish model. COX deficiency was induced using morpholinos to reduce expression of CoxVa, a structural subunit, and Surf1, an assembly factor, both of which impaired COX assembly. Reduction of COX activity to 50% resulted in developmental defects in endodermal tissue, cardiac function, and swimming behavior. Cellular investigations revealed different underlying mechanisms. Apoptosis was dramatically increased in

the hindbrain and neural tube, and secondary motor neurons were absent or abnormal, explaining the motility defect. In contrast, the heart lacked apoptotic cells but showed increasingly poor performance over time, consistent with energy deficiency. The zebrafish model has revealed tissue-specific responses to COX deficiency and holds promise for discovery of new therapies to treat mitochondrial diseases in humans.
Descriptors: zebrafish , deficiency, cytochrome C oxidase, disease model, pathology, mitochondrial diseases in humans.

Bassett, D. and P.D. Currie (2004). **Identification of a zebrafish model of muscular dystrophy.** *Clinical and Experimental Pharmacology and Physiology* 31(8): 537-40. ISSN: 0305 1870.
Abstract: 1. Large-scale mutagenic screens of the zebrafish genome have identified a number of different classes of mutations that disrupt skeletal muscle formation. Of particular interest and relevance to human health is a class of recessive lethal mutations in which muscle differentiation occurs normally, but is followed by tissue-specific degeneration reminiscent of human muscular dystrophies. 2. We have shown that one member of this class of mutations, sapje (sap), results from mutations within the zebrafish orthologue of the human Duchenne muscular dystrophy (DMD) gene. Mutations in this locus cause Duchenne or Becker muscular dystrophies in human patients and are thought to result in a dystrophic pathology by disrupting the link between the actin cytoskeleton and the extracellular matrix in skeletal muscle cells. 3. We have found that the progressive muscle degeneration phenotype of sapje-mutant zebrafish embryos is caused by the failure of somitic muscle attachments at the embryonic myotendinous junction (MTJ). 4. Although a role for dystrophin at the MTJ has been postulated previously and MTJ structural abnormalities have been identified in the dystrophin-deficient mdx mouse model, in vivo evidence of pathology based on muscle attachment failure is thus far lacking. Therefore, the sapjre mutation may provide a model for a novel pathological mechanism of Duchenne muscular dystrophy and other muscle diseases. In the present review, we discuss this finding in light of previously postulated models of dystrophin function.
Descriptors: zebrafish as disease models, muscular dystrophy genetics, muscular dystrophy pathology, Duchenne, Becker, mutation.

Bassett, D.I. and P.D. Currie (2003). **The zebrafish as a model for muscular dystrophy and congenital myopathy.** *Human Molecular Genetics* 12(Spec No 2): R265-70. ISSN: 0964-6906.
NAL Call Number: RB155.5.H87
Abstract: The muscular dystrophies and congenital myopathies are inherited diseases of the skeletal muscle, which lead to a loss of muscle function and are often fatal. While many of the loci involved are already known, these conditions remain incurable, and genetic models are being developed in an effort to understand the pathological mechanisms involved. Recently several papers have shown that the zebrafish, which is now widely used in developmental genetic studies, will provide a useful addition to our toolkit in this regard. Here we describe these studies, including a zebrafish model of what is potentially the novel pathological mechanism of muscle attachment failure in Duchenne and other muscular dystrophies.
Descriptors: zebrafish, mice, disease models, muscular diseases genetics, muscular dystrophies genetics, congenital muscular diseases.

Beattie, C.E., T.L. Carrel, and M.L. McWhorter (2007). **Fishing for a mechanism: using zebrafish to understand spinal muscular atrophy.** *Journal of Child Neurology* 22(8): 995-1003. ISSN: 0883-0738.
Abstract: Motoneuron diseases cause paralysis and death due to loss of motoneurons that innervate skeletal muscle. Spinal muscular atrophy is a human motoneuron disease that is genetically

linked to the survival motor neuron gene (SMN). Although SMN was identified more than a decade ago, it remains unclear how decreased levels of the SMN protein cause spinal muscular atrophy. The use of animal models, however, offers a crucial tool in determining the function of SMN in this disease. In this review, we discuss our efforts to develop a zebrafish model of spinal muscular atrophy.

Descriptors: zebrafish embryos, cyclic AMP response element binding protein genetics, genetic predisposition to disease genetics, muscular atrophy, spinal genetics, muscular atrophy, spinal metabolism , nerve tissue RNA binding cell differentiation genetics, cyclic AMP response element binding protein metabolism, disease models, motor neurons metabolism and pathology, skeletal muscle embryology, innervation and physiopathology, muscular atrophy, spinal physiopathology, mutation genetics, nerve tissue proteins metabolism, RNA binding proteins metabolism.

Notes: A review.

Beis, D. and D.Y. Stainier (2006). **In vivo cell biology: following the zebrafish trend.** *Trends in Cell Biology* 16(2): 105-12. ISSN: 0962-8924.

NAL Call Number: QH573.T73

Abstract: A deeper understanding of the mechanisms of cell behavior is essential if we want to comprehend how an organism develops and functions. Changes in cellular processes, including the orientation of cell divisions, cell shape, polarity, differentiation and migration, account for tissue rearrangements during development and homeostasis. The in vivo relevance of in vitro findings is being constantly debated and the need for in vivo systems becoming more pressing. The zebrafish (*Danio rerio*) might become the vertebrate system of choice for a wide spectrum of biological questions that need to be investigated in vivo at cellular and subcellular resolutions. Here, we discuss some recent studies in which the zebrafish was used to gain insight into cell-biological mechanisms. Although this model system has been predominantly appreciated for its amenability to forward genetics, current advances in imaging technology and an increasing number of transgenic lines are bringing it closer to its full potential.

Descriptors: zebrafish, biology trends, cells chemistry, research models, cell biology, imaging potential.

Benyumov, A.O., T.K. Venkatachalam, O.O. Grigoriants, A.O. Vassilev, H.E. Tibbles, S. Downs, D. Dumezb, and F.M. Uckun (2005). **Anti-proliferative and anti-leukemic activity of DDE46 (compound WHI-07), a novel bromomethoxylated arylphosphate derivative of zidovudine, and related compounds. Studies using human acute lymphoblastic leukemia cells and the zebrafish model.** *Arzneimittel Forschung* 55(2): 114-22. ISSN: 0004 4172.

NAL Call Number: RS1 A7

Abstract: The anti-proliferative effects of a novel bromomethoxylated arylphosphate derivative of zidovudine (compound DDE46, CAS 213982-96-8) were first examined in a zebra fish embryo model. DDE46 blocked the cell division at the 2-cell stage of the embryonic development followed by total cell fusion. DDE46 also inhibited the proliferation of the leukemic cell lines NALM-6 and MOLT-3. DDE46 enhanced the activity of the pro-apoptotic enzymes Caspase-3, Caspase-6, Caspase-8, and Caspase-9 leading to the apoptotic death of the leukemic cell line Jurkat. These results justify the further development of this agent as a new anti-leukemic drug candidate.

Descriptors: zebrafish, antineoplastic agents pharmacology, leukemia drug therapy, thymidine monophosphate analogs, derivatives and pharmacology, zidovudine analogs, derivatives and pharmacology, antineoplastic agents therapeutic use, apoptosis drug effects, caspases antagonists and inhibitors, cell proliferation drug effects, drug screening assays, antitumor, jurkat cells, magnetic

resonance spectroscopy, nephelometry and turbidimetry, prodrugs pharmacology, tetrazolium salts, thiazoles, tubulin biosynthesis and genetics.

Berghmans, S., J. Hunt, A. Roach, and P. Goldsmith (2007). **Zebrafish offer the potential for a primary screen to identify a wide variety of potential anticonvulsants.** *Epilepsy Research* 75(1): 18-28. ISSN: 0920 1211.

Abstract: The search for novel anticonvulsants requires appropriate model systems in which to test hypotheses through focused compound screening or genetic manipulation, or conduct black box screening of large numbers of compounds or potential genetic modifiers. Many models are currently in existence that subserve particular roles in achieving these aims, but all have their limitations. Zebrafish have been suggested as an additional model of epilepsy, but their optimum role is unclear. They are more amenable to high throughput analysis, but are more genetically removed from humans than rodents. We therefore sought to develop assay methodology applicable to medium/high throughput screening using an automated tracking system to measure the amount of movement induced by exposure to the proconvulsant, pentylene tetrazole (PTZ). We then used this system to explore how many known anti-epileptic drugs (AEDs) would be detected when running such a screen. We were able to detect suppression of PTZ-induced excessive movements with 13 out of 14 standard AEDs. A parallel sedation and toxicity screen suggested these effects were due to direct anti-epileptic effect, although non-specific effects cannot be fully excluded. These results suggest zebrafish may be a useful high throughput primary screen to pick up potential novel AEDs.

Descriptors: zebrafish embryo, anticonvulsants therapeutic use, epilepsy models, epilepsy drug therapy, behavior , drug dose response relationship, preclinical drug evaluation, drug interactions chemically induced epilepsy, movement drug effects, pentylenetetrazole, time factors.

Berman, J., K. Hsu, and A.T. Look (2003). **Zebrafish as a model organism for blood diseases.** *British Journal of Haematology* 123(4): 568-76. ISSN: 0007 1048.

Descriptors: zebrafish, hematologic diseases, blood disease models, genetically modified, DNA mutational analysis, hematopoiesis, morphogenesis physiology, vertebrates embryology.

Berman, J.N., J.P. Kanki, and A.T. Look (2005). **Zebrafish as a model for myelopoiesis during embryogenesis.** *Experimental Hematology* 33(9): 997-1006. ISSN: 0301 472X.

NAL Call Number: RB145.E97

Abstract: Zebrafish produce nearly identical hematopoeitic cell lineages to those found in mammals and other higher vertebrates. As in mammals, blood cell development proceeds in distinct waves, constituting embryonic (primitive) and adult (definitive) hematopoiesis. The conservation of genes such as scl, pu.1, c/ebpalpha, mpo, l-plastin, and lysozyme C in myelopoiesis and the corresponding expression patterns in zebrafish suggests that shared genetic pathways regulate this complex developmental process. In the zebrafish model system, experimental approaches have been applied, including RNA in situ hybridization, morpholino injections, and the analysis of mutant and transgenic fish lines, leading to improved understanding of the regulation in vivo of key molecular pathways with conserved roles in vertebrate myelopoiesis.

Descriptors: zebrafish, embryonic development genetics, myelopoiesis physiology, embryonic development physiology, developmental gene expression regulation, hematopoiesis genetics and physiology, models, myelopoiesis genetics.

Bretaud, S., C. Allen, P.W. Ingham, and O. Bandmann (2007). **P-53-dependent neuronal cell death in a DJ-1-deficient zebrafish model of Parkinson's disease.** *Journal of Neurochemistry* 100(6): 1626-35. ISSN: 0022-3042.

NAL Call Number: QP351.J6
Abstract: Mutations in DJ-1 lead to early onset Parkinson's disease (PD). The aim of this study was to elucidate further the underlying mechanisms leading to neuronal cell death in DJ-1 deficiency in vivo and determine whether the observed cell loss could be prevented pharmacologically. Inactivation of DJ-1 in zebrafish, *Danio rerio*, resulted in loss of dopaminergic neurons after exposure to hydrogen peroxide and the proteasome inhibitor MG132. DJ-1 knockdown by itself already resulted in increased p53 and Bax expression levels prior to toxin exposure without marked neuronal cell death, suggesting subthreshold activation of cell death pathways in DJ-1 deficiency. Proteasome inhibition led to a further increase of p53 and Bax expression with widespread neuronal cell death. Pharmacological p53 inhibition either before or during MG132 exposure in vivo prevented dopaminergic neuronal cell death in both cases. Simultaneous knockdown of DJ-1 and the negative p53 regulator mdm2 led to dopaminergic neuronal cell death even without toxin exposure, further implicating involvement of p53 in DJ-1 deficiency-mediated neuronal cell loss. Our study demonstrates the utility of zebrafish as a new animal model to study PD gene defects and suggests that modulation of downstream mechanisms, such as p53 inhibition, may be of therapeutic benefit.
Descriptors: zebrafish Parkinson's disease model, nerve tissue proteins deficiency, neurons physiology, Parkinson disease pathology, tumor suppressor protein p53 physiology, proteins deficiency, genetically modified, cell death drug effects and genetics, disease models, embryo, developmental gene expression regulation drug effects and genetics, hydrogen peroxide pharmacology, in situ hybridization methods and nick end labeling, leupeptins pharmacology, neurons drug effects, neurotoxins pharmacology, Parkinson disease genetics, messenger RNA biosynthesis, reverse transcriptase PCR methods, tyrosine 3 monooxygenase metabolism, Bcl 2 associated x protein metabolism.

Brown, S.B., C.S. Tucker, C. Ford, Y. Lee, D.R. Dunbar, and J.J. Mullins (2007). **Class III antiarrhythmic methanesulfonanilides inhibit leukocyte recruitment in zebrafish.** *Journal of Leukocyte Biology* 82(1): 79-84. ISSN: 0741-5400.
NAL Call Number: QP185.R4
Abstract: Understanding fundamental molecular mechanisms that govern the transmigration and interstitial migration of leukocytes to sites of tissue damage and infection is of potential significance in identifying novel therapeutic targets for the management of chronic inflammatory disorders. CD31 is a mammalian cell adhesion molecule that regulates the recruitment of leukocytes from the circulation. Our recent unpublished work has suggested that homophilic ligation of CD31 can negatively regulate the ether-a-go-go-related gene (ERG) current within leukocytes to regulate cell-cell adhesion. To validate and probe the functional significance of ERG in leukocytes, we developed an infected wound model of inflammation in zebrafish and assessed the efficacy of two ERG-specific inhibitors, dofetilide and E4031, as well as an ERG-specific antisense RNA morpholino on neutrophil recruitment. Our data confirm a hitherto undescribed role for ERG in leukocytes, where inhibition or translational knockdown of ERG resulted in significant attenuation of the inflammatory response to an infectious stimulus. Inhibition of ERG was verified independently by a decrease in the ventricular heart rate, where ERG also functions in the repolarization of the cardiac action potential. Our results suggest that ERG-specific Class III antiarrhythmic drugs can modulate inflammatory responses to infection.
Descriptors: zebrafish infected wound model , anilides pharmacology, anti arrhythmia agents pharmacology, chemotaxis, leukocyte drug effects, ether a go go potassium channels physiology, inflammation immunology, antisense RNA pharmacology, cell adhesion, disease models ether a go go potassium channels antagonists and inhibitors, ether a go go potassium channels genetics,

heart rate, inflammation drug therapy, antisense RNA therapeutic use, wound infection immunology and pathology.

Burgess, S. (2006). **New techniques for using zebrafish as a model for development**. *Methods* 39(3): 181-182. ISSN: 1046 2023.
NAL Call Number: QP519.7.M47
Descriptors: zebrafish development model, cell transplantation, therapeutic and prophylactic techniques, mosaic analysis, genetic techniques, tilling, targeting induced local lesions in genomes, large scale enhancer trapping, small region electroporation, transgenesis.

Carney, S.A., A.L. Prasch, W. Heideman, and R.E. Peterson (2006). **Understanding dioxin developmental toxicity using the zebrafish model.** *Birth Defects Research* 76(1): 7-18. ISSN: 1542 0752.
NAL Call Number: QL991.T4
Descriptors: zebrafish embryos, toxin models, teratogens toxicity, tetrachlorodibenzodioxin toxicity, zebrafish growth and development and metabolism, aryl hydrocarbon hydroxylases metabolism, cytochrome P450 CYPA1A1 genetics and metabolism, drug toxicity and effects, pathology, developmental gene expression regulation drug effects, larva drug effects and metabolism, morpholines pharmacology, aryl hydrocarbon receptors antagonists, inhibitors and metabolism.

Carr, A.J., T. Katherine Tamai, L.C. Young, V. Ferrer, M.P. Dekens, and D. Whitmore (2006). **Light reaches the very heart of the zebrafish clock.** *Chronobiology International* 23(1-2): 91-100. ISSN: 0742 0528.
NAL Call Number: QP84.6.C47
Abstract: Zebrafish are typically used as a model system to study various aspects of developmental biology, largely as a consequence of their ex vivo development, high degree of transparency, and, of course, ability to perform forward genetic mutant screens. More recently, zebrafish have been developed as a model system with which to study circadian clocks. Cell lines generated from early-stage zebrafish embryos contain clocks that are directly light-responsive. We describe recent experiments using single-cell luminescent imaging approaches to study clock function in this novel cell line system. Furthermore, studies examining the process of entrainment to light pulses within this cell population are described in this review, as are experiments examining light-responsiveness of early-stage zebrafish embryos.
Descriptors: zebrafish, circadian clock model, biological clocks, light, cell cycle, cell line, cell lineage, developmental gene expression regulation, mutation.

Cheesman, S.E. and K. Guillemin (2007). **We know you are in there: conversing with the indigenous gut microbiota**. *Research in Microbiology* 158(1): 2-9. ISSN: 0923 2508.
NAL Call Number: QR1.A55
Abstract: The vertebrate gut harbours a coevolved consortium of microbes that plays critical roles in the development and health of this organ. Here we discuss recent insights into the microbial-host molecular dialogs that shape the digestive tracts of the model vertebrates, mice and zebrafish, and consider the parallels between vertebrate-microbial mutualisms and the well-studied squid-Vibrio symbiosis. Copyright © 2008 CABI
Descriptors: zebrafish , animal models, health, intestinal microorganisms, intestines, digestive tract models, *Danio rerio*, mice.

Chen, E. and S.C. Ekker (2004). **Zebrafish as a genomics research model.** *Current Pharmaceutical Biotechnology* 5(5): 409-13. ISSN: 1389 2010.

Abstract: The zebrafish (*Danio rerio*) is a recent addition to the genomic scientists' repertoire of vertebrate animal model systems. Unlike simple invertebrates such as the fly or the nematode, this teleost maintains the biological and genomic complexity found in higher vertebrates. Furthermore, the zebrafish has many advantageous technical and genomic properties that open the door to experimental approaches not practical using more classical models. The zebrafish genome can be functionally accessed using both forward and reverse genetics based approaches. A notable recent addition to the zebrafish genomics toolbox is the development of morpholino-based antisense gene inhibition for sequence-based 'knockdown' screening. This method offers the opportunity to examine the role of significant subsets of the vertebrate genome for specific gene function in vivo. The zebrafish embryo can rapidly provide critical information for drug target discovery purposes when examined with an emphasis on clinically-relevant biological processes. Finally, the advent of chemical genetics in zebrafish suggests that, in addition to the identification and understanding of drug targets and their biology, this system will be a powerful tool in the direct development of novel pharmaceuticals in the near future.

Descriptors: zebrafish embryos, genomics, genetically modified gene targeting, genetic models.

Chitnis, A.B. and M. Itoh (2004). **Exploring alternative models of rostral-caudal patterning in the zebrafish neurectoderm with computer simulations.** *Current Opinion in Genetics and Development* 14(4): 415-21. ISSN: 0959 437X.

NAL Call Number: QH426.C88

Descriptors: zebrafish embryology, body patterning physiology, ectoderm physiology, developmental gene expression regulation, computer simulation, morphogenesis.

Choi, T.Y., J.H. Kim, D.H. Ko, C.H. Kim, J.S. Hwang, S. Ahn, S.Y. Kim, C.D. Kim, J.H. Lee, and T.J. Yoon (2007). **Zebrafish as a new model for phenotype-based screening of melanogenic regulatory compounds.** *Pigment Cell Research* 20(2): 120-7. ISSN: 0893-5785.

Abstract: Although many hypo-pigmenting agents are currently available, the demand for novel whitening agents is increasing, in part due to the weak effectiveness and unwanted side effects of currently available compounds. To screen for novel hypo-pigmenting agents, many methodologies such as cell culture and enzymatic assays are routinely used. However, these models have disadvantages in terms of physiological and economic relevance. In this study, we validated zebrafish as a whole-animal model for phenotype-based screening of melanogenic inhibitors or stimulators. We used both the well-known melanogenic inhibitors (1-phenyl-2-thiourea, arbutin, kojic acid, 2-mercaptobenzothiazole) and newly developed small molecule compounds (haginin, YT16i). All the tested compounds produced inhibitory effects on the pigmentation of zebrafish, most likely due to their inhibitory potential on tyrosinase activity. In simultaneous in vivo toxicity tests, a newly developed melanogenic inhibitor YT16i showed massive abnormalities in terms of deformed morphologies and cardiac function. Together, these results provide a rationale in screening and evaluating the putative melanogenic regulatory compounds. We suggest that the zebrafish system is a novel alternative to mammalian models, with several advantages including the rapidity, cost-effectiveness, and physiological relevance.

Descriptors: zebrafish embryology, preclinical drug evaluation methods, melanins metabolism, models, pigmentation , antioxidants pharmacology, arbutin pharmacology, benzimidazoles pharmacology melanins antagonists and inhibitors, monophenol monooxygenase metabolism, phenotype, pyrones pharmacology.

Chong, M., Y.H. Gui, L. Cheng, and D.R. Lu (2007). **Establishment of model of congenital heart disease with Tbx2 gene knockdown: experiment with zebrafish**. *Zhonghua Yi Xue Za Zhi* 87(14): 991-4. ISSN: 0578-1337.

Abstract: OBJECTIVE: To establish a model of congenital heart disease with Tbx2 gene knockdown. METHODS: 1200 fertilized eggs of zebrafish (*Danio rerio*) were divided into 3 groups: wild type group (n = 100), Tbx2-morpholino (MO) group (n = 600, to be injected with Tbx2-MO so as to knock down the Tbx2 gene), and control MO group (n = 500, to be injected with control MO). 12, 24, 36, 48, 72, and 96 hours after fertilization the heart structure, heart rate, rhythm, contractibility, and blood flow of the embryos were observed under anatomic microscope and digital camera was used to record the findings. Whole-mount in situ hybridization was conducted to observe the expression of versican in the heart. RESULTS: Eight hours after fertilization 27.2% of the fertilized eggs of the Tbx2-MO group died, and 21.3%, 32.8%, and 18.7% of them showed mild, moderate, or severe developmental abnormality of heart, including hypogenetic ventricle, dilatation of atria, abnormal atrioventricular canal, slow heartbeat, arrhythmia, and blood regurgitation. Whole-mount in situ hybridization showed that versican did not decrease with the passage of time but was expressed abnormally in the atria and ventricles, and did not disappear 72 hours after fertilization; however, it was expressed normally in the statolith. CONCLUSION: MO antisense oligonucleotide knockdown is a good method for the study of heart development. Tbx2 gene plays an important role in the differentiation of atria and ventricles and formation of atrioventricular canal.

Descriptors: zebrafish, congenital heart disease model, congenital heart disease, CHD, Tbx2 gene, knockdown.

Language of Text: Chinese.

Collery, R.F., M.L. Cederlund, V.A. Smyth, and B.N. Kennedy (2006). **Applying transgenic zebrafish technology to study the retina.** *Advances in Experimental Medicine and Biology* 572: 201-7. ISSN: 0065 2598.

NAL Call Number: QP901.A33

Descriptors: genetically modified zebrafish, genetic techniques, retina metabolism, retinal diseases genetics and pathology, disease models, time factors.

Concha, M.L. (2004). **The dorsal diencephalic conduction system of zebrafish as a model of vertebrate brain lateralisation.** *Neuroreport* 15(12): 1843-6. ISSN: 0959-4965.

Abstract: Lateralisation is an attractive and intriguing feature of the vertebrate CNS studied for decades in the different disciplines of the neurosciences. Due to the complexity of the phenomena and intrinsic limitations of the approaches used to date, it has been difficult to establish useful links between the different, and usually distant, levels of lateralisation e.g. between genetics, morphology, physiology and behaviour. Recently, the dorsal diencephalon of the teleost zebrafish has emerged as a valuable model to begin addressing this issue and as a result unravel the role of vertebrate CNS lateralisation. Zebrafish is a well-established genetic system that allows a 'bottom up' ('gene to behaviour') approach to the study of lateralisation. In fact, it is the single vertebrate system to date in which asymmetric gene expression in the brain has been directly linked to asymmetric morphology. Zebrafish offers several experimental advantages that allow the study of brain lateralisation using a wide range of experimental tools, from study of gene function through in vivo analysis of morphology and physiology to behavioural assessments. Altogether, these features will allow the establishment of operational links between lower (genetics and morphology) and upper (physiology and behaviour) levels of brain lateralisation.

Descriptors: zebrafish, brain physiology, diencephalon physiology, functional laterality physi-

ology, brain development model, behavior, developmental gene expression regulation, systems theory, vertebrates.

Costache, A.D., P.K. Pullela, P. Kasha, H. Tomasiewicz, and D.S. Sem (2005). **Homology-modeled ligand-binding domains of zebrafish estrogen receptors alpha, beta1, and beta2: from in silico to in vivo studies of estrogen interactions in *Danio rerio* as a model system.** *Molecular Endocrinology* 19(12): 2979-90. ISSN: 0888-8809.
NAL Call Number: QP187.3M64M6
Abstract: Homology models were constructed for the ligand-binding domains of zebrafish estrogen receptors (zfERs) alpha, beta(1), and beta(2). Estradiol-binding sites are nearly identical in zfERs and their human homologs, suggesting that zebrafish will serve as a good model system for studying human ER-binding drugs. Conversely, studies of endocrine disruptor effects on zebrafish will benefit from the wealth of data available on xenoestrogen interactions with human ERs. Compounds flagged by the Interagency Coordinating Committee on the Validation of Alternative Methods for endocrine disruptor screening were docked into our zfER homology models. Ideally, these in silico docking studies would be complemented with in vivo binding studies. To this end, fluorescently tagged estradiol was docked into zfERalpha and found to bind in the same manner as in human ERalpha, with fluorescein preferentially occupying a region between helices 11 and 12. Fluorescently tagged estradiol was synthesized and was found to localize along the path of primordial germ cell migration in the developing zebrafish embryo 3 d after fertilization, consistent with previous reports of 1) a role for estradiol in sex determination, and 2) the first appearance of ERs 2 d after fertilization. These data provide a foundation for future in silico and in vivo binding studies of estrogen agonists and antagonists with zebrafish ERs.
Descriptors: zebrafish estrogen receptor model, estrogen receptor alpha chemistry, estrogens metabolism, proteins chemistry, amino acid sequence, computer simulation, estradiol analysis, estrogen receptor alpha metabolism, estrogen receptor beta chemistry and metabolism, fluorescence, germ cells chemistry, ligands, tagged estradiol, molecular sequence data, protein Conformation, sequence alignment, amino acid sequence homology, zebrafish growth and development.

Daggett, M.A.F. (2005). **The use of adult and embryonic zebrafish in undergraduate independent research projects**. *Federation of American Societies for Experimental Biology Journal* 19(4, Suppl. S, Part 1): A783. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: Zebrafish have become an accepted model organism for the study of embryonic development and genetic based diseases. Zebrafish are also becoming an established organism for use in undergraduate teaching laboratories. The advantages of using zebrafish include ease of culturing and maintaining the fish in the lab and the availability of information on the Internet for performing various scientific investigations using zebrafish. Courses in Animal Physiology and Molecular Cell Biology at Missouri Western State College have utilized the zebrafish model in both independent and ongoing class-based research projects. Independent research projects offer all opportunity for students to design, perform and report on their own research question. Independent projects in the past have been mostly limited to pharmocodynamic assays measuring such dependent variables as mortality, heart rate and gross developmental abnormalities as determined in real time while observing the fish through a microscope. Future directions for projects, made possible by the acquisition of physiological recording equipment will include the ability to measure more quantitative physiological variables including, but not limited to, changes in electrocardiogram wave forms, pulse rate, oxygen consumption, nerve cell electrophysiology and force generation during muscle contraction. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, education, undergraduate teaching research project, embryonic development, genetic disease, future potential with equipment and technology.

Dahm, R. (2006). **The zebrafish exposed**. *American Scientist* 94(5): 446-453. ISSN: 0003-0996.
Descriptors: zebrafish, embryogenesis, embryonic development and cell cycle, freshwater fish, mutations, *Danio rerio.*

Dahm, R. and R. Geisler (2006). **Learning from small fry: the zebrafish as a genetic model organism for aquaculture fish species.** *Marine Biotechnology* 8(4): 329-45. ISSN: 1436-2228.
NAL Call Number: TP248.27.M37M37
Abstract: In recent years, the zebrafish has become one of the most prominent vertebrate model organisms used to study the genetics underlying development, normal body function, and disease. The growing interest in zebrafish research was paralleled by an increase in tools and methods available to study zebrafish. While zebrafish research initially centered on mutagenesis screens (forward genetics), recent years saw the establishment of reverse genetic methods (morpholino knock-down, TILLING). In addition, increasingly sophisticated protocols for generating transgenic zebrafish have been developed and microarrays are now available to characterize gene expression on a near genome-wide scale. The identification of loci underlying specific traits is aided by genetic, physical, and radiation hybrid maps of the zebrafish genome and the zebrafish genome project. As genomic resources for aquacultural species are increasingly being generated, a meaningful interaction between zebrafish and aquacultural research now appears to be possible and beneficial for both sides. In particular, research on nutrition and growth, stress, and disease resistance in the zebrafish can be expected to produce results applicable to aquacultural fish, for example, by improving husbandry and formulated feeds. Forward and reverse genetics approaches in the zebrafish, together with the known conservation of synteny between the species, offer the potential to identify and verify candidate genes for quantitative trait loci (QTLs) to be used in marker-assisted breeding. Moreover, some technologies from the zebrafish field such as TILLING may be directly transferable to aquacultural research and production.
Descriptors: transgenic zebrafish, genetic techniques, gene expression models, fisheries, genomics methods, mutagenesis genetics, phylogeny.

Dambly Chaudiere, C. (2004). **Imagerie et developpement de la ligne laterale chez le Poisson-zebre. [Imaging the development of the zebrafish line]**. *Journal De La Societe De Biologie* 198(2): 153-55. ISSN: 1295-0661.
Abstract: The lateral line system of the zebrafish offers the qualities of simplicity, accessibility and reproducibility which are most appropriate for the analysis of neural development and pattern formation in vertebrates. Furthermore, the transparency of the fish embryos allows the application of a variety of optical and molecular methods in the living animal, which is essential for a more complete analysis of the system.
Descriptors: zebrafish embryos, developmental models, neural development, pattern formation.
Language of Text: French.

Dambly Chaudiere, C., D. Sapede, F. Soubiran, K. Decorde, N. Gompel, and A. Ghysen (2003). **The lateral line of zebrafish: A model system for the analysis of morphogenesis and neural development in vertebrates.** *Biology of the Cell* 95(9): 579-587. ISSN: 0248 4900.
NAL Call Number: QH201.J6
Abstract: The lateral line of the zebrafish has many of the advantages that made the sensory organs of *Drosophila* a very productive model system: 1) it comprises a set of discrete sense organs (neuromasts) arranged in a defined, species-specific pattern, such that each organ can be indi-

vidually recognized; 2) the neuromasts are superficial and easy to visualize, and the innervating neurons are easy to label; 3) the sensory projection is simple yet reproducibly organized. Here we describe some of the tools that can be used to investigate the development of this system, and we illustrate their usefulness with specific exemples. We conclude that the lateral line is uniquely suited among vertebrate sensory systems for a molecular, cellular and genetic analysis of pattern formation and of neural development.
Descriptors: zebrafish, *Danio rerio*, lateral line, model system for analysis of development, development.
Notes: A review.

Dlugos, C.A. and R.A. Rabin (2007). **Ocular deficits associated with alcohol exposure during zebrafish development.** *Journal of Comparative Neurology* 502(4): 497-506. ISSN: 0021-9967.
Descriptors: zebrafish embryos, nervous system alcohol induced disorders physiopathology, ethanol toxicity, eye drug effects and physiopathology, chemically induced eye abnormalities and physiopathology, nervous system alcohol induced disorders chemically induced and nervous system pathology, apoptosis drug effects, cell differentiation drug effects and physiology, cell survival drug effects and physiology, central nervous system depressants toxicity, drug dose response relationship, eye pathology and abnormalities pathology, electron transmission microscopy, neurons drug effects and pathology, ovum drug effects and pathology, retina abnormalities, drug effects and physiopathology.

Drummond, I.A. (2005). **Kidney development and disease in the zebrafish.** *Journal of the American Society of Nephrology* 16(2): 299-304. ISSN: 1555-9041.
Abstract: Unraveling the molecular pathogenesis of human disease presents many experimental challenges, not the least of which is that experiments on humans are generally frowned upon. Model organisms, including the zebrafish, allow for experimental analysis of gene function and the detailed characterization of disease processes. Zebrafish have matured as a vertebrate model organism now that genetic tools for targeted "knockdowns" and unbiased mutagenesis approaches are in hand. The fish larval pronephros is a relevant kidney in which to pursue many aspects of human kidney development and disease. This short review outlines recent progress in applying the zebrafish pronephros to issues of human health and development.
Descriptors: zebrafish embryos, embryonic structures ultrastructure, kidney embryology, nephrons embryology, organogenesis physiology, polycystic kidney diseases genetics, disease models, genomics models, nephrons ultrastructure, polycystic kidney diseases embryology, species specificity.

Dufourcq, P., M. Roussigne, P. Blader, F. Rosa, N. Peyrieras, and S. Vriz (2006). **Mechano-sensory organ regeneration in adults: the zebrafish lateral line as a model.** *Molecular and Cellular Neuroscience* 33(2): 180-7. ISSN: 1044-7431.
Abstract: In this report, we present a study of regeneration of the lateral line, a collection of mechano-sensory organ, in the adult zebrafish caudal fin. As all neuromasts are innervated by axon fibers, neuronal regeneration is a key issue in the regenerating process. We first show that support cells from the last neuromast adjacent to the amputation plane divide and migrate to colonize the blastema in order to reform the missing part of the lateral line. We then show that nerve re-growth takes place later than neuromast progenitor cell migration. We also provide evidence that new growth cones form at the amputation plane and subsequently follow the migrating placode-like structure to re-innervate regenerated neuromasts as they differentiate. Altogether, our observations indicate that caudal lateral line regeneration is not a mere recapitulation of the ontogenic process.

Descriptors: zebrafish caudal fin, models, nerve regeneration physiology, sensory receptors cytology and physiology, animal structures cytology and innervation , and physiology, biological markers, cell division physiology.

Dufourcq, P. and S. Vriz (2005). **Zebrafish to help the human diseases.** *Biofutur* 254: 33-37. ISSN: 0294-3506.
Descriptors: zebrafish disease models, genomes, human diseases, karyotypes, phylogenetics, *Danio rerio.*

Elo, B., C.M. Villano, D. Govorko, and L.A. White (2007). **Larval zebrafish as a model for glucose metabolism: expression of phosphoenolpyruvate carboxykinase as a marker for exposure to anti-diabetic compounds.** *Journal of Molecular Endocrinology* 38(4): 433-40. ISSN: 0952-5041.
NAL Call Number: QP187.3.M64J68
Abstract: The zebrafish model system is one of the most widely used animal models for developmental research and it is now becoming an attractive model for drug discovery and toxicological screening. The completion of sequencing the zebrafish genome and the availability of full-length cDNAs and DNA microarrays for expression analysis, in addition to techniques for generating transgenic lines and targeted mutations, have made the zebrafish model even more attractive to researchers. Recent data indicate that the regulation of glucose metabolism in zebrafish, through the production of insulin, is similar to mammalian models, and many of the genes involved in regulating blood glucose levels have been identified in zebrafish. The data presented here show that adult zebrafish respond to anti-diabetic drugs similarly to mammalian models, by reducing blood glucose levels. Furthermore, we show that the expression of phosphoenolpyruvate carboxykinase (PEPCK), which catalyzes a rate-limiting step in gluconeogenesis and is transcriptionally regulated by glucagon and insulin, is regulated in larval zebrafish similarly to that seen in mammalian systems, and changes in PEPCK expression can be obtained through real-time PCR analysis of whole larval RNA. Taken together, these data suggest that larval zebrafish may be an appropriate model for the examination of glucose metabolism, using PEPCK as an indicator of blood glucose levels.
Descriptors: zebrafish larvae, blood glucose drug effects, glipizide pharmacology, hypoglycemic agents pharmacology, metformin pharmacology, phosphoenolpyruvate carboxykinase GTP metabolism, thiazolidinediones pharmacology, metabolism, cyclic AMP pharmacology, dexamethasone pharmacology, larva, glucose metabolism model, tretinoin pharmacology.

Esnault, Y., O. Jaillon, J.M. Aury, J. Weissenbach, and H. Roest Crollius (2005). **Tetraodon, fish with a genome model.** *Biofutur* 254: 23-28. ISSN: 0294-3506.
Descriptors: zebrafish, genomes, karyotypes, phylogenetics, *Danio rerio*, *Oryzias latipes*, *Takifugu rubripes*, *Tetraodon nigroviridis*, medaka.
Language of Text: French.

Fan, L., A. Alestrom, P. Alestrom, and P. Collodi (2004). **Development of cell cultures with competency for contributing to the zebrafish germ line.** *Critical Reviews in Eukaryotic Gene Expression* 14(1-2): 43-51. ISSN: 1045 4403.
NAL Call Number: QH450.C74
Abstract: The zebrafish is an established model for the genetic analysis of vertebrate development. Forward-genetic screens have generated thousands of mutations, and antisense-based methods have been used to transiently knockdown gene expression during embryogenesis. Although these methods have made the zebrafish a valuable system for the identification and functional characterization of developmentally important genes, one deficiency of the zebrafish

model is the absence of methods to introduce targeted mutations to generate knockout lines of fish. Application of gene-targeting methods has been limited in nonmurine species due to the absence of germ-line competent embryonic stem (ES) cell lines. Recently, progress was made in addressing this problem by the derivation of zebrafish embryo cell lines that remain pluripotent and germ-line competent for multiple passages in culture. Zebrafish germ-line chimeras were generated using cultures derived from embryos at two different developmental stages, and targeted insertion of vector DNA by homologous recombination was demonstrated in both cultures. Several strategies are being used to optimize the production and identification of germ-line chimeras. The zebrafish embryo cell culture system should provide the basis of a gene-targeting approach that will complement other genetic strategies and improve the utility of the zebrafish model for studies of development and disease.
Descriptors: zebrafish embryology, gene targeting methods, germ cells cytology, cell culture techniques methods, cytology, insertional mutagenesis.

Feng, J., H.H. Ip, S.H. Cheng, and P.K. Chan (2004). **A relational-tubular (ReTu) deformable model for vasculature quantification of zebrafish embryo from microangiography image series.** *Computerized Medical Imaging and Graphics* 28(6): 333-44. ISSN: 0895 6111.
Abstract: Embryonic cardiovascular system plays a vital role in embryonic development of human and animal. In this work, we introduce a novel deformable model, which we called Relational-tubular (ReTu) deformable model for segmenting and quantifying the embryonic vasculature of zebrafish embryo from microangiography image series. Particularly, to incorporate additional constraints on the spatial relationships among vessel branches, we introduce a new energy term called relation energy into the model energy function. This energy item acts as a repulsion force between neighboring vessels during the deformation to encourage them to move towards their respective volume data. Using the ReTu deformable model, the deformation process is an iterative two-stage procedure: vascular axis deformation and vascular surface deformation. The efficiency and robustness of this approach are demonstrated by experiments which show that satisfactory quantifications of the vasculature can be obtained after 3-4 iterations.
Descriptors: zebrafish embryology, angiography methods, cardiovascular system anatomy and histology, cardiovascular model.

Fetcho, J.R. (2007). **The utility of zebrafish for studies of the comparative biology of motor systems.** *Journal of Experimental Zoology. Part B. Molecular and Developmental Evolution* 308(5): 550-62. ISSN: 1552-5007.
NAL Call Number: QL1.J87
Descriptors: zebrafish, locomotion physiology, motor systems models, motor neurons physiology, physiology, comparative methods, genetic inborn diseases physiopathology, interneurons physiology, synapses physiology.

Fisher, S., E.A. Grice, R.M. Vinton, S.L. Bessling, and A.S. McCallion (2006). **Conservation of RET regulatory function from human to zebrafish without sequence similarity.** *Science* 312(5771): 276-9. ISSN: 0036-8075.
NAL Call Number: 470Sci2
Abstract: Evolutionary sequence conservation is an accepted criterion to identify noncoding regulatory sequences. We have used a transposon-based transgenic assay in zebrafish to evaluate noncoding sequences at the zebrafish ret locus, conserved among teleosts, and at the human RET locus, conserved among mammals. Most teleost sequences directed ret-specific reporter gene expression, with many displaying overlapping regulatory control. The majority of human RET noncoding sequences also directed ret-specific expression in zebrafish. Thus, vast amounts

of functional sequence information may exist that would not be detected by sequence similarity approaches.
Descriptors: zebrafish embryology, conserved sequence, enhancer elements genetics, gene expression regulation, proto oncogene proteins C RET genetics, regulatory sequences, nucleic acid, genetic models, neurons metabolism, DNA sequence analysis, Takifugu genetics, transgenes.

Flanagan-Steet, H.R., R.A. Steet, and S. Kornfeld (2006). **A zebrafish model for mucolipidosis II**. *Glycobiology* 16(11): 1132. ISSN: 0959 6658.
NAL Call Number: QP552.G59G593
Descriptors: zebrafish model, nervous system, metabolism, enzymology, mucolipidosis II, nervous system disease, genetic disease, behavioral and mental disorders, metabolic disease, etiology, phenotype, pectoral fin development, neuromuscular junction organization, motoneuron migration.

Frank, P.G. and M.P. Lisanti (2006). **Zebrafish as a novel model system to study the function of caveolae and caveolin-1 in organismal biology.** *American Journal of Pathology* 169(6): 1910-2. ISSN: 0002-9440.
Descriptors: zebrafish embryology, caveolae physiology, caveolin 1 physiology, models, amino acid sequence, molecular sequence data, protein isoforms, amino acid sequence homology, signal transduction, tissue distribution , metabolism.

Garcia Ximenez, F.U.P.d.V.E.T.S.d.I.A., M.F. Simao, and M. Perez Camps (2007). **El desarrollo de embriones de pez cebra en estadio MBT puede ser detenido reversiblemente a 16 grados C. (Comunicacion corta.) [zebrafish embryo development can be reversibly arrested at the MBT (mid blastula transition) stage by exposure to a water temperature of 16 deg C. (Short communication.)]**. *Spanish Journal of Agricultural Research* 5(2): 181-185. ISSN: 1695 971X.
NAL Call Number: S3.S636
Descriptors: zebrafish embryos, *Danio rerio*, hybridization, embryo culture, gametes, chimaeras, embryonic development, cold water temperature, fish culture, animal models, experimentation, animal developmental stages, aquaculture, biological development, breeding methods, cells, culture techniques, developmental stages, in vitro culture, temperature effects.

Ge, W. (2005). **Intrafollicular paracrine communication in the zebrafish ovary: the state of the art of an emerging model for the study of vertebrate folliculogenesis.** *Molecular and Cellular Endocrinology* 237(1-2): 1-10. ISSN: 0303-7207.
NAL Call Number: QP187.A1M6
Abstract: The development and function of vertebrate ovary are primarily controlled by the gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), from the pituitary. However, most of the activities of FSH and LH are mediated or modulated by a variety of locally produced factors that form an intimate regulatory network within and between the follicles. As a top vertebrate model for genetic and developmental studies, the zebrafish has caught tremendous attention in the past two decades; however, its utility has quickly been extended to other areas including physiology. In the past few years, a variety of peptide growth factors have been identified and characterized in the zebrafish ovary including activin and epidermal growth factor (EGF), and lines of evidence point to the existence of an ovarian network of communication involving these factors. This article provides the state of the art of zebrafish as a model for analyzing ovarian development and its regulation.
Descriptors: zebrafish ovarian development model, epidermal growth factor pharmacology,

ovarian follicle growth and development, paracrine communication physiology, oocytes cytology and metabolism, ovarian follicle metabolism.

Geiger, G.A., S.E. Parker, A.P. Beothy, J.A. Tucker, M.C. Mullins, and G.D. Kao (2006). **Zebrafish as a "biosensor"? effects of ionizing radiation and amifostine on embryonic viability and development.** *Cancer Research* 66(16): 8172-81. ISSN: 0008 5472.
Abstract: The zebrafish (*Danio rerio*) has emerged as a popular vertebrate model system for cancer and treatment-related research. Benefits include ease of care, rapid development, optical clarity of embryos, which allows visualization of major organ systems, and opportunities for genetic manipulation. However, specific parameters of radiation sensitivity have not been systematically documented. We investigated the effects of radiation and a radiomodifier on zebrafish viability and embryonic development. Embryos were exposed to gamma-radiation (5, 10, or 20 Gy) at sequential times postfertilization and serially assessed for viability and morphologic abnormalities. As expected, lethality and morphologic perturbations were more pronounced earlier in embryogenesis and with higher radiation doses and were partially reversed by amifostine. The effects of radiation and concurrent treatment with amifostine on the developmental organization of the eye and brain were striking. Radiation resulted in hypocellularity and disorganization of the cellular layers of the retina, effects partially reversed by amifostine, as well as lens opacification. Radiation strikingly reduced the volume of brain, but the volume loss was substantially blocked by amifostine. Increased terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labeling signal was noted in both the irradiated eye and brain, but reduced by amifostine. Finally, irradiating embryos resulted in caspase activation detectable in 96-well microplates, which was proportional to the number of embryos and radiation dose; the degree of activation was markedly reduced by amifostine. These results together suggest the power and versatility of the zebrafish in assessing the effects of radiation and radiomodifiers on organ and tissue development.
Descriptors: zebrafish embryology, amifostine pharmacology, cell survival radiation effects, physiology, embryonic development radiation effects, radiation effects , embryonic development drug effects, ionizing radiation, radiation protective agents pharmacology.

Gerhard, G.S. (2003). **Comparative aspects of zebrafish (*Danio rerio*) as a model for aging research.** *Experimental Gerontology* 38(11-12): 1333-41. ISSN: 0531 5565.
NAL Call Number: QP86.E85
Abstract: The zebrafish has emerged over the past decade as a major model system for the study of development due to its invertebrate-like advantages coupled with its vertebrate biology. These features also make it a potentially valuable organism for gerontological research. The main advantages of zebrafish include its economical husbandry, small yet accessible size, high reproductive capacity, genetic tractability, and a large and growing biological database. Although zebrafish life span is longer than rodents, it shares the feasibility of large-scale mutational analysis with the extremely short-lived invertebrate models. This review compares zebrafish with the more widely used model organisms used for aging research, including yeast, worms, flies, mice, and humans.
Descriptors: zebrafish, aging physiology, aging research model, cell aging physiology, *Drosophila* physiology, mice.

Gerhard, G.S., R.L. Malek, E. Keller, J. Murtha, and K.C. Cheng (2004). **Zebrafish, killifish, neither fish, both fish?** *Journals of Gerontology Series A, Biological Sciences and Medical Sciences* 59(9): B873-5. ISSN: 1079-5006.
Descriptors: zebrafish, aging physiology, Fundulidae physiology, fishes, models for biological research.

Glass, A.S. and R. Dahm (2004). **The zebrafish as a model organism for eye development.** *Ophthalmic Research* 36(1): 4-24. ISSN: 0030-3747.

Descriptors: zebrafish model for eye embryology, eye growth and development, eye metabolism, crystalline lens embryology and metabolism, retina embryology and metabolism, transcription factors metabolism.

Gleeson, M., V. Connaughton, and L.S. Arneson (2007). **Induction of hyperglycaemia in zebrafish (*Danio rerio*) leads to morphological changes in the retina.** *Acta Diabetologica* 44(3): 157-63. ISSN: 0940 5429.

Abstract: Diabetes affects over 16 million Americans yearly, resulting in hyperglycaemia and microvascular complications, including retinopathy, neuropathy and nephropathy. Animal models have been developed to examine the immunological aspects of type 1 diabetes and the pathogenic mechanisms associated with diabetic retinopathy, but the methods of diabetes induction raise concerns regarding these models. Zebrafish (*Danio rerio*) have been used extensively to study developmental processes and mutant zebrafish strains have been used to examine vision disease present in humans. In this paper, we have induced hyperglycaemia in zebrafish by alternately immersing the fish in glucose solution or water. Eyes from untreated fish or fish exposed to alternating glucose/water solutions for 28 days were dissected, sectioned and stained to visualise cell bodies in the retina. In untreated fish retinas, the inner plexiform layer (IPL) and inner nuclear layer (INL) were approximately the same thickness, whereas in fish repeatedly exposed to glucose solutions the IPL was approximately 55% the thickness of the INL. Both the IPL and INL were significantly reduced in retinas of treated fish, compared to untreated fish, similar to that seen in other animal models of diabetes and in diabetic patients. These results suggest that zebrafish may be used as an animal model in which to study diabetic retinopathy.

Descriptors: zebrafish model for diabetic retinopathy, hyperglycemia pathology, retina pathology, blood glucose metabolism, disease models, vision physiology, vision disorders blood, vision disorders pathology, vision disorders physiopathology.

Guan, J.L. (Guan,Jun,Lin) (2005). ***Methods in Molecular Biology***, 294, Humana Press Inc.: Totowa, NJ , 364 p. ISBN: 1588293823.

NAL Call Number: QH506.M45V.294

Abstract: This 357-page book is volume 294 of the series Methods in Molecular Biology and this volume focuses on the developmental methods and protocols used in the study of cell migration. Central to the better understanding of both molecular mechanisms and disease, cell migration plays an essential role in a variety of biological processes and is now the subject of intense study using an array of powerful new technologies. In this volume researchers describe in step-by-step detail their most successful techniques for studying the macromolecular machinery of cell movement. These readily reproducible protocols include a wide range of novel and state-of-the-art methodologies, as well as many classic methods, for use in cultured cells, different model organisms, and specialized cells in both normal development and disease. Highlights include the basic assays that apply to all cell migration studies in vitro, assays in various model organisms, and assays for cancer cells, endothelial cells, and neurons both in vitro and in animal models. The authors also offer several novel approaches to the study of cell migration, as well as extensive coverage of cell migration studies in developmental and disease models. The protocols follow the format of the successful Methods in Molecular Biology series, with each offering step-by-step laboratory instructions, an introduction outlining the principle behind the technique, lists of necessary equipment and reagents, as well as tips on troubleshooting and avoiding known pitfalls. The book is

structured into 5 parts and contains 24 individually-authored chapters written in English. The book highlights a list of contributors and their respective institutions. This book will be useful resource for researchers in cell biology. Copyright©Thomson Reuters 2009
Descriptors: zebrafish disease, cell biology, movement and support, time lapse microscopy, imaging and microscopy techniques, multiphoton microscopy, imaging and microscopy techniques, cell adhesion assay, wound healing assay, Boyden chamber assay, Dunn chemotaxis chamber assay, cell scatter assay, tumor cell invasion assay, microscope based fourier resonance energy transfer, spectrum analysis techniques, microfluidic channel networks, angiogenesis, cell migration, dorsal closure, protein tyrosine phosphorylation.

Gupta, T. and M.C. Mullins (2010). **Dissection of organs from the adult zebrafish**. *Journal of Visualized Experiments* 37
Online: http://www.jove.com/index/Details.stp?ID=1717
Descriptors: zebrafish, dissection, organs, larva, remodeling of larval organs, morphogenesis of adult organs.
Notes: Open access article. DOI: 10.3791/1717.

Guyon, J.R., L.S. Steffen, M.H. Howell, T.J. Pusack, C. Lawrence, and L.M. Kunkel (2007). **Modeling human muscle disease in zebrafish.** *Biochimica Et Biophysica Acta* 1772(2): 205-15. ISSN: 0006 3002.
NAL Call Number: 381B522
Abstract: Zebrafish reproduce in large quantities, grow rapidly, and are transparent early in development. For these reasons, zebrafish have been used extensively to model vertebrate development and disease. Like mammals, zebrafish express dystrophin and many of its associated proteins early in development and these proteins have been shown to be vital for zebrafish muscle stability. In dystrophin-null zebrafish, muscle degeneration becomes apparent as early as 3 days post-fertilization (dpf) making the zebrafish an excellent organism for large-scale screens to identify other genes involved in the disease process or drugs capable of correcting the disease phenotype. Being transparent, developing zebrafish are also an ideal experimental model for monitoring the fate of labeled transplanted cells. Although zebrafish dystrophy models are not meant to replace existing mammalian models of disease, experiments requiring large numbers of animals may be best performed in zebrafish. Results garnered from using this model could lead to a better understanding of the pathogenesis of the muscular dystrophies and the development of future therapies.
Descriptors: zebrafish, muscle disease models, muscular dystrophies genetics, pathology, etiology and therapy, metabolism.

Hall, T.E., R.J. Bryson Richardson, S. Berger, A.S. Jacoby, N.J. Cole, G.E. Hollway, J. Berger, and P.D. Currie (2007). **The zebrafish candyfloss mutant implicates extracellular matrix adhesion failure in laminin l2-deficient congenital muscular dystrophy.** *Proceedings of the National Academy of Sciences of the United States of America* 104(17): 7092-97. ISSN: 0027 8424.
NAL Call Number: 500N21P
Abstract: Mutations in the human laminin l2 (LAMA2) gene result in the most common form of congenital muscular dystrophy (MDC1A). There are currently three models for the molecular basis of cellular pathology in MDC1A: (i) lack of LAMA2 leads to sarcolemmal weakness and failure, followed by cellular necrosis, as is the case in Duchenne muscular dystrophy (DMD); (ii) loss of LAMA2-mediated signaling during the development and maintenance of muscle tissue results in myoblast proliferation and fusion defects; (iii) loss

of LAMA2 from the basement membrane of the Schwann cells surrounding the peripheral nerves results in a lack of motor stimulation, leading to effective denervation atrophy. Here we show that the degenerative muscle phenotype in the zebrafish dystrophic mutant, candyfloss (caf) results from mutations in the laminin l2 (lama2) gene. In vivo time-lapse analysis of mechanically loaded fibers and membrane permeability assays suggest that, unlike DMD, fiber detachment is not initially associated with sarcolemmal rupture. Early muscle formation and myoblast fusion are normal, indicating that any deficiency in early Lama2 signaling does not lead to muscle pathology. In addition, innervation by the primary motor neurons is unaffected, and fiber detachment stems from muscle contraction, demonstrating that muscle atrophy through lack of motor neuron activity does not contribute to pathology in this system. Using these and other analyses, we present a model of lama2 function where fiber detachment external to the sarcolemma is mechanically induced, and retracted fibers with uncompromised membranes undergo subsequent apoptosis.
Descriptors: zebrafish candyfloss mutant, laminin l2-deficient, muscular dystrophy.

Hamm, E.E., D.E. Voth, and J.D. Ballard (2006). **Identification of *Clostridium difficile* toxin B cardiotoxicity using a zebrafish embryo model of intoxication.** *Proceedings of the National Academy of Sciences of the United States of America* 103(38): 14176-81. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: *Clostridium difficile* toxin B (TcdB) has been studied extensively by using cell-free systems and tissue culture, but, like many bacterial toxins, the in vivo targets of TcdB are unknown and have been difficult to elucidate with traditional animal models. In the current study, the transparent *Danio rerio* (zebrafish) embryo was used as a model for imaging of in vivo TcdB localization and organ-specific damage in real time. At 24 h after treatment, TcdB was found to localize at the pericardial region, and zebrafish exhibited the first signs of cardiovascular damage, including a 90% reduction in systemic blood flow and a 20% reduction in heart rate. Within 72 h of exposure to TcdB, the ventricle chamber of the heart became deformed and was unable to contract or pump blood, and the fish exhibited extensive pericardial edema. In line with the observed defects in ventricle contraction, TcdB was found to directly disrupt coordinated contractility and rhythmicity in primary cardiomyocytes. Furthermore, using a caspase-3 inhibitor, we were able to block TcdB-related cardiovascular damage and prevent zebrafish death. These findings present an insight into the in vivo targets of TcdB, as well as demonstrate the strength of the zebrafish embryo as a tractable model for identification of in vivo targets of bacterial toxins and evaluation of novel candidate therapeutics.
Descriptors: zebrafish embryology, bacterial toxins metabolism and toxicity, drug effects, heart drug effects, caspase 3 antagonists and inhibitors, cultured cells, anatomy and histology, metabolism, fluorescent dyes metabolism, heart anatomy, histology, embryology and physiology, heart rate drug effects, cardiac myocytes cytology, drug effects and physiology, rats, zebrafish anatomy and histology.

Hill, S.A., X.P. Liu, M.A. Borla, J.V. Jose, and D.M. O'Malley (2005). **Neurokinematic modeling of complex swimming patterns of the larval zebrafish**. *Neurocomputing* S 65-66: 61-68. ISSN: 0925-2312.
NAL Call Number: QA76.87
Descriptors: zebrafish, activity patterns, behavior, brain, circuits, freshwater fish, kinematics, modelling, N-methyl-d-aspartic acid, neurons, oscillations, oscillators, prey, spinal cord, swimming, synapses, synaptic strength, neurocomputing, *Danio rerio.*

Hirschner, W., H.M. Pogoda, C. Kramer, U. Thiess, B. Hamprecht, K.H. Wiesmuller, M. Lautner, and S. Verleysdonk (2007). **Biosynthesis of Wdr16, a marker protein for kinocilia-bearing cells, starts at the time of kinocilia formation in rat, and wdr16 gene knockdown causes hydrocephalus in zebrafish.** *Journal of Neurochemistry* 101(1): 274-88. ISSN: 0022 3042.

NAL Call Number: QP351.J6

Abstract: The rat ortholog of the WD40 repeat protein Wdr16 is abundantly expressed in testis and cultured ependymal cells. Low levels are found in lung and brain, respectively, while it is absent from kinocilia-free tissues. In testis and ependymal primary cultures, Wdr16 messenger RNA appears concomitantly with the messages for sperm-associated antigen 6, a kinocilia marker, and for hydin, a protein linked to ciliary function and hydrocephalus. In testis, ependyma and respiratory epithelium, the Wdr16 protein is up-regulated together with kinocilia formation. The wdr16 gene is restricted to genera in possession of kinocilia, and it is strongly conserved during evolution. The human and zebrafish proteins are identical in 62% of their aligned amino acids. On the message level, the zebrafish Wdr16 ortholog was found exclusively in kinocilia-bearing tissues by in situ hybridisation. Gene knockdown in zebrafish embryos by antisense morpholino injection resulted in severe hydrocephalus formation with unaltered ependymal morphology or ciliary beat. Wdr16 can be considered a differentiation marker of kinocilia-bearing cells. In the brain, it appears to be functionally related to water homeostasis or osmoregulation.

Descriptors: zebrafish embryos, gene knockdown, brain effects, cilia metabolism, hydrocephalus genetics, microfilament nerve tissue zebrafish abnormalities, amino acid and base sequence, biological markers analysis and metabolism, cultured cells, ciliary motility disorders genetics, metabolism and physiopathology, down regulation drug effects and physiology, ependyma abnormalities, cytology and metabolism, molecular evolution, hydrocephalus metabolism and physiopathology, lateral ventricles abnormalities, metabolism and physiopathology, microfilament proteins biosynthesis, isolation and purification, molecular sequence data, nerve tissue proteins biosynthesis, isolation and purification, antisense oligonucleotides pharmacology, phylogeny, messenger RNA metabolism, rats, amino acid sequence homology, water electrolyte balance genetics and physiology, proteins biosynthesis, isolation and purification.

Ho, S.Y., M. Pack, and S.A. Farber (2003). **Analysis of small molecule metabolism in zebrafish.** *Methods in Enzymology* 364: 408-26. ISSN: 0076-6879.

NAL Call Number: QP601M49

Abstract: Recent work has shown that it is possible to assay phospholipid metabolism and prostanoid synthesis in zebrafish. These preliminary studies suggest that important questions of lipid biology are amenable to large-scale high-throughput analyses in this model system. Lipid metabolism can now be added to the growing list of vertebrate developmental and physiological processes that can be assayed in zebrafish. The potential to identify new genes, or novel functions of known genes that regulate dietary lipid metabolism, or the generation of lipid signaling molecules, may lead to the development of treatment strategies for common human diseases.

Descriptors: zebrafish embryology, metabolism, disease models, lipid metabolism, time factors.

Ho, S.Y., J.L. Thorpe, Y. Deng, E. Santana, R.A. DeRose, and S.A. Farber (2004). **Lipid metabolism in zebrafish.** *Methods in Cell Biology* 76: 87-108. ISSN: 0091-679X.

NAL Call Number: 442P92

Abstract: Forward genetics is an unbiased methodology to discover new genes or functions of genes. At the present, the zebrafish is one of the few vertebrate systems where large-scale forward genetic studies are practical. Fluorescent lipid labeling of zebrafish larvae derived from families created from ENU-mutagenized fish enabled us to perform a large scale in vivo screen to identify mutants with perturbed lipid processing. With the aid of the zebrafish genome project, positional cloning of mutated genes with abnormal lipid metabolism can be accelerated. MO- and gripNA-based transient gene silencing is feasible in zebrafish embryos and provides a reverse genetic screening strategy to search for important lipid regulators. The advantages of using zebrafish as a vertebrate model to study lipid metabolism include its rapid external development and its optical clarity that enables the monitoring of biological processes. Large scale, high-throughput drug screening in vivo, especially for drugs that inhibit lipid absorption, can be easily achieved in this model. These zebrafish-based assays are important tools to understand aspects of lipid biology with significant clinical implications.
Descriptors: zebrafish, 4 chloro 7 nitrobenzofurazan analogs and derivatives, cholesterol analogs and derivatives, lipid metabolism, 4 chloro 7 nitrobenzofurazan metabolism, alkyl and aryl transferases antagonists and inhibitors, annexin a2 metabolism, azetidines pharmacology, boron compounds chemistry, caveolin 1, caveolins metabolism, cell movement drug effects, cholesterol metabolism, DNA drug effects, DNA genetics, diterpenes pharmacology, drug effects, metabolism, ethylnitrosourea pharmacology, farnesol pharmacology, farnesyltranstransferase, geranyltranstransferase, germ cells cytology, germ cells drug effects, heptanoic acids pharmacology, hydroxymethylglutaryl coa reductase inhibitors pharmacology, lipids analysis, lovastatin pharmacology, mevalonic acid pharmacology, fluorescence microscopy, site directed mutagenesis mutation, phosphatidylethanolamines chemistry, phosphatidylethanolamines metabolism, protein bindingdrug effects, protein isoprenylation drug effects, pyrroles pharmacology, simvastatin pharmacology.

Hsu, C.H., Z.H. Wen, C.S. Lin, and C. Chakraborty (2007). **The zebrafish model: use in studying cellular mechanisms for a spectrum of clinical disease entities.** *Current Neurovascular Research* 4(2): 111-20. ISSN: 1567 2026.
Abstract: Although the zebrafish model provides an important platform for the study of developmental biology, recent work with the zebrafish model has extended its application to a wide variety of experimental studies relevant to human disease. Currently, the zebrafish model is used for the study of human genetic disease, caveolin-associated muscle disease, homeostasis, kidney development and disease, cancer, cardiovascular disorders, oxidative stress, caloric restriction, insulin-like pathways, angiogenesis, neurological diseases, liver disease, hemophilia, bacterial pathogenesis, apoptosis, osteoporosis, immunological studies, germ cell study, Bardet-Biedl syndrome gene (BBS11), Alzheimer's disease, virology studies and vaccine development. Here we describe the essential use of the zebrafish model that applies to several clinical diseases. With increased understanding of the cellular mechanisms responsible for disease, we can use knowledge gained from the zebrafish model for the development of therapeutics.
Descriptors: zebrafish, biomedical research, disease models, anatomy, histology and metabolism, genetically modified.

Hsu, H.J., N.C. Hsu, M.C. Hu, and B.C. Chung (2006). **Steroidogenesis in zebrafish and mouse models.** *Molecular and Cellular Endocrinology* 248(1-2): 160-3. ISSN: 0303-7207.
NAL Call Number: QP187.A1M6
Abstract: Steroid hormones regulate physiological homeostasis for salt, sugar, and sex differentiation. All steroids are synthesized from a common precursor, cholesterol, in a step that

converts cholesterol to pregnenolone. The enzyme carrying out this first conversion step is CYP11A1. To further investigate the importance of steroid biosynthesis, animal models with defects in the Cyp11a1 gene are used. Mice with targeted disruption of the Cyp11a1 gene produce no steroids with severe adrenal defects. These mice survive during embryogenesis, but die after birth. Zebrafish with a block in cyp11a1 gene function has an earlier defect, presumably because it lacks adequate maternal steroid supply. When cyp11a1 activity was compensated by the injection of antisense morpholino oligos, the embryos have shortened axis and a defect of epibolic cell movement during early embryogenesis. The discovery of steroid function in cell movement is novel, and should provide new insights into our understanding of diverse functions of steroids.
Descriptors: zebrafish and mouse animal models, cholesterol side chain cleavage enzyme metabolism, hormones biosynthesis, mice metabolism, steroids biosynthesis, metabolism, cholesterol side chain cleavage enzyme genetics, embryonic development, mice genetics, mice, mutant strains.

Hu, M.C., H.J. Hsu, I.C. Guo, and B.C. Chung (2004). **Function of CYP11A1 in animal models**. *Molecular and Cellular Endocrinology* 215(1-2): 95-100. ISSN: 0303-7207.
NAL Call Number: QP187.A1M6
Descriptors: zebrafish embryonic development model, freshwater fish, gene expression, glands, gonads, mutagenesis, secretion, sex hormones, steroids, *Danio rerio.*

Irwin, D.M. (2004). **A second insulin gene in fish genomes**. *General and Comparative Endocrinology* 135(1): 150-158. ISSN: 0016-6480.
NAL Call Number: 444.8G28
Descriptors: zebrafish, endocrinology, evolution, freshwater fish, genomes, human diseases, insulin gene, models, phylogenetics, *Danio rerio*, fugu, evolution.

Jacqueline, F.W. and F.S. Thomas (2006). **Zebrafish in comparative context: A symposium**. *Integrative and Comparative Biology* 46(5): 569-576. ISSN: 1540-7063.
NAL Call Number: QL.I67
Descriptors: zebrafish, phylogeny, cypriniform fishes, genome evolution, evolution of gastrulation, dentition, pigmentation, craniofacial development, nervous system structure, function.

Jagadeeswaran, P. (2005). **Zebrafish: a tool to study hemostasis and thrombosis.** *Current Opinion in Hematology* 12(2): 149-52. ISSN: 1065 6251.
Abstract: PURPOSE OF REVIEW: In the past eight years our laboratory has developed the zebrafish model to study hemostasis and thrombosis. The purpose of this review is to explore current developments involving the zebrafish model in the study of hemostasis and thrombosis because the time is now ripe to apply this model to identify novel players that participate in hemostasis and thrombosis. RECENT FINDINGS: In the past twelve months, three papers appeared in the hemostasis and thrombosis area using the zebrafish model. The first one is a review article that summarizes establishment of the zebrafish model to study hemostasis and thrombosis. The second study is a methodological paper describing assays for measuring hemostasis and thrombosis by inducing vascular occlusion in zebrafish larvae. The third paper describes a knockdown of prothrombin in zebrafish, which recapitulates knockout studies in mouse, and marks the beginning of studies in the hemostasis and thrombosis area by this new knockdown technology. In addition to the above papers, there is one abstract that describes kinetics of thrombocyte and thrombocyte-microparticle recruitment

in laser-induced arterial thrombus formation in zebrafish. SUMMARY: With the above advances, the zebrafish model has now matured to the point that it can address more important questions in the hemostasis and thrombosis area using genetic approaches. This review therefore summarizes the issues described in the above papers along with thoughts about future progress of the zebrafish model as a tool to study hemostasis and thrombosis.
Descriptors: zebrafish, hemostasis and thrombosis model, genetically modified.
Notes: A review.

Jagadeeswaran, P., M. Gregory, K. Day, M. Cykowski, and B. Thattaliyath (2005). **Zebrafish: a genetic model for hemostasis and thrombosis.** *Journal of Thrombosis and Haemostasis* 3(1): 46-53. ISSN: 1538-7933.
Abstract: Here we review the zebrafish hemostatic system, its relevance to mammalian hemostasis, and its efficacy as a vertebrate genetic model to further the understanding of hemostasis and thrombosis.
Descriptors: zebrafish research models, hemostasis, thrombosis, genetically modified, blood platelets cytology, lasers, mutation, transgenes.
Notes: A review.

Jin, P., T. Tian, Z. Sun, and A. Meng (2004). **Generation of mutants with developmental defects in zebrafish by N-ethyl-N-nitrosurea mutagenesis**. *Chinese Science Bulletin* 49(20): 2154-2158. ISSN: 1001-6538.
Descriptors: zebrafish development, defects, embryonic development, freshwater fish, mutagenesis, resource development, *Danio rerio*.

Kainz, P.M., A.R. Adolph, K.Y. Wong, and J.E. Dowling (2003). **Lazy eyes zebrafish mutation affects Muller glial cells, compromising photoreceptor function and causing partial blindness.** *Journal of Comparative Neurology* 463(3): 265-80. ISSN: 0021-9967.
Descriptors: mutant zebrafish larvae, visual function deficits, amblyopia genetics, neuroglia physiology, vertebrate photoreceptors physiology, retina physiology, adaptation, ocular physiology , blindness etiology and physiopathology, cell death physiology, dark adaptation physiology, electroretinography, immunohistochemistry, in situ nick end labeling, neuroglia ultrastructure, vertebrate photoreceptors pathology, retina pathology and ultrastructure.

Kari, G., U. Rodeck, and A.P. Dicker (2007). **Zebrafish: an emerging model system for human disease and drug discovery.** *Clinical Pharmacology and Therapeutics* 82(1): 70-80. ISSN: 0009 9236.
NAL Call Number: RM1.C52
Abstract: In vivo studies represent an essential step in drug development and currently rely largely on mice, yet limitations of mammalian models motivated the search for complementary vertebrate model systems. This review focuses on zebrafish, *Danio rerio,* as a facile model system to study human disease and drug responses. Zebrafish are particularly suited for this purpose because they represent a vertebrate species, their genome is sequenced, and a large number of synchronously developing, transparent embryos can be produced. Zebrafish embryos are permeable to drugs and can easily be manipulated using well-established genetic and molecular approaches. Here, we summarize recent work on drug discovery and toxicity in zebrafish embryos. In addition, we provide a synopsis of current efforts to establish disease models in zebrafish focusing on neoplasia. The results of these studies highlight the potential of zebrafish as a viable addition to established animal models by offering medium and, potentially, high throughput capabilities.

Descriptors: zebrafish embryology, drug design and evaluation methods, research models, toxicity tests methods, antineoplastic agents pharmacology and therapeutic use, carcinogenicity tests methods, carcinogens toxicity, cell transformation, neoplastic drug effects, DNA damage, drug effects, pathology, developmental gene expression regulation drug effects and neoplastic drug effects, mutagenicity tests methods, mutagens toxicity, neoplasms, experimental drug therapy and experimental embryology, reproducibility of results.
Notes: A review.

Kasuga, A., J.H. Park, S.H. Seok, M.W. Baek, H.Y. Lee, D.J. Kim, and J.H. Park (2004). **An Atlas of zebrafish (*Danio rerio*) histology.** *Korean Journal of Laboratory Animal Science* 20(2): 118-127. ISSN: 1225 813X.
Descriptors: zebrafish, histology, tissue, microscopy, an atlas of anatomy and structure.
Language of Text: Korean.
Notes:
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Keller, E.T. and J.M. Murtha (2004). **The use of mature zebrafish (*Danio rerio*) as a model for human aging and disease.** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 138(3): 335-41. ISSN: 1532 0456.
NAL Call Number: QP901.C6
Abstract: Zebrafish *(Danio rerio*) have been extensively utilized for understanding mechanisms of development. These studies have led to a wealth of resources including genetic tools, informational databases, and husbandry methods. In spite of all these resources, zebrafish have been underutilized for exploring pathophysiology of disease and the aging process. Zebrafish offer several advantages over mammalian models for these studies, including the ability to perform saturation mutagenesis and the capability to contain thousands of animals in a small space. In this review, we will discuss the use of mature zebrafish as an animal model and provide specific examples to support this novel use of zebrafish. Examples include demonstrating that clinical pathology can be performed in mature zebrafish and that age-associated changes in heat shock response can be observed in zebrafish. These highlights demonstrate the utility of zebrafish as a model for disease and aging.
Descriptors: zebrafish, aging physiology, disease models for aging, fish diseases metabolism and pathology, heat shock proteins metabolism, .

Key, B. and C.A. Devine (2003). **Zebrafish as an experimental model: strategies for developmental and molecular neurobiology studies.** *Methods in Cell Science* 25(1-2): 1-6. ISSN: 1381-5741.
NAL Call Number: QH573.T52
Abstract: Zebrafish provide a rapid and effective means for assessing gene function in the vertebrate nervous system. By employing gain- and loss-of-function techniques it is possible to obtain insights into the roles of both wild-type and heterologously expressed genes. Such approaches enable rapid progression from gene discovery to gene expression and finally to gene function even when examining development of a tissue as complex as the nervous system. Exploiting the full potential of zebrafish as a bioassay for the nervous system will require, not only an understanding of the molecular and cellular basis of normal zebrafish development, but also an appreciation of comparative processes in other species. When applied to mutant animals, classic morphological approaches and contemporary molecular genetic techniques are providing a wealth of information on the development of the nervous system at the molecular, cell, system and behavioural levels. Zebrafish are now emerging as an

important tool, supporting mouse genetical approaches for understanding neural function in vertebrates.

Descriptors: zebrafish, models, genetic models, neurobiology, cytology, physiology, zebrafish anatomy, histology and physiology.

Khuchua, Z., Z. Yue, L. Batts, and A.W. Strauss (2006). **A zebrafish model of human Barth syndrome reveals the essential role of tafazzin in cardiac development and function.** *Circulation Research* 99(2): 201-8. ISSN: 0009 7330.

Abstract: Barth syndrome is an X-linked disorder characterized by cardiomyopathy, skeletal myopathy, neutropenia, organic aciduria, and growth retardation caused by mutations in tafazzin. The sequence similarity of tafazzin to acyltransferases suggests a role in mitochondrial phospholipid metabolism. To study the role of tafazzin in heart function and development, we created a knockdown zebrafish model. Zebrafish tafazzin mRNA is first evident at 7 hours post-fertilization (hpf). At 10 and 24 hpf, tafazzin mRNA is ubiquitous, with highest levels in the head. By 51 hpf, expression becomes cardiac restricted. The tafazzin knockdown created by antisense morpholino yolk injection resulted in dose-dependent lethality, severe developmental and growth retardation, marked bradycardia and pericardial effusions, and generalized edema, signs that resemble human Barth syndrome heart failure. This knockdown phenotype was rescued by concomitant injection of normal tafazzin mRNA. Abnormal cardiac development, with a linear, nonlooped heart, and hypomorphic tail and eye development proves that tafazzin is essential for overall zebrafish development, especially of the heart. The tafazzin knockdown zebrafish provides an animal model similar to Barth syndrome to analyze the severity of human mutants and to test potential treatments.

Descriptors: knockdown zebrafish model, dilated congenital cardiomyopathy and etiology, heart growth and development, transcription factors deficiency, proteins deficiency, cardiomyopathy, dilated genetics, child development, disease models, developmental gene expression regulation, heart physiology, infant, antisense RNA pharmacology, messenger RNAantagonists and inhibitors, syndrome, transcription factors genetics and physiology.

Khuchua, Z., Z. Yue, and A.W. Strauss (2004). **Zebrafish (*danio rerio*) model of barth syndrome**. *Circulation* 110(17, Suppl. S): 46. ISSN: 0009 7322.

NAL Call Number: RC681.A1 C8

Abstract: Background: Barth syndrome is an X-linked genetic disorder characterized by dilated cardiomyopathy, skeletal myopathy, cyclic neutropenia, organic aciduria and growth retardation. Human Barth syndrome is associated with mutations in the G4.5 (tafazzin) gene located on Xq28. The function of tafazzin is unknown, although the protein has a high degree of homology with acyl-transferases, suggesting a role in phospholipid metabolism. To study the role of the G4.5 gene in heart development, we employed a zebrafish *(Danio rerio* model. Methods: The zebrafish G4.5 gene was inactivated at the early stages of development by injecting the yolk sac of freshly fertilized embryos with antisense morpholino oligonucleotides (MO). Results: Microinjection of morpholino oligonucleotides resulted in severe developmental and growth retardation, marked bradycardia (heart rates: 142 +/- 10 and 38 +/- 2 beats/min for WT and MO respectively), and dysmorphic hearts (h) with large pericardial effusions and generalized edema (e), consistent with congestive heart failure. In the range of 0.6-3.0 pmol MO/embryo, the resulting phenotypes were dose-dependent. Microinjection of 3 pmoles of tafazzin MO resulted in 90 +/- 5% lethality rate vs. 41 +/- 4% for nonspecific control MO. These symptoms resemble Barth syndrome in humans. Following injection of morpholino oligonucleotides under identical experimental conditions, the phenotype was completely rescued by injecting freshly fertilized embryos with full length of G4.5 mRNA.

Our results show that the G4.5 gene is essential for normal cardiac development in zebrafish and provide a model for more detailed characterization of the biochemical phenotype. [GRAPHICS] Copyright©Thomson Reuters 2009
Descriptors: zebrafish, molecular genetics, cardiovascular system, model for Human Barth syndrome, heart disease, genetic disease, phenotype, microinjection of morpholino oligonucleotides heart rate, lethality rate.

Kishi, S. (2004). **Functional aging and gradual senescence in zebrafish.** *Annals of the New York Academy of Sciences* 1019: 521-6. ISSN: 0077 8923.
NAL Call Number: 500 N484
Abstract: Zebrafish (*Danio rerio*) has been recognized as a powerful model for genetic studies in developmental biology. Recently, the zebrafish system also has given insights into several human diseases such as neurodegenerative, hematopoietic, and cardiovascular disease, and cancer. Because aging processes affect these and various other human disorders, it is important to compare zebrafish and mammalian senescence. However, the aging process of zebrafish remains largely unexplored, and little is known about functional aging and senescence in zebrafish. In our initial studies to assess aging phenotypes in zebrafish, we have identified several potential aging biomarkers in an ongoing search for suitable ones on zebrafish aging. In aging zebrafish, we detected senescence-associated beta-galactosidase activity in skin and oxidized protein accumulation in muscle. On the other hand, we did not observe lipofuscin granules (aging pigments), which accumulate in postmitotic cells, in muscle of zebrafish with advancing age. Consistently, there were continuously proliferating myocytes that incorporated BrdU in muscle tissues of the aged fish. Moreover, we demonstrated that zebrafish have constitutively abundant telomerase activity in adult somatic tissues implicating unlimited replicative ability of cells throughout their lives. Although some stress-associated markers are upregulated and minor histological changes are observed during the aging process of zebrafish, our studies together with other evidence of remarkable reproductive and regenerative abilities suggest that zebrafish show very gradual senescence. By using those biological and biochemical aging markers already characterized in normal zebrafish, transgenic fish analyses and genetic mutant fish screens can be readily performed. These efforts will help to elucidate the role and molecular mechanisms of common or different pathways of aging among vertebrates from fish to humans and also will contribute to the discovery of potential drugs applicable to age-associated diseases in the future.
Descriptors: transgenic zebrafish aging model, cell aging, genetically modified, bromodeoxyuridine pharmacology, coloring agents pharmacology, muscles metabolism, Poecilia, reproduction, telomerase metabolism and ultrastructure.

Kishi, S., J. Uchiyama, A.M. Baughman, T. Goto, M.C. Lin, and S.B. Tsai (2003). **The zebrafish as a vertebrate model of functional aging and very gradual senescence.** *Experimental Gerontology* 38(7): 777-86. ISSN: 0531 5565.
NAL Call Number: QP86.E85
Abstract: The zebrafish (*Danio rerio*) has been developed as a powerful model for genetic studies in developmental biology, which also gives insights into several diseases of adult humans such as cardiovascular disease and cancer. Because aging processes affect these and many other human diseases, it is important to compare zebrafish and other mammalian aging. However, the aging process of zebrafish remains largely unexplored, and little is known about its functional aging and senescence. In a survey of aging in zebrafish, we detected senescence-associated beta-galactosidase activity in skin and oxidized protein accumulation in muscle. However, we did not observe lipofuscin granules ('aging pigments'), which com-

monly accumulate in postmitotic cells of other vertebrates. This absence of lipofuscins may be consistent with the existence of continuously proliferating myocytes that incorporated BrdU in muscle tissues of aged zebrafish. Moreover, we demonstrated that zebrafish have constitutively abundant telomerase activity in somatic tissues from embryos to aged adults. Although some stress-associated markers are upregulated and minor histological changes are observed during the aging process of zebrafish, our studies together with other evidence of remarkable reproductive and regenerative abilities suggest that zebrafish show very gradual or sub-negligible senescence in vivo.

Descriptors: zebrafish aging model, aging physiology , biological markers analysis, bromodeoxyuridine metabolism, cell division, lipofuscin analysis, muscles cytology, muscles metabolism, oxidation reduction, Poecilia physiology, skin enzymology, species specificity, telomerase metabolism, vertebrates physiology, beta galactosidase analysis.

Krock, B.L., J. Bilotta, and B.D. Perkins (2007). **Noncell-autonomous photoreceptor degeneration in a zebrafish model of choroideremia.** *Proceedings of the National Academy of Sciences of the United States of America* 104(11): 4600-5. ISSN: 0027-8424.

NAL Call Number: 500N21P

Abstract: Choroideremia is an X-linked hereditary retinal degeneration resulting from mutations in the Rab escort protein-1 (REP1). The Rep1 protein facilitates posttranslational modification of Rab proteins, which regulate intracellular trafficking in the retinal pigment epithelium (RPE) and photoreceptors and are likely involved in the removal of outer segment disk membranes by the RPE. A critical question for potential treatment of choroideremia is whether photoreceptor degeneration results from autonomous defects in opsin transport within the photoreceptor or as a nonautonomous and secondary consequence of RPE degeneration. To address this question, we have characterized the retinal pathology in zebrafish rep1 mutants, which carry a recessive nonsense mutation in the REP1 gene. Zebrafish rep1 mutants exhibit degeneration of the RPE and photoreceptors and complete loss of visual function as measured by electroretinograms. In the mutant RPE, photoreceptor outer segment material was not effectively eliminated, and large vacuoles were observed. However, opsin trafficking in photoreceptors occurred normally. Mosaic analysis revealed that photoreceptor degeneration was nonautonomous and required contact with the mutant RPE as mutant photoreceptors were rescued in wild-type hosts and wild-type photoreceptors degenerated in mutant hosts. We conclude that mutations in REP1 disrupt cellular processes in the RPE, which causes photoreceptor death as a secondary consequence. These results suggest that therapies that correct the RPE may successfully rescue photoreceptor loss in choroideremia.

Descriptors: zebrafish REP1 mutation, choroideremia genetics, vertebrate photoreceptors pathology, alleles, choroideremia pathology, disease models, electroretinography, electron transmission microscopy, mosaicism, opsin metabolism, pigment epithelium of eye metabolism, retinal degeneration genetics, time factors.

Kunkel, L.M., E. Bachrach, R.R. Bennett, J. Guyon, and L. Steffen (2006). **Diagnosis and cell-based therapy for Duchenne muscular dystrophy in humans, mice, and zebrafish.** *Journal of Human Genetics* 51(5): 397-406. ISSN: 1434-5161.

Abstract: The muscular dystrophies are a heterogeneous group of genetically caused muscle degenerative disorders. The Kunkel laboratory has had a longstanding research program into the pathogenesis and treatment of these diseases. Starting with our identification of dystrophin as the defective protein in Duchenne muscular dystrophy (DMD), we have continued our work on normal dystrophin function and how it is altered in muscular dystrophy. Our

work has led to the identification of the defective genes in three forms of limb girdle muscular dystrophy (LGMD) and a better understanding of how muscle degenerates in many of the different dystrophies. The identification of mutations causing human forms of dystrophy has lead to improved diagnosis for patients with the disease. We are continuing to improve the molecular diagnosis of the dystrophies and have developed a high-throughput sequencing approach for the low-cost rapid diagnosis of all known forms of dystrophy. In addition, we are continuing to work on therapies using available animal models. Currently, there are a number of mouse models of the human dystrophies, the most notable being the mdx mouse with dystrophin deficiency. These mice are being used to test possible therapies, including stem-cell-based approaches. We have been able to systemically deliver human dystrophin to these mice via the arterial circulation and convert 8% of dystrophin-deficient fibers to fibers expressing human dystrophin. We are now expanding our research to identify new forms of LGMD by analyzing zebrafish models of muscular dystrophy. Currently, we have 14 different zebrafish mutants exhibiting various phenotypes of muscular dystrophy, including muscle weakness and inactivity. One of these mutants carries a stop codon mutation in dystrophin, and we have recently identified another carrying a mutation in titin. We are currently positionally cloning the disease-causative mutation in the remaining 12 mutant strains. We hope that one of these new mutant strains of fish will have a mutation in a gene not previously implicated in human muscular dystrophy. This gene would become a candidate gene to be analyzed in patients which do not carry a mutation in any of the known dystrophy-associated genes. By studying both disease pathology and investigating potential therapies, we hope to make a positive difference in the lives of people living with muscular dystrophy.
Descriptors: zebrafish, cell transplantation methods, Duchenne muscular dystrophy diagnosis, therapy and pathology, disease models, mice, .

Kuo, P.D. and C. Eliasmith (2005). **Integrating behavioral and neural data in a model of zebrafish network interaction.** *Biological Cybernetics* 93(3): 178-87. ISSN: 0340 1200.
Abstract: The spinal neural networks of larval zebrafish *(Danio rerio*) generate a variety of movements such as escape, struggling, and swimming. Various mechanisms at the neural and network levels have been proposed to account for switches between these behaviors. However, there are currently no detailed demonstrations of such mechanisms. This makes determining which mechanisms are plausible extremely difficult. In this paper, we propose a detailed biologically plausible model of the interactions between the swimming and escape networks in the larval zebrafish, while taking into account anatomical and physiological evidence. We show that the results of our neural model generate the expected behavior when used to control a hydrodynamic model of carangiform locomotion. As a result, the model presented here is a clear demonstration of a plausible mechanism by which these distinct behaviors can be controlled. Interestingly, the networks are anatomically overlapping, despite clear differences in behavioral function and physiology.
Descriptors: zebrafish larva, neurological models, nerve net physiology, swimming physiology, larva physiology, models for swimming and escape, neural pathways physiology, neurons physiology.

Kwan, T.T.F., C.L. Cheung, R. Liang, and A.Y.H. Leung (2005). **Function of survivin during embryonic angiogenesis and hematopoiesis in a zebrafish model**. *Biomedicine and Pharmacotherapy* 59(Suppl. 2): S348-S349. ISSN: 0753 3322.
NAL Call Number: R41.B52
Descriptors: zebrafish embryos, development, molecular genetics, microangiography, imaging and microscopy techniques, hematopoiesis, embryonic angiogenesis, polypoidy.

Lage, C.R., A. Nayak, and C.H. Kim (2006). **Arsenic ecotoxicology and innate immunity**. *Integrative and Comparative Biology* 46(6): 1040-1054. ISSN: 1540 7063.
NAL Call Number: QL1.I67
Abstract: Understanding the ecotoxicological effects of arsenic in the environment is paramount to mitigating its deleterious effects on ecological and human health, particularly on the immune response. Toxicological and long-term health effects of arsenic exposure have been well studied. Its specific effects on immune function, however, are less well understood. Eukaryotic immune function often includes both general (innate) as well as specific (adaptive) responses to pathogens. Innate immunity is thought to be the primary defence during early embryonic development, subsequently potentiating adaptive immunity in jawed vertebrates, whereas all other eukaryotes must rely solely on the innate immune response throughout their life cycle. Here, we review the known ecotoxicological effects of arsenic on general health, including immune function, and propose the adoption of zebrafish as a vertebrate model for studying such effects on innate immunity. Copyright © 2008 CABI
Descriptors: zebrafish model for arsenic toxicity, cell mediated immunity, embryonic development, immune response, *Danio rerio*.
Notes: A review.

Langenau, D.M. and L.I. Zon (2005). **The zebrafish: a new model of T-cell and thymic development.** *Nature Reviews. Immunology* 5(4): 307-17. ISSN: 1474-1733.
NAL Call Number: QR180
Abstract: T-cell and thymic development are processes that have been highly conserved throughout vertebrate evolution. Mammals, birds, reptiles and fish share common molecular signalling pathways that regulate the development of the adaptive immune system. This Review article focuses on defining the similarities and differences between zebrafish and mammalian T-cell immunobiology, and it highlights the advantages of using the zebrafish as a genetic model to uncover mutations that affect T-cell and thymic development. Finally, we summarize the use of the zebrafish as a new model for assessing stem-cell function and for drug discovery.
Descriptors: zebrafishgenetic models, T lymphocytes immunology, thymus gland embryology, cytology and immunology, zebrafish immunology, genetically modified, cell differentiation immunology, reporter genes, mutation, T lymphocytes cytology.
Notes: A review.

Langheinrich, U. (2003). **Zebrafish: a new model on the pharmaceutical catwalk.** *BioEssays* 25(9): 904-12. ISSN: 0265 9247.
NAL Call Number: QH506.B365
Descriptors: zebrafish, preclinical drug evaluation methods, apoptosis, drug industry, heart physiology, hemostasis, inflammation, lipid metabolism, drug evaluation models, pathologic neovascularization.
Notes: A review.

Law, J.M. (2003). **Issues related to the use of fish models in toxicologic pathology: session introduction**. *Toxicologic Pathology* 31(1): 49-52. ISSN: 0192-6233.
Descriptors: zebrafish and others as research models, conferences, freshwater fish, literature reviews, marine fish, models, pathology, reviews, toxicology, *Danio rerio*, *Oncorhynchus mykiss*, *Oryzias laticeps*, pisces, Japanese killifish, Japanese medaka, rainbow trout.
Notes: A review.

Liang, J.O. and A.L. Rubinstein (2003). **Patterning of the zebrafish embryo by nodal signals.** *Current Topics in Developmental Biology* 55: 143-71. ISSN: 0070 2153.
NAL Call Number: QL951.C8
Descriptors: zebrafish embryos, body patterning, signal transduction, endoderm metabolism, developmental gene expression regulation, mesoderm metabolism, mutation, nervous system embryology, transcription factors metabolism, transforming growth factor beta genetics and metabolism.

Liu, T.X., N.G. Howlett, M. Deng, D.M. Langenau, K. Hsu, J. Rhodes, J.P. Kanki, A.D. D'Andrea, and A.T. Look (2003). **Knockdown of zebrafish Fancd2 causes developmental abnormalities via p53-dependent apoptosis.** *Developmental Cell* 5(6): 903-14. ISSN: 1534 5807.
NAL Call Number: QH573.C42
Abstract: Mechanisms underlying the multiple developmental defects observed in Fanconi anemia (FA) patients are not well defined. We have identified the zebrafish homolog of human FANCD2, which encodes a nuclear effector protein that is monoubiquitinated in response to DNA damage, targeting it to nuclear foci where it preserves chromosomal integrity. Fancd2-deficient zebrafish embryos develop defects similar to those found in children with FA, including shortened body length, microcephaly, and microophthalmia, which are due to extensive cellular apoptosis. Developmental defects and increased apoptosis in Fancd2-deficient zebrafish were corrected by injection of human FANCD2 or zebrafish bcl2 mRNA, or by knockdown of p53, indicating that in the absence of Fancd2, developing tissues spontaneously undergo p53-dependent apoptosis. Thus, Fancd2 is essential during embryogenesis to prevent inappropriate apoptosis in neural cells and other tissues undergoing high levels of proliferative expansion, implicating this mechanism in the congenital abnormalities observed in human infants with FA.
Descriptors: zebrafish , multiple abnormalities genetics, apoptosis physiology, Fanconi anemia genetics, nuclear tumor suppressor protein p53 genetics, amino acid sequence, cross linking reagents pharmacology, epoxy compounds pharmacology, Fanconi anemia complementation group D2 protein, developmental gene expression regulation, molecular sequence data, antisense oligonucleotides pharmacology, proto oncogene proteins C Bcl 2 genetics, messenger RNA pharmacology, up regulation genetics, animal disease model.

Lorent, K., S.Y. Yeo, T. Oda, S. Chandrasekharappa, A. Chitnis, R.P. Matthews, and M. Pack (2004). **Inhibition of Jagged-mediated Notch signaling disrupts zebrafish biliary development and generates multi-organ defects compatible with an Alagille syndrome phenocopy.** *Development* 131(22): 5753-66. ISSN: 0950 1991.
NAL Call Number: QL951.D38
Abstract: The Alagille Syndrome (AGS) is a heritable disorder affecting the liver and other organs. Causative dominant mutations in human Jagged 1 have been identified in most AGS patients. Related organ defects occur in mice that carry jagged 1 and notch 2 mutations. Multiple jagged and notch genes are expressed in the developing zebrafish liver. Compound jagged and notch gene knockdowns alter zebrafish biliary, kidney, pancreatic, cardiac and craniofacial development in a manner compatible with an AGS phenocopy. These data confirm an evolutionarily conserved role for Notch signaling in vertebrate liver development, and support the zebrafish as a model system for diseases of the human biliary system.
Descriptors: zebrafish embryos, Alagille syndrome metabolism, biliary tract embryology and metabolism, calcium binding proteins metabolism, membrane proteins metabolism, signal transduction, proteins metabolism, Alagille syndrome embryology and pathology, genetically modified, bile ducts embryology and metabolism, calcium binding proteins antagonists,

inhibitors and genetics, intercellular signaling peptides and proteins , ligands, liver anatomy, histology, cytology, embryology and metabolism, mammals anatomy and histology, electron transmission microscopy, Notch receptors, zebrafish abnormalities and metabolism, proteins antagonists, inhibitors and genetics.

Love, D.R., C.C. Lan, A. Dodd, A.N. Shelling, W.C. McNabb, and L.R. Ferguson (2007). **Modeling inflammatory bowel disease: the zebrafish as a way forward.** *Expert Review of Molecular Diagnostics* 7(2): 177-93. ISSN: 1473 7159.

Abstract: The zebrafish has proved to be an informative model of vertebrate development and, more recently, an emerging model of human disease. The realization of the full potential of the zebrafish as a disease model lies in two interdependent areas. The first is an appreciation that the often overlooked strength of this species lies in allowing the design of experiments that address the interplay of genetics and the environment in a manipulable manner. The second is in the application and further development of gene targeting approaches. These twin features will be addressed in this review in the context of modeling inflammatory bowel disease.

Descriptors: zebrafish vertebrate development, disease models, inflammatory bowel diseases genetics and metabolism, gene targeting.

Love, D.R., F.B. Pichler, A. Dodd, B.R. Copp, and D.R. Greenwood (2004). **Technology for high-throughput screens: the present and future using zebrafish.** *Current Opinion in Biotechnology* 15(6): 564-71. ISSN: 0958 1669.

NAL Call Number: TP248.13.C87

Abstract: The zebrafish is a popular vertebrate model organism with similar organ systems and gene sequences to humans. Zebrafish embryos are optically transparent enabling organ visualisation, which can be complemented with gene expression analysis at the transcript and protein levels. Furthermore, zebrafish can be treated with small molecules and drugs in a microtitre plate format for high-throughput analysis and for the identification and validation of drugs. High-throughput methodologies for use in zebrafish include phenotype-based visualisation, transcript studies using low-density DNA microarrays and proteomic analysis. These technologies offer significant whole-organism biological value in the drug discovery and drug development pipeline.

Descriptors: zebrafish embryos, oligonucleotide array sequence analysis, proteomics, phenotype, PCR, .

Lumsden, A.L., T.L. Henshall, S. Dayan, M.T. Lardelli, and R.I. Richards (2007). **Huntingtin-deficient zebrafish exhibit defects in iron utilization and development.** *Human Molecular Genetics* 16(16): 1905-20. ISSN: 0964-6906.

NAL Call Number: RB155.5.H87

Abstract: Huntington's disease (HD) is one of nine neurodegenerative disorders caused by expansion of CAG repeats encoding polyglutamine in their respective, otherwise apparently unrelated proteins. Despite these proteins having widespread and overlapping expression patterns in the brain, a specific and unique subset of neurons exhibits particular vulnerability in each disease. It has been hypothesized that perturbation of normal protein function contributes to the specificity of neuronal vulnerability; however, the normal biological functions of many of these proteins including the HD gene product, Huntingtin (Htt), are unclear. To explore the roles of Htt, we have used antisense morpholino oligonucleotides to observe the effects of Htt deficiency in early zebrafish development. Knockdown of Htt expression resulted in a variety of developmental defects. Most notably, Htt-deficient zebrafish had

hypochromic blood due to decreased hemoglobin production, despite the presence of iron within blood cells. Furthermore, transferrin receptor 1 transcripts were increased, suggesting cellular iron starvation. Provision of iron to the cytoplasm in a bio-available form restored hemoglobin production in Htt-deficient embryos. Since erythroid cells acquire iron via receptor-mediated endocytosis of transferrin, these results suggest a role for Htt in making endocytosed iron accessible for cellular utilization. Iron is required for oxidative energy production, and defects in iron homeostasis and energy metabolism are features of HD pathogenesis that are most pronounced in the major region of neurodegeneration. It is therefore plausible that perturbation of Htt's normal role in the iron pathway (by polyglutamine tract expansion) contributes to HD pathology, and particularly to its neuronal specificity.
Descriptors: zebrafish embryology, iron metabolism, developmental gene expression regulation, dominant genes, hemoglobins biosynthesis, Huntington disease genetics and metabolism, phenotype, messenger RNA metabolism, transferrin receptor genetics, proteins deficiency and genetics.

Maaswinkel, H., J.Q. Ren, and L. Li (2003). **Slow-progressing photoreceptor cell degeneration in night blindness c mutant zebrafish.** *Journal of Neurocytology* 32(9): 1107-16. ISSN: 0030-4864.
Abstract: We describe here the morphological and functional alterations of the retina of mutant zebrafish, night blindness c (nbc). The nbc mutant was isolated from the F1 generation of N -ethyl- N -nitrosourea mutagenized founders. Visual sensitivity of wildtype and heterozygous (nbc (+/-)) mutant fish was determined using a behavioral assay based on visually mediated escape responses. Histology, immunocytochemistry, and electroretinography were used to study structural and functional changes of the outer retina. The behavioral visual response of nbc (+/-) mutants started to deteriorate at 12 months of age. Considerable variations existed between the extents of retinal degeneration of individual fish. In the most severe cases, both rod and cone outer segments were degenerated. In moderate cases, only rod outer segments were affected. Yet in other cases, no degeneration was detected. The retina of homozygous (nbc (-/-)) larvae had a normal appearance. However, they develop abnormally and died before 9 days post fertilization. In conclusion, nbc causes late-onset and progressive dominant retinal degeneration of both rod and cone photoreceptor cells. However, nbc is not a retina-specific gene, as the homozygous fish displayed extra-retinal defects.
Descriptors: zebrafish mutants, night blindness pathology, photoreceptors pathology, retina pathology, retinal degeneration pathology, aging genetics and pathology, cell division genetics, retina cones pathology and ultrastructure, contrast sensitivity genetics, disease models and progression, dominant genes, lethal heterozygote, homozygote, larva genetics, growth, development and metabolism, electron microscopy, mutation genetics, night blindness genetics and physiopathology, penetrance, photoreceptors ultrastructure, retina metabolism, retinal degeneration genetics and physiopathology , retinal rods pathology and ultrastructure, zebrafish abnormalities.

Maaswinkel, H., L.E. Riesbeck, M.E. Riley, A.L. Carr, J.P. Mullin, A.T. Nakamoto, and L. Li (2005). **Behavioral screening for nightblindness mutants in zebrafish reveals three new loci that cause dominant photoreceptor cell degeneration.** *Mechanisms of Ageing and Development* 126(10): 1079-89. ISSN: 0047-6374.
NAL Call Number: QP84.M4
Abstract: Here we report three dominant nightblindness mutations in zebrafish: nightblindness e (nbe), nightblindness f (nbf) and nightblindness g (nbg). The mutants were isolated in the F1 generation of N-ethyl-N-nitrosourea (ENU) mutagenized zebrafish using a behavioral

assay based on visually mediated escape responses. Subsequently, electroretinographic (ERG) recordings were made, and histological sections were screened for degenerative processes. For each mutant line, correlation analysis between behavioral, ERG and histological parameters was performed, and their relationships were determined by either calculating the Pearson correlation coefficient or by ANOVA. nbe is characterized by severe rod outer segments (ROS) degeneration. The degeneration correlates weakly with behavioral threshold and ERG b-wave amplitude, however, behavioral threshold correlates strongly with ERG b-wave. nbf is characterized by a dual histological pathology: patchy ROS-degeneration and 'gaps' homogeneously distributed over the outer nuclei layer (ONL) and between cone outer segments (COS). The correlations between histological pathology and behavioral threshold, and between behavioral threshold and ERG b-wave amplitude are obvious, but the correlation between histology and b-wave amplitude is less prominent. nbg is characterized by moderate ROS degeneration and moderate correlation between histology and behavioral threshold. Interestingly, behavioral threshold correlated inversely with ERG b-wave amplitude and threshold. Thus, contrary to what is normally seen in other nightblindness mutants, in nbg, the fish with the lowest behavioral threshold had the smallest b-waves amplitudes and the highest b-wave threshold. In our interpretation, the major impairment in nbe is photoreceptor-specific. In nbf, both photoreceptor degeneration and altered post-photoreceptor signaling are responsible for the behavioral deficit. In nbg, we find hypersensitivity at a post-photoreceptoral level concurrently with behavioral impairment.

Descriptors: zebrafish nightblindness mutants, behavior, night blindness genetics, photoreceptors metabolism, quantitative trait loci genetics, neurodegenerative diseases genetics, metabolism and pathology, night blindness metabolism and pathology, photoreceptors pathology.

Madsen, E. and J.D. Gitlin (2007). **Copper deficiency**. *Current Opinion in Gastroenterology* 23(2): 187-192 . ISSN: 0267 1379.

Abstract: Purpose of review: Reports of the neurologic findings in adults with acquired copper deficiency as well as the development of novel models for Menkes disease have permitted a greater understanding of the role of copper in the central nervous system. A role of mitochondrial copper homeostasis in cellular energy metabolism suggests roles for this metal in cellular differentiation and biochemical adaptation. Recent findings: Acquired copper deficiency in adults is reported with increasing frequency, often without any identifiable cause. Chemical genetic studies identified a zebrafish model of Menkes disease that can be used for high-throughput therapeutics and revealed a hierarchy of copper distribution during development. Studies in mice reveal that the copper transport protein Ctr1 is essential for intestinal copper absorption and suggest a unique role for copper in axonal extension, excitotoxic cell death and synaptic plasticity in the central nervous system. Lastly, recent biochemical studies indicate a central role for the mitochondrial matrix in cellular copper metabolism. Summary: The recent developments in our understanding of copper deficiency and copper homeostasis outlined in this review provide an exciting platform for future investigations intended to elucidate the role of copper in central nervous system development and disease. Copyright © 2008 CABI

Descriptors: zebrafish Menkes disease model, animal models, biochemistry, central nervous system, copper, deficiency, energy metabolism, copper homeostasis, intestines, mineral deficiencies, mitochondria, nervous system, research, reviews, therapy, *Danio rerio*, mice.

Notes: A review.

Madsen, E.C., P.A. Morcos, B.A. Mendelsohn, and J. Gitlin (2008). **In vivo correction of a Menkes disease model using antisense oligonucleotides**. *Proceedings of the National Academy of Sciences, USA* 105(10): 3909-3914. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish Menkes disease model, fatal, neurodegenerative disorder, ATP7A gene, copper-transport, p-type ATPase, embryonic-recessive, mutants.

Mari Beffa, M., J.A. Santamaria, C. Murciano, L. Santos Ruiz, J.A. Andrades, E. Guerado, and J. Becerra (2007). **Zebrafish fins as a model system for skeletal human studies.** *The Scientific World Journal* 7: 1114-27. ISSN: 1537-744X.
Abstract: Recent studies on the morphogenesis of the fins of *Danio rerio* (zebrafish) during development and regeneration suggest that a number of inductive signals involved in the process are similar to some of those that affect bone and cartilage differentiation in mammals and humans. Akimenko et al. (2002) has shown that bone morphogenetic protein-2b (BMP2b) is involved in the induction of dermal bone differentiation during fin regeneration. Many other groups have also shown that molecules from the transforming growth factor-beta superfamily (TGFb), including BMP2, are effective in promoting chondrogenesis and osteogenesis in vivo in higher vertebrates, including humans. In the present study, we review the state of the art of this topic by a comparative analysis of skeletal tissue development, regeneration and renewal processes in tetrapods, and fin regeneration in fishes. A general conclusion of this study states that lepidotrichia is a special skeletal tissue different to cartilage, bone, enamel, or dentine in fishes, according to its extracellular matrix (ECM) composition. However, the empirical analysis of inducing signals of skeletal tissues in fishes and tetrapods suggests that lepidotrichia is different to any responding features with main skeletal tissues. A number of new inductive molecules are arising from fin development and regeneration studies that might establish an empirical basis for further molecular approaches to mammal skeletal tissues differentiation. Despite the tissue dissimilarity, this empirical evidence might finally lead to clinical applications to skeletal disorders in humans.
Descriptors: zebrafish fins, bone and bones embryology and physiology, cartilage embryology and physiology, morphogenesis physiology, osteogenesis physiology, human skeletal system model.

Mathias, J.R., M.E. Dodd, K.B. Walters, J. Rhodes, J.P. Kanki, A.T. Look, and A. Huttenlocher (2007). **Live imaging of chronic inflammation caused by mutation of zebrafish Hai1.** *Journal of Cell Science* 120(Pt 19): 3372-83. ISSN: 0021-9533.
NAL Call Number: QH301.J6
Abstract: The hallmark of chronic inflammation is the infiltration and persistence of leukocytes within inflamed tissue. Here, we describe the first zebrafish chronic inflammation mutant identified in an insertional mutagenesis screen for mutants that exhibit abnormal tissue distribution of neutrophils. We identified a mutant line with an insertion in the hepatocyte growth factor activator inhibitor 1 gene (hai1; also known as Spint1) that showed accumulation of neutrophils in the fin. The mutant embryos exhibited inflammation in areas of epidermal hyperproliferation that was rescued by knock-down of the type II transmembrane serine protease Matriptase 1 (also known as St14), suggesting a novel role for Hai1-Matriptase 1 pathway in regulating inflammation. Using time-lapse microscopy of mutant embryos that express GFP from a neutrophil-specific promoter, we found that individual neutrophils in inflamed tissue displayed random motility characterized by periods of pausing alternating with periods of motility. During periods of persistent movement the cells were highly polarized, while the pausing modes were characterized by a loss of cell polarity. In

contrast to responses to acute injury, neutrophils did not exhibit clear retrograde chemotaxis or resolution of inflammation in the mutant. These findings illustrate the utility of zebrafish as a new model system to study chronic inflammation and to visualize immune responses with high resolution in vivo.

Descriptors: zebrafish mutant, chronic inflammation, Type II transmembrane serine protease Matriptase 1, St14, Sprint1, Hai1-Matriptase 1, inflammation, time-lapse, neutrophils.

Matsui, J.I., A.L. Egana, K. Koo, and J.E. Dowling (2005). **Ethanol-induced microphthalmia is mediated by changes in cell death and proliferation in the developing zebrafish retina**. *Investigative Ophthalmology & Visual Science* 46(Suppl. S): 583. ISSN: 0146 0404.

Abstract: Purpose: Microphthalmia is a component of fetal alcohol syndrome (FAS), a collection of morphological and cognitive abnormalities exhibited by children bornto mothers that consumed alcohol during their pregnancy. The mechanisms that underlie the decrease in eye size identified in FAS children remain largely unexplored. Recently, the reduction in the eye size phenotype has been recapitulated in zebra fish (Bilotta et al., Neurotoxicology Teratology, 46: 737-43). The goal of this study is to determine if the microphthalmia observed in ethanol-exposed zebra fish embryos was caused by an increase in apoptotic cell deathin the retina and/or a down regulation in cell proliferation in the ciliary marginal zone. Methods: To study the effect of ethanol on cell proliferation and death independently from the initial process of retinal cell specification and differentiation, zebra fish embryos were raised in fish water supplemented with ethanol (1%-1.75% per volume) from 2 days post-fertilization (dpf) and fixed on 3, 4, or 5 dpf. The area and diameter of eye and length of body from nose to tail were measured. TUNEL staining and antibodies to detect activated caspase-3 were used to determine if the ethanol triggered apoptosis in the retina. BrdU immunohistochemistry using a pulse/fix paradigm and transmission electron microscopy (TEM) were used to study the patterns of cell proliferation. Results: Analysis of the ratio between eye diameter and body length indicated that ethanol treatment caused a decrease in eye size that was not proportional to the concomitant reduction in body length observed. Based on TEM morphological data, we hypothesized that the ethanol activated an apoptotic-signaling pathway. A concentration dependent increase in the number of apoptotic cells was observed in the retina by TUNEL staining and activated caspase-3 immunohistochemistry. TEM and BrdU analysis of proliferation at the ciliary marginal zone indicated a decrease in cell proliferation as a consequence of ethanol treatment. Conclusions: These results indicate that the decrease in eye size observed in ethanol-treated embryos is not proportional to the decrease in body length, and thus there are ethanol-dependent mechanisms at play in the retina that underlie the microphthalmia phenotype. Studies with TUNEL and activated caspase-3immunohistochemistry show that caspase-dependent apoptosis may be one factor regulating the size. A second factor is the inhibition in cell proliferation at the ciliary marginal zone based on the TEM and BrdU studies. Copyright©Thomson Reuters 2009

Descriptors: zebrafish, fetal alcohol syndrome model, toxicology, development, sensory reception, microphthalmia, eye disease, toxicity, cell proliferation, phenotype, cell death, body length.

McGonnell, I.M. and R.C. Fowkes (2006). **Fishing for gene function--endocrine modelling in the zebrafish.** *Journal of Endocrinology* 189(3): 425-39. ISSN: 0022-0795.

Abstract: The use of zebrafish (*Danio rerio*) in scientific research is growing rapidly. It initially became popular as a model of vertebrate development because zebrafish embryos develop rapidly and are transparent. In the past 5 years, the sequencing of the zebrafish genome has increased the profile of zebrafish research even further, expanding into other

areas such as pharmacology, cancer research and drug discovery. The use of zebrafish in endocrine research has mainly been confined to the study of the development of endocrine organs. However, it is likely to be a useful model in other areas of endocrinology, as there are a wide variety of both forward and reverse genetic techniques that can be employed in the zebrafish to decipher gene function in disease states. In this review, we compare the endocrine system of the zebrafish to mouse and human, demonstrating that the systems are sufficiently similar for zebrafish to be employed as a model for endocrine research. We subsequently review the repertoire of genetic techniques commonly employed in the zebrafish model to understand gene function in vertebrate development and disease. We anticipate that the use of these techniques will make the zebrafish a prominent model in endocrine research in the coming years.
Descriptors: zebrafish model, mice, humans, endocrine glands physiology, endocrine research, genetically modified, genomics, species comparison.
Notes: A review.

McMahon, C., E.V. Semina, and B.A. Link (2004). **Using zebrafish to study the complex genetics of glaucoma.** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 138(3): 343-50. ISSN: 1532 0456.
NAL Call Number: QP901.C6
Abstract: The overall goal of this review is to highlight the power of zebrafish as a model system for studying complex diseases which involve multiple genetic loci. We are interested in identifying and characterizing genes implicated in the blinding condition of glaucoma. Glaucoma is a complex disease that often involves multiple genetic loci. Most disease causing and modifying genes for glaucoma remain unidentified. However, several genes that regulate various aspects of ocular development have been shown to associate with glaucoma. With zebrafish, forward and reverse genetic approaches can be combined in order to identify critical genetic interactions required for normal and pathological events in the development and maintenance of the eye.
Descriptors: zebrafish , ocular development model, fish diseases genetics and metabolism, glaucoma metabolism, genetics and pathology, homeodomain proteins genetics and metabolism, transcription factors.
Notes: A review.

Miller, J.D. and M.N. Neely (2004). **Zebrafish as a model host for streptococcal pathogenesis.** *Acta Tropica* 91(1): 53-68. ISSN: 0001 706X.
NAL Call Number: 475Ac8
Abstract: Streptococcal pathogens continue to evade concerted efforts to determine clear-cut virulence mechanisms, although numerous genes have been implicated in pathogenesis. A single species can infect a diversity of tissues, suggesting the expression of specific virulence factors based on the local tissue environment or stage of infection. In an effort to identify the interactions that occur between the host and pathogen that lead to activation of virulence mechanisms and contribute to specific streptococcal disease states, we have developed a unique animal model, the zebrafish (*Danio rerio*), to characterize specific virulence mechanisms utilized within various tissues in vivo. We are using this model host to study infection by two streptococcal species that represent two forms of streptococcal disease: a natural pathogen of fish and humans, *Streptococcus iniae* and a human-specific pathogen, *Streptococcus pyogenes*. *S. iniae* primarily causes a fatal systemic disease in the zebrafish following intra-muscular injection, with similar pathologies to that seen in human infections caused by *Streptococcus agalactiae* and *S. pneumoniae*. While the fatal infection by *S. pyogenes*

causes a locally spreading necrotic disease confined to the muscle with pathology similar to what is observed in a human infection of necrotizing fasciitis. By studying pathogens that are virulent for both fish and humans and that mediate disease states in the zebrafish that are identical to those found in human streptococcal infections, we will be able to identify common virulence strategies shared by a number of Gram-positive pathogens.
Descriptors: zebrafish, streptococcal disease models, infections physiopathology, streptococcus species pathogenicity, zebrafish microbiology, cell line, macrophages microbiology, mice, streptococcal infections microbiology, virulence.

Montanez, G.E., M.N. Neely, and Z. Eichenbaum (2005). **The streptococcal iron uptake (Siu) transporter is required for iron uptake and virulence in a zebrafish infection model.** *Microbiology Reading* 151(11): 3749-57. ISSN: 1350-0872.
NAL Call Number: QR1.J64
Abstract: A limited understanding of iron uptake mechanisms is available for *Streptococcus pyogenes*, a haemolytic human pathogen capable of using a variety of haemoproteins in addition to ferric and ferrous iron. This study characterizes a transporter named siu (for streptococcal iron uptake), which consists of an ATP-binding protein (SiuA), a substrate-binding protein (SiuD), and two membrane permease subunits (SiuBG). An siuG mutant was constructed and characterized. The mutant demonstrated growth reduction in comparison to the parent strain when grown in complex medium containing iron in the form of blood, haemoglobin or serum. Only a small reduction in the growth of the siuG mutant was observed in medium containing ferric iron. However, in iron uptake assays the siuG mutant showed a decrease of approximately 30 % in Fe3+ incorporation. Addition of 6 microM haem to the medium inhibited Fe3+ uptake by the wild-type by 76 %, while addition of protoporphyrin IX did not, suggesting that utilization of haem as an iron source is responsible for the inhibition of Fe3+ uptake. Inactivation of siuG moderately reduced the ability of haem to inhibit Fe3+ incorporation by the cells. Inactivation of siaB (encoding a membrane permease of a second iron transporter) had a similar outcome, and inactivation of both transporters had a cumulative effect. These observations implicate both the siu and sia transporters in haem utilization by *Strep. pyogenes*. Studies in a zebrafish infection model revealed that the siuG mutant was attenuated in both intramuscular and intraperitoneal routes of infection. Together these observations show that the siu system is an iron acquisition pathway in *Strep. pyogenes* that is important both in vitro and in vivo.
Descriptors: zebrafish infection model, bacterial proteins metabolism, gene expression regulation, bacterial, iron metabolism, streptococcal infections microbiology, *Streptococcus pyogenes* pathogenicity, bacterial culture media, membrane transport membrane transport proteins metabolism, *Streptococcus pyogenes* growth, development and metabolism, virulence, zebrafish microbiology.

Moorman, S.J. (2005). **Zebrafish as a model organism**. *Journal of Neurochemistry* 94(Suppl. 1): 123. ISSN: 0022 3042.
NAL Call Number: QP351.J6
Descriptors: zebrafish, molecular biophysics, developmental disease, pathology, genetics , genetic screening, genetic techniques, phenotype, physiology, gene function, developmental process, gene position, vertebrate biology.

Murtha, J.M., W. Qi, and E.T. Keller (2003). **Hematologic and serum biochemical values for zebrafish (*Danio rerio*).** *Comparative Medicine* 53(1): 37-41. ISSN: 1532 0820.
NAL Call Number: SF77.C65

Abstract: The zebrafish (*Danio rerio*) has proven an excellent model for study of vertebrate development and genetics. Mutagenesis studies have produced many blood mutants with defects ranging from hematopoiesis to coagulation. The overwhelming majority of zebrafish studies have focused on development and mutational effects in embryos, whereas effects in mature zebrafish have gone largely unexplored. We believe that zebrafish will prove a valuable model for study of aging and age-related diseases, and we have sought to characterize some of the basic features of mature zebrafish. Accordingly, blood was collected from adult zebrafish and was analyzed to determine reference hematologic and biochemical parameters. White blood cell differential counts indicated predominantly lymphocytes, with mean proportion of 82.95%. Total red blood cell counts averaged 3.02 x 106 cells/microliter. Except for increases in alanine transaminase (ALT), amylase, and phosphorus values, serum biochemical analytes were within the range of reported values for mammals and other species of fish. Accurate analysis of the many zebrafish mutants generated requires determination of normal characteristics of zebrafish. We believe results such as these will help define normal adult zebrafish, which have a tremendous potential for use in the study of human disease and aging.
Descriptors: zebrafish, *Danio rerio*, laboratory animals, animal models, normal values, blood chemistry, lymphocytes, monocytes, neutrophils, eosinophils, basophils, cytoplasm, serum albumin, alkaline phosphatase, alanine transaminase, enzyme activity, alpha amylase, bilirubin, urea, phosphorus, creatinine, blood glucose, potassium, globulins, inorganic ions, calcium, hematologic tests, leukocyte count, blood protein, nuclei, differential counts.

Mykytyn, K. (2003). **Study of complex inheritance of Bardet-Biedl syndrome.** *Journal of Medical Genetics* 40(Supplement 1): S18. ISSN: 0022 2593.
NAL Call Number: 442.8 J8224
Descriptors: zebrafish, molecular genetics, sense organs, sensory reception, Bardet Biedl syndrome, behavioral and mental disorders, congenital disease, endocrine disease, gonads, eye disease, nutritional disease, complex inheritance, genetic mutations.

Ninkovic, J. and L. Bally Cuif (2006). **The zebrafish as a model system for assessing the reinforcing properties of drugs of abuse.** *Methods* 39(3): 262-74. ISSN: 0882-4010.
NAL Call Number: QP519.7.M47
Abstract: Recent reports make use of the zebrafish to study complex behavior such as addiction, anxiety, or learning and memory. We have established reliable tests and appropriate controls to measure these behavioral parameters in the zebrafish adult. Our assays are robust enough to permit the detection of dominant mutations affecting drug-induced reward, and therefore can be used in forward genetic screens. We provide the reader with the technical details of these tests, as well as their appropriate and crucial, although often overlooked, control assays. In particular, our results make it possible to use the zebrafish as a promising model to identify new genetic components of the reward pathway, or other measurable behaviors.
Descriptors: zebrafish, drug abuse model, reinforcement psychology, substance related disorders genetics, addictive behavior genetics, conditioning psychology, dextroamphetamine toxicity, mutation, street drugs toxicity, toxicity tests instrumentation and methods, .

Nixon, S.J., J. Wegner, C. Ferguson, P.F. Mery, J.F. Hancock, P.D. Currie, B. Key, M. Westerfield, and R.G. Parton (2005). **Zebrafish as a model for caveolin-associated muscle disease; caveolin-3 is required for myofibril organization and muscle cell patterning.** *Human Molecular Genetics* 14(13): 1727-43. ISSN: 0964-6906.
NAL Call Number: RB155.5.H87

Abstract: Caveolae are an abundant feature of many animal cells. However, the exact function of caveolae remains unclear. We have used the zebrafish, *Danio rerio*, as a system to understand caveolae function focusing on the muscle-specific caveolar protein, caveolin-3 (Cav3). We have identified caveolin-1 (alpha and beta), caveolin-2 and Cav3 in the zebrafish. Zebrafish Cav3 has 72% identity to human CAV3, and the amino acids altered in human muscle diseases are conserved in the zebrafish protein. During embryonic development, cav3 expression is apparent by early segmentation stages in the first differentiating muscle precursors, the adaxial cells and slightly later in the notochord. cav3 expression appears in the somites during mid-segmentation stages and then later in the pectoral fins and facial muscles. Cav3 and caveolae are located along the entire sarcolemma of late stage embryonic muscle fibers, whereas beta-dystroglycan is restricted to the muscle fiber ends. Down-regulation of Cav3 expression causes gross muscle abnormalities and uncoordinated movement. Ultrastructural analysis of isolated muscle fibers reveals defects in myoblast fusion and disorganized myofibril and membrane systems. Expression of the zebrafish equivalent to a human muscular dystrophy mutant, CAV3P104L, causes severe disruption of muscle differentiation. In addition, knockdown of Cav3 resulted in a dramatic up-regulation of eng1a expression resulting in an increase in the number of muscle pioneer-like cells adjacent to the notochord. These studies provide new insights into the role of Cav3 in muscle development and demonstrate its requirement for correct intracellular organization and myoblast fusion.
Descriptors: zebrafish, Cav3 muscle development model, caveolins metabolism, skeletal muscle embryology, skeletal myoblasts metabolism and ultrastructure, myofibrils metabolism, amino acid sequence, caveolins genetics, cell differentiation genetics and physiology, cell fusion, embryology, molecular sequence data, skeletal muscle ultrastructure, myofibrils geneticsm and ultrastructure.

Novoa, B., A. Romero, V. Mulero, I. Rodriguez, I. Fernandez, and A. Figueras (2006). **Zebrafish (*Danio rerio)* as a model for the study of vaccination against viral haemorrhagic septicemia virus (VHSV)**. *Vaccine* 24(31/32): 5806-5816. ISSN: 0264 410X.
NAL Call Number: QR189.V32
Abstract: The rhabdovirus viral haemorrhagic septicemia virus (VHSV) is the etiological agent of one of the most important salmonid viral diseases. In the present work, the ability of VHSV to infect and replicate in zebrafish at low temperature (15 degrees C) was demonstrated. Zebrafish was also used to determine the effectiveness of the recombinant virus rIHNV-Gvhsv GFP as a live attenuated vaccine against the virulent VHSV strain. Fish intraperitoneally injected with 3x106 to 3x105 TCID50/ml of the wild type VHSV showed a 100% of cumulative mortality, meanwhile only 57% of mortality was obtained in bath infections. Infected fish showed external clinical signs and histological observations revealed the appearance of small haemorrhages in the muscle, kidney, liver and dermis. Neither mortalities nor clinical signs were recorded in fish infected with a live attenuated recombinant virus. By RT-PCR technique, VHSV was detected in all the organs as early as 24 h, but the recombinant virus was not detected in all the sampled days. VHSV was able to replicate "in vitro" in head kidney cells but the replication capacity of the attenuated viral strain was limited. The recombinant virus rIHNV-Gvhsv GFP was able to protect against VHSV with a survival rate ranging from 20% to 60% depending of the vaccine dose. The increase of TLR3, IFN alpha beta , Mx, IFN gamma and TNF alpha expression at 72 h post-infection in the kidney of VHSV-infected fish contrasted with the results obtained with the avirulent virus, which did not induce an increment of this expression in infected fish. Zebrafish is a suitable animal model to study VHSV infection and immune (innate and adaptive) responses and, more importantly, we demonstrate for the first time the usefulness of the zebrafish as

a vaccination model to viral diseases. In addition, the high protection obtained with the live attenuated virus demonstrates that the zebrafish is able to mount an efficient antiviral immune response at 15 degrees C. Copyright © 2008 CABI
Descriptors: zebrafish, brain, clinical aspects, experimental infection, gills, VHSV, immune response, immunization, kidneys, live vaccines, liver, muscles, vaccination model, vaccines, *Danio rerio*.

Ny, A., M. Autiero, and P. Carmeliet (2006). **Zebrafish and *Xenopus* tadpoles: small animal models to study angiogenesis and lymphangiogenesis.** *Experimental Cell Research* 312(5): 684-93. ISSN: 0014 4827.
NAL Call Number: 442.8EX7
Abstract: Small vertebrate organisms have emerged as key players in the post-genomic era for the functional characterization of novel genes on a high-throughput scale. In this context, the zebrafish embryos and *Xenopus* tadpoles represent attractive and valuable models to rapidly identify and characterize novel genes involved in angiogenesis and lymphangiogenesis-a significant task with a consequent impact on the design of more effective therapeutic strategies. The advantages of these two models will be discussed in the present review.
Descriptors: zebrafish embryology and genetics, *Xenopus*, lymphangiogenesis physiology, physiologic neovascularization physiology, Xenopus physiology, physiology, disease models, research trends, *Xenopus* embryology and genetics.
Notes: A review.

O'Brien, G.S., S. Rieger, S.M. Martin, A.M. Cavanaugh, C. Portera-Cailliau, and A. Sagasti (2009). **Two-photon axotomy and time-lapse confocal imaging in live zebrafish embryos**. *Journal of Visualized Experiments* 24
Online: http://www.jove.com/index/Details.stp?ID=1129
Descriptors: zebrafish, time-lapse imaging, embryo, confocal microscope, two-photon axotomy, axonal recovery.
Notes: Free Access article. DOI: 10.3791/1129.

Petrie-Hanson, L., C.L. Romano, R.B. Mackey, P. Khosravi, C.M. Hohn, and C.R. Boyle (2007). **Evaluation of zebrafish *Danio rerio* as a model forentericsepticemia of catfish (ESC)**. *Journal of Aquatic Animal Health* 19(3): 151-158. ISSN: 0899-7659.
NAL Call Number: SH171.J68
Descriptors: zebrafish infection model, aquatic animals, chronic infection, colony-forming cells, encephalitis, fish diseases, freshwater fish, heart, histopathology, immersion, kidney, liver, models, mortality, mortality causes, necrosis, septicemia, spleen, swimming behavior, symptoms, *Danio rerio*, *Edwardsiella ictaluri*, *Ictalurus punctatus*, channel catfish.

Phelps, H.A., D.L. Runft, and M.N. Neely (2009). **Adult zebrafish model of Streptococcal infection**. *Current Protocols in Microbiology* 13: 9D.1.1-9D.1.27.
Descriptors: zebrafish, animal model, streptococcal infection, necrotizing fasciitis, *Streptococcus pyogenes*, *Streptococcus iniae*, histology.
Notes: DOI: 10.1002/9780471729259.mc09d01s13.

Poss, K.D. (2004). **A zebrafish model of germ cell aneuploidy.** *Cell Cycle* 3(10): 1225-6. ISSN: 1538 4101.
Abstract: The high frequency of chromosomal nondisjunction in human germ cells impacts society in many ways. Yet, the etiology of chromosome disorders remains unclear. Using

a zebrafish strain with a hypomorphic mutation in the kinase Mps1, a genetic association between reduced germ cell mitotic checkpoint activity and aneuploid progeny was recently established. This work highlights the exquisite sensitivity of vertebrate germ cells to disruptions in Mps1 function and mitotic checkpoint activity. In addition, it introduces the zebrafish as a promising tool with which to further investigate the origins of aneuploidy.
Descriptors: zebrafish, mutant kinase, aneuploidy, germ cells metabolism and pathology, models.

Pozos, T.C. and L. Ramakrishan (2004). **New models for the study of *Mycobacterium*-host interactions**. *Current Opinion in Immunology* 16(4): 499-505. ISSN: 0952 7915.
NAL Call Number: QR180.C87
Descriptors: zebrafish, immune system, chemical coordination, *Mycobacterium* infection, infectious disease, tuberculosis, bacterial disease, genetic screen, real time imaging, clinical techniques, diagnostic techniques, imaging and microscopy techniques, complex interaction.

Pyati, U.J., A.T. Look, and M. Hammerschmidt (2007). **Zebrafish as a powerful vertebrate model system for in vivo studies of cell death.** *Seminars in Cancer Biology* 17(2): 154-65. ISSN: 1044-579X.
NAL Call Number: RC261.A1
Abstract: Understanding and manipulating cell death pathways are critical to our ability to treat human degenerative diseases and cancer. The zebrafish *Danio rerio*, a common aquatic pet, has evolved as a powerful tool for the discovery of genes regulating cellular suicide both during normal vertebrate development and after genetic or environmental insult. In this review, we describe the techniques that can be applied to studying cell death in zebrafish as well as highlighting what has been discovered so far. Finally, we discuss future perspectives in the field and how they relate to human disease.
Descriptors: zebrafish embryology, cell death physiology, models , metabolism, gene expression regulation, neoplastic genetics.

Qian, L., Y. Wang, Q. Jiang, T. Zhong, and H. Song (2005). **Ethanol disrupts the formation of hypochord and dorsal aorta during the development of embryonic zebrafish.** *Science in China. Series C, Life Sciences* 48(6): 608-15. ISSN: 1006-9305.
Abstract: Exposure to ethanol during human embryonic period has severe teratogenic effects on the cardiovascular system. In our study, we demonstrated that ethanol of gradient concentrations can interfere with the establishment of circulatory system in embryonic zebrafish. The effective concentration to cause 50% malformations (EC50) was 182.5 mmol/L. The ethanol pulse exposure experiment displayed that dome stage during embryogenesis is the sensitive time window to ethanol. It is found that 400 mmol/L ethanol pulse exposure can induce circulatory defects in 43% treated embryos. We ruled out the possibility that ethanol can interfere with the process of hematopoiesis in zebrafish. By employing in situ hybridization with endothelial biomarker (Flk-1), we revealed that ethanol disrupts the establishment of trunk axial vasculature, but has no effect on cranial vessels. Combined with the results of semi-thin histological sections, the in situ hybridization experiments with arterial and venous biomarkers (ephrinB2, ephB4) suggested that ethanol mainly interrupts the development of dorsal aorta while has little effect on axial vein. Further study indicated the negative influence of ethanol on the development of hypochord in zebrafish. The consequent lack of vasculogenic factors including Radar and Ang-1 partly explains the defects in formation and integrity of dorsal aorta. These results provide important clues to the study of adverse effects of ethanol on the cardiovascular development in human fetus.

Descriptors: zebrafish embryos, aorta drug effects, cardiovascular system drug effects, ethanol toxicity, teratogens toxicity, aorta embryology, blood circulation drug effects and physiology, cardiovascular system embryology, drug dose response relationship, embryo blood supply and drug effects, GATA1 transcription factor analysis and metabolism, hematopoiesis drug effects, in situ hybridization methods, models, time factors, vascular endothelial growth factor receptor 2 analysis and metabolism, veins embryology, proteins analysis and metabolism.

Quarto, N. and M.T. Longaker (2005). **The zebrafish (*Danio rerio*): a model system for cranial suture patterning.** *Cells, Tissues, Organs* 181(2): 109-18. ISSN: 1422 6405.
Descriptors: zebrafish, body patterning, cranial sutures growth and development, cranial suture patterning model, osteogenesis, cranial sutures anatomy and histology.

Ramsay, J.M., G.W. Feist, Z.M. Varga, M. Westerfield, M.L. Kent, and C.B. Schreck (2006). **Whole-body cortisol is an indicator of crowding stress in adult zebrafish, *Danio rerio*.** *Aquaculture* 258(1/4): 565-574. ISSN: 0044 8486.
NAL Call Number: SH1.A6
Abstract: Plasma cortisol levels have been used to evaluate the stress response in a variety of cultured fish species. However, little is known about the stress response of zebrafish, *Danio rerio*, despite its extensive use as a laboratory research organism. Due to its small size, evaluation of whole-body cortisol has provided a means to assess zebrafish stress levels Understanding the role of crowding on zebrafish whole-body cortisol would allow researchers to optimize various rearing procedures. The aim of this study was to determine the zebrafish whole-body cortisol response to crowding stress. Baseline cortisol values have not been established for zebrafish; therefore, we compared specific treatment groups acclimated to similar conditions. We crowded zebrafish at a high density (40 fish/L) for 3 h (acute stress), or 5 days (chronic stress). Crowding resulted in a four-fold increase in mean whole-body cortisol level in both groups compared to zebrafish maintained at a much lower density (0.25 fish/L). Additional experiments demonstrated that the cortisol response to crowding was modulated by fasting, feeding and density. In large glass aquaria (76 L), fasted, crowded fish (40 fish/L) had significantly higher cortisol compared to fasted, control fish (0.2 fish/L). Furthermore, weight was inversely related to cortisol level in fasted, crowded fish held in large glass aquaria. For fed fish, crowding did not significantly increase cortisol level, suggesting an interaction between feeding and crowding. In small tanks (4 L), crowding (40 fish/L) did not significantly increase cortisol compared to control fish held at 4 fish/L. Our results suggest that whole-body cortisol is a useful indicator of crowding stress in fasted, adult zebrafish. Understanding how crowding and other environmental conditions affect zebrafish fitness could aid in optimizing zebrafish growth, health and reproduction as well as improving the consistency and reproducibility of in vivo studies that use this popular vertebrate model. Copyright © 2008 CABI
Descriptors: zebrafish, animal models, crowding stress indicators, fish culture, hydrocortisone, restricted feeding, stress response, *Danio rerio.*

Renn, J., C. Winkler, M. Schartl, R. Fischer, and R. Goerlich (2006). **Zebrafish and medaka as models for bone research including implications regarding space-related issues.** *Protoplasma* 229(2-4): 209-14. ISSN: 0033-183X.
NAL Call Number: 442.8 P94
Abstract: Teleost fish develop bones directly from mesenchymal condensations and from cartilage precursors. At the cellular level, the involved cell populations share many features

with their mammalian counterparts. In addition, several genes are already described in fish showing high homology in amino acid sequence and expression with the corresponding genes of tetrapods that are involved in bone metabolism. Therefore, analysis of the underlying molecular mechanism in fish, in particular zebrafish and medaka, will increase the knowledge in teleosts. Furthermore, it will help to identify novel genes and regulatory pathways of bone homeostasis and skeletal disorders also in higher vertebrates, including disorders caused by altered gravity.

Descriptors: zebrafish, bone development genetics, fish proteins metabolism, developmental gene expression regulation, altered gravity, *Oryzias* physiology, physiological adaptation, cytokines genetics and metabolism, extracellular matrix proteins genetics and metabolism, fish gravity perception, *Oryzias* genetics and metabolism, peptide hormones genetics and metabolism, transcription factors genetics and metabolism, metabolism.

Robison, B.D. and W. Rowland (2005). **A potential model system for studying the genetics of domestication: behavioral variation among wild and domesticated strains of zebra danio (*Danio rerio*)**. *Canadian Journal of Fisheries and Aquatic Sciences* 62(9): 2046-2054. ISSN: 0706 652X.

NAL Call Number: 442.9C16J

Abstract: The process of domestication in fish is fundamentally important to conservation efforts because of the extensive use of hatcheries to mitigate population declines. Research into the genetic changes associated with the domestication process in many endangered species is impeded by a lack of genomic tools, long generation times, and large space requirements. The study of the genetics of fish domestication could therefore benefit from the introduction of a model system. In this paper, we document behavioral and growth rate differences observed between a domesticated laboratory strain of zebra danio (*Danio rerio*) and a strain newly introduced into the laboratory from its native habitat in India. Domesticated zebra danio showed a higher degree of surface orientation, a reduced startle response, and higher growth rate compared with wild zebra danio. Wild-domesticated interstrain hybrids were intermediate in phenotype for all traits. When strains were reared together, most interstrain behavioral differences were maintained, indicating a genetic basis underlying the interstrain phenotypic variation. Phenotypic differences observed in this study are consistent with the effects of domestication in other fish species, indicating that the zebra danio can be used as a model system for studying the genetics of the domestication process in fish. Copyright © 2008 CABI

Descriptors: zebrafish, behavior, genetic analysis, change and models, genomes, genotypes, growth rate, phenotypes, strains, *Danio rerio*.

Language of Text: French.

Rubinstein, A.L. (2003). **Zebrafish: from disease modeling to drug discovery.** *Current Opinion in Drug Discovery and Development* 6(2): 218-23 . ISSN: 1367 6733.

Abstract: The study of zebrafish, a leading model organism for developmental biology, is rapidly expanding to include human disease. Zebrafish models based on known disease mechanisms have been developed in several therapeutic areas, including blood diseases, diabetes, muscular dystrophy, neurodegenerative disease, angiogenesis and lipid metabolism. This review summarizes recent progress in disease model development, and outlines the potential of zebrafish to contribute to drug discovery through the identification of novel drug targets, validation of those targets and screening for new therapeutic compounds.

Descriptors: zebrafish disease models, pharmacology trends, preclinical drug evaluation, .

Notes: A review.

Rubinstein, A.L., S.Y. Ho and S.A. Farber (2005). **Zebrafish model of lipid metabolism for drug discovery**. *Unraveling Lipid Metabolism With Microarrays,* Marcel Dekker: New York, NY 10016 USA, p. 423-431. ISBN: 0824758110.
Descriptors: zebrafish, methods and techniques, lipid metabolism model, pharmaceuticals, pharmacology, molecular genetics, genetic screening, genetic techniques, lipid metabolism, drug discovery.

Salzmann, J., O. Anichtchik, J. Best, F. Richards, A. Fleming, A. Roach, and P. Goldsmith (2006). **Development of a mptp model of Parkinson's disease in zebrafish**. *Behavioural Pharmacology* 17(5-6): 541. ISSN: 0955 8810.
Descriptors: zebrafish, behavior, toxicology, nervous system, Parkinson's disease model, nervous system disease, behavioral effect.

Sar, A.M.v.d., B.J. Appelmelk, C.M.J.E. Vandenbroucke Grauls, and W. Bitter (2004). **A star with stripes: zebrafish as an infection model**. *Trends in Microbiology* 12(10): 451-457. ISSN: 0966 842X.
NAL Call Number: QR1.T74
Abstract: The zebrafish, one of the favourite animals of developmental biologists, is rapidly gaining ground in infection models. Various experimental infection models have been set up to study both human and fish pathogens in more detail. The most interesting of these pathogens is perhaps *Mycobacterium marinum*, a close relative of *M. tuberculosis*, and causes fish tuberculosis. With the zebrafish, genetic screens in a vertebrate host with a fully developed immune system are possible. In addition, bacterial infections can be analysed in real-time in zebrafish embryos. An article is presented discussing the use of the zebrafish as a host for studying infectious diseases, and also the challenges, benefits and hurdles associated with using this model. Copyright © 2008 CABI
Descriptors: zebrafish infection model, bacterial diseases, fish diseases, tuberculosis, fishes, *Mycobacterium marinum.*

Schiffer, N.W., S.A. Broadley, T. Hirschberger, P. Tavan, H.A. Kretzschmar, A. Giese, C. Haass, F.U. Hartl, and B. Schmid (2007). **Identification of anti-prion compounds as efficient inhibitors of polyglutamine protein aggregation in a zebrafish model.** *Journal of Biological Chemistry* 282(12): 9195-203. ISSN: 0021-9258.
NAL Call Number: 381J824
Abstract: Several neurodegenerative diseases, including Huntington disease (HD), are associated with aberrant folding and aggregation of polyglutamine (polyQ) expansion proteins. Here we established the zebrafish, *Danio rerio*, as a vertebrate HD model permitting the screening for chemical suppressors of polyQ aggregation and toxicity. Upon expression in zebrafish embryos, polyQ-expanded fragments of huntingtin (htt) accumulated in large SDS-insoluble inclusions, reproducing a key feature of HD pathology. Real time monitoring of inclusion formation in the living zebrafish indicated that inclusions grow by rapid incorporation of soluble htt species. Expression of mutant htt increased the frequency of embryos with abnormal morphology and the occurrence of apoptosis. Strikingly, apoptotic cells were largely devoid of visible aggregates, suggesting that soluble oligomeric precursors may instead be responsible for toxicity. As in nonvertebrate polyQ disease models, the molecular chaperones, Hsp40 and Hsp70, suppressed both polyQ aggregation and toxicity. Using the newly established zebrafish model, two compounds of the N'-benzylidene-benzohydrazide class directed against mammalian prion proved to be potent inhibitors of polyQ aggregation, consistent with a common structural mechanism of aggregation for prion and polyQ disease

proteins.
Descriptors: zebrafish, apoptosis, mutation, peptides chemistry, green fluorescent proteins chemistry, hsp40 heat shock proteins chemistry, HSP70 heat shock proteins chemistry, chemical models, molecular models, peptides metabolism, protein binding, tertiary protein structure, subcellular fractions, thermodynamics.

Schilling, T.F. and J. Webb (2007). **Considering the zebrafish in a comparative context.** *Journal of Experimental Zoology. Part B. Molecular and Developmental Evolution* 308(5): 515-22. ISSN: 1552-5007.
NAL Call Number: QL1.J87
Descriptors: zebrafish, embryonic development, evolution models, developmental biology, physiology, molecular evolution, fishes embryology and genetics, genome, phylogeny.

Shafizadeh, E. and B.H. Paw (2004). **Zebrafish as a model of human hematologic disorders.** *Current Opinion in Hematology* 11(4): 255-61. ISSN: 1065 6251.
Abstract: This review summarizes the status of zebrafish as a genetic model to study human hematological disorders. Much of our current understanding of the function of genes modulating the process of hematopoietic stem cell generation, specification, and differentiation has come from mutant analysis. Because of the transparency of zebrafish embryos that allows for direct visualization of circulating erythroid cells, mutations affecting zebrafish erythropoiesis were among the first characterized mutants through positional cloning and candidate gene strategies. RECENT FINDINGS: New technologies have evolved that allow for generation, detection, and characterization of lineage specific alterations in the hematopoietic system. We will also briefly discuss the applications of several of these technologies such as targeted gene knockdown using antisense morpholinos, small molecule screen, transgenesis, and cell transplantation as related to blood disorders and hematopoietic development. SUMMARY: The combination of phenotype-driven forward genetic analyses and innovative technical advances has conferred zebrafish as a powerful genetic model to further dissect the function of hematopoietic genes. Through the use of available resources, the identification of novel genes or novel function for known hematopoietic genes will have important implications for our understanding of human disease pathogenesis, treatment, and prevention.
Descriptors: zebrafish, disease models, erythropoiesis genetics, hematologic diseases genetics, hematopoietic system physiology, genetically modified erythropoiesis physiology, mutation.
Notes: Review article.

Sherwood, N.M. and S. Wu (2005). **Developmental role of GnRH and PACAP in a zebrafish model.** *General and Comparative Endocrinology* 142(1-2): 74-80. ISSN: 0016-6480.
NAL Call Number: 444.8G28
Abstract: GnRH is expressed early in development long before reproduction begins. To determine whether GnRH has a role in development, gene knockdown with morpholinos was used in one-cell zebrafish embryos to block translation of gnrh mRNA into protein. Gene knockdown of gnrh2, gnrh3 or both at the one-cell stage resulted in a high percentage of embryos at 24-48 h with a defective mid-hindbrain boundary and underdeveloped eyes; a small percentage of embryos at 72 h had a defective heart. In similar studies on GHRH-PACAP, gene knockdown resulted in a smaller brain and eyes, but a normal-appearing heart. The evidence supports a role for the three neuropeptides in early development.
Descriptors: zebrafish embryos, gonadotropin releasing hormone physiology, nerve growth factors physiology, neuropeptides physiology, neurotransmitter agents physiology, genetically

modified, gonadotropin releasing hormone genetics, nerve growth factors genetics, neuropeptides genetics, neurotransmitter agents genetics, pituitary adenylate cyclase activating polypeptide, vasoactive intestinal peptide receptors.

Song, Y.S. and R.D. Cone (2007). **Creation of a genetic model of obesity in a teleost**. *Federation of American Societies for Experimental Biology Journal* 21(9): 2042-2049. ISSN: 0892 6638.
NAL Call Number: QH301.F3
Abstract: The adipostat is the mechanism by which the brain detects and maintains constant levels of energy stored in adipocytes in the form of lipids. Key elements of the adipostat include the adipocyte-derived hormone leptin that is expressed in proportion to energy levels and serves to communicate this information to the central nervous system and the central circuits, which sense and respond to leptin. Blocking one of these circuits, the central melanocortin system, disrupts leptin action and causes a distinct obesity syndrome in mice and humans, characterized by increased adiposity as well as increased linear growth. We show here that transgenic zebrafish overexpressing the endogenous melanocortin antagonist agouti-related protein (AgRP) also exhibit obesity, increased linear growth, and adipocyte hypertrophy. These findings demonstrate that key elements of the adipostat originated before the evolution of mammals. Furthermore, transgenic overexpression of AgRP in zebrafish yields a new model system for the genetic analysis of energy homeostasis in a simple vertebrate system. Copyright © 2008 CABI
Descriptors: zebrafish, adipocytes, animal model of the genetics of energy homeostasis, antagonists, body fat, gene expression, genes, homeostasis, leptin, molecular genetics , obesity, *Danio rerio.*

Spitsbergen, J. (2007). **Imaging neoplasia in zebrafish.** *Nature Methods* 4(7): 548-9. ISSN: 1548-7091.
NAL Call Number: QH315
Descriptors: zebrafish, disease models, acoustic microscopy methods, neoplasms, experimental ultrasonography, experimental neoplasms pathology.

Spitsbergen, J.M. and M.L. Kent (2003). **The state of the art of the zebrafish model for toxicology and toxicologic pathology research--advantages and current limitations.** *Toxicologic Pathology* 31(Suppl): 62-87. ISSN: 0192-6233.
Abstract: The zebrafish (*Danio rerio*) is now the pre-eminent vertebrate model system for clarification of the roles of specific genes and signaling pathways in development. The zebrafish genome will be completely sequenced within the next 1-2 years. Together with the substantial historical database regarding basic developmental biology, toxicology, and gene transfer, the rich foundation of molecular genetic and genomic data makes zebrafish a powerful model system for clarifying mechanisms in toxicity. In contrast to the highly advanced knowledge base on molecular developmental genetics in zebrafish, our database regarding infectious and noninfectious diseases and pathologic lesions in zebrafish lags far behind the information available on most other domestic mammalian and avian species, particularly rodents. Currently, minimal data are available regarding spontaneous neoplasm rates or spontaneous aging lesions in any of the commonly used wild-type or mutant lines of zebrafish. Therefore, to fully utilize the potential of zebrafish as an animal model for understanding human development, disease, and toxicology we must greatly advance our knowledge on zebrafish diseases and pathology.
Descriptors: zebrafish, environmental carcinogens toxicity, toxicology models, genetically

modified, carcinogenicity tests, pathology methods and trends, toxicology methods and trends.

Sprague, J., L. Bayraktaroglu, D. Clements, T. Conlin, D. Fashena, K. Frazer, M. Haendel, D.G. Howe, P. Mani, S. Ramachandran, K. Schaper, E. Segerdell, P.R. Song, B. Sprunger, S. Taylor, C.E.V. Slyke, and M. Westerfield (2006). **The Zebrafish Information Network: the zebrafish model organism database**. *Nucleic Acids Research* 34(Suppl. 1): D581-D585. ISSN: 0305 1048.
NAL Call Number: QD341.A2N8
Abstract: The Zebrafish Information Network (ZFIN; http://zfin.org) is a web based community resource that implements the curation of zebrafish genetic, genomic and developmental data. ZFIN provides an integrated representation of mutants, genes, genetic markers, mapping panels, publications and community resources such as meeting announcements and contact information. Recent enhancements to ZFIN include (i) comprehensive curation of gene expression data from the literature and from directly submitted data, (ii) increased support and annotation of the genome sequence, (iii) expanded use of ontologies to support curation and query forms, (iv) curation of morpholino data from the literature, and (v) increased versatility of gene pages, with new data types, links and analysis tools. Copyright © 2008 CABI
Descriptors: zebrafish , models, databases, gene expression, gene mapping, genes, genetic markers, genomes, nucleotide sequences, *Danio rerio*, Zebrafish Information Network, fishes.

Sprague, J., D. Clements, T. Conlin, P. Edwards, K. Frazer, K. Schaper, E. Segerdell, P. Song, B. Sprunger, and M. Westerfield (2003). **The Zebrafish Information Network (ZFIN): the zebrafish model organism database.** *Nucleic Acids Research* 31(1): 241-3. ISSN: 0305-1048.
NAL Call Number: QD341.A2N8
Abstract: The Zebrafish Information Network (ZFIN) is a web based community resource that serves as a centralized location for the curation and integration of zebrafish genetic, genomic and developmental data. ZFIN is publicly accessible at http://zfin.org. ZFIN provides an integrated representation of mutants, genes, genetic markers, mapping panels, publications and community contact data. Recent enhancements to ZFIN include: (i) an anatomical dictionary that provides a controlled vocabulary of anatomical terms, grouped by developmental stages, that may be used to annotate and query gene expression data; (ii) gene expression data; (iii) expanded support for genome sequence; (iv) gene annotation using the standardized vocabulary of Gene Ontology (GO) terms that can be used to elucidate relationships between gene products in zebrafish and other organisms; and (v) collaborations with other databases (NCBI, Sanger Institute and SWISS-PROT) to provide standardization and interconnections based on shared curation.
Descriptors: zebrafish, genetic databases, gene expression, genome, models, phylogeny, terminology, zebrafish anatomy, histology, growth and development, Zebrafish Information Network.

Sprague, J., L. Bayraktaroglu, Y. Bradford, T.o.m. Conlin, N. Dunn, D. Fashena, K.e.n. Frazer, M. Haendel, D.G. Howe, J. Knight, P. Mani, S.A. Moxon, C. Pich, S. Ramachandran, K. Schaper, E. Segerdell, X. Shao, A.m.y. Singer, P. Song, B. Sprunger, C.E. Van Slyke, and M. Westerfield (2008). **The Zebrafish Information Network: the zebrafish model organism database provides expanded support for genotypes and phenotypes**. *Nucleic Acids Research* 36(suppl 1): D768-D772. ISSN: 0305-1048.
NAL Call Number: QD341.A2N8

Descriptors: zebrafish, Zebrafish Information Network, ZFIN, gene and allele nomenclature, anatomical ontology, (AO), gene ontology, (GO) , phenotypic quality ontology, (PATO).

Steffen, L.S., J.R. Guyon, E.D. Vogel, R. Beltre, T.J. Pusack, Y. Zhou, L.I. Zon, and L.M. Kunkel (2007). **Zebrafish orthologs of human muscular dystrophy genes.** *BioMed Central Medical Genomics* 8: 79. ISSN: 1755 8794.

Abstract: BACKGROUND: Human muscular dystrophies are a heterogeneous group of genetic disorders which cause decreased muscle strength and often result in premature death. There is no known cure for muscular dystrophy, nor have all causative genes been identified. Recent work in the small vertebrate zebrafish *Danio rerio* suggests that mutation or misregulation of zebrafish dystrophy orthologs can also cause muscular degeneration phenotypes in fish. To aid in the identification of new causative genes, this study identifies and maps zebrafish orthologs for all known human muscular dystrophy genes. RESULTS: Zebrafish sequence databases were queried for transcripts orthologous to human dystrophy-causing genes, identifying transcripts for 28 out of 29 genes of interest. In addition, the genomic locations of all 29 genes have been found, allowing rapid candidate gene discovery during genetic mapping of zebrafish dystrophy mutants. 19 genes show conservation of syntenic relationships with humans and at least two genes appear to be duplicated in zebrafish. Significant sequence coverage on one or more BAC clone(s) was also identified for 24 of the genes to provide better local sequence information and easy updating of genomic locations as the zebrafish genome assembly continues to evolve. CONCLUSION: This resource supports zebrafish as a dystrophy model, suggesting maintenance of all known dystrophy-associated genes in the zebrafish genome. Coupled with the ability to conduct genetic screens and small molecule screens, zebrafish are thus an attractive model organism for isolating new dystrophy-causing genes/pathways and for use in high-throughput therapeutic discovery.

Descriptors: zebrafish, gene expression regulation, muscular dystrophies genetics, mutation, calpain genetics, genetic databases, disease model for orthologs of human muscular dystrophy, expressed sequence tags, gene duplication, genome, muscle phenotype, physical chromosome mapping, software.

Su, F., M.A. Juarez, C.L. Cooke, L. Lapointe, J.A. Shavit, J.S. Yamaoka, and S.E. Lyons (2007). **Differential Regulation of Primitive Myelopoiesis in the zebrafish by Spi-1/Pu.1 and C/ebp1.** *Zebrafish* 4(3): 187-99. ISSN: 1545-8547.

Abstract: The zebrafish has become a powerful tool for analysis of vertebrate hematopoiesis. Zebrafish, unlike mammals, have a robust primitive myeloid pathway that generates both granulocytes and macrophages. It is not clear how this unique primitive myeloid pathway relates to mammalian definitive hematopoiesis. In this study, we show that the two myeloid subsets can be distinguished using RNA in situ hybridization. Using a morpholino-antisense gene knockdown approach, we have characterized the hematopoietic defects resulting from knockdown of the myeloid transcription factor gene pu.1 and the unique zebrafish gene c/ebp1. Severe reduction of pu.1 resulted in complete loss of primitive macrophage development, with effects on granulocyte development only with maximal knockdown. Reduction of c/ebp1 did not ablate initial macrophage or granulocyte development, but resulted in loss of expression of the secondary granule gene lys C. These data reveal strong functional conservation of pu.1 between zebrafish primitive myelopoiesis and mammalian definitive myelopoiesis. Further, these results are consistent with a conserved role between c/ebp1 and mammalian C/EBPE, whose ortholog in zebrafish has not been identified. These studies validate the examination of zebrafish primitive myeloid development as a model for human

myelopoiesis, and form a framework for identification and analysis of myeloid mutants.
Descriptors: zebrafish, model for human myelopoiesis, Spi-1, pu.1, Spi-1, pu.1, regulation.

Taylor, M.R., J.B. Hurley, H.A. Van Epps, and S.E. Brockerhoff (2004). **A zebrafish model for pyruvate dehydrogenase deficiency: rescue of neurological dysfunction and embryonic lethality using a ketogenic diet.** *Proceedings of the National Academy of Sciences of the United States of America* 101(13): 4584-9. ISSN: 0027-8424.
NAL Call Number: 500N21P
Abstract: Defects in the pyruvate dehydrogenase (PDH) complex result in severe neurological dysfunction, congenital lactic acidosis, growth retardation, and early death. Current treatments for PDH deficiency are administered postnatally and are generally unsuccessful. Because many patients with this disease are born with irreversible defects, a model system for the development of effective pre- and postnatal therapies would be of great value. In a behavioral genetic screen aimed to identify zebrafish with visual function defects, we previously isolated two alleles of the recessive lethal mutant no optokinetic response a (noa). Here we report that noa is deficient for dihydrolipoamide S-acetyltransferase (Dlat), the PDH E2 subunit, and exhibits phenotypes similar to human patients with PDH deficiency. To rescue the deficiency, we added ketogenic substrates to the water in which the embryos develop. This treatment successfully restored vision, promoted feeding behavior, reduced lactic acidosis, and increased survival. Our study demonstrates an approach for establishing effective therapies for PDH deficiency and other congenital diseases that affect early embryonic development.
Descriptors: zebrafish, model for pyruvate dehydrogenase deficiency, diet, physiology, ketones administration and dosage, nervous system embryology, pyruvate dehydrogenase complex genetics, adenosine diphosphate metabolism, adenosine triphosphate metabolism, amino acid and base sequence, chromosome mapping, molecular cloning, disease models, fetal death, ketones therapeutic use, lactates metabolism, molecular sequence data, nervous system diseases genetics, prevention and control, pyruvates metabolism, vision disorders genetics, prevention and control.

Teh, C., S. Parinov, and V. Korzh (2005). **New ways to admire zebrafish: progress in functional genomics research methodology.** *BioTechniques* 38(6): 897-906. ISSN: 0736 6205.
NAL Call Number: RB37.1B56
Abstract: The main challenge of the post-genomic era is to functionally characterize genes identified by the genome sequencing projects. Model organisms, including zebrafish, are indispensable for this demanding task. Zebrafish has been successfully incorporated into large-scale genetic screens due to the optical clarity of the embryos and their accessibility to various experimental techniques throughout development. The attractiveness of the zebrafish as a model organism is enhanced by the availability of continuously improving genomic tools and methodologies for functional characterization of the gene. This article will highlight the current techniques used in the field, with the focus on transgenesis.
Descriptors: zebrafish genomics model, molecular cloning , DNA transposable elements, electroporation, HSP70 heat shock mutagenesis, research, spermatozoa ultrastructure, transgenes.

Teraoka, H., W. Dong, and T. Hiraga (2003). **Zebrafish as a novel experimental model for developmental toxicology.** *Congenital Anomalies* 43(2): 123-32. ISSN: 0914 3505.
Abstract: It is widely believed that embryos and infants during development are highly sensitive to chemicals that cause serious damage to growth. However, knowledge on the

mechanisms of developmental toxicity is scarce. One reason for this is limited convenient model system other than organ cultures using rodents to study the various aspects of developmental toxicology. Cultured cells are not always adequate for this purpose, since events in morphogenesis are processed through interactions with other tissues. We focused on zebrafish embryo (*Danio rerio*), one of the most important organisms in developmental biology. Saturation mutagenesis, applied to drosophila and nematode to define the functions of genes, has been carried out in zebrafish but almost no other vertebrate, and several thousand lines are available due to the rapid growth and transparent body of this embryo. Enhanced databases for the genome and ESTs are available at websites with abundant genetic and biological background. By targeted gene knock-down with morpholino-modified antisense oligonucleotieds (morpholinos), the translation of a specific protein can be transiently blocked for several days. Many reporter systems in vivo have been established mainly as GFP-transgenic fish for environmental chemicals. Although several excellent studies have been performed with zebrafish embryos on the effects of chemicals, the developmental toxicology of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been most extensively studied to date. We have found that TCDD induces apoptosis in dorsal midbrain with a concomitant decrease in local blood flow, using developing zebrafish. TCDD seems to produce oxidative stress through CYP1A induction in vascular endothelium, resulting in local circulation failure and apoptosis in the dorsal midbrain. In addition to applications in toxicology, an experimental system with zebrafish embryos could help to clarify the mechanism of congenital anomaly, which arises from genetic mutation.
Descriptors: zebrafish toxicology models, apoptosis drug effects, dioxins toxicity, drug effects, mesencephalon blood supply, tetrachlorodibenzodioxin toxicity.

Ton, C., Y. Lin, and C. Willett (2006). **Zebrafish as a model for developmental neurotoxicity testing.** *Birth Defects Research* 76(7): 553-67. ISSN: 1542 0752.
NAL Call Number: QL991.T4
Abstract: BACKGROUND: To establish zebrafish as a developmental toxicity model, we used 7 well-characterized compounds to examine several parameters of neurotoxicity during development. METHODS: Embryos were exposed by semistatic immersion from 6 hrs post-fertilization (hpf). Teratogenicity was assessed using a modified method previously developed by Phylonix. Dying cells in the brain were assessed by acridine orange staining (these cells are likely to be apoptotic). Motor neurons were assessed by antiacetylated tubulin staining and catecholaminergic neurons were visualized by antityrosine hydroxylase staining. RESULTS: Atrazine, dichlorodiphenyltrichloroethane (DDT), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were primarily teratogenic and not specifically neurotoxic. 2,4-dichlorophenoxyacetic acid (2,4-D), dieldrin, and nonylphenol showed specific neurotoxicity; dieldrin and nonylphenol were specifically toxic to catecholaminergic neurons. Malathion, although not teratogenic, showed some nonspecific toxicity. CONCLUSIONS: Teratogenicity measured in 96-hpf zebrafish is predictive of mammalian teratogenicity and is useful in determining whether a compound causes specific neurotoxicity or general developmental toxicity. Induction of apoptosis or necrosis is an indicator of neurotoxicity. An effect on motor neurons in the caudal third of the embryo correlates with expected defects in motility. Overall, our results showed a strong correlation with mammalian data and suggest that zebrafish is a predictive animal model for neurotoxicity screening.
Descriptors: zebrafish, models, malathion, developmental neurotoxins toxicity testing, teratogens toxicity, toxicity tests methods, apoptosis drug effects, axons metabolism and ultrastructure, drug dose response relationship, drug effects, metabolism, developmental gene

expression regulation drug effects, lethal dose 50, motor neurons drug effects and metabolism, phenotype, zebrafish growth and development.

Ton, C. and C. Parng (2005). **The use of zebrafish for assessing ototoxic and otoprotective agents.** *Hearing Research* 208(1-2): 79-88. ISSN: 0378-5955.

Abstract: Zebrafish and other fish exhibit hair cells in the lateral-line neuromasts which are structurally and functionally similar to mammalian inner ear hair cells. To facilitate drug screening for ototoxic or otoprotective agents, we report a straightforward, quantitative in vivo assay to determine potential ototoxicity of drug candidates and to screen otoprotective agents in zebrafish larva. In this study, a fluorescent vital dye, DASPEI (2-(4-(dimethylamino)styryl)-N-ethylpyridinium iodide), was used to stain zebrafish hair cells in vivo and morphometric analysis was performed to quantify fluorescence intensity and convert images to numerical endpoints. Various therapeutics, including gentamicin, cisplatin, vinblastine sulfate, quinine, and neomycin, which cause ototoxicity in humans, also resulted in hair cell loss in zebrafish. In addition, protection against cisplatin-induced ototoxicity was observed in zebrafish larva co-treated with cisplatin and different antioxidants including, glutathione (GSH), allopurinol (ALO), N-acetyl l-cysteine (l-NAC), 2-oxothiazolidine-4-carboxylate (OTC) and d-methionine (d-MET). Our data indicate that results of ototoxicity and otoprotection in zebrafish correlated with results in humans, supporting use of zebrafish for preliminary drug screening.

Descriptors: zebrafish larva, preclinical drug evaluation methods, inner ear drug effects, anti bacterial agents toxicity, antineoplastic agents toxicity, antioxidants pharmacology, cisplatin toxicity, inner ear pathology, fluorescent dyes, gentamicins toxicity, glutathione pharmacology, hair cells drug effects and pathology, larva drug effects models, pyridinium compounds, species specificity.

Traver, D., P. Herbomel, E.E. Patton, R.D. Murphey, J.A. Yoder, G.W. Litman, A. Catic, C.T. Amemiya, L.I. Zon, and N.S. Trede (2003). **The zebrafish as a model organism to study development of the immune system**. *Advances in Immunology* 81: 254-330. ISSN: 0065-2776.

NAL Call Number: 448.8 AD93

Descriptors: zebrafish, animal models, biological development, defence mechanisms, development, disease resistance, fish immunity, freshwater fish, immune system, immunology, literature reviews, modelling, test organisms, *Danio rerio.*

Notes: A review.

Tropepe, V. and H.L. Sive (2003). **Can zebrafish be used as a model to study the neurodevelopmental causes of autism?** *Genes, Brain, and Behavior* 2(5): 268-81. ISSN: 1601-1848.

Abstract: The zebrafish has proven to be an excellent model for analyzing issues of vertebrate development. In this review we ask whether the zebrafish is a viable model for analyzing the neurodevelopmental causes of autism. In developing an answer to this question three topics are considered. First, the general attributes of zebrafish as a model are discussed, including low cost maintenance, rapid life cycle and the multitude of techniques available. These techniques include large-scale genetic screens, targeted loss and gain of function methods, and embryological assays. Second, we consider the conservation of zebrafish and mammalian brain development, structure and function. Third, we discuss the impressive use of zebrafish as a model for human disease, and suggest several strategies by which zebrafish could be used to dissect the genetic basis for autism. We conclude that the zebrafish system could be used to make important contributions to understanding autistic disorders.

Descriptors: zebrafish, autistic disorder model and genetics, brain growth and development, disease models, genetic screening.
Notes: A review.

Tsujikawa, M. and J. Malicki (2004). **Ovl is essential for differentiation and survival of vertebrate sensory neurons**. *Investigative Ophthalmology & Visual Science* 45(Suppl. 1): U382. ISSN: 0146 0404.
Abstract: Purpose: Mutation in the zebrafish oval (ovl) locus causes photoreceptor cell loss and kidney cysts by 3 days after fertilization. This phenotype is very reminiscent of the human Bardet-Biedl Syndrome. Our purpose is to identify and characterize ovl gene. Methods: Positional candidate cloning was followed by biochemical assays.Results: Using a positional candidate approach, we succeeded in identifying the gene affected in ovl. ovl encodes an 824 amino acid-polypeptide and the ovl iz288b mutation causes a truncation of two thirds of its sequence. The injection of anti-ovl morpholinos reproduces the ovl phenotype in wild-type embryos and ovl mRNA injection rescues the defects. These results indicate that we cloned the correct locus. Immunostaining revealed that ovl is concentrated in connecting cilia. To investigate ovl function further, we examined whether cilia and basal bodies are present in mutant photoreceptors by immunostaining. This experiment indicates that the loss of ovl affects either the assembly or the maintenance of photoreceptor cilia but leaves the basal body intact at 3 dpf Thelack of cilia could be due to the lack of cilia maintenance or to the absenceof cilia formation. To test which of the above is the case, we performed staining with anti-acetylated-alpha-tubutin antibodies at earlier stages of development. In the retina of the ovl mutant homozygotes, acetylated-alpha-tubulin staining reveals the presence of cilia in neuroepithelial cells at 30 hpf. The staining is indistinguishable from the wild type. These results indicate that ovl function is not necessary for cilia formation in sensory organs but is required for their maintenance. Because cilia are also found in sensory cells of the vertebrate auditory and olfactory systems, we investigated the generation and maintenance of cilia in these sensory organs. The immunostaining experiments revealed that cilia are also generated but not maintained also in other sensory organs. Moreover, the loss of cilia is followed by the loss of sensory neurons in the sense organs.Conclusions: We conclude that ovl function is not necessary for cilia formation in sensory organs but is required for their maintenance and for the survivalof sensory neurons. Copyright©Thomson Reuters 2009
Descriptors: mutant zebrafish behavior, endocrine system , chemical coordination and homeostasis, sense organs, sensory reception, Bardet Biedl syndrome, congenital disease, endocrine disease, gonads, nutritional disease, behavioral and mental disorders, immunostaining, histology and cytology techniques, immunologic techniques.

Tsujikawa, M., Y. Omori, J. Biyanwila, and J. Malicki (2007). **Mechanism of positioning the cell nucleus in vertebrate photoreceptors**. *Proceedings of the National Academy of Sciences, USA* 104(37): 14819-14824. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish, Bardet-Biedl syndrome, cell death, cytoplasm, dynein, gene families, mutation, neurons, nuclear membranes, organelles, phenotypes, photoreceptors, polarity, polypeptides, dynactin, *Danio rerio.*

Van Der Sar, A.M., R.J. Musters, F.J. Van Eeden, B.J. Appelmelk, C.M. Vandenbroucke Grauls, and W. Bitter (2003). **Zebrafish embryos as a model host for the real time analysis of *Salmonella typhimurium* infections.** *Cellular Microbiology* 5(9): 601-11. ISSN: 1462 5814.
NAL Call Number: QR1 .C45

Abstract: Bacterial virulence is best studied in animal models. However, the lack of possibilities for real time analysis and the need for laborious and invasive sample analysis limit the use of experimental animals. In the present study 28 h-old zebrafish embryos were infected with DsRed-labelled cells of *Salmonella typhimurium.* Using multidimensional digital imaging microscopy we were able to determine the exact location and fate of these bacterial pathogens in a living vertebrate host during three days. A low dose of wild-type *S. typhimurium* resulted in a lethal infection with bacteria residing and multiplying both in macrophage-like cells and at the epithelium of blood vessels. Lipopolysaccharide (LPS) mutants of *S. typhimurium*, known to be attenuated in the murine model, proved to be non-pathogenic in the zebrafish embryos and were partially lysed in the bloodstream or degraded in macrophage-like cells. However, injection of LPS mutants in the yolk of the embryo resulted in uncontrolled bacterial proliferation. Heat-killed, wild-type bacteria were completely lysed extracellularly within minutes after injection, which shows that the blood of these zebrafish embryos does already contain lytic activity. In conclusion, the zebrafish embryo model allows for rapid, non-invasive and real time analysis of bacterial infections in a vertebrate host.
Descriptors: zebrafish embryos, microbiology, *salmonella* infections microbiology, *salmonella typhimurium* physiology, colony count, microbial, metabolism, *Escherichia coli* physiology, *Escherichia coli* infections microbiology, macrophages cytology and microbiology, *salmonella typhimurium* cytology, *salmonella typhimurium* genetics and pathogenicity, time factors.

Vasta, G.R., H. Ahmed, S. Du, and D. Henrikson (2004). **Galectins in teleost fish: zebrafish (*Danio rerio*) as a model species to address their biological roles in development and innate immunity.** *Glycoconjugate Journal* 21(8-9): 503-21. ISSN: 0282-0080.
NAL Call Number: QP752.G56
Abstract: Cell surface glycans, such as glycocoproteins and glycolipids, encode information that modulates interactions between cells, or between cells and the extracellular matrix, by specifically regulating the binding to cell surface-associated or soluble carbohydrate-binding receptors, such as lectins. Rapid modifications of exposed carbohydrate moieties by glycosidases and glycosyltransferases, and the equally dynamic patterns of expression of their receptors during early development, suggest that both play important roles during embryogenesis. Among a variety of biological roles, galectins have been proposed to mediate developmental processes, such as embryo implantation and myogenesis. However, the high functional "redundancy" of the galectin repertoire in mammals has hindered the rigorous characterization of their specific roles by gene knockout approaches in murine models. In recent years, the use of teleost fish as alternative models for addressing developmental questions in mammals has expanded dramatically, and we propose their use for the elucidation of biological roles of galectins in embryogenesis and innate immunity. All three major galectin types, proto, chimera, and tandem-repeat, are present in teleost fish, and phylogenetic topologies confirm the expected clustering with their mammalian orthologues. As a model organism, the zebrafish (*Danio rerio)* may help to overcome limitations imposed by the murine models because it offers substantial advantages: external fertilization, transparent embryos that develop rapidly in vitro, a diverse toolbox of established methods to manipulate early gene expression, a growing collection of mutations that affect early embryonic development, availability of cell lines, and most importantly, an apparently less diversified galectin repertoire.
Descriptors: zebrafish immune system model, galectins physiology, amino acid sequence, chemistry and genetics, gene expression regulation physiology, molecular sequence data, phylogeny, amino acid sequence homology.

Villava, C.E., A. Arellano Torres, R.E. Navarro, and E. Maldonado (2007). **Freeze-crack technique to study epidermal development in zebrafish using differential interference contrast microscopy and fluorescent markers.** *BioTechniques* 43(3): 313-20 . ISSN: 0736 6205.
NAL Call Number: RB37.1B56
Abstract: The zebrafish is a model organism used to study organogenesis during vertebrate development; however epidermis development has been the focus of only a few studies. Thus, new methodologies to highlight and study epidermal cells could be valuable to deepen our understanding of skin development. Large-scale mutagenic screenings have already identified many zebrafish mutants, which are models for human developmental diseases, however only four epidermis mutants have been isolated. Novel screening techniques are needed to improve this collection. We designed and tested a novel freeze-crack technique to obtain, fix, and stain epidermal cells from 5 days postfertilization zebrafish larvae. Using commercially available fluorescent markers and differential interference contrast (DIC) microscopy, we were able to label and highlight subcellular structures such as microridges, cell boundaries, nuclei, and the Golgi complex from epidermis cells. Acquiring and processing epidermis samples from 15 to 75 larvae takes about 2-4 h, respectively. Therefore this method could be used as part of large-scale screenings. In addition, we present a more extensive protocol for antibody staining, which could be employed for more specific studies.
Descriptors: zebrafish epidermal development model, freeze fracturing methods, fluorescence microscopy methods, microscopy, phase contrast methods, skin cytology, skin embryology, fluorescent dyes, image enhancement methods.

Yeh, H.C. and L.H. Wang (2006). **Profiling of prostanoids in zebrafish embryonic development.** *Prostaglandins, Leukotrienes, and Essential Fatty Acids* 75(6): 397-402. ISSN: 0952-3278.
NAL Call Number: QP752.F3PF76
Abstract: Prostanoids (PG) play important roles in vascular, pulmonary, reproductive and renal physiology. Little is known about their roles in the embryonic development. Using the oviparous zebrafish embryo as a model, we determined the temporal expression of PGs synthesized from exogenous prostaglandin H(2). Prostaglandin E(2) is the major PG throughout first 120 h post-fertilization (hpf), whereas prostaglandin F(2)(alpha) is at a lower but also a constant level. Reverse transcription-polymerase chain reaction (RT-PCR) showed that transcripts of cytosolic and membrane-bound PGE synthases were evident during the 120 hpf period. Compared with thromboxane A(2), the level of prostacyclin (PGI(2))is higher at first 24 hpf, the stage before the formation of blood vessel. RT-PCR showed that transcript of prostacyclin synthase appeared at 7 hpf whereas thromboxane synthase appeared at 48 hpf, suggesting that PGI(2) has additional functions besides hemostasis. Interestingly, level of prostaglandin D(2) (PGD(2)) followed an exponential decay over 120 hpf with a rate constant of 0.048 h(-1) and transcript of lipocalin-type PGD synthase was expressed at a higher level at early stage of development, suggesting that PGD(2) is highly regulated during embryogenesis.
Descriptors: zebrafish, embryonic development genetics, prostaglandins metabolism, endoperoxide synthases genetics, endoperoxide synthases metabolism and analysis, messenger RNA analysis and metabolism, reverse transcriptase PCR.

Yimlamai, D., L. Konnikova, L.G. Moss, and D.G. Jay (2005). **The zebrafish down syndrome cell adhesion molecule is involved in cell movement during embryogenesis.** *Developmental Biology* 279(1): 44-57. ISSN: 0012 1606.
NAL Call Number: 442.8D49
Abstract: The Down syndrome cell adhesion molecule (Dscam) is a protein overexpressed

in the brains of Down syndrome patients and implicated in mental retardation. Dscam is involved in axon guidance and branching in *Drosophila*, but cellular roles in vertebrates have yet to be elucidated. To understand its role in vertebrate development, we cloned the zebrafish homolog of Dscam and showed that it shares high amino acid identity and structure with the mammalian homologs. Zebrafish dscam is highly expressed in developing neurons, similar to what has been described in *Drosophila* and mouse. When dscam expression is diminished by morpholino injection, embryos display few neurons and their axons do not enter stereotyped pathways. Zebrafish dscam is also present at early embryonic stages including blastulation and gastrulation. Its loss results in early morphogenetic defects. dscam knockdown results in impaired cell movement during epiboly as well as in subsequent stages. We propose that migrating cells utilize dscam to remodel the developing embryo.
Descriptors: zebrafish Dscam protein, cell adhesion molecules physiology, amino acid and base sequence, body patterning, cell adhesion molecules genetics, molecular cloning, DNA primers, physiology, molecular sequence data, morphogenesis, reverse transcriptase PCR.

Yoo, K.W. and C.H. Kim (2003). **Zebrafish -a new animal model.** *Korean Journal of Laboratory Animal Science* 19(4): 196-208. ISSN: 1225 813X.
Abstract: ..
Descriptors: zebrafish, animal research and development models.

Yu, L., V. Tucci, S. Kishi, and I.V. Zhdanova (2006). **Cognitive aging in zebrafish.** *Public Library of Science One* 1: E14. ISSN: 1817-101X.
Abstract: BACKGROUND: Age-related impairments in cognitive functions represent a growing clinical and social issue. Genetic and behavioral characterization of animal models can provide critical information on the intrinsic and environmental factors that determine the deterioration or preservation of cognitive abilities throughout life. METHODOLOGY/PRINCIPAL FINDINGS: Behavior of wild-type, mutant and gamma-irradiated zebrafish *(Danio rerio*) was documented using image-analysis technique. Conditioned responses to spatial, visual and temporal cues were investigated in young, middle-aged and old animals. The results demonstrate that zebrafish aging is associated with changes in cognitive responses to emotionally positive and negative experiences, reduced generalization of adaptive associations, increased stereotypic and reduced exploratory behavior and altered temporal entrainment. Genetic upregulation of cholinergic transmission attenuates cognitive decline in middle-aged achesb55/+ mutants, compared to wild-type siblings. In contrast, the genotoxic stress of gamma-irradiation accelerates the onset of cognitive impairment in young zebrafish. CONCLUSIONS/SIGNIFICANCE: These findings would allow the use of powerful molecular biological resources accumulated in the zebrafish field to address the mechanisms of cognitive senescence, and promote the search for therapeutic strategies which may attenuate age-related cognitive decline.
Descriptors: zebrafish cognitive aging, response, spatial, visual, temporal, young, middle-aged, old animals, cognitive senescence.

Zdanowicz, M., J.K. Sicklick, A.M. Diehl, and Y.X. Li (2005). **A zebrafish model of fetal alcohol syndrome parallels Hedgehog signaling defects**. *Alcoholism Clinical and Experimental Research* 29(5, Suppl. S): 86A. ISSN: 0145 6008.
Descriptors: zebrafish fetal alcohol syndrome model, cardiovascular system, transport and circulation, toxicology, nervous system, neural coordination, skeletal system, movement and support, reproductive system, reproduction, mental retardation, nervous system disease, behavioral and mental disorders, ongenital disease, toxicity craniofacial malformation, bone

disease, cardiac malformation, heart disease, immunochemical staining, histology and cytology techniques, immunologic techniques.

Zhang, L., T. Zhong, Y. Wang, Q. Jiang, H. Song, and Y. Gui (2006). **Tbx1, a DiGeorge syndrome candidate gene, is inhibited by retinoic acid**. *International Journal of Developmental Biology* 50(1): 55-61. ISSN: 0214 6282.

Abstract: Both retinoic acid (RA) and Tbx1 are definitively indispensable for the development of the pharyngeal arches. The defects produced by a loss of Tbx1 highly resemble those induced by hyper- and hypo- RA. Based on these similarities, the effects of RA on Tbx1 expression pattern were explored during pharyngeal arch development in zebrafish. Whole-mount in situ hybridization and real-time quantitative PCR were used. Zebrafish embryos were treated with 5x10(-8) mol/L and 10(-7) mol/L RA at 12.5 hours post fertilization for 1.5 hours, respectively. Whole-mount in situ hybridization showed that Tbx1 was expressed in the cardiac region, pharyngeal arch and otic vesicle between 24 hpf and 72 hpf in zebrafish. Tbx1 expression was obviously reduced, even lost, in the pharyngeal arch and outflow tract in RA treated groups. Real-time quantitative PCR analysis showed that Tbx1 expression rose to a peak level at 36 hpf in wild type group. Repression of Tbx1 expression was most evident at 36 hpf, 24 hours after RA treatment. 10(-7) mol/L RA caused a more severe effect on the Tbx1 expression level than 5x10(-8) mol/L RA.The results suggested that RA could produce an altered Tbx1 expression pattern in zebrafish. In addition, RA could repress Tbx1 expression in a dose-dependant manner. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, cardiovascular system, transport and circulation, neural coordination, immune system, chemical coordination and homeostasis, molecular genetics, biochemistry and molecular biophysics, development, DiGeorge syndrome, heart disease, genetic disease, immune system disease, pathology, genetics, cardiac development.

Zhao, X.C., R.W. Yee, E. Norcom, H. Burgess, A.S. Avanesov, J.P. Barrish, and J. Malicki (2006). **The zebrafish cornea: structure and development.** *Investigative Ophthalmology and Visual Science* 47(10): 4341-8. ISSN: 0146-0404.

Abstract: PURPOSE: To evaluate the zebrafish as a model for the studies of corneal development and disease. METHODS: Zebrafish embryos and larvae at various stages of development were used for documenting corneal morphogenesis and differentiation. Corneal samples were collected from embryos, larvae, and adult zebrafish for histologic and electron microscopy analysis. Expression patterns of corneal polypeptides were investigated by immunostaining of sections. RESULTS: The zebrafish cornea develops rapidly during embryogenesis, so that its three major layers, the epithelium, the stroma, and the endothelium, are well formed by day 3 postfertilization. The subsequent steps of corneal differentiation, such as the thickening of the corneal stroma, proceed relatively slowly. Several polypeptides are highly enriched in the epithelium or the stroma of the larval and adult zebrafish cornea and are excellent markers of corneal differentiation. CONCLUSIONS: Development and differentiation of the zebrafish cornea are easily accessible to analysis. Anatomic and ultrastructural characterization of the zebrafish cornea demonstrates many similarities to the human cornea and provides the basis for the use of the zebrafish model both to analyze the basic genetic mechanisms of corneal development and to study the causes of corneal disease.

Descriptors: zebrafish embryos, cornea embryology, cornea growth and development, cell differentiation, cornea metabolism, corneal stroma cytology, embryonic development, corneal endothelium cytology, corneal epithelium cytology, eye proteins metabolism, immunohistochemistry, morphogenesis.

Ziegler, I. (2003). **The pteridine pathway in zebrafish: regulation and specification during the determination of neural crest cell-fate.** *Pigment Cell Research* 16(3): 172-82. ISSN: 0893-5785.

Abstract: This review describes pteridine biosynthesis and its relation to the differentiation of neural crest derivatives in zebrafish. During the embryonic development of these fish, neural crest precursor cells segregate into neural elements, ectomesenchymal cells and pigment cells; the latter then diversifying into melanophores, iridophores and xanthophores. The differentiation of neural cells, melanophores, and xanthophores is coupled closely with the onset of pteridine synthesis which starts from GTP and is regulated through the control of GTP cyclohydrolase I activity. De novo pteridine synthesis in embryos of this species increases during the first 72-h postfertilization, producing H4biopterin, which serves as a cofactor for neurotransmitter synthesis in neural cells and for tyrosine production in melanophores. Thereafter, sepiapterin (6-lactoyl-7,8-dihydropterin) accumulates as yellow pigment in xanthophores, together with 7-oxobiopterin, isoxanthopterin and 2,4,7-trioxopteridine. Sepiapterin is the key intermediate in the formation of 7-oxopteridines, which depends on the availability of enzymes belonging to the xanthine oxidoreductase family. Expression of the GTP cyclohydrolase I gene (gch) is found in neural cells, in melanoblasts and in early xanthophores (xanthoblasts) of early zebrafish embryos but steeply declines in xanthophores by 42-h postfertilization. The mechanism(s) whereby sepiapterin branches off from the GTP-H4biopterin pathway is currently unknown and will require further study. The surge of interest in zebrafish as a model for vertebrate development and its amenability to genetic manipulation provide powerful tools for analysing the functional commitment of neural crest-derived cells and the regulation of pteridine synthesis in mammals.

Descriptors: zebrafish embryos, neural crest embryology, pteridines metabolism, biopterin metabolism, cell lineage, GTP cyclohydrolase metabolism, developmental gene expression regulation, guanosine triphosphate chemistry and metabolism, melanophores metabolism, biological and chemical models, mutation, neurons metabolism, phenotype, biological pigments metabolism, pterins metabolism, signal transduction, time factors, tyrosine metabolism, xanthine dehydrogenase metabolism, xanthine oxidase metabolism, xanthopterin metabolism.

Toxicology

Ahlgren, S.C. (2004). **Effects of ethanol on zebrafish growth.** *Birth Defects Research* 70(5): 313. ISSN: 1542 0752.
NAL Call Number: QL991.T4
Descriptors: zebrafish, development, toxicology, zebrafish, cyclopia, growth, ethanol effects .

Airhart, M.J., D.H. Lee, T.D. Wilson, B.E. Miller, M.N. Miller, and R.G. Skalko (2007). **Movement disorders and neurochemical changes in zebrafish larvae after bath exposure to fluoxetine (PROZAC).** *Neurotoxicology and Teratology* 29(6): 652-64. ISSN: 0892-0362.
NAL Call Number: RA1224
Abstract: This study examines the effects of the selective serotonin reuptake inhibitor (SSRI), fluoxetine (PROZAC), on the ontogeny of spontaneous swimming activity (SSA) in developing zebrafish. The development of zebrafish motor behavior consists of four sequential locomotor patterns that develop over 1-5 days post fertilization (dpf), with the final pattern, SSA, established at 4-5 dpf. In stage specific experiments, larvae were exposed to 4.6 muM fluoxetine for 24 h periods beginning at 24 h post fertilization (hpf) and extending through 5 dpf. From 1-3 dpf, there was no effect on SSA or earlier stages of motor development, i.e., spontaneous coiling, evoked coiling and burst swimming. Fluoxetine exposure at 3 dpf for 24 h resulted in a transient decrease in SSA through 7 dpf with a complete recovery by 8 dpf. Larvae exposed to 4.6 muM fluoxetine for 24 h on 4 or 5 dpf showed a significant decrease in SSA by day 6 with no recovery through 14 dpf. Although SSA was significantly affected 24 h after fluoxetine exposure, there was little or no effect on pectoral fin movement. These results demonstrate both a stage specific and a long term effect of 4.6 muM fluoxetine exposure in 4 and 5 dpf larvae. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed to determine the relative levels of a serotonin transporter protein (SERT) transcript and the serotonin 1A (5-HT(1A)) receptor transcript in developing embryos/larvae over 1-6 dpf. Both transcripts were present at 24 hpf with the relative concentration of SERT transcript showing no change over the developmental time range. The relative concentration of the 5-HT(1A) receptor transcript, however, showed a two-tiered pattern of concentration. RT-PCR was also used to detect potential changes in the SERT and 5-HT(1A) receptor transcripts in 6 dpf larvae after a 24 h exposure to 4.6 muM fluoxetine on 5 dpf. Three separate regions of the CNS were individually analyzed, two defined brain regions and spinal cord. The two brain regions showed no effect on transcript levels subsequent to fluoxetine exposure, however, the spinal cord showed a significant decrease in both transcripts. These results suggest a correlation between decreased concentration of SERT and 5-HT(1A) receptor transcripts in spinal cord and decreased SSA subsequent to fluoxetine exposure.
Descriptors: zebrafish larvae, movement, disorders, neurochemistry, exposure, fluoxetine, prozac exposure.

Allebrandt, K.V., V. Rajesh, and P.G. Layer (2005). **Expression of acetylcholinesterase (AChE) and aryl acylamidase (AAA) during early zebrafish embryogenesis.** *Chemico Biological Interactions* 157-158: 353-5. ISSN: 0009 2797.
NAL Call Number: QH345.C5
Abstract: In zebrafish, the onset of acetylcholinesterase (AChE) expression was detected by RT-PCR at 4 hpf (hours post-fertilization). The aryl acylamidase (AAA) associated with AChE, a serotonin sensitive activity with unknown physiological function, was significantly higher than the esterase activity on zebrafish embryos homogenates at 4-12 h development (test-t = 3.523; d.f. = 4). Remarkably, the ratio of AAA/AChE activity decreased 210-fold from 4 to 144 h development, indicating a distinct embryonic role of AAA during early embryogenesis. The AAA activity

was sensitive to eserine and serotonin, ensuring its association with AChE. This is the first report of AAA activity on fish, establishing zebrafish as a model to study AAA on development.
Descriptors: zebrafish embryos, aryl acylamidase (AAA), acetylcholinesterase metabolism, amidohydrolases metabolism, developmental gene expression regulation drug effects, enzymologic gene expression regulation drug effects, acetylcholinesterase genetics, amidohydrolases genetics, physostigmine pharmacology, serotonin pharmacology, time factors.

Alsop, D., S. Brown, and G. Van Der Kraak (2007). **The effects of copper and benzo[a]pyrene on retinoids and reproduction in zebrafish.** *Aquatic Toxicology* 82(4): 281-95. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: This study examines whether a link exists between toxicant exposure, retinoids and reproduction in fish. Zebrafish were fed a control diet (8.1 microg Cu/g diet, 0 microg benzo[a] pyrene/g diet) or diets containing elevated copper (100 microg, 500 microg and 1000 microg Cu/g diet) or benzo[a]pyrene (B[a]P; 30 and 150 microg B[a]P/g diet) for 260 days. Toxicant-supplemented diets did not affect growth or mortality rates. While whole body retinoid levels in control zebrafish decreased during the experiment, females exposed to Cu or B[a]P for 200 days or more experienced additional losses of retinyl esters (45-100% depleted) and retinal (45% depleted in B[a]P-fed fish). Despite the reduced retinoids, Cu and B[a]P did not effect reproduction with respect to the number of eggs spawned, fertilization rates or egg retinal content (retinal was instead increased 55-65% in eggs from B[a]P-fed fish). There were no apparent deformities observed in 36 h post fertilization embryos from any treatment. It appears that although internal retinoid stores were depleted in adults, dietary retinoids were sufficient to meet the daily requirement for retinal deposition in the eggs and retinoic acid synthesis. This study has shown that retinoid levels in female zebrafish are sensitive to Cu and B[a]P, and are a good indicator of long-term exposure. It also brings to light the resiliency of the retinoid system in fish and the importance of the diet on the toxicological response. Specifically that dietary retinoids appear to support normal reproduction in the absence of internal retinoid stores.
Descriptors: zebrafish, benzoapyrene toxicity, copper toxicity, retinoids analysis, chemical water pollutants toxicity, animal feed analysis, body size drug effects, carboxylic ester hydrolases analysis and drug effects, diet veterinary, esters analysis, gene expression drug effects, lipid peroxidation drug effects, ovum chemistry, oxidative stress drug effects, random allocation, reproduction drug effects, retinaldehyde analysis, tocopherols analysis, vitamin A analogs and derivatives, vitamin A analysis, proteins biosynthesis.

An, L., J. Hu, M. Yang, F. Jin, Q. Du, and Z. Ke (2006). **Enhanced vitellogenin induction of secondary effluents by chlorination**. *Bulletin of Environmental Contamination and Toxicology* 77(1): 67-73. ISSN: 0007 4861.
NAL Call Number: RA1270.P35A1
Abstract: In this study, zebrafish (*Danio rerio*), vitellogenin (VTG, zf-VTG) and medaka *(Oryzias latipes*) larval development were used to monitor the effects of oestrogenic activity in chlorinated secondary effluents from Fangzhuang sewage treatment plants (STP) in Beijing, China, compared to secondary effluents only. Eleven polycyclic aromatic hydrocarbons (PAHs, naphthalene, acenaphthene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene and dibenzo(a,h)anthracene) were used. The effluents were chlorinated with 5 mg/litre NaOH for 2 h. 20 mature male zebrafish were exposed to control and chlorinated secondary effluents for 2 weeks, after which VTG was analysed. 40 larval medaka were also exposed to the effluents before and after chlorination for 1, 2, 3 and 4 weeks. PAH levels were also measured in the effluent samples. It was shown that VTG concentration was 1362.1+or-221.7 ng/ml in the chlorinated effluents compared to the control

effluent, which was 2236.1+or-1067.6 ng/ml. Acenaphthene, phenanthrene, anthracene, fluoranthene and dibenzo(a,h)anthracene were detected at a total concentration of 887 ng/litre in secondary effluents. Fish larval weights and lengths exposed to chlorinated secondary effluents were lower compared to those in the control group. VTG concentration in chlorinated effluents was 6481.8+or-36825.5 ng/ml. Larval growth in chlorinated effluents was more rapid than in secondary effluents after 3 weeks of exposure, although it was significantly lower than in the control after 4 weeks of exposure. These results show that the water quality of effluents is improved after chlorination. Copyright © 2008 CABI

Descriptors: zebrafish, medaka, aromatic hydrocarbons, adults and larva exposure, effects on growth, larva migrans, naphthalene, estrogenic properties, phenanthrene, poisoning, polycyclic hydrocarbons, sewage effluent, toxicity, vitellogenins, water pollution and quality, *Danio rerio*, *Oryzias latipes.*

Andersen, L., R. Goto Kazeto, J.M. Trant, J.P. Nash, B. Korsgaard, and P. Bjerregaard (2006). **Short-term exposure to low concentrations of the synthetic androgen methyltestosterone affects vitellogenin and steroid levels in adult male zebrafish (Danio rerio).** *Aquatic Toxicology* 76(3-4): 343-52. ISSN: 0166 445X.

NAL Call Number: QH541.5.W3A6

Abstract: Short-term effects of methyltestosterone (MT) on the endocrine system of adult male zebrafish (*Danio rerio*) were examined. Males were exposed to 0, 4.5, 6.6, 8.5, 19.8, 35.9, 62.3 ng MT/l and ethinylestradiol (EE2) (26.4 ng/l) for 7 days. Several physiological endpoints that may be affected by endocrine disrupters were analysed, specifically vitellogenin (VTG) concentration, estradiol (E2), testosterone (T), and 11-ketotestosterone (KT) content, brain aromatase activity and gene expression of CYP19A1 and CYP19A2 in the testis. Exposure to the lowest MT concentration (4.5 ng MT/l), and the EE2 increased the concentration of VTG significantly compared to solvent control group. Exposure to higher concentrations of MT did not increase VTG levels. Endogenous KT and T levels decreased significantly in a concentration-dependent manner in response to the MT exposure and the lowest effective concentrations were 6.4 and 8.5 ng MT/l, respectively. The levels of KT and T were also significantly suppressed by EE2 when compared to the solvent control group. Significant decreases in endogenous E2 levels were found in some MT groups but it was not possible to distinguish a simple concentration-response relationship. No effects of MT or EE2 on the brain aromatase activity or on testicular gene expression of CYP19A1 and CYP19A2 were detected. The results show that androgens such as MT can act as endocrine disrupters even at very low concentrations.

Descriptors: zebrafish, gonadal steroid hormones analysis, methyltestosterone toxicity, vitellogenins drug effects, analysis of variance, aromatase analysis and biosynthesis, body size, brain drug effects and enzymology, drug dose response relationship, ELISA veterinary, ethinyl estradiol and synthetic hormone toxicity, reverse transcriptase PCR veterinary, testis drug effects and metabolism, vitellogenins analysis, chemical water pollutants toxicity, proteins biosynthesis.

Andersen, L., H. Holbech, A. Gessbo, L. Norrgren, and G.I. Petersen (2003). **Effects of exposure to 17alpha-ethinylestradiol during early development on sexual differentiation and induction of vitellogenin in zebrafish (*Danio rerio*).** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 134(3): 365-74. ISSN: 1532 0456.

NAL Call Number: QP901.C6

Abstract: To determine the critical stage of zebrafish development where exposure to xenoestrogens can affect sex ratio and vitellogenin induction, zebrafish (*Danio rerio*) were exposed to 17alpha-ethinylestradiol (actual concentration 15.4+/-1.4 ng EE2/l) during early development: from fertilisation to hatch; hatch to 10 days post hatch (dph); 10-20 dph; 20-30 dph; 20-40 dph;

20-60 dph; fertilisation to 25 dph; or hatch to 60 dph. Vitellogenin was measured in whole body homogenate 30 dph by ELISA and sex ratio was determined 60 dph by histological examination of the gonads. All exposure periods significantly induced vitellogenin synthesis and affected the sex differentiation leading to development of ovo-testis or complete feminisation of the exposed fish depending on exposure period. Complete sex reversal was obtained in groups exposed from 20 to 60 dph and hatch to 60 dph. The half-life for degradation of vitellogenin was calculated. Juvenile zebrafish were exposed to 15.4+/-1.4 ng EE2/l (actual concentration) from fertilisation to 25 dph and transferred to clean water, after which weekly measurements of vitellogenin concentration in whole body homogenate were performed until day 46 post hatch. The half-life of vitellogenin was 2.4 days.

Descriptors: zebrafish embryology, estrogens toxicity, ethinyl estradiol toxicity, sex differentiation drug effects, vitellogenins biosynthesis, feminization chemically induced, fertilization, larva drug effects, ovary drug effects and growth and development, sex ratio, testis drug effects and growth and development, time factors, water analysis.

Andreasen, E.A., L.K. Mathew, C.V. Lohr, R. Hasson, and R.L. Tanguay (2007). **Aryl hydrocarbon receptor activation impairs extracellular matrix remodeling during zebra fish fin regeneration.** *Toxicological Sciences* 95(1): 215-26. ISSN: 1096-6080.

NAL Call Number: RA1190.F8

Abstract: Adult zebra fish completely regenerate their caudal (tail) fin following partial amputation. Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) inhibits this regenerative process. Proper regulation of transcription, innervation, vascularization, and extracellular matrix (ECM) composition is essential for complete fin regeneration. Previous microarray studies suggest that genes involved in ECM regulation are misexpressed following activation of the aryl hydrocarbon receptor. To investigate whether TCDD blocks regeneration by impairing ECM remodeling, male zebra fish were i.p. injected with 50 ng/g TCDD or vehicle, and caudal fins were amputated. By 3 days postamputation (dpa), the vascular network in the regenerating fin of TCDD-exposed fish was disorganized compared to vehicle-exposed animals. Furthermore, immunohistochemical staining revealed that axonal outgrowth was impacted by TCDD as early as 3 dpa. Histological analysis demonstrated that TCDD exposure leads to an accumulation of collagen at the end of the fin ray just distal to the amputation site by 3 dpa. Mature lepidotrichial-forming cells (fin ray-forming cells) were not observed in the fins of TCDD-treated fish. The capacity to metabolize ECM was also altered by TCDD exposure. Quantitative real-time PCR studies revealed that the aryl hydrocarbon pathway is active and that matrix-remodeling genes are expressed in the regenerate following TCDD exposure.

Descriptors: zebrafish, caudal (tail) fin regeneration, environmental carcinogens toxicity, extracellular matrix metabolism, aryl hydrocarbon receptors agonists, regeneration drug effects, tetrachlorodibenzodioxin toxicity, wound healing drug effects, proteins metabolism, collagen metabolism, cytochrome P450 CYPA1A1 biosynthesis, polyacrylamide gel electrophoresis, enzyme induction drug effects, extremities anatomy and histology, extremities physiology, gene expression regulation drug effects, immunohistochemistry, matrix metalloproteinases metabolism, PCR, proteoglycans metabolism, messenger RNA metabolism, aryl hydrocarbon receptors metabolism, time factors, tissue plasminogen activator metabolism, transforming growth factor beta1 metabolism.

Andreasen, E.A., L.K. Mathew, and R.L. Tanguay (2006). **Regenerative growth Is Impacted by TCDD: Gene Expression analysis Reveals Extracellular Matrix modulation**. *Toxicological Sciences* 92(1): 254-269. ISSN: 1096-6080.

NAL Call Number: RA1190.F8

Descriptors: zebrafish fins, abundance, amputation, angiogenesis, differentiation, extracellular matrix, fins, freshwater fish, gene expression, hydrocarbons, mesenchyme, metabolism, nerves, nucleotide sequence, pollution effects, PCR, regeneration, Tcdd, wound healing , wounding, *Danio rerio.*

Antkiewicz, D.S., C.G. Burns, S.A. Carney, R.E. Peterson, and W. Heideman (2005). **Heart malformation is an early response to TCDD in embryonic zebrafish.** *Toxicological Sciences* 84(2): 368-77. ISSN: 1096-6080.

NAL Call Number: RA1190.F8

Abstract: The zebrafish (*Danio rerio*) has become an attractive vertebrate model for studying developmental processes, and is emerging as a model system for studying the mechanisms by which toxic compounds perturb normal development. When exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) shortly after fertilization, zebrafish embryos exhibit pericardial edema and reduced blood flow by 72 h post fertilization (hpf). To better understand the progression of dioxin toxicity in zebrafish, we have examined the effects of TCDD on heart development. At 72 hpf, TCDD-treated embryos exhibited altered looping, with the atria positioned distinctly posterior to the ventricles, contrary to the looping of control hearts, where the two chambers lied side by side. Moreover, the ventricles in dioxin-exposed hearts became more compact, and the atria elongated in comparison to controls. These defects are not secondary to pericardial edema because they were observed when edema formation was suppressed with osmotic support. In addition to morphological changes, TCDD produced functional deficits in the developing hearts, including blood regurgitation and a striking ventricular standstill that became prevalent by 120 hpf. We also assessed the effect of TCDD on the heart size using stereological measurements, which demonstrated significant reduction in heart tissue volume at 72 hpf. Perhaps our most significant finding was a decrease in the total number of cardiomyocytes in TCDD-exposed embryos by 48 hpf, one day prior to observable effects on peripheral blood flow. We conclude that the developing heart is an important target for TCDD in zebrafish.

Descriptors: zebrafish embryos, drug induced abnormalities etiology, embryonic heart development drug effects, environmental pollutants toxicity, chemically induced congenital heart defects , teratogens toxicity, dioxin toxicity, drug induced abnormalities pathology, cardiac myosins metabolism, cell count, disease models, embryonic development physiology, indirect fluorescent antibody technique, congenital heart defects pathology, heart rate drug effects, myocardium metabolism and pathology , cardiac myocytes drug effects and pathology, myosin heavy chains metabolism.

Antkiewicz, D.S., R.E. Peterson, and W. Heideman (2006). **Blocking expression of AHR2 and ARNT1 in zebrafish larvae protects against cardiac toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin.** *Toxicological Sciences* 94(1): 175-182. ISSN: 1096 6080.

NAL Call Number: RA1190.F8

Abstract: The zebrafish (*Danio rerio*) has become an attractive vertebrate model for studying developmental processes, and is emerging as a model system for studying the mechanisms by which xenobiotic compounds perturb normal development. Embryos treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) shortly after fertilization exhibit a range of adverse effects on the heart: an early reduction in cardiac myocyte number, followed by a change in heart looping and morphology, with an apparent compaction of the ventricle and overall decrease in heart size. These changes are accompanied by impaired cardiac function including a decrease in cardiac output and eventually irreversible ventricular standstill. The mechanisms involved in mediating effects of TCDD on the heart remain unknown. However, it is widely accepted that aryl hydrocarbon receptor (AHR) activation mediates endpoints of TCDD toxicity in vertebrates.

In zebrafish, there are multiple forms of AHR and AHR nuclear translocator protein (ARNT) raising the question about whether different endpoints of TCDD toxicity are mediated by different components of the AHR/ARNT pathway. To address this question we used morpholino oligonucleotide technology to specifically block the expression of zfAHR2, zfARNT1, zfARNT2, and zfCYP1A, and assessed the previously described effects of TCDD on heart morphology, size, and function in the developing morphants. We report that blocking zfAHR2 and zfARNT1 expression provided protection against the TCDD-mediated alteration in heart morphology, reduced cardiac myocyte number, decreased cardiac output and ventricular standstill in zebrafish larvae, while the zfarnt2 and zfcyp1a morpholinos did not block the TCDD-induced cardiac toxicity..
Descriptors: zebrafish embryo, adverse effects, dioxin toxicity models, cardiovascular system, gene expression, genes, heart, xenobiotics, *Danio rerio.*

Arenzana, F.J., M.J.3. Carvan, J. Aijon, R. Sanchez Gonzalez, R. Arevalo, and A. Porteros (2006). **Teratogenic effects of ethanol exposure on zebrafish visual system development.** *Neurotoxicology and Teratology* 28(3): 342-8. ISSN: 0892-0362.
NAL Call Number: RA1224
Abstract: Ethanol intake during pregnancy can produce a wide range of adverse effects on nervous system development including fetal alcohol syndrome (FAS). The most severe congenital malformation observed in newborns with FAS is cyclopia. In this study, we have exposed zebrafish embryos to different ethanol concentrations (2.4%, 1.5% or 1.0%) during eye morphogenesis in four zebrafish strains (AB, EK, GL and TL). In addition, we have studied the survival rate of the cyclopic animals to the end of larval development. The zebrafish strains GL and AB generated the higher percentage of cyclopic animals after exposure to 2.4% ethanol, while EK showed the higher percent cyclopic animals using 1.5% and 1.0% ethanol. The EK strain showed the higher percent survival during the larval period at all ethanol concentrations (2.4%, 1.5% and 1.0%). Moreover, we have investigated cytoarchitectural alterations in the main components of the visual pathway-retina and optic tectum-and ethanol treatment affects both the retina and the optic tectum. The lamination of neural retina is clearly delayed in treated larvae 3 days postfertilization and the thickness of the pigmented epithelium is considerably reduced. With regard to the optic tectum, treatment with ethanol alters the normal pattern of tectal lamination. The use of zebrafish EK strain is a suitable in vivo vertebrate model system for analyzing the teratogenic effect of ethanol during vertebrate visual system morphogenesis as it relates to both cyclopia and FAS.
Descriptors: zebrafish embryos, ethanol toxicity, chemically induced eye abnormalities, organogenesis drug effects, teratogens toxicity, drug dose response relationship, embryo culture techniques, drug effects, eye abnormalities embryology and pathology, retina embryology and pathology, species specificity, superior colliculus embryology and pathology.

Baez Ramirez, O.A., F. Prieto Garcia, and C.A. Galan Vidal (2004). **Bioacumulacion y danos genotoxicos en Pez Cebra (*Danio rerio*) por arsenico en aguas de Zimpan, Hidalgo (Mexico). [Bioaccumulation and genotoxics damages in zebra fish (*Danio rerio*) by arsenic in Zimapan waters, Hidalgo (Mexico)].** *AquaTIC*(21): 62-70. ISSN: 1578 4541.
Abstract: In bicarbonate calcic-magnesic waters from UAEH and Zimapan V wells, the last recorded arsenic (As) content of 0.50 mg/l was studied and its bioacumulation in Zebra Fish (*Danio rerio*) and the probability of genotoxic damages in gill cells, evaluating micronuclei (MNs) induction. The animals were studied for 30 days in three separated groups (negative control with UAEH wellwater, positive control with 5 mg As/l, and Zimapan V wellwater) with 50 individuals per group. In waters were As diminished its concentration with time, accumulation of As in fish

had an increase. After 30 days it had produced a decrease in the water of positive control of 1 mg As/l (0.035 mg As/l/day), and in fish had a bioacumulation of 0.015 mg As/kg (0.0005 mg As/kg/day). In Zimapan V wellwater had a drop of 0.2 mg As/L (0.0067 mg As/l/day), and in fish an increase of 0.0005 mg As/kg (0.00017 mg As/kg/day). On the other side, in negative control was detected a spontaneous micronuclei induction (MI) in gill cells of 8 MNs/1000 cells. In positive control had a MI 15.38 times greater than negative control, and in fish exposed to Zimapan V wellwater were appreciated a MI increase of 2.38 times more than negative control. All of this reveals a genotoxicity effect due to As. Copyright © 2008 CABI
Descriptors: zebrafish, arsenic, genotoxicity, gills, toxicity, well water pollution, *Danio rerio*.
Language of Text: Spanish.

Baganz, D., R. Siegmund, G. Staaks, S. Pflugmacher, and C.E.W. Steinberg (2005). **Temporal pattern in swimming activity of two fish species (*Danio rerio* and *Leucaspius delineatus*) under chemical stress conditions**. *Biological Rhythm Research* 36(3): 263-276. ISSN: 0929 1016.
NAL Call Number: Q176.J6
Abstract: Circadian periodicity of swimming activity was investigated in two fish species, the zebrafish (*Danio rerio*) and the sunbleak (*Leucaspius delineatus*) under sublethal long-term exposure to the cyanobacteria toxin microcystin-LR (nominal concentrations of 0.5 micro g l-1, 5 micro g l-1, 15 micro g l-1, 50 micro g l-1) in 15-litre tanks. Swimming activity of fish was monitored continuously by using an automated video-monitoring and object-tracing system over a period of 17 days. Influenced by long-term exposure to microcystin-LR, *Leucaspius delineatus* reversed their significant diurnal swimming activity and the fish became statistically significant nocturnal. *Danio rerio* remained diurnal active, but a significant phase shift was registered. In both *Danio rerio* and *Leucaspius delineatus* analysis of time series by cosinor regression revealed microcystin-LR induced dose-dependent alterations of the mean of oscillation, amplitude, acrophase and period length in a different extent. For *Danio rerio* the periodogram analysis revealed a significant circadian component of swimming activity for control as well as exposure groups, whereby the spectral amplitude clearly decreased at microcystin-LR concentrations of 15 and 50 micro g l-1. For *Leucaspius delineatus* the amplitude of circadian rhythm was decreased at all exposure concentrations of MC-LR. Furthermore the dominance of circadian rhythm was clearly reduced, whereas the rate of ultradian rhythms increased at elevated MC-LR concentrations of 5 micro g l-1, 15 micro g l-1 and 50 micro g l-1. The studied temporal aspects of behaviour clearly indicated stress symptoms in both fish species, therefore it proved to be a relevant method to characterise the impact of toxic substances in the environment and for biomonitoring. Copyright © 2008 CABI
Descriptors: zebrafish, sunbleak, circadian rhythm, diurnal activity, locomotion, periodicity, temporal variation, toxic substances , toxicity, environmental toxins, water pollution and quality, cyanobacteria, *Danio rerio*, *Leucaspius*.

Baganz, D., G. Staaks, S. Pflugmacher, and C.E.W. Steinberg (2004). **Comparative study of microcystin-LR-induced behavioral changes of two fish species, *Danio rerio* and *Leucaspius delineatus***. *Environmental Toxicology* 19(6): 564-570. ISSN: 1520 4081.
NAL Call Number: RA1221.T69
Abstract: The spontaneous locomotor behavior separated into day/night activity phases of two fish species *Danio rerio* and *Leucaspius delineatus* was recorded and quantified continuously under sublethal long-term exposure to microcystin-LR in tanks. Microcystin-LR was applied in concentrations of 0.5, 5, 15, and 50 micro g L-1. By using an automated video-monitoring and object-tracing system, the average motility (swimming velocity) and the average number of turns were assessed. Clear dose-dependent effects of microcystin-LR on the behavior of both test fish

were measured. During the daytime, the motility of *Danio rerio* as well as *Leucaspius delineatus* increased significantly by exposure to the lowest concentrations, whereas higher concentrations led to significantly decreased motility. Influenced by microcystin-LR, the swimming time of *Leucaspius delineatus* reversed, going from a prominently diurnal activity to a nocturnal one; Danio rerio remained active during the daytime. Most of the relative changes in the behavioral patterns of *Danio rerio* and *Leucaspius delineatus* suggest these fish have comparable susceptibility to microcystin-LR and may indicate some adverse consequences for fish populations, for example, in connection with reproduction and predator-prey interactions. Copyright © 2008 CABI
Descriptors: zebrafish, *Leucaspius delineatus*, sunbleak, behavior, diurnal activity and variation, nocturnal activity, physical activity, toxicity, toxins, cyanobacteria, *Danio rerio.*

Barillet, S., C. Adam, O. Palluel, and A. Devaux (2007). **Bioaccumulation, oxidative stress, and neurotoxicity in *Danio rerio* exposed to different isotopic compositions of uranium.** *Environmental Toxicology and Chemistry* 26(3): 497-505. ISSN: 0730 7268.
NAL Call Number: QH545A1E58
Abstract: Experiments were carried out on adult male zebrafish (*Danio rerio*) to assess early changes induced by waterborne exposure to different isotopic compositions of uranium (depleted uranium associated or not with 233U). Oxidative stress and neurotoxicity were selected as effect endpoints to characterize uranium chemo- and radiotoxicity. Catalase, glutathione peroxidase, and superoxide dismutase activities and total glutathione content of hepatic extracts, as well as brain acetylcholinesterase activity and uranium bioaccumulation, were measured. Oxidative stress induced by uranium exposure led to decreases in superoxide dismutase and catalase activity levels as well as total glutathione content in liver extracts. These perturbations were significantly more marked in 233U-exposed fish. Furthermore, significant increase in acetylcholinesterase activity was observed in brain extracts at the same level, whatever the isotopic composition of uranium.
Descriptors: zebrafish, uranium toxicity, oxidative stress, uranium pharmacokinetics, water pollutants, radioactive pharmacokinetics, metabolism, brain, enzymes, isotopes, liver, pharmacokinetics, tissue distribution.

Behra, M., C. Etard, X. Cousin, and U. Strahle (2004). **The use of zebrafish mutants to identify secondary target effects of acetylcholine esterase inhibitors.** *Toxicological Sciences* 77(2): 325-33. ISSN: 1096-6080.
NAL Call Number: RA1190.F8
Abstract: We are confronted with a large and steadily growing number of bioactive compounds, including drugs, pesticides, and industrial by-products. The assessment of target specificity and potential toxic effect on human health and the environment generates a strong demand for robust and cost-effective models with high predictive power. We investigated the potential of the zebrafish embryo as a whole organism, vertebrate model to assess the specificity of compounds that are known to inhibit acetylcholinesterase (AChE). Inhibitors of AChE are widely used as drugs and pesticides. By application of simple assays and comparison with the phenotype of embryos with genetic lesions in the ache gene, we demonstrate that only one of the AChE inhibitors (galanthamine) reproduces the phenotype of ache mutant embryos. The other compounds produced additional effects indicating secondary targets. Our work demonstrates the power of a genetic system for toxicological evaluations. The combination of genetics and transgenesis with the other experimental virtues of the zebrafish embryo, such as small size and low cost, offers a whole organism platform for medium to high throughput compound testing.
Descriptors: zebrafish embryo mutants, genetically modified, cholinesterase inhibitors toxicity, drug effects, acetylcholinesterase analysis and genetics, gene transfer techniques, models, motor activity drug effects, toxicity tests economics and methods, metabolism.

Bello, S.M., W. Heideman, and R.E. Peterson (2004). **2,3,7,8-Tetrachlorodibenzo-p-dioxin inhibits regression of the common cardinal vein in developing zebrafish.** *Toxicological Sciences* 78(2): 258-66. ISSN: 1096-6080.

NAL Call Number: RA1190.F8

Abstract: A role for the aryl hydrocarbon receptor (AHR) pathway in vascular maturation has been implicated by studies in Ahr-null mice. In this study the hypothesis that activation of AHR signaling by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alters common cardinal vein (CCV) development in the zebrafish embryo was investigated. The CCV is a paired vessel that grows across the yolk, connecting to the heart. It is extensively remodeled and regresses as the heart migrates dorsally within the pericardium. TCDD significantly reduced CCV growth as early as 44 h post fertilization (hpf), and CCV area was reduced to 63% of control at 62 hpf. This vascular response to TCDD was at least as sensitive as previously defined endpoints of TCDD developmental toxicity in zebrafish. TCDD also blocked regression of the CCV (by 80 hpf), possibly contributing to the "string-like" heart phenotype seen in TCDD-exposed zebrafish larvae. Dependence of the block in CCV regression on zebrafish (zf) AHR2 was investigated using a zfahr2 specific morpholino to knock down expression of AHR2. The zfahr2 morpholino had no effect on CCV regression in the absence of TCDD, but did protect against the TCDD-induced block of CCV regression. This demonstrates that the TCDD-induced block in CCV regression is AHR2 dependent. It is significant that decreased CCV growth occurs before and inhibition of CCV regression occurs concurrent with overt signs of TCDD developmental toxicity. This suggests that alterations of vascular growth and remodeling may play a role in TCDD developmental toxicity in zebrafish.

Descriptors: zebrafish larvae, dioxin toxicity, cardinal vein, environmental pollutants toxicity, tetrachlorodibenzodioxin toxicity, genetically modified, fish edema, larva drug effects, growth, development and metabolism, morpholines pharmacology, pericardium drug effects, pathology, aryl hydrocarbon receptors deficiency, genetics and metabolism, regional blood flow drug effects, veins drug effects, growth, development and pathology, zebrafish proteins deficiency, genetics and metabolism.

Benya, J.P., M. Gantar, R.E. Gawley, M. Wang, and K.S. Rein (2004). **Pharmacology and toxicology of pahayokolide a, a bioactive metabolite from a freshwater species of *lyngbya* isolated from the florida everglades**. *Comparative Biochemistry and Physiology: Part C, Toxicology and Pharmacology* 139(4): 231-238. ISSN: 1532 0456.

NAL Call Number: QP901.C6

Abstract: The genus of filamentous cyanobacteria, *Lyngbya*, has been found to be a rich source of bioactive metabolites. However, identification of such compounds from *Lyngbya* has largely focused on a few marine representatives. Here, we report on the pharmacology and toxicology of pahayokolide A from a freshwater isolate, *Lyngbya* sp. strain 15-2, from the Florida Everglades. Specifically, we investigated inhibition of microbial representatives and mammalian cell lines, as well as toxicity of the compound to both invertebrate and vertebrate models. Pahayokolide A inhibited representatives of *Bacillus*, as well as the yeast, *Saccharomyces cerevisiae*. Interestingly, the compound also inhibited several representatives of green algae that were also isolated from the Everglades. Pahayokolide A was shown to inhibit a number of cancer cell lines over a range of concentrations (IC50 varied from 2.13 to 44.57 muM) depending on the cell-type. When tested against brine shrimp, pahayokolide was only marginally toxic at the highest concentrations tested (1 mg/mL). The compound was, however, acutely toxic to zebrafish embryos (LC50=2.15 muM). Possible biomedical and environmental health aspects of the pahayokolides remain to be investigated; however, the identification of bioactive metabolites such as these demonstrates the potential of the Florida Everglades as source of new toxins and drugs. Copyright 2004 Elsevier

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Descriptors: zebrafish toxicology of pahayokolide, biogeography, population studies, freshwater ecology, environmental sciences, infection, pharmacology, toxicology *Bacillus* infection, bacterial disease, drug therapy, etiology, *Saccharomyces cerevisiae* infection, agal toxin, drug therapy.

Berry, J.P., M. Gantar, P.D. Gibbs, and M.C. Schmale (2007). **The zebrafish (*Danio rerio*) embryo as a model system for identification and characterization of developmental toxins from marine and freshwater microalgae.** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 145(1): 61-72. ISSN: 1532 0456.
NAL Call Number: QP901.C6
Abstract: The zebrafish (*Danio rerio*) embryo has emerged as an important model of vertebrate development. As such, this model system is finding utility in the investigation of toxic agents that inhibit, or otherwise interfere with, developmental processes (i.e. developmental toxins), including compounds that have potential relevance to both human and environmental health, as well as biomedicine. Recently, this system has been applied increasingly to the study of microbial toxins, and more specifically, as an aquatic animal model, has been employed to investigate toxins from marine and freshwater microalgae, including those classified among the so-called "harmful algal blooms" (HABs). We have developed this system for identification and characterization of toxins from cyanobacteria (i.e. "blue-green algae") isolated from the Florida Everglades and other freshwater sources in South and Central Florida. Here we review the use of this system as it has been applied generally to the investigation of toxins from marine and freshwater microalgae, and illustrate this utility as we have applied it to the detection, bioassay-guided fractionation and subsequent characterization of developmental toxins from freshwater cyanobacteria.
Descriptors: zebrafish embryo microbial toxins, blue-green algae chemistry, drug effects, embryonic development drug effects, marine toxins toxicity, teratogens toxicity, cyanobacteria chemistry, dinoflagellida chemistry, eutrophication, fresh water, water microbiology.

Biales, A.D., D.C. Bencic, J.i.m.L. Lazorchak, and D.L. Lattier (2007). **A quantitative real-time polymerase chain reaction method for the analysis of vitellogenin transcripts in model and nonmodel fish species**. *Environmental Toxicology and Chemistry* 26(12): 2679-2686. ISSN: 0730-7268.
NAL Call Number: QH545A1E58
Descriptors: zebrafish, habitat, aquatic ecosystems, estrogens, fresh water pollution, *Danio rerio*, *Oncorhynchus mykiss*, *Oryzias latipes.*

Billiard, S.M., A.R. Timme Laragy, D.M. Wassenberg, C. Cockman, and R.T. Di Giulio (2006). **The role of the aryl hydrocarbon receptor pathway in mediating synergistic developmental toxicity of polycyclic aromatic hydrocarbons to zebrafish.** *Toxicological Sciences* 92(2): 526-36. ISSN: 1096-6080.
NAL Call Number: RA1190.F8
Abstract: Planar halogenated aromatic hydrocarbons (pHAHs), such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin), show strong binding affinity for the aryl hydrocarbon receptor (AHR) and are potent inducers of cytochrome P4501A (CYP1A). It is widely accepted that dioxin toxicity is largely AHR mediated; however, the role of CYP1A activity in causing that toxicity is less clear. Another class of AHR agonists of increasing concern because of their known toxicity and ubiquity in the environment is the polycyclic aromatic hydrocarbons (PAHs). Like dioxin, some PAHs also cause toxicity to early life stages of vertebrates. Symptoms include increased cardiovascular dysfunction, pericardial and yolk sac edemas, subcutaneous hemorrhages, craniofacial deformities, reduced growth, and increased mortality rates. Although developmental effects are

comparable between these two types of AHR agonists, the roles of both the AHR and CYP1A activity in PAH toxicity are unknown. As observed in previous studies with killifish (*Fundulus heteroclitus*), we demonstrate here that coexposure of zebrafish (*Danio rerio*) embryos to the PAH-type AHR agonist beta-naphthoflavone (BNF) and the CYP1A inhibitor alpha-naphthoflavone (ANF) significantly enhanced toxicity above that observed for single-compound exposures. In order to elucidate the role of the AHR pathway in mediating synergistic toxicity of PAH mixtures to early life stages, we used a morpholino approach to knock down expression of zebrafish AHR2 and CYP1A proteins during development. We observed that while knock down of AHR2 reduces cardiac toxicity of BNF combined with ANF to zebrafish embryos, CYP1A knockdown markedly enhanced toxicity of BNF alone and BNF + ANF coexposures. These data support earlier chemical inducer/inhibitor studies and also suggest that mechanisms underlying developmental toxicity of PAH-type AHR agonists are different from those of pHAHs. Identifying the pathways involved in PAH toxicity will provide for more robust, mechanistic-based tools for risk assessment of single compounds and complex environmental mixtures.

Descriptors: zebrafish embryos, aromatic polycyclic hydrocarbons toxicity, aryl hydrocarbon receptors metabolism, benzoflavones toxicity , cytochrome P450 CYPA1A1 metabolism, antagonists, inhibitors and genetics, drug effects, embryology, metabolism, enzyme inhibitors toxicity, antisense oligonucleotides genetics, aryl hydrocarbon receptors agonists, aryl hydrocarbon receptors genetics, beta naphthoflavone toxicity.

Bilotta, J., J.A. Barnett, L. Hancock, and S. Saszik (2004). **Ethanol exposure alters zebrafish development: a novel model of fetal alcohol syndrome.** *Neurotoxicology and Teratology* 26(6): 737-43. ISSN: 0892-0362.

NAL Call Number: RA1224

Abstract: Prenatal exposure to alcohol has been shown to produce the overt physical and behavioral symptoms known as fetal alcohol syndrome (FAS) in humans. Also, it is believed that low concentrations and/or short durations of alcohol exposure can produce more subtle effects. The purpose of this study was to investigate the effects of embryonic ethanol exposure on the zebrafish (*Danio rerio*) in order to determine whether this species is a viable animal model for studying FAS. Fertilized embryos were reared in varying concentrations of ethanol (1.5% and 2.9%) and exposure times (e.g., 0-8, 6-24, 12-24, and 48-72 h postfertilization; hpf); anatomical measures including eye diameter and heart rate were compared across groups. Results found that at the highest concentration of ethanol (2.9%), there were more abnormal physical distortions and significantly higher mortality rates than any other group. Embryos exposed to ethanol for a shorter duration period (0-8 hpf) at a concentration of 1.5% exhibited more subtle effects such as significantly smaller eye diameter and lower heart rate than controls. These results indicate that embryonic alcohol exposure affects external and internal physical development and that the severity of these effects is a function of both the amount of ethanol and the timing of ethanol exposure. Thus, the zebrafish represents a useful model for examining basic questions about the effects of embryonic exposure to ethanol on development.

Descriptors: zebrafish, nervous system alcohol induced disorders physiopathology, abnormalities and drug effects, ethanol toxicity, chemically induced bradycardia , disease models, drug dose response relationship, drug administration schedule, physiology, chemically induced eye abnormalities, pathology, mortality, time factors, growth and development.

Blechinger, S.R., R.C. Kusch, K. Haugo, C. Matz, D.P. Chivers, and P.H. Krone (2007). **Brief embryonic cadmium exposure induces a stress response and cell death in the developing olfactory system followed by long-term olfactory deficits in juvenile zebrafish.** *Toxicology and Applied Pharmacology* 224(1): 72-80. ISSN: 0041-008X.

NAL Call Number: 391.8T662

Abstract: The toxic effects of cadmium and other metals have been well established. A primary target of these metals is known to be the olfactory system, and fish exposed to a number of different waterborne metals display deficiencies in olfaction. Importantly, exposure over embryonic/larval development periods can cause deficits in chemosensory function in juvenile fish, but the specific cell types affected are unknown. We have previously characterized a transgenic zebrafish strain expressing the green fluorescent protein (eGFP) gene linked to the hsp70 gene promoter, and shown it to be a useful tool for examining cell-specific toxicity in living embryos and larvae. Here we show that the hsp70/eGFP transgene is strongly and specifically upregulated within the olfactory sensory neurons (OSNs) of transgenic zebrafish larvae following a brief 3-h exposure to water-borne cadmium. This molecular response was closely correlated to an endpoint for tissue damage within the olfactory placode, namely cell death. Furthermore, cadmium-induced olfactory cytotoxicity in zebrafish larvae gives rise to more permanent effects. Juvenile zebrafish briefly exposed to cadmium during early larval development display deficits in olfactory-dependent predator avoidance behaviors 4-6 weeks after a return to clean water. Lateral line neuromasts of exposed zebrafish larvae also activate both the endogenous hsp70 gene and the hsp70/eGFP transgene. The data reveal that even a very brief exposure period that gives rise to cell death within the developing olfactory placode results in long-term deficits in olfaction, and that hsp70/eGFP may serve as an effective indicator of sublethal cadmium exposure in sensory cells.

Descriptors: zebrafish larvae, cadmium toxicity, cell death drug effects, physiology, olfaction disorders chemically induced, smell drug effects , stress pathology, behavior drug effects, endpoint determination, HSP70 heat shock proteins biosynthesis and genetics, computer assisted image processing, in situ hybridization, in situ nick end labeling, indicators and reagents, larva physiology, lateral line system pathology, afferent neurons drug effects and metabolism, olfaction disorders psychology, olfactory mucosa pathology, predatory behavior drug effects, up regulation drug effects, chemical water pollutants toxicity.

Bogers, R., E. Mutsaerds, J. Druke, D.F. De Roode, A.J. Murk, B. Van Der Burg, and J. Legler (2006). **Estrogenic endpoints in fish early life-stage tests: luciferase and vitellogenin induction in estrogen-responsive transgenic zebrafish.** *Environmental Toxicology and Chemistry* 25(1): 241-7. ISSN: 0730 7268.

NAL Call Number: QH545A1E58

Abstract: This study incorporated specific endpoints for estrogenic activity in the early life-stage (ELS) test, as described in Guideline 210 of the Organization for Economic Cooperation and Development and traditionally used for toxicity screening of chemicals. A transgenic zebrafish model expressing an estrogen receptor-mediated luciferase reporter gene was exposed to ethinylestradiol (EE2), and luciferase activity as well as vitellogenin (VTG) was measured. Concentrations of EE2 were tested at 1, 3, or 10 ng/L for 30 d from fertilization or during only the last 4 d with dimethylsulfoxide (DMSO) as presolvent (0.01%). Exposure to EE2 induced no toxic effects. Mean body weights were significantly higher in groups exposed for 30 d in the presence of DMSO, but condition factors were not affected. Significant luciferase and VTG induction occurred following 30-d exposure (3 and 10 ng EE2/L), while only VTG levels were affected in the 4-d exposure (10 ng EE2/L). This study demonstrated the usefulness of incorporating estrogenic endpoints in the OECD ELS test, fitting the requirements for screening estrogenic activity of chemicals. Quantitative measurement of both VTG and luciferase activity proved to be rapid and sensitive. Additional value of using transgenic zebrafish lies in combining VTG measurement with the more mechanistic approach of luciferase induction in one experiment.

Descriptors: transgenic zebrafish model, ethinyl estradiol toxicity, luciferases metabolism,

vitellogenins metabolism, genetically modified embryology and metabolism, reporter genes, luciferases genetics, estrogen receptors, metabolism, toxicity tests methods.

Bolcome, R.E., S.E. Sullivan, R. Zeller, A.P. Barker, R. Collier, and J. Chan (2008). **Anthrax lethal toxin induces cell death-independent permeability in zebrafish vasculature**. *Proceedings of the National Academy of Sciences, USA* 105(7): 2439-2444. ISSN: 0027-8424.
NAL Call Number: 500N21P
Descriptors: zebrafish bacterial toxin model, acridine orange, anthrax lethal toxin, blood vessels, cell death, embryos, endothelial cells, extravasation, permeability, signal transduction, vascular endothelial growth factor, vascular endothelial growth factor receptors, vascular permeability factor, vascular system, imaging, microspheres, *Bacillus anthracis*, *Danio rerio.*

Bradfield, J.Y., J.R. West, and S.E. Maier (2005). **Measurement of zebrafish embryo ethanol concentration**. *Alcoholism Clinical and Experimental Research* 29(5, Suppl. S): 128A. ISSN: 0145 6008.
Descriptors: zebrafish embryo, toxicology, development, ethanol toxicity model.

Bradfield, J.Y., J.R. West, and S.E. Maier (2006). **Uptake and elimination of ethanol by young zebrafish embryos.** *Neurotoxicology and Teratology* 28(5): 629-33. ISSN: 0892-0362.
NAL Call Number: RA1224
Abstract: Among animal models being explored to understand ethanol-induced teratogenesis, the zebrafish (*Danio rerio*) is attracting attention because its embryonic development is well characterized and readily visualized. Despite the potential of the zebrafish embryo in research on developmental anomalies produced by ethanol exposure, little is known about the relationship between embryonic ethanol content and the nature/severity of ethanol-mediated deficits. Here, using gas chromatography and radiometry of labeled ethanol carbon, we examine accumulation and clearance of ethanol by dechorionated zebrafish embryos during blastulation/gastrulation. Our data indicate that: (a) rates of uptake and loss of ethanol are directly proportional to the extra-/intra-embryonic ethanol concentration gradient and (b) ethanol in the water fraction of embryos reaches near equimolarity with ethanol in the exposure medium. It appears that, within a wide range of exposure concentrations, embryonic ethanol content can be predicted accurately according to exposure time. Furthermore, it appears that embryonic ethanol can be adjusted rapidly to and maintained at a targeted concentration.
Descriptors: zebrafish, ethanol drug effects, metabolism and pharmacology, analysis of variance, body weight drug effects, carbon isotopes metabolism, gas chromatography methods, drug dose response relationship, radiometry methods, time factors.

Bresolin, T., M. de Freitas Rebelo, and A. Celso Dias Bainy (2005). **Expression of PXR, CYP3A and MDR1 genes in liver of zebrafish.** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 140(3-4): 403-7. ISSN: 1532 0456.
NAL Call Number: QP901.C6
Abstract: The Pregnane X Receptor (PXR) is a nuclear receptor involved in the transcriptional regulation of drug-metabolizing enzymes and transporters. In mammals, many xenobiotics induce the expression of cytochrome P4503A (CYP3A) and the multiple drug resistance 1 (MDR1) genes via the PXR pathway. Little attention has been given to studies about the identification and biological function of PXR homologues in non-mammalian species. Zebrafish is being widely used and accepted as model for toxicological and pharmacological studies to understand the mechanisms of human diseases and identify conserved signaling pathways. The aim of this study was to evaluate the in vivo expression of PXR, CYP3A and MDR1 genes in liver of zebrafish treated with the synthetic steroid pregnenolone 16alpha-carboninitrile (PCN), the antimycotic

clotrimazole (CTZ) and the antianginal drug nifedipine (NIF). The liver of fish treated with PCN showed a 1.9-fold induction in the PXR followed by 1.8-fold induction in the CYP3A and 1.6-fold induction in the MDR1 mRNA. CTZ and NIF did not affect statistically the expression of PXR, CYP3A and MDR1. The similar pattern of mRNA expression of PXR, CYP3A and MDR1 genes found in fish treated with different PXR inducers suggests that the intrinsic association between these three genes is conserved in zebrafish.

Descriptors: zebrafish PXR homologus, aryl hydrocarbon hydroxylases genetics, gene expression regulation drug effects, liver metabolism, oxidoreductases, N demethylating genetics, P glycoprotein genetics, cytoplasmic receptors and nuclear genetics, steroid receptors genetics, clotrimazole pharmacology, cytochrome P450 CYP3A, liver drug effects, nifedipine pharmacology, pregnenolone carbonitrile pharmacology.

Bretaud, S., S. Lee, and S. Guo (2004). **Sensitivity of zebrafish to environmental toxins implicated in Parkinson's disease.** *Neurotoxicology and Teratology* 26(6): 857-64. ISSN: 0892-0362.

NAL Call Number: RA1224

Abstract: Parkinson's disease (PD) is characterized by the progressive loss of dopaminergic (DA) neurons in the substantia nigra and movement defects, including bradykinesia, tremor, and postural imbalance. Whereas the etiology and pathogenesis of PD is still poorly understood, studies in animal models are providing important insights. One valuable type of animal model for PD is established by treating animals with PD-inducing neurotoxins, including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, and paraquat. These neurotoxins are thought to inhibit mitochondrial complex I activity leading to oxidative stress, impaired energy metabolism, proteasomal dysfunction, and, eventually, dopamine neuronal loss. However, the genes and pathways that underlie the neurotoxicity of these agents are not known. In this study, we explored the effect of MPTP, rotenone, and paraquat in both adult and larval zebrafish, which are highly amenable to genetic analysis that can lead to the identification of the underlying genes and pathways. Here, we report that adult zebrafish display behavioral alterations, including decreased locomotor activity in response to MPTP, whereas larval zebrafish exhibited developmental, behavioral, and DA sensitivity to these agents. Taken together, these findings suggest that zebrafish could be a valuable model for genetically dissecting the molecular mechanisms underlying the neurotoxicity of PD-inducing agents.

Descriptors: zebrafish, central nervous system drug effects, motor activity drug effects, neurotoxins toxicity, secondary chemically induced Parkinson disease, 1 methyl 4 phenyl 1,2,3,6 tetrahydropyridine adverse effects, 1 methyl 4 phenylpyridinium toxicity, behavior drug effects and physiology, cardiovascular abnormalities chemically induced, catecholamines metabolism, central nervous system metabolism and physiopathology, drug dose response relationship and synergism, abnormalities, drug effects, physiopathology, larva drug effects, growth and development, motor activity genetics, neurons drug effects, metabolism and pathology, paraquat toxicity, pigmentation drug effects and genetics, rotenone toxicity, MPTP toxicity.

Brion, F., C.R. Tyler, X. Palazzi, B. Laillet, J.M. Porcher, J. Garric, and P. Flammarion (2004). **Impacts of 17beta-estradiol, including environmentally relevant concentrations, on reproduction after exposure during embryo-larval-, juvenile- and adult-life stages in zebrafish (*Danio rerio*).** *Aquatic Toxicology* 68(3): 193-217. ISSN: 0166 445X.

NAL Call Number: QH541.5.W3A6

Abstract: Zebrafish *(Danio rerio*) were exposed for 3 weeks to low concentrations of estradiol including environmentally relevant concentrations (5, 25 and 100 ng/l), encompassing either their embryo-larvae (from fertilization to 21 day post-fertilization (dpf)), juvenile (from 21 to 42 dpf) or adult life stages (>200 dpf) with a view to investigating the most sensitive life stage of the

zebrafish to 17beta-estradiol (E2). At all sampling points, whole-body vitellogenin concentrations and gonadal development were analyzed in order to investigate the effects of estrogen exposure on these endpoint in the zebrafish. In the adult stage, additional endpoints were measured including secondary sexual characteristics (manifestation of the uro-genital papillae (UGP) in males), gonadal growth (the gonado-somatic index (GSI)) and sex ratio. For all the different life stage exposures, reproductive performance of the F0 generation was assessed (egg production) and survival and development of the F1 embryo-larvae. Exposure to low concentrations of E2 resulted in vitellogenin induction whatever the life stage exposed but these effects were reversible after depuration. The effective concentration for vitellogenin induction in zebrafish early life stages was 100 ng E2/l, and in adult male zebrafish the effective concentration for vitellogenin induction (between 5 and 25 ng/l) was lower than for the early life stage fish. Exposure to E2 prior to (from fertilization to 21 dpf) and during the time of sex differentiation (from 21 to 42 dpf) also caused disruptions in the process of sexual differentiation (resulting in formation of a retrogonadal cavity in presumptive male, germ cell development and leading to a significant change of the sex ratio towards the female sex at the dose of 100 ng E2/l for the fish exposure as embryo-larvae) and altered patterns of egg production in the subsequent adults. Exposure of adult fish to E2 resulted in a modification of the secondary sexual characteristic in males at 25 and 100 ng E2/l as well as a dose-dependent inhibition of egg production. The findings from this study show that the nature and intensity of the reproductive effects of E2 are dependent of the time and concentration of exposures of zebrafish to E2, some of these effects being permanent (effect on the sexual differentiation) while others being reversible (effect on the Vtg induction). This study demonstrated that early life stages of zebrafish are sensitive to low concentrations of E2 and provides relevant data that could be used for the adaptation of existing fish early life stage test for the in vivo testing of estrogenic compounds. The data presented raise further concerns about the effects of steroid estrogens in the environment on fish reproductive health.

Descriptors: zebrafish toxicity model, environmental exposure, estradiol toxicity, reproduction drug effects, age factors, analysis of variance, biometry, drug dose response relationship, gonads drug effects, growth and development, histological techniques, larva physiology, reproduction physiology, sex characteristics, sex ratio, time factors, vitellogenins metabolism.

Burnison, B.K., T. Meinelt, R. Playle, M. Pietrock, A. Wienke, and C.E. Steinberg (2006). **Cadmium accumulation in zebrafish (*Danio rerio*) eggs is modulated by dissolved organic matter (DOM).** *Aquatic Toxicology* 79(2): 185-91. ISSN: 0166 445X.

NAL Call Number: QH541.5W3A6

Abstract: Experiments were conducted to investigate factors influencing the accumulation of cadmium (Cd(2+)) into zebrafish (*Danio rerio*) eggs. The accumulation of (109)Cd was affected by: (1) concentration, (2) time, (3) presence of dissolved organic material (DOM), (4) different origin of DOM and (5) different parts of fish eggs. Over a 5-h exposure, zebrafish eggs showed a steady increase in Cd-accumulation. DOM-concentrations over 15ppm carbon (C) decreased Cd-uptake significantly. Both samples of DOM, brown water marsh (LM) and a eutrophic pond (SP), at 16.9ppmC, reduced the Cd-accumulation in the chorion, perivitelline liquid and the embryo. Cd was mainly accumulated in the egg's outer shell chorion (61%) and only small amounts passed through the chorion into the perivitelline liquid (38%) and embryo (1%). In the presence of LM-DOM, the accumulation of Cd into the egg components was decreased by 43% (chorion), 52% (perivitelline liquid) and 52% (embryo), respectively, compared with the control group. Similarly, the presence of SP-DOM reduced the Cd-accumulation by 29% (chorion), 61% (perivitelline liquid) and 60% (embryo), respectively, compared with the controls. DOM-concentration should be taken into consideration when determining ecotoxicological effects of Cd on fish populations.

Descriptors: zebrafish eggs, embryos, cadmium pharmacokinetics, humic substances toxicity, ovum metabolism, chemical water pollutants toxicity, metabolism, cadmium toxicity, cadmium radioisotopes diagnostic use, chorion chemistry and physiology, drug dose response relationship, chemistry, physiology, fresh water chemistry, ovum chemistry, ovum drug effects, scintillation counting, time factors.

Bury, N.R. and M. Grosell (2003). **Waterborne iron acquisition by a freshwater teleost fish, zebrafish *Danio rerio*.** *Journal of Experimental Biology* 206(Pt 19): 3529-35. ISSN: 0022-0949.
NAL Call Number: 442.8B77
Abstract: Waterborne iron accumulation by the gills of the zebrafish *Danio rerio* was assessed in ion-poor water. Branchial iron uptake, which comprises both the iron that has entered the gill cells and iron that is strongly bound to the epithelia, has high- and low-affinity components. At low nominal [Fe] (<40 nmol l(-1)) the high-affinity component demonstrated saturation kinetics, with an apparent K(m) of 5.9 nmol l(-1) Fe and V(max) of 2.1 pmol g(-1) h(-1). Over a range of higher nominal [Fe] (40-200 nmol l(-1)), branchial uptake was linear. In the presence of 2 micro mol l(-1) of the reducing agent dithiothreitol (DTT), branchial iron accumulation was significantly enhanced at [Fe]>15 nmol l(-1). The proton pump inhibitor bafilomycin A significantly reduced iron uptake in the presence of DTT. On the basis of these observations we conclude that branchial iron uptake at low [Fe] shows characteristics similar to those of other iron-transporting epithelia, coupling an apical membrane ferric reductase to a Fe(2+)/H(+) symporter. Zebrafish branchial iron transport at 18.6 nmol l(-1) was inhibited by 200 nmol l(-1) Cd(2+). But, unlike other Fe(2+)/H(+) symporters, iron uptake was not affected by other divalent metals (Co(2+), Ni(2+), Pb(2+), Cu(2+), Zn(2+) and Mn(2+)). Zebrafish loaded with (59)Fe from the water showed a loss of 7.9 pmol Fe g(-1) body mass over the first day and a further loss of 5.7 pmol Fe g(-1) body mass over the following 28 days. The depuration kinetics followed a two-component exponential model; for the short-lived component, t(1/2)=0.31 days, and for the long-lived component, t(1/2)=13.2 days. The daily iron loss by zebrafish can be compensated by iron uptake at exceedingly low water iron concentrations (uptake rate at 1.625 nmol l(-1) Fe=0.425 pmol g(-1) h(-1)), demonstrating that uptake of iron from the water is potentially an important source of this nutritive metal in freshwater teleost fish.
Descriptors: zebrafish, gills metabolism, iron pharmacokinetics, metabolism, biological transport, active drug effects, cadmium pharmacology, dithiothreitol pharmacology, fresh water, iron metabolism, macrolides pharmacology.

Campagna, A.F., M.N. Eler, R. Fracacio, B.K. Rodrigues, and N.F. Verani (2007). **The toxic potential of aldrin and heptachlor on *Danio rerio* juveniles (Cypriniformes, Cyprinidae)**. *Ecotoxicology* 16(3): 289-298. ISSN: 0963 9292.
NAL Call Number: RA565.A1E27
Abstract: With the objective of evaluating the effects of organochlorine pesticides (aldrin and heptachlor) on the survival, growth and gill morphology of juvenile zebrafish (*Danio rerio*), four partial chronic toxicity bioassays were conducted (seven days' duration) with both compounds in a semi-static system with renewal every 24 h. The results did not show any effects on the fish's survival, but did on their growth and gill morphology. Aldrin was more toxic than heptachlor, since the chronic value (CV) for growth was nearly 8.7 times less, a result confirmed by analyzing the histology of the gills, in which the changes detected were considered more severe for the former substance. In general, they were found branchial lesions of first stage, in other words, cell proliferation between the secondary lamellae, hyperplasia, lifting of respiratory epithelial cells; fusion of several secondary lamellae and dilation of blood vessels. Aneurysms (alterations of second stage) they were verified in the exposed organisms to the aldrin, which also presented

about 10% of reduction in the standard length and 30% of reduction in the total weight in relation to the exposed organisms to the control. For heptachlor these values were of 8% and 25%, respectively. The intensity of the gill lesions and growth of the fish did not depend on the pesticide concentration, suggesting different modes of action of the products. Copyright © 2008 CABI

Descriptors: zebrafish pesticide toxicity model, aldrin, heptachlor, insecticide residues, insecticides, morphology, nontarget effects, nontarget organisms, survival, growth toxicity, *Danio rerio.*

Cao, Z., R.L. Tanguay, D. McKenzie, R.E. Peterson, and J.M. Aiken (2003). **Identification of a putative calcium-binding protein as a dioxin-responsive gene in zebrafish and rainbow trout.** *Aquatic Toxicology* 63(3): 271-82. ISSN: 0166 445X.

NAL Call Number: QH541.5.W3A6

Abstract: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD, dioxin) is a widespread environmental contaminant that causes multiple effects in vertebrates. TCDD elicits its toxicity through aryl hydrocarbon receptor (AhR)-mediated modulation of gene regulation, increasing intracellular free calcium, and inducing calcium-mediated apoptosis in cell culture. Two TCDD-responsive cDNAs, which encode putative calcium-binding proteins, have been isolated from zebrafish and rainbow trout. The zebrafish and rainbow trout sequences are 88% similar to each other at the amino acid level and are orthologs of the human S100A4 calcium-binding protein. In zebrafish liver cell culture, treatment with TCDD increases S100A4a mRNA abundance. In juvenile rainbow trout, S100A4 mRNA was constitutively expressed in the heart, kidney, intestine, and spleen, but not in the liver. Exposure to TCDD significantly increased rainbow trout S100A4 mRNA abundance in the rainbow trout kidney. Taken together, these findings demonstrate in zebrafish and rainbow trout that dioxin increases expression of this EF-hand calcium-binding protein gene in a tissue-dependent fashion. However, demonstration that the encoded S100A4 proteins actually bind calcium and play a role in dioxin toxicity will require further study.

Descriptors: zebrafish, rainbow trout, dioxin containment models, calcium binding proteins metabolism, environmental pollutants toxicity, *Oncorhynchus mykiss* genetics and metabolism, tetrachlorodibenzodioxin toxicity, amino acid and base sequence, calcium binding proteins drug effects, genetics, cultured cells, gene expression physiology, kidney metabolism, liver cytology, metabolism, molecular sequence data, S100 proteins drug effects, genetics, metabolism, isolation and purification, sequence homology, species specificity, tissue distribution.

Capps, T., S. Mukhi, J.J. Rinchard, C.W. Theodorakis, V.S. Blazer, and R. Patino (2004). **Exposure to perchlorate induces the formation of macrophage aggregates in the trunk kidney of zebrafish and mosquitofish**. *Journal of Aquatic Animal Health* 16(3): 145-151. ISSN: 0899 7659.

NAL Call Number: SH171.J68

Abstract: Environmental contamination of ground and surface waters by perchlorate, derived from ammonium perchlorate (AP) and other perchlorate salts, is of increasing concern. Exposure to perchlorate can impair the thyroid endocrine system, which is thought to modulate renal and immune function in vertebrates. This study with zebrafish *Danio rerio* and eastern mosquitofish *Gambusia holbrooki* examined the histological effects of perchlorate on the trunk kidney, which in teleosts serves excretory and hemopoietic functions and therefore may be a target of perchlorate effects. Adult zebrafish of both sexes were exposed in the laboratory to waterborne, AP-derived perchlorate at measured concentrations of 18

mg/L for 8 weeks. Adult male mosquitofish were exposed to waterborne sodium perchlorate at measured perchlorate concentrations of 1-92 mg/L for 8 weeks. Control fish were kept in untreated water. The region of the body cavity containing the trunk kidney was processed from each fish for histological analysis. Macrophage aggregates (MAs), possible markers of contaminant exposure or immunotoxic effect, were present in the hemopoietic region of the kidney in both species exposed to perchlorate. The estimated percent area of kidney sections occupied by MAs was greater in zebrafish exposed to perchlorate at 18 mg/L (P<0.05) than in controls. In male mosquitofish, the incidence of renal MAs increased proportionally with sodium perchlorate concentration and was significantly different from that of controls at 92 mg/L (P<0.05). These observations confirm that in fish the kidney is affected by exposure to perchlorate. The concentrations of perchlorate at which the effects were noted are relatively high but within the range reported in some contaminated habitats. Copyright © 2008 CABI
Descriptors: zebrafish, eastern mosquitofish, kidneys, perchlorate exposure, macrophages, toxicity, toxicology, water pollution, *Danio rerio*, *Gambusia holbrooki.*

Carlsson, G. and L. Norrgren (2004). **Synthetic musk toxicity to early life stages of zebrafish (*Danio rerio*).** *Archives of Environmental Contamination and Toxicology* 46(1): 102-5. ISSN: 0090 4341.
NAL Call Number: TD.172.A7
Abstract: Synthetic musk substances have been found in a number of environmental samples. Some of these chemicals have been detected in concentrations above 1 microg/L in water, which raises concern about possible effects on aquatic life. The toxicity of four synthetic musks, 4-tert-butyl-2,6-dimethyl-3,5-dinitrophenylethanone (musk ketone), 1-tert-butyl-3,5-dimethyl-2,4,6-trinitrobenzene (musk xylene), 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)ethanone (AHTN, tonalide) and 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-[g]-2-benzopyrane (HHCB, galaxolide), were studied in zebrafish by the use of two different early life-stage methods. In the first method, specific developmental characteristics during the first 48 hours were studied. In the second method, hatching and survival times were studied on eggs and larvae. The results on heart rate in the first test gave the following LOECs: musk ketone 10 microg/L, musk xylene and AHTN 33 microg/L, and HHCB showed no effect up to 1000 microg/L. In the study of survival time, LOEC for musk ketone was 100 microg/L, for musk xylene 33 microg/L, and AHTN gave no effect on survival time up to 100 microg/L. The LOECs for musk ketone, musk xylene, and AHTN in this study are in the range of what has been measured in sewage effluents and recipients, and consequently these substances may have adverse impact on wild fish.
Descriptors: zebrafish, monounsaturated fatty acids toxicity and chemical synthesis, chemical water pollutants toxicity, lethal dose 50, odors, ovum growth and development, survival analysis.

Carvan, M.J.3., E. Loucks, D.N. Weber, and F.E. Williams (2004). **Ethanol effects on the developing zebrafish: neurobehavior and skeletal morphogenesis.** *Neurotoxicology and Teratology* 26(6): 757-68. ISSN: 0892-0362.
NAL Call Number: RA1224
Abstract: Exposure to ethanol during development can lead to a constellation of congenital anomalies, resulting in prenatal and postnatal failure to thrive, central nervous system (CNS) deficits, and a number of patterning defects that lead to defects in the cardiovascular system, facial structures, and limbs. The cellular, biochemical, and molecular mechanisms by which ethanol exerts its developmental toxicity and the genes that influence sensitivity

to developmental ethanol exposure have yet to be discovered, despite being one of the more common nongenetic causes of birth defects. The zebrafish undergoes much the same patterning and morphogenesis as other vertebrate embryos do--including humans--that are distinct and cannot be studied in invertebrates. Developmental processes in zebrafish are affected by ethanol exposure in a dose-dependent manner, resulting in learning and memory deficits, cell death in the CNS, skeletal dysmorphogenesis, and alterations in startle reflex responses. Interestingly, significant ethanol effects on learning and behavioral endpoints occurred at concentrations well below those that induced cell death in the CNS. This work provides the foundation for identifying genes and pathways involved in developmental alcohol toxicity in vertebrates, leading to a more complete mechanistic understanding of fetal alcohol disorders in humans.

Descriptors: zebrafish, nervous system alcohol induced disorders physiopathology, chemically induced craniofacial abnormalities , ethanol toxicity model, abnormalities, growth and development, nervous system alcohol induced disorders pathology, behavior drug effects and physiology, bone and bones abnormalities and drug effects, cell death drug effects and genetics, craniofacial abnormalities pathology, disease models, drug dose response relationship, abnormalities, drug effects, larva drug effects, growth and development, learning and memory disorders chemically induced and physiopathology, startle reaction drug effects and genetics.

Cazenave, J., M.D.l.A. Bistoni, E. Zwirnmann, D.A. Wunderlin, and C. Wiegand (2006). **Attenuating effects of natural organic matter on microcystin toxicity in zebra fish (*Danio rerio*) embryos - benefits and costs of microcystin detoxication**. *Environmental Toxicology* 21(1): 22-32. ISSN: 1520 4081.

NAL Call Number: RA1221.T69

Abstract: To contribute to the understanding of joined factors in the environment, impact of pure microcystins (-RR and -LF) on zebra fish (*Danio rerio*) embryos were investigated individually and in combination with a natural organic matter (NOM). The applied NOM was a reverse osmosis isolate from Lake Schwarzer See (i.e., Black Lake, BL-NOM). Teratogenic effects were evaluated through changes in embryonic development within 48 h of exposure. Detoxication activities were assessed by the activities of phase II biotransformation enzymes, soluble and microsomal glutathione S-transferase (s, mGST). Oxidative stress was assessed by determining both the production of hydrogen peroxide and by analyzing the activities of the antioxidative enzymes, guajacol peroxidase (POD), catalase (CAT), glutathione peroxidase (GPx), and the glutathione restoring enzyme glutathione reductase (GR). Energetic costs were evaluated by determining contents of fat, carbohydrates, and proteins in both exposed and control embryos. BL-NOM attenuated toxic effects of MC-LF and MC-RR verified by less pronounced teratological effects within 24 h, in particular, as well as less rise in the activity of s-GST, when compared with embryos exposed to either pure toxins or in combination with organic matter. BL-NOM also diminished oxidative effects caused by MC-LF; however, it failed to attenuate oxidative stress caused by MC-RR. Content of lipids was significantly reduced in exposed embryos following a trend similar to that obtained with teratological and enzymatic assays confirming the attenuating effect of BL-NOM. Physiological responses to microcystins and NOM required energetic costs, which were compensated to the expense of the energy resources of the yolk, which in turn might affect the normal development of embryos. Copyright © 2008 CABI

Descriptors: zebrafish embryos, attenuation, bacterial toxins, carbohydrates, catalase, detoxification, embryonic development, enzyme activity, enzymes, fat, glutathione peroxidase,

glutathione reductase NADPH, glutathione transferase, hydrogen peroxide, lipids, organic matter, peroxidases, protein Content, teratogenesis, toxicity, *Danio rerio.*

Chan, P.K. and S.H. Cheng (2003). **Cadmium-induced ectopic apoptosis in zebrafish embryos.** *Archives of Toxicology* 77(2): 69-79. ISSN: 0340 5761.

Abstract: In this study, we tested the hypothesis that cadmium-induced developmental toxicity was mediated via ectopic occurrence of apoptosis during embryonic development. We employed confocal microscopy to acquire images of whole-mount staining of apoptotic cells in zebrafish embryo exposed to 100 micro M cadmium from 5 hours post fertilisation (hpf) to 28 hpf. Three-dimensional reconstruction of the images was performed and the spatial and temporal distributions of apoptotic cells in the embryos were compared. In cadmium-treated embryos with varying degrees of gross developmental malformations, significantly higher numbers of apoptotic cells were detected with this method. In order to detect the precise locations of apoptotic cells, we performed terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) assay in sectioned embryos. In the degenerating neural tube of cadmium-treated embryos apoptotic cells were detected, while in the healthy neural tube of the untreated controls no apoptotic cells were found. We then employed flow cytometry to investigate whether cadmium exposure would affect the dynamics of apoptosis or induce any abnormalities in cell-cycle progression. It appeared that cadmium did not induce cell-cycle arrest. The percentages of apoptotic cells did not differ in the two groups at 13, 16 or 19 hpf. At 28 hpf, however, a significantly higher percentage of apoptotic cells were found in the cadmium-treated group. Exposure to cadmium, therefore, induced ectopic apoptosis at 28 hpf without affecting the dynamics of apoptosis at earlier developmental stages.

Descriptors: zebrafish embryology, apoptosis drug effects, cadmium toxicity, drug effects, chemical water pollutants toxicity, cell count, DNA analysis, DNA drug effects, pathology, flow cytometry, in situ nick end labeling , confocal microscopy, time factors.

Chan, P.K. and S.H. Cheng (2003). **Fractal analysis of vascular complexity in cadmium-treated zebrafish embryos.** *Aquatic Toxicology* 63(1): 83-7. ISSN: 0166 445X.

NAL Call Number: QH541.5.W3A6

Descriptors: zebrafish embryology, blood vessels abnormalities, cadmium toxicity, fractals, zebrafish abnormalities, angiography, blood vessels drug effects, computer assisted image processing, biological models.

Chang, B.E., M.H. Liao, M.Y. Kuo, and C.H. Chen (2004). **Developmental toxicity of arecoline, the major alkaloid in betel nuts, in zebrafish embryos.** *Birth Defects Research* 70(1): 28-36. ISSN: 1542 0752.

NAL Call Number: QL991.T4

Descriptors: zebrafish embryos, arecoline toxicity, morphogenesis drug effects, areca, base sequence, DNA primers , drug effects, physiology, in situ hybridization, RNA genetics, isolation and purification, reverse transcriptase PCR.

Chen Chang (2003). **Molluscicides and their application in China**. *Chinese Journal of Schistosomiasis Control* 15(5): 321-322. ISSN: 1005 6661.

Abstract: This general account of *Oncomelania hupensis* control in China refers to the use of the molluscicide niclosamide, the results of laboratory and field trials using different concentrations of molluscicides, and the use of biological control agents such as the freshwater fish *Brachydanio rerio*. Copyright © 2008 CABI

Descriptors: zebrafish, aquatic animals, biological control, biological control agents, disease

control, freshwater fishes and molluscs, human diseases, intermediate hosts, molluscicides, niclosamide, predators, schistosomiasis, cyprinidae, *Danio rerio*, fishes, man, mollusca, *Oncomelania hupensis*, *Schistosoma japonicum*, snails.
Language of Text: Chinese.

Chen, H., Z. Li, Y.u.n. Xu, M. Qu, and Z. Kong (2006). **Ecotoxicological effects of the veterinary additive olaquindox**. *Journal of Agro-Environment Science* 25(4): 885-889. ISSN: 1672-2043.
NAL Call Number: S589.75.N86
Descriptors: zebrafish embryos, acute toxicity, additives, carp, DNA, ecological effects, electrophoresis, environmental factors, enzymes, fish, freshwater fish, genotoxicity, kidneys, testing procedures, toxicity tests, veterinary medicine, water pollution effects, acute toxicity, earthworms, toxicity testing, *Cyprinus carpio*, *Danio rerio*, *Eisenia fetida*, *Eisenia foetida*.

Chen Sheng, X., M. Yang Xiao, Wu Liang, G. Li Long, X. Zhang Rong, Xia Lei, and H. Shao Shi (2007). **Study on molluscicidal effect of the extracts from sarcotesta of Ginkgo biloba**. *Chinese Journal of Parasitology and Parasitic Diseases* 25(1): 45-48. ISSN: 1000 7423.
Abstract: This study evaluated the molluscicidal activity of extracts from sarcotesta from *G. biloba*. *Oncomelania hupensis* snails were immersed in different concentrations of dry powder sarcotesta of *G. biloba* (DPGB), extract of G. biloba sarcotesta by water (EGSW) and EGSB for molluscicide screening to observe the molluscicidal activity and the inhibiting effect on the snails' climbing ability as well as acute toxicity to *Brachydanio rerio*. Niclosamide was used as control. Extractions from *G. biloba* all showed molluscicidal activity, with EGSB as the best. Its 24 h LC50 and LC90 were 0.65 mg/L, and the 48 h LC50 and LC90 were 0.07 mg/L and 0.85 mg/L, respectively. The combination of EGSB and ARE showed better effect than EGSB alone. Its 24 h LC50 and LC90 were 0.26 mg/L and 0.56 mg/L respectively, a sharp decrease by 60% and 90% compared to EGSB ($P<0.05$). Under the concentration of 2.50 mg/L of EGSB, the rate of snails' climbing was 10%, while under the concentration of 0.16 mg/L of the EGSB + ARE combination, the rate was 8%. The inhibition on the snails' climbing of the combination was stronger than EGSB. The fish survived for 24 h and 10 h respectively at the concentration of 1 x LC90 and 2 x LC90 of EGSB. Under the concentration of 2 x LC90 of the combination, only 50% of the fish died and no fish died at the concentration of 1 x LC90. The toxicity of the combination was lower than EGSB alone. In conclusion, EGSB shows adequate molluscicidal activity and it is worth of further investigation. Copyright © 2008 CABI
Descriptors: zebrafish toxicity testing model, molluscicidal plants, molluscicidal properties, molluscicides, toxicology, *Danio rerio*, *Ginkgo biloba*, *Oncomelania hupensis*, plants, snails.
Language of Text: Chinese.

Chen, W.Y., J.A. John, C.H. Lin, and C.Y. Chang (2007). **Expression pattern of metallothionein, MTF-1 nuclear translocation, and its dna-binding activity in zebrafish (*Danio rerio*) induced by zinc and cadmium.** *Environmental Toxicology and Chemistry* 26(1): 110-7. ISSN: 0730 7268.
NAL Call Number: QH545A1E58
Abstract: Metallothionein is a small (6-kDa), cysteine-rich protein expressed by a six-zinc finger protein called metal-responsive element-binding transcription factor-1 (MTF-1) in response to Zn and Cd. Our previous reports have shown the basal expression of metallothionein (mt) and MTF-I (mtf-1) genes in embryo and early larval stages of zebrafish (*Danio rerio*). In the present study, we investigated the mt expression in zebrafish early larvae

induced by exposure to Cd and Zn (48, 72, 96, and 120 h postfertilization). Whole-mount in situ hybridization showed that Zn induced mt expression in the olfactory pit, cerebellum, ceratobranchials, liver, chloride cells, and neuromasts of the lateral line. Cadmium also induced mt expression in all the above regions except the cerebellum. Using fluorescence techniques, we have shown that Zn and Cd mediate cytoplasmic and nuclear translocation of MTF- 1-enhanced green fluorescent protein fusion protein in zebrafish liver cell line. The MTF-1 protein was produced recombinantly by inserting zebrafish mtf-1 cDNA (1.8 kb) into pET-20b(+) expression vector and expressing in *Escherichia coli* BL21 (DE3) pLysS host strain competent cell on induction with isopropyl-beta-D-thiogalactopyranoside. The protein was then purified by affinity chromatography on a nickel-nitrilotriacetic acid column. Electrophoretic mobility shift assay revealed binding of the recombinant MTF-1 in response to Zn and Cd at the putative metal-responsive elements (MREs) in the promoter region of the mt gene. Taken together, these results suggest that Zn and Cd are efficiently involved with mt expression induced in zebrafish embryos and with MTF-1 nuclear translocation and that this induction is achieved through the activation of MTF-1 binding at the MREs.
Descriptors: zebrafish larvae, heavy metal toxicity model, cadmium toxicity, DNA binding proteins genetics, proteins metabolism and genetics, gene expression regulation drug effects, metallothionein genetics, transcription factors metabolism, zinc toxicity, base sequence, DNA primers, electrophoretic mobility shift assay, protein transport, recombinant proteins genetics and metabolism, transcription factors genetics.

Chen, W., J. John, C. Lin, H. Lin, S. Wu, C. Lin, and C. Chang (2004). **Expression of metallothionein gene during embryonic and early larval development in zebrafish.** *Aquatic Toxicology* 69(3): 215-27. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: Metallothionein (Mt) has been considered as a molecular marker of metal pollution in aquatic ecosystems. Less is known about the expression of mt gene during embryogenesis. Here, we report the cloning, sequencing, and the expression pattern of mt gene during developmental stages in zebrafish. The zebrafish embryogenesis when takes place in a medium containing a dosage of 1000 micromolar zinc resulted in high mortality, indicating the deleterious effect of zinc on development. The zebrafish mt gene consists of three exons encoding 60 amino acids with 20 conserved cysteine residues. RT-PCR result indicates the maternal contribution of Mt transcripts. Using digoxigenin (DIG)-labeled anti-sense RNA probe, whole-mount in situ hybridization was performed to observe the expression pattern of zebrafish mt gene during embryonic and early larval stages. Stronger as well as ubiquitous expression of mt gene during early embryonic stages narrowed to specific expression after hatching. The mt promoter region contains seven copies of putative metal-responsive elements (MREs), which are shown to be important for the high level activity by deletion analysis. The expression of mt gene during embryogenesis implies its significant role on development.
Descriptors: zebrafish embryos, water pollution, metal pollution model, *Danio rerio*, laboratory animals, metallothionein, genes, biomarkers, zinc, bioaccumulation, gene expression, genetic markers, developmental stages, embryogenesis, in situ hybridization, cloning (DNA), DNA primers, nucleotide sequences, sequence analysis, exons, amino acids, reverse transcriptase PCR , plasmids, transfection, mortality, molecular sequence data.

Chen, Y., H. Chen, Y. Wu, Z. Li, L. Sun, M. Qu, and Z. Kong (2007). **Toxicity evaluation of Meiliang Bay, Lake Taihu, China-a drinking water source**. *Hydrobiologia* 581(1): 297-303. ISSN: 0018-8158.

Descriptors: zebrafish embryos, bioaccumulation, coagulation, comet assay, DNA, DNA damage, edema, embryonic development, freshwater fish, genotoxicity, heart, rivers, lakes, lymphocytes, pollutants, pollution effects, rivers, solvents, statistical analysis, teratogenesis, testes, testing procedures, toxicity testing model, water pollution effects, *Danio rerio*, Peoples Republic of China.

Cheng, J., E. Flahaut, and S.H. Cheng (2007). **Effect of carbon nanotubes on developing zebrafish (*Danio rerio*) embryos.** *Environmental Toxicology and Chemistry* 26(4): 708-16. ISSN: 0730 7268.

NAL Call Number: QH545A1E58

Abstract: The impact of carbon nanotubes (CNTs) on the aquatic environment was investigated by examining the properties of raw CNTs under several environmental conditions and using developing zebrafish (*Danio rerio*) embryos. The agglomerate size for single-walled CNTs (SWCNTs) was significantly larger at pH 11 or greater and was stable at temperatures from 4 to 40 degrees C and salinities from 0 to 30 ppt. Exposure to SWCNTs induced a significant hatching delay in zebrafish embryos between 52 to 72 h postfertilization (hpf) at concentrations of greater than 120 mg/L, but 99% of the exposed embryos hatched by 75 hpf. Double-walled CNTs also induced a hatching delay at concentrations of greater than 240 mg/L, but carbon black did not affect hatching at the concentrations tested. Molecular and cellular analysis showed that the embryonic development of the exposed embryos up to 96 hpf was not affected at SWCNT concentrations of up to 360 mg/L. Scanning-electron microscopic inspection showed that the size of the pores on the embryo chorion was nanoscaled and that the size of SWCNT agglomerates was microscaled or larger, indicating that the chorion of zebrafish embryos was an effective protective barrier to SWCNT agglomerates. The hatching delay observed in this study likely was induced by the Co and Ni catalysts used in the production of SWCNTs that remained at trace concentrations after purification. This study suggests that materials associated with raw SWCNTs (perhaps metal contaminants) have the potential to affect aquatic life when released into the aquatic environment.

Descriptors: zebrafish embryos, embryonic development drug effects, carbon nanotubes toxicity, analysis of variance, apoptosis drug effects, blood vessels drug effects, chorion drug effects and ultrastructure, drug dose response relationship, drug effects, hydrogen ion concentration, in situ hybridization, scanning electron microscopy, particle size, soot toxicity, temperature, toxicity model.

Cheshenko, K., F. Brion, Y. Le Page, N. Hinfray, F. Pakdel, O. Kah, H. Segner, and R.I. Eggen (2007). **Expression of zebra fish aromatase cyp19a and cyp19b genes in response to the ligands of estrogen receptor and aryl hydrocarbon receptor.** *Toxicological Sciences* 96(2): 255-67. ISSN: 1096-6080.

NAL Call Number: RA1190.F8

Abstract: Many endocrine-disrupting chemicals act via estrogen receptor (ER) or aryl hydrocarbon receptor (AhR). To investigate the interference between ER and AhR, we studied the effects of 17beta-estradiol (E2) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the expression of zebra fish cyp19a (zfcyp19a) and cyp19b (zfcyp19b) genes, encoding aromatase P450, an important steroidogenic enzyme. In vivo (mRNA quantification in exposed zebra fish larvae) and in vitro (activity of zfcyp19-luciferase reporter genes in cell cultures in response to chemicals and zebra fish transcription factors) assays were used. None of the treatments affected zfcyp19a, excluding the slight upregulation by E2 observed in vitro. Strong upregulation of zfcyp19b by E2 in both assays was downregulated by TCDD. This effect could be rescued by the addition of an AhR antagonist. Antiestrogenic effect of TCDD

on the zfcyp19b expression in the brain was also observed on the protein level, assessed by immunohistochemistry. TCDD alone did not affect zfcyp19b expression in vivo or promoter activity in the presence of zebra fish AhR2 and AhR nuclear translocator 2b (ARNT2b) in vitro. However, in the presence of zebra fish ERalpha, AhR2, and ARNT2b, TCDD led to a slight upregulation of promoter activity, which was eliminated by either an ER or AhR antagonist. Studies with mutated reporter gene constructs indicated that both mechanisms of TCDD action in vitro were independent of dioxin-responsive elements (DREs) predicted in the promoter. This study shows the usefulness of in vivo zebra fish larvae and in vitro zfcyp19b reporter gene assays for evaluation of estrogenic chemical actions, provides data on the functionality of DREs predicted in zfcyp19 promoters and shows the effects of cross talk between ER and AhR on zfcyp19b expression. The antiestrogenic effect of TCDD demonstrated raises further concerns about the neuroendocrine effects of AhR ligands.
Descriptors: zebrafish larvae endocrine disruption model, aromatase genetics, enzymologic gene expression regulation drug effects, aryl hydrocarbon receptors agonists, estrogen receptors agonists, aromatase metabolism, aryl hydrocarbon receptor nuclear translocator pharmacology, benzoapyrene pharmacology, benzoflavones pharmacology, cell line, estradiol analogs and derivatives, estradiol pharmacology, immunohistochemistry, larva drug effects and metabolism, ligands, luciferases genetics and metabolism, neuroglia cytology, drug effects and metabolism, promoter regions genetics, messenger RNA genetics and metabolism, aryl hydrocarbon receptors antagonists and inhibitors, estrogen receptors antagonists and inhibitors, tetrachlorodibenzodioxin pharmacology, transcription factors pharmacology.

Christianson Heiska, I., P. Smeds, N. Granholm, E. Bergelin, and B. Isomaa (2007). **Endocrine modulating actions of a phytosterol mixture and its oxidation products in zebrafish (*Danio rerio*).** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 145(4): 518-27. ISSN: 1532 0456.
NAL Call Number: QP901.C6
Abstract: In this study, a phytosterol preparation ("ultrasitosterol"; 80% beta-sitosterol) and an oxidized ultrasitosterol preparation were evaluated for reproductive effects in zebrafish. Adults were exposed in a continuous flow to 10 microg/L and 100 microg/L ultrasitosterol and oxidized ultrasitosterol, and to 0.27 microg/L 17beta-estradiol and 0.28 microg/L testosterone for 3 weeks. Biomarkers analysed included plasma vitellogenin, testosterone, 11-ketotestosterone, 17beta-estradiol, and gonadal histopathology. Ovarian steroid production of testosterone and 17beta-estradiol was examined in isolated zebrafish follicles exposed in vitro to the compounds at the same concentrations as in vivo. Vtg was induced in males exposed to ultrasitosterol, and in males and females exposed to 17beta-estradiol. Males exposed to oxidized phytosterols showed increased levels of testosterone and 11-ketotestosterone, and accelerated spermatogenesis. Increased follicular atresia was observed in females exposed to oxidized phytosterols and 17beta-estradiol. Correlation analyses between biomarkers revealed more intercorrelated values for females than for males, and the strongest associations were found in females exposed to oxidized phytosterols. Testosterone production was significantly increased in follicles exposed to the oxidized phytosterol preparations. These findings indicate that the phytosterol mixture is weakly estrogenic in male fish, and that the oxidized phytosterol mixture contains substances that may interfere with spermatogenesis, oogenesis and gonadal steroidogenesis in zebrafish.
Descriptors: zebrafish, gonads drug effects, phytosterol effects , sitosterols pharmacology, biological markers, gonadal steroid hormones biosynthesis and blood, gonads metabolism and pathology, oxidation reduction, reproduction drug effects, vitellogenins blood.

Christianson Heiska, I., P. Wahteristo, E.L. Kastilan, E. Bergelin, G. Bylund, and B. Isomaa (2004). **Effects of the wood extractive betulinol and 17beta-oestradiol on reproduction in zebrafish, *Danio rerio* (Hamilton)--complications due to a bacterial infection.** *Journal of Fish Diseases* 27(5): 267-76. ISSN: 0140-7775.

NAL Call Number: SH171.A1J68

Abstract: Zebrafish were exposed to the wood extractive betulinol (5 microg L(-1)) and to 17beta-oestradiol (E2, 0.27 microg L(-1)) for 8 weeks in an attempt to study the possible endocrine-disrupting activity of betulinol. Females exposed to betulinol showed increased spawning intensity, while males exposed to betulinol and E2 had increased incidences of structural alterations in the testes. However, histological examination of the fish revealed that they were infected by acid-fast bacteria suspected to be *Mycobacterium* sp. despite a careful examination of their health state prior to the onset of the experiment. Fish exposed to betulinol and E2 showed more serious consequences of the bacterial infection than control fish indicating that the test chemicals had weakened the immune defence of the fish. When the exposure was repeated with healthy fish, an increase in the proportion of spermatogonia was seen in the testes of betulinol-treated males. A similar alteration, although not statistically significant, was also seen in the first experiment. However, no increase in the incidences of structural alterations in the testes was seen in betulinol- and E2-treated fish in the second experiment. Our study indicates that betulinol might have an endocrine-disrupting effect in zebrafish, but the increase in incidences of structural alterations in the testes might have been caused by a synergistic action between the test compounds and the bacterial infection. Our study stresses the importance of carefully checking the health of experimental fish, not only prior to the onset of an experiment but also upon termination of the experiment, in order to avoid misinterpretation of the results.

Descriptors: zebrafish betulinol exposure, estradiol pharmacology, fish diseases microbiology, *Mycobacterium* infections veterinary, reproduction drug effects, triterpenes toxicity, analysis of variance, fish diseases physiopathology, histological techniques, natural immunity drug effects, *Mycobacterium* infections physiopathology, reproduction physiology, spermatogonia drug effects and pathology, testis drug effects and pathology.

Christianson-Heiska, I.L., T. Haavisto, J. Paranko, E. Bergelin, and B. Isomaa (2008). **Effects of the wood extractives dehydroabietic acid and betulinol on reproductive physiology of zebrafish (*Danio rerio*)-A two-generation study**. *Aquatic Toxicology* 86(3): 388-396. ISSN: 0166-445X.

NAL Call Number: QH541.5.W3A6

Descriptors: zebrafish papermill effluent effects, 17 beta -estradiol, condition factor, effluents, eggs, endocrine disruptors, oocytes, paper mills, pulp, spawning, statistical analysis, steroid hormones, testosterone, vitellogenin, biomarkers, dehydroabietic acid, *Danio rerio.*

Chun, C.Z. and T.T. Chen (2007). **Microinjecting recombinant rainbow trout Ea4-peptide of pro-IGF-I into zebrafish embryos causes abnormal development in heart, red blood cells, and vasculature.** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 145(1): 39-44. ISSN: 1532 0456.

NAL Call Number: QP901.C6

Abstract: E-peptides and mature insulin-like growth factors (IGFs) are produced from pre-pro-IGFs during post-translational processing and co-secreted into the circulation. Previously, we reported that introduction of a transgene encoding the secreted form of rainbow trout (rt) Ea4-peptide or human (h) Eb-peptide into newly fertilized eggs of medaka (*Oryzias latipes*) and zebrafish (*Danio rerio*) resulted in developmental defects in heart, red blood

cells and vasculature. In addition to vasculature and red blood cell developmental defects, multiple phenocopies of heart developmental defects categorized by developmental arrest at cardiomyocyte, heart tube and heart looping stages were also observed. These results raise a question of whether rtEa4- or hEb-peptide exerts pleiotropic inhibitory effects on heart, vasculature and red blood cell development in fish embryos. To answer this question, various amounts of recombinant rtEa4-peptide were microinjected into zebrafish eggs at 1.5, 2.5 and 5.5 h post-fertilization (hpf). Although a dose-dependent developmental defect in heart, vasculature and red blood cells was observed in embryos microinjected with rtEa4-peptide at 1.5 and 2.5 hpf, the heart development in all of the microinjected embryos was arrested at the cardiomyocyte stage. Furthermore, the mRNA levels of Nkx2.5, GATA5, VEGF, GATA1 and GATA2 genes in defective embryos were significantly reduced by rtEa4-peptide. These results confirm our previous findings that rtEa4- or hEb-peptide exhibits pleiotropic effects in inhibiting heart, vasculature and red blood cell development in zebrafish embryos.
Descriptors: zebrafish eggs rtEa4-peptide injections, blood vessels abnormalities, drug effects and embryology, erythrocytes drug effects, heart embryology, peptide fragments pharmacology, protein precursors pharmacology, DNA primers, drug dose response relationship, abnormalities, developmental gene expression regulation drug effects, heart drug effects, chemically induced congenital heart defects and pathology, insulin like growth factor I administration, dosage, chemistry and pharmacology, microinjections, *Oncorhynchus mykiss* , peptide fragments administration and dosage, protein precursors administration, dosage and chemistry, messenger RNA biosynthesis, recombinant proteins administration, dosage and pharmacology, reverse transcriptase PCR.

Chung, W.G., A. Sen, J.L. Wang Buhler, Y.H. Yang, N. Lopez, G.F. Merrill, C.L. Miranda, C.H. Hu, and D.R. Buhler (2004). **cDNA-directed expression of a functional zebrafish CYP1A in yeast.** *Aquatic Toxicology* 70(2): 111-21. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: A cytochrome P450 1A (CYP1A) cDNA was isolated from an adult zebrafish (*Danio rerio*) library. The 2580-bp clone (GenBank Accession No. AF210727) contained a 62-bp 5'-unstranslated region (UTR), 1557-bp coding region and 962-bp 3'-UTR. The deduced 519-residue protein (calculated molecular weight 58,556, pI = 7.58) shared 74% identity with rainbow trout CYP1A and 57 and 54% identities with mouse and human CYP1A1s, respectively. The zebrafish CYP1A protein coding region was cloned into the pDONR201 entry vector and then transferred to a yeast expression vector pYES-DEST52. Expression of zebrafish CYP1A in *Saccharomyces cerevisiae* transformants was induced by galactose to a maximum level of 493 pmol CYP1A per mg microsomal protein or about 8 nmol/l of culture. Recombinant CYP1A protein expressed in yeast was mainly in the denatured P420 form under normal microsomal preparation conditions but when the oxygen concentration was reduced in the buffer by degassing and the yeast cells were maintained at less than 10 degrees C, the integrity of the CYP1A was preserved and it exhibited a characteristic reduced CO-difference spectrum maximum at 448 nm. The recombinant zebrafish CYP1A demonstrated 7-ethoxyresorufin O-deethylase (EROD) activity with an apparent Km (Km(app)) and Vmax values at 30 degrees C of 0.31 +/- 0.04 microM and 0.70 +/- 0.10 nmol/min/nmol CYP, respectively. The recombinant protein also metabolized benzo(a) pyrene with a Km(app) and Vmax values of 5.34 +/- 0.58 microM and 1.16 +/- 0.13 nmol/min/nmol CYP, respectively. These results show the recombinant expression of a functional zebrafish CYP in yeast and validated yeast as a host for heterologous expression of zebrafish CYP1A and potentially for other zebrafish CYPs.
Descriptors: zebrafish cytochrome clone, benzoapyrene metabolism , molecular cloning,

cytochrome P450 CYPA1A1 biosynthesis, genetics, and metabolism, complementary DNA genetics, gene library, liver microsomes enzymology, RNA chemistry and genetics, recombinant proteins, reverse transcriptase PCR veterinary, *Saccharomyces cerevisiae* enzymology and genetics.

Cook, L.W., C.J. Paradise, and B. Lom (2005). **The pesticide malathion reduces survival and growth in developing zebrafish**. *Environmental Toxicology and Chemistry* 24(7): 1745-1750. ISSN: 0730 7268.

NAL Call Number: QH545A1E58

Abstract: Malathion is an organophosphorous pesticide widely used to control mosquitoes in urban areas and pests, such as boll weevils, in agricultural areas. Zebrafish, Danio rerio, are model organisms for developmental toxicology research because they are readily available, produce large numbers of clear embryos, and are sensitive to environmental changes. The nonlethal effects of malathion on developing zebrafish embryos, however, previously have not been analysed quantitatively. We exposed zebrafish embryos to sublethal malathion concentrations to determine malathion's effects on a developing vertebrate. Zebrafish exposed to 0.5, 1.0, or 1.5 mg/L of malathion consistently elicited more rapid hatching from the chorion than zebrafish exposed to 2.0-, 2.5-, or 3.0-mg/L malathion concentrations. In addition, exposure to 2.0, 2.5, or 3.0 mg/L of malathion resulted in significantly shorter body length and eye diameters, indicating that malathion had teratogenic effects on zebrafish embryos. Malathion's action as an acetylcholinesterase inhibitor and the toxicity of the metabolites of malathion may be responsible for malathion's teratogenic effects on fish development. Copyright © 2008 CABI

Descriptors: zebrafish embryos, acetylcholinesterase, biological development, enzyme inhibitors, enzymes, growth, malathion, nontarget effects, pesticide residues, pesticides, survival, toxic substances, toxicity, *Danio rerio.*

Cooper, C.A., R.D. Handy, and N.R. Bury (2006). **The effects of dietary iron concentration on gastrointestinal and branchial assimilation of both iron and cadmium in zebrafish (*Danio rerio*).** *Aquatic Toxicology* 79(2): 167-75. ISSN: 0166 445X.

NAL Call Number: QH541.5.W3A6

Abstract: Zebrafish (*Danio rerio*) were fed either a diet containing 33mgFekg(-1) (low) or 95mgFekg(-1) (normal) for 10 weeks, after which short-term Cd and Fe uptake by the gastrointestinal tract and gill was assessed. Carcass metal content and transcript levels of the iron importer, Divalent Metal Transporter 1 (DMT1) and an iron exporter, ferroportin1, in both the gastrointestinal tract and gill were also measured. Fish fed the low Fe diet accumulated 13 times more Cd into their livers via the gastrointestinal tract than those fed the normal Fe diet. However, no significant increase in liver Fe accumulation was measured. Concomitantly, when exposed to 48nmolCdL(-1) fish fed the low Fe diet exhibited a approximately 4-fold increase in Cd accumulation on the gill and in the liver, compared to those fed a normal diet. In addition, fish fed the low Fe diet also significantly accumulated more Fe on the gill (nine-fold increase) and into the carcass (four-fold increase) when exposed to 96nmolFeL(-1), compared to fish fed a normal diet. Surprisingly, carcass Fe, Ca and Mg concentrations were increased in fish fed the low Fe diet, which suggests that Fe body levels may not be a good indicator of whether a fish is more or less susceptible to increased non-essential metal accumulation via an Fe uptake pathway. However, significantly elevated transcript levels of DMT1 and ferroportin1 (2.7- and 3.8-fold induction, respectively) were seen in the gastrointestinal tract, and DMT1 in the gills (1.8-fold induction) of zebrafish fed a low Fe diet. The correlation between Cd uptake and DMT1 expression suggests that one route of uptake

of Cd, either from the diet or from the water, could be via DMT1.
Descriptors: zebrafish, cadmium pharmacokinetics, gastrointestinal tract metabolism, gills metabolism, dietary iron administration and dosage, metabolism, calcium analysis, cation transport proteins biosynthesis and drug effects, DNA primers chemistry, gene expression drug effects, gills chemistry, dietary iron pharmacokinetics, liver metabolism, manganese analysis, heavy metals analysis and metabolism, ribosomal RNA 18s biosynthesis, chemical water pollutants pharmacokinetics.

Correa-Reyes, G., M.T. Viana, F.J. Marquez-Rocha , A.F. Licea, E. Ponce, and R. Vazquez-Duhalt (2007). **Nonylphenol algal bioaccumulation and its effect through the trophic chain**. *Chemosphere* 68(4): 662-670. ISSN: 0045-6535.
NAL Call Number: TD172.C54
Descriptors: zebrafish, acute toxicity, acute toxicity, algae, bioaccumulation, crustaceans, cytochrome, cytochrome p450, detergents, diets, endocrine disruptors, enzymes, food, fresh-water fish, growth rate, hormones, marine crustaceans, nonylphenol, phenols, physiology, pollutants, pollution effects on organisms, risk, toxicity, transformation, trophic structure, vitellogenin, water pollution effects, acute toxicity, crustaceans, endocrine disruptors, algae, artemia, *Danio rerio*, *Isochrysis galbana.*

Craig, P.M., C.M. Wood, and G.B. McClelland (2007). **Oxidative stress response and gene expression with acute copper exposure in zebrafish (*Danio rerio*).** *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 293(5): R1882-92. ISSN: 0363 6119.
Abstract: In fish, environmental pollution is one factor that induces oxidative stress, and this can disturb the natural antioxidant defense system. Oxidative stress has been well characterized in vitro, yet the in vivo effects of metal-induced oxidative stress have not been extensively studied. In two experiments we examined the impacts of copper (Cu) on gene expression, oxidative damage, and cell oxidative capacity in liver and gill of zebrafish. In the first experiment, soft water-acclimated zebrafish were exposed to 8 and 15 mug/l Cu for 48 h. This exposure resulted in significant increases in gene expression of cytochrome c oxidase subunit 17 (COX-17) and catalase, associated with both increased Cu load and protein carbonyl concentrations in the gill and liver after 48 h. In addition, we examined the potential protective effects of increased waterborne Ca(2+) (3.3 mM) and Na(+) (10 mM) on acute Cu toxicity. While both treatments were effective at reducing liver and/or gill Cu loads and attenuating oxidative damage at 48 h, 10 mM Na(+) was more protective than 3.3 mM Ca(2+). There were variable changes in the maximal activities of COX and citrate synthase (CS), indicating possible alterations in cell oxidative capacity. Moreover, Cu affected COX-to-CS ratios in both gill and liver, suggesting that Cu alters normal mitochondrial biogenic processes, possibly though metallochaperones like COX-17. Overall, this study provides important steps in determining the transcriptional and physiological endpoints of acute Cu toxicity in a model tropical species.
Descriptors: zebrafish copper toxicity model, gene expression drug effects, oxidative stress drug effects, catalase biosynthesis and genetics, citrate si synthase biosynthesis and genetics, copper analysis and metabolism, electron transport complex IV biosynthesis and genetics, gills drug effects and enzymology, liver drug effects and enzymology, peptide elongation factor 1 metabolism, messenger RNA biosynthesis and genetics, reverse transcriptase PCR, superoxide dismutase biosynthesis and genetics, genetic transcription drug effects.

DeCarvalho, S., J.V. Lombardi, M.J.T.R. Paiva, J.G. DeFranca-Monkolski, and J.R. Ferreira (2006). **Bioaccumulation of mercury in fish exposed to experimentally contaminated water and sediment**. *Bulletin of Environmental Contamination and Toxicology* 77(6): 854-860. ISSN: 0007 4861.

NAL Call Number: RA1270.P35A1

Abstract: This study investigated the role of sediment in the deposit and release of mercury (Hg), using bioassays for toxicity in fish based on indicators of mortality and Hg bioaccumulation. The fish utilized were tilapia fingerlings, *Oreochromis niloticus* (0.53 g+or-0.29 g) and zebrafish, *Danio rerio* (0.30 g +or-0.03 g), obtained from commercial fish farms. Sediment samples were collected from the bottom of a natural lake. The two experiments were carried out simultaneously, with five treatments and five simultaneous replicates: C - control (only dilution water, without sediment); SC - sediment control (sediment + dilution water); SSHg - sediment experimentally contaminated (spiked) with 1.0 mg Hg/litre; SSHg0.2 - sediment maintained in continuous contact with dilution water, contaminated with mercury at a concentration of 0.2 mg/litre; and Hg0.2 - only dilution water, contaminated with mercury at a concentration of 0.2 mg/litre. Results showed that the mortality rates observed over the course of the experiments were relatively low and did not differ significantly among treatments. The mortality used for the control groups (C and SC) were below 10%. Treatment SSHg also showed mean mortality values of 10%. Higher mortality indices were recorded for treatment Hg0.2, that is, 23.33 and 16.66% for *Oreochromis niloticus and Danio rerio*, respectively. No Hg was detected in the samples of control sediment. Treatment SSHg adsorbed 0.06 and 0.23 micro g Hg/g, in experiments 1 and 2, respectively. Analysis of the sediment in treatment SHg0.2 showed that part of the Hg that was in the water (0.2 mg/litre) became incorporated into the sediment after 7 days exposure (0.01 micro g/g) in experiment 1 and 0.1 micro g/g in experiment 2. Bioaccumulation of Hg demonstrated that there was a statistically significant difference between treatment Hg0.2 and SHg0.2 for the two fish species. In all experiments, fish exposed to a concentration of 0.2 mg Hg/litre without sediment accumulated more Hg when compared with fish submitted to treatment containing sediment. Fish exposed for 7 days showed bioaccumulation level of 1.0 micro g Hg/g tissue. Copyright © 2008 CABI

Descriptors: zebrafish and tilapia fingerlings, bioassays, biological indicators, exposure, indicator species, mercury, mortality, polluted water, sediment, toxicity, water pollution, water quality, *Danio rerio*, *Oreochromis niloticus*.

Deeds, J.R., R. Reimschuessel, and A.R. Place (2006). **Histopathological effects in fish exposed to the toxins from Karlodinium micrum**. *Journal of Aquatic Animal Health* 18(2): 136-148. ISSN: 0899-7659.

NAL Call Number: SH171.J68

Descriptors: zebrafish, aquatic animals, biological poisons, chloride, fish kill, fish larvae, freshwater fish, gills, histopathology, hypertrophy, injuries, lamellae, microscopy, mortality, mortality causes, necrosis, pathology, phytoplankton, toxicants, toxicity tests, natural toxins, transmission electron microscopy, *Cyprinodon variegatus*, *Danio rerio*, dinophyceae, *Karlodinium micrum*, sheepshead minnow.

DeOliveira Filho, E.C., R.M. Lopes, and F.J.R. Paumgartten (2004). **Comparative study on the susceptibility of freshwater species to copper-based pesticides**. *Chemosphere* 56(4): 369-374. ISSN: 0045 6535.

NAL Call Number: TD172.C54

Abstract: Copper compounds have been intentionally introduced into water bodies as

aquatic plant herbicides, algicides and molluscicides. Copper-based fertilizers and fungicides have been widely used in agriculture as well. Despite the fact that copper is an essential element for all biota, elevated concentrations of this metal have been shown to affect a variety of aquatic organisms. Nonetheless, comparative studies on the susceptibility of different freshwater species to copper compounds have seldom been performed. This study was conducted to compare toxicity of copper-based pesticides (copper oxychloride, cuprous oxide and copper sulfate) to different freshwater target (*Raphidocelis subcapitata*, a planktonic alga and *Biomphalaria glabrata*, a snail) and non-target (*Daphnia similis*, a planktonic crustacean and *Danio rerio*, a fish) organisms. Test water parameters were as follows: pH=7.4+or-0.1; hardness 44+or-1 mg/l as CaCO3; DO 8-9 mg/l at the beginning and >4 mg/l at the end; temperature, fish and snails 25+or-1 degrees C, Daphnia 20+or-2 degrees C, algae 24+or-1 degrees C. *D. similis* (immobilization), 48-h EC50s (95% CLs) ranging from 0.013 (0.011-0.016) to 0.043 (0.033-0.057) mg Cu/l, and R. subcapitata (growth inhibition), 96-h IC50s from 0.071 (0.045-0.099) to 0.137 (0.090-0.174) mg Cu/l, were the most susceptible species. B. glabrata (lethality), 48-h LC50s from 0.179 (0.102-0.270) to 0.854 (0.553-1.457) mg Cu/l, and *D. rerio* (lethality), 48-h LC50s 0.063 (0.045-0.089), 0.192 (0.133-0.272) and 0.714 (0.494-1.016) mg Cu/l, were less susceptible than Daphnia to copper-based pesticides. Findings from the present study therefore suggest that increased levels of copper in water bodies is likely to adversely affect a variety of aquatic species. Copyright © 2008 CABI
Descriptors: zebrafish copper toxicty model, copper oxychloride, copper sulfate, cuprous oxide, nontarget effects, nontarget organisms , polluted water, toxicity, water pollution, *Biomphalaria glabrata*, *Danio rerio*, *Daphnia.*

Diekmann, M., V. Hultsch, and R. Nagel (2004). **On the relevance of genotoxicity for fish populations I: effects of a model genotoxicant on zebrafish (*Danio rerio*) in a complete life-cycle test.** *Aquatic Toxicology* 68(1): 13-26. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Descriptors: zebrafish genotoxicant model, 4 nitroquinoline 1 oxide toxicity, growth drug effects, mutagens toxicity, reproduction drug effects, analysis of variance, body weights and measures , computer simulation, drug dose response relationship, gas chromatography mass spectrometry, mutagenicity tests, toxicity tests, chronic, .

Diekmann, M., P. Waldmann, A. Schnurstein, T. Grummt, T. Braunbeck, and R. Nagel (2004). **On the relevance of genotoxicity for fish populations II: genotoxic effects in zebrafish (*Danio rerio*) exposed to 4-nitroquinoline-1-oxide in a complete life-cycle test.** *Aquatic Toxicology* 68(1): 27-37. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Descriptors: zebrafish, 4 nitroquinoline 1 oxide toxicity, drug effects on DNA repair and replication , mutagens toxicity, comet assay, drug dose response relationship, micronucleus tests, mutagenicity tests, toxicity tests, chronic, .

DiGiulio, R., S. Billiard, J. Meyer, D. Wassenberg, and P. Hodson (2006). **Synergistic developmental toxicity of polycyclic aromatic hydrocarbons: towards a mechanistic understanding**. *Marine Environmental Research* 62(Suppl. S): S45-S46. ISSN: 0141 1136.
NAL Call Number: QH545.W3M36
Descriptors: zebrafish toxicology model, marine ecology, polycyclic aromatic hydrocarbons, environmental sciences.

Dlugos, C.A. and R.A. Rabin (2004). **Behavioral effects of chronic ethanol treatment on three strains of adult zebrafish.** *Alcoholism Clinical and Experimental Research* 28(5, Suppl. S): 142A. ISSN: 0145 6008.

Descriptors: zebrafish ethanol exposure, behavior, biochemistry and molecular biophysics, nervous system, toxicology, computer imaging, imaging and microscopy techniques.

Dominique, Y., B. Muresan, R. Duran, S. Richard, and A. Boudou (2007). **Simulation of the chemical fate and bioavailability of liquid elemental mercury drops from gold mining in Amazonian freshwater systems**. *Environmental Science & Technology* 41(21): 7322-7329. ISSN: 0013-936X.

NAL Call Number: TD420.A1E5

Descriptors: zebrafish, elemental mercury, gold amalgamation, South America, ecosystems, indoor microcosms, bioavailability , gold mining, (Hg(II)), methylmercury (MeHg), *Danio rerio*.

Dong, W., W. Muramoto, Y. Nagai, K. Takehana, J.J. Stegeman, H. Teraoka, and T. Hiraga (2006). **Retinal neuronal cell is a toxicological target of tributyltin in developing zebrafish.** *Journal of Veterinary Medical Science* 68(6): 573-79. ISSN: 0916-7250.

NAL Call Number: SF604.J342

Abstract: Organotins are among the most common marine pollutants in the world, as they are widely used as antifouling paint on ships and fishing nets. It has been reported that organotin preferentially accumulates in the central nervous system, especially in the retinal neurons of marine organisms including fish. In this study, we investigated the effects of waterborne tributyltin (TBT) on early-stage developing zebrafish *(Danio rerio)*. Below the lethal concentrations, TBT specifically increased the number of apoptotic cells in the retina as well as some cells near trigeminal neurons, detected by terminal transferase-mediated nick-end-labeling staining. Apoptosis peaked at 60 hpf and decreased by 72 hpf, which was associated with macrophage accumulation. Furthermore, the effect of TBT was markedly inhibited by antioxidants, ascorbic acid or trolox. These results suggest that TBT preferentially induces apoptosis in the retinal neuron of developing zebrafish. Oxidative stress may be involved in this toxicological response.

Descriptors: zebrafish marine pollution model, afferent neurons drug effects, retina cytology, trialkyltin compounds toxicity, tributyltin pollution, water pollutants toxicity, apoptosis drug effects, drug dose response relationship, retina embryology.

Dong, W., H. Teraoka, Y. Tsujimoto, J.J. Stegeman, and T. Hiraga (2004). **Role of aryl hydrocarbon receptor in mesencephalic circulation failure and apoptosis in zebrafish embryos exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin.** *Toxicological Sciences* 77(1): 109-16. ISSN: 1096-6080.

NAL Call Number: RA1190.F8

Abstract: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a persistent and potent developmental toxicant in various animals, with developing fish being the most sensitive organisms. Although the expression of aryl hydrocarbon receptor (AHR) as well as the partner molecule, AHR nuclear translocator (ARNT) in the brain has been reported, the effect of TCDD on the brain remains to be clarified in detail. Previously, we reported local circulation failure and apoptosis in dorsal midbrain caused by TCDD in developing zebrafish. In the present experiments, we investigated the effects of morpholino antisense oligos against aryl hydrocarbon receptor 2 (zfAHR2) (AHR2-MO) on toxicological endpoints caused by TCDD in developing zebrafish. AHR2-MO but not its negative homologue (4mis-AHR2-MO) improved

TCDD-evoked circulation failure in mesencephalic vein and reduced the occurrence of apoptosis in dorsal midbrain, with concomitant inhibition of CYP1A induction in vascular endothelium. Injection of bovine serum albumin (BSA) into the general circulation, followed by immunohistochemistry with anti-BSA, showed that TCDD raised vascular permeability to albumin in dorsal midbrain, which was blocked by AHR2-MO and N-acetlycystein. In the absence of TCDD, development of embryos injected with AHR2-MO appeared normal at least until 60 h after fertilization. It is concluded that AHR2 activation in the vascular endothelium of the zebrafish embryo midbrain is involved in the mesencephalic circulation failure and apoptosis elicited by TCDD. This is the further evidence that vascular endothelium is the target of TCDD in relation to local circulation failure and apoptosis in dorsal midbrain.

Descriptors: zebrafish embryos, drug induced abnormalities physiopathology, apoptosis drug effects, drug effects, mesencephalon drug effects, aryl hydrocarbon receptors genetics, tetrachlorodibenzodioxin toxicity, drug induced abnormalities genetics , blood circulation drug effects, capillary permeability drug effects, metabolism, pathology, vascular endothelium drug effects, metabolism and pathology, mesencephalon blood supply and physiopathology, morpholines, antisense oligonucleotides pharmacology, veins drug effects and physiopathology.

Dong, W., J.f. Yang, Y. Cao, S. Wang, H. Teraoka , and T. Hiraga (2005). **Correlation between short lower jaw in zebrafish embryos induced by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (tcdd) and shh gene**. *Hupo Kexue* 17(2): 162-168. ISSN: 1003 5427.

Abstract: Dioxins including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are persistent, bioaccumulative toxicants that are widely distributed in the environment. TCDD induced cleft palate in the mice and short lower jaw in the zebrafish (*Danio rerio*) by aryl hydrocarbon receptor (AhR) which is most toxic in the Dioxins. In this experiment, we investigated the correlation between TCDD induced short lower jaw and Sonic hedgehog (Shh) a development gene in the zebrafish embryos during early development, it has many advantages as a vertebrate toxicological model. Embryos is exposed to TCDD (0 - 1 mu g/L) at 24 h post fertilization (24 hpf) until the time of observation for studying by morphology and in situ hybridization. Embryos treated with TCDD showed marked short lower jaw and reduction of shh and twhh expression in lower developing jaw. This inhibitory effect was dependence on exposed concentration. TCDD concentrations 0.3, 0.5 and 1.0 g/L exerted significant effects that were first detected at 84, 72, and 60 hpf, respectively. But 0.1 mu g/L TCDD did not have any effect until 120 hpf. Shh function defective mutant embryos (Syu) with relatively normal circulation around developing jaw showed marked retardation of jaw growth. When cyclopamine, an inhibitor for shh, was applied, lower jaw growth was significantly inhibited. It was found that TCDD affected chondrogenesis in the head in later stages of zebrafish development by alcian blue stain. TCDD shortened the length of Mechel's cartilage. Similarly, the short Mechel's cartilage was found in the sync embryos and cyclopamine treated embryos. These results indicate the close correlation between hedgehog expression and jaw growth retardation by TCDD in developing zebrafish embryos. It is also suggested zebrafish may become a biomarker of dioxin toxicity. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, pharmacology, toxicology, pollution assessment control, skeletal system, development, dental and oral system, cleft palate, chemically induced congenital disease, in situ hybridization, histology and cytology techniques, Sonic hedgehog (Shh), genetic techniques, chondrogenesis.

Language of Text: Chinese.

Drastichova, J., Z. Svobodova, M. Groenland, R. Dobsikova, V. Zlabek, D. Weissova, and M. Szotkowska (2005). **Effect of exposure to bisphenol A and 17 beta -estradiol on the sex differentiation in zebrafish (*Danio rerio*)**. *Acta Veterinaria (Brno)* 74(2): 287-291. ISSN: 0001 7213.

NAL Call Number: SF604.B7

Abstract: The effects of bisphenol A and 17 beta -estradiol on sex differentiation were investigated in the zebrafish, *Danio rerio*. The 20-day-old fry with undifferentiated gonads were fed with food containing bisphenol A at the dose of 500, 1000, 2000 mg.kg-1 and 17 beta -estradiol at the dose of 20 mg.kg-1 diet for 45 days. Sex ratio and gonadal development after the chemical treatment were determined by histological examination of gonads. In the control group, the sex ratio of fry was 1:1 (female:male), i.e. 50% of females and 50% of males appeared. The sex of fry fed with 17 beta -estradiol at the dose of 20 mg.kg-1 diet was all females. Feminization of the fry was induced by exogenic 17 beta -estradiol. The sex of fry fed with bisphenol A at the dose of 500, 1000, 2000 mg.kg-1 diet was 1.4:1 (p=0.31), 3.8:1 (p=0.01) and 11.5:1 (p<0.01). Bisphenol A induced feminization of the fry at the two highest doses tested. Copyright © 2008 CABI

Descriptors: zebrafish feminization model, bisphenol A and 17 beta -estradiol dosage effects, estradiol, fry, gonads, histology, sex ratios and differentiation, sexual development, treatment, *Danio rerio*.

Language of Text: Czech.

Duan Zheng, H., Zheng Min, and Zhu Lin (2006). **Toxicity of joint action of pentachlorophenol and bisphenol A on the growth of zebrafish (*Danio rerio*) embryo.** *China Environmental Science* 26(Suppl.): 121-124. ISSN: 1000 6923.

Abstract: The joint toxicity test of environmental oestrogen material of pentachlorophenol and bisphenol A were made adopting zebrafish embryo technique and the methods of 1:1 toxicity ratio and different toxicity ratios. At zero contamination and the toxicity effect ratio of 1:1, the 24-h death of PCP and BPA showed synergistic action; and the additive action at 72-h hatch prohibitive rate. At the joint toxicities of PCP and BPA toxicity effect rates antagonistic action existed at 24 h blood balk to the 6 hpf contamination; while synergistic action existed at 32 h cyst of 0 hpf contamination embryos. Besides, 6 hpf joint action compared with that of 0 hpf, the 3 kinds of toxicity effect of 24 h blood balk, 32 h blood balk and 32 h cyst all lowered somewhat. Copyright © 2008 CABI

Descriptors: zebrafish, embryos, estrogens, bisphenol A, pentachlorophenol, polluted water, synergism, toxicity, water pollution, *Danio rerio.*

Language of Text: Chinese with an English summary.

Duan, Z.H. and L. Zhu (2006). **Toxicity of bisphenol A on the growth of zebrafish embryos**. *Acta Hydrobiologica Sinica* 30(6): 638-642. ISSN: 1000 3207.

Abstract: In order to evaluate the toxicity of the bisphenol A (BPA) on the growth of zebrafish embryos, fertilized eggs were exposed to the concentration of 2.00, 4.00, 6.00, 8.00, 10.00, 15.00, 18.00, 22.00 and 25.00 mg/L BPA for 72 h at 26 +/- I T. The results revealed that the sublethal toxicological endpoints induced by BPA were: delayed hatch > blood balk > cyst > altered axial curvature and tail malformation. The median embryo lethal concentration (LC50) after 24 h was 16.36 mg/L. We concluded that the BPA toxicity on zebrafish embryos were caused before 8h exposure and it was not the result of long-term accumulation. Therefore, BPA maybe cause altered gene expression at the early stage of zebrafish embryos. In the further studies, we will use the technology of genetic chips to look for the toxic mechanism of BPA. Copyright©Thomson Reuters 2009

Descriptors: zebrafish toxicity model, pollution assessment, toxicity evaluation, bisphenol A, genetic chips.

Ekanem, A.P., T. Meinelt, W. Kloas, and K. Knopf (2004). **Early life stage toxicity of extracts from the african fish poison plants *tephrosia vogelii Hook* and *asystasia vogeliana benth* on zebrafish embryos**. *Journal of Fish Biology* 65(2): 489-494. ISSN: 0022 1112.
NAL Call Number: QL614.J68
Abstract: Embryos of *Danio rerio* are highly susceptible to extracts of the plants *Tephrosia vogelii* and *Asystasia vogeliana*. The concentration of the dried extracts at which 50% of the embryos were affected (EC50) after 24 h exposure were 320 and 572 mug l-1, respectively; corresponding 50% mortality (LC50) values after 48 It exposure were 493 and 869 mug l-1. Results indicate that the use of these ichthyotoxic plants might have a severe impact on the survival of fish larvae in the field. Copyright 2004 The Fisheries Society of the British Isles. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryo toxicology model, *Tephrosia vogelii*, *Asystasia vogeliana*, icthyotoxic plants.

Elskus, A. (2006). **Studying toxicity**. *International Water Power and Dam Construction* 58(3): 30-33. ISSN: 0306-400X.
Descriptors: zebrafish, fathead minnows, salmon, anadromous species, dam effects, dams, endangered species, fish, freshwater fish, international waters, marine fish, model studies, pollutants, resuspended sediments, rivers, salmon, sediment contamination, sediment chemistry, sediment pollution, toxicity, toxicity tests, *Danio rerio*, *Pimephales promelas*, *Salmo salar*, Penobscot River, Maine, USA.

Fenske, M., G. Maack, C. Schafers, and H. Segner (2005). **An environmentally relevant concentration of estrogen induces arrest of male gonad development in zebrafish, *Danio rerio.*** *Environmental Toxicology and Chemistry* 24(5): 1088-98. ISSN: 0730 7268.
NAL Call Number: QH545A1E58
Abstract: The aim of the present study was to elucidate how full life-cycle exposure to estrogens impacts zebrafish development and reproduction, compared to partial life-cycle exposure only, and whether the estrogen-induced effects in zebrafish are reversible or irreversible. Zebrafish were exposed in a flow-through system to an environmentally relevant concentration (3 ng/L) of the synthetic estrogen 17alpha-ethinylestradiol (EE2) either from fertilization until the all-ovary stage of gonad development (i.e., 42 d postfertilization [DPF] in our experiment) or from fertilization until the reproductive stage (i.e., 118 DPF). Reversibility of the estrogen-induced effects was assessed after 58 d of depuration in EE2-free water until 176 DPE Early life exposure led to a lasting induction of plasma vitellogenin (VTG) in adult females but altered neither the sex ratio nor the reproductive capabilities. Full life-cycle exposure resulted in elevated VTG concentrations and caused gonadal feminization in 100% of exposed fish and thus inhibited reproduction. Two types of ovaries were observed in continuously exposed adult fish, immature ovaries with primary growth stage oocytes only and mature ovaries containing the full range of all oocyte maturation stages. Fish with immature ovaries had plasma VTG levels like control males, while fish with mature ovaries had female-like VTG levels. The effects of full life cycle exposure were at least partly reversible, and 26% of fish of the previous all-female cohort developed fully differentiated testes. These findings suggest that continuous estrogen exposure had arrested the developmental transition of the gonads of genetic males from the early all-ovary stage to functional testes. After the exposure had ceased, however, these males apparently were able to accomplish testicular differentia-

tion.
Descriptors: zebrafish feminization model, estrogens toxicity, gonads drug effects, sexual development drug effects on gonads, chemical water pollutants toxicity, drug dose response relationship, fertilization, gonads growth and development, life cycle stages, norethynodrel analogs and derivatives, norethynodrel pharmacology and toxicity, reproduction, time factors, vitellogenins metabolism.

Fenske, M. and H. Segner (2004). **Aromatase modulation alters gonadal differentiation in developing zebrafish (*Danio rerio*).** *Aquatic Toxicology* 67(2): 105-26. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: This study investigated whether gonadal sex differentiation of zebrafish *(Danio rerio*) is susceptible to compounds that interfere with cytochrome P450 aromatase (P450arom). Treatment of zebrafish during the period of gonadal differentiation with either the non-steroidal aromatase inhibitor fadrozole or 17alpha-methyltestosterone (MT) changed gonad morphological differentiation and altered the pattern of P450arom gene (CYP19) expression. Application of fadrozole (500 microg/g of food) between days 35 and 71 post-fertilisation (pf) resulted in 100% masculinisation, i.e. the gonads of all individuals examined (n = 40) showed testicular morphology. At the same time, fadrozole treatment suppressed gonadal CYP19A (gonad-derived CYP19 gene) mRNA expression, as assessed by means of semi-quantitative RT-PCR. After termination of fadrozole treatment at day 71 pf and subsequent rearing of zebrafish under control conditions until 161 days pf, the gonads of all individuals still displayed testicular morphology. Gonadal CYP19A expression, however, showed a dimorphic pattern, with 14 out of 22 individuals having low CYP19A mRNA levels similar to those found in testes of control fish, while eight fish showed high, ovary-like levels of gonadal CYP19A mRNA. MT treatment (10 microg/l) during the period of gonadal differentiation (days 35 to 71 pf) resulted in phenological feminisation, i.e., all fish examined (n = 28) showed an ovarian gonadal morphology. While gonadal CYP19A expression was suppressed compared to control fish, cerebral CYP19B (brain-derived CYP19 gene) mRNA expression was increased in 71-day-old MT-treated fish. The results from this study provide evidence that exogenous compounds interfering with the P450arom system are able to disrupt, even persistently, gonadal sex differentiation of the protogynic zebrafish.
Descriptors: zebrafish embryos, cytochrome P450, aromatase inhibitors, estrogen antagonists pharmacology, gonads embryology, sex differentiation drug effects, aromatase genetics, DNA primers, estrogens genetics, fadrozole pharmacology, gene expression, gonads anatomy, histology and drug effects, histological techniques, methyltestosterone pharmacology, messenger RNA genetics, reverse transcriptase PCR.

Fracacio, R., N.F. Verani, E.L.G. Espindola, O. Rocha, O. Rigolin Sa, and C.A. Andrade (2003). **Alterations on growth and gill morphology of *Danio rerio* (Pisces, Ciprinidae) exposed to the toxic sediments**. *Brazilian Archives of Biology and Technology* 46(4): 685-695. ISSN: 1516 8913.
NAL Call Number: QH301.A73
Abstract: The objective of this work was to assess the toxicity of sediment samples from six cascade reservoirs in Tiete river system, in Sao Paulo State, Brazil, through chronic-partial toxicity bioassays with *Danio rerio* larvae as test organisms. Histology of gills and biometric measurements were used to determine the existence of toxicity problems. The alterations on gill morphology here detected were considered of first stage (hyperplasia, lamellar junction and excess of mucous cells) in the first reservoirs and very slight in the last ones. The biometric analysis pointed to inadequate conditions for the growth of the test-organisms when

exposed to the sediment of the rivers and upstream reservoirs and also indicated an improvement of environmental conditions along the system. Copyright © 2008 CABI
Descriptors: zebrafish, gills, growth and morphology, polluted water, sediment, toxicity, water pollution, water quality, *Danio rerio*.
Language of Text: Portuguese.

Giacomini, N.J., B. Rose, K. Kobayashi, and S. Guo (2006). **Antipsychotics produce locomotor impairment in larval zebrafish.** *Neurotoxicology and Teratology* 28(2): 245-50. ISSN: 0892-0362.
NAL Call Number: RA1224
Abstract: Zebrafish has been a favored vertebrate genetic model organism for studying developmental processes. It also holds a great potential for understanding the genetic basis of behavior and associated behavioral disorders. Despite such potential, their use in the study of behavior is greatly under-explored. It is well known that multiple classes of drugs used to treat psychiatric diseases produce extrapyramidal side (EPS) effects and consequent movement disorders in humans. The underlying molecular causes of these drug-induced movement disorders are poorly understood. Here we report that zebrafish treated with the antipsychotics fluphenazine and haloperidol (both of which can induce severe EPS in humans) develop movement defects. In contrast, another antipsychotic olanzapine, which produces mild to little EPS in humans, leads to minimal movement defects in zebrafish. These results establish a rapid assay system in which the effects of EPS-inducing agents can be assessed. Thus, future genetic screening in zebrafish shall identify genes and pathways that elucidate drug-induced movement disorder in human as well as provide insights into the brain control of locomotor activity. Future chemical screening in zebrafish may act as a preclinical test for the EPS effect of certain drugs, as well as a test used to researching drugs made to counteract the effects of EPS.
Descriptors: zebrafish larvae, antipsychotic agents toxicity, fluphenazine toxicity, haloperidol toxicity, motor activity drug effects, movement disorders etiology, anti-Parkinson agents therapeutic use, behavior drug effects, disease models, drug interactions, larva drug effects, levodopa therapeutic use, movement disorders drug therapy, swimming.

Gonzalez, P., M. Baudrimont, A. Boudou, and J.P. Bourdineaud (2006). **Comparative effects of direct cadmium contamination on gene expression in gills, liver, skeletal muscles and brain of the zebrafish (*Danio rerio*).** *Biometals* 19(3): 225-35. ISSN: 0966 0844.
NAL Call Number: QP532
Abstract: The effects of cadmium (Cd) on gene expression were examined in four organs (gills, liver, skeletal muscles and brain) of the zebrafish. Adult male fish were subjected to three different water contamination pressures over periods of 7 and 21 days: control medium (C(0): no Cd added) and two contaminated media (C(1): 1.9 +/- 0.6 microg Cd l(-1), and C(2): 9.6 +/- 2.9 microg Cd l(-1)). Fourteen genes involved in antioxidant defences, metal chelation, active efflux of organic compounds, mitochondrial metabolism, DNA repair and apoptosis were selected and their expression levels investigated by quantitative real-time PCR. Cadmium concentrations were determined in the four organs and metallothionein (MT) protein levels investigated in brain, liver and gills. Although skeletal muscle was a poor Cd-accumulating tissue, many genes were up-regulated at day 7: mt1, cyt, bax, gadd and rad51 genes. Three additional genes, c-jun, pyc and tap, were up-regulated in muscles at day 21 whereas bax, gadd and rad51 had returned to basal levels. Surprisingly, mt1 and c-jun were the only genes displaying a differential induction after 21 days in liver, although this organ accumulated the highest cadmium concentration. In brain, only mt1, mt2 and c-jun genes

were up-regulated after 21 days. In gills, the highest response was observed after 7 days, featuring the differential expression of oxidative stress-response hsp70 and mitochondrial sod genes, along with genes involved in mitochondrial metabolism and metal detoxification. Then, after 21 days, the expression of almost every genes returned to basal levels while both mt1 and mt2 genes were up-regulated.

Descriptors: zebrafish cadmium toxicity model, cadmium poisoning metabolism, gene expression, brain, gills, liver and skeletal muscle drug effects, proteins biosynthesis, brain, skeletal muscle, gill and liver metabolism, metallothionein biosynthesis, up regulation.

Gonzalez, P., Y. Dominique, J.C. Massabuau, A. Boudou, and J.P. Bourdineaud (2005). **Comparative effects of dietary methylmercury on gene expression in liver, skeletal muscle, and brain of the zebrafish (*Danio rerio*).** *Environmental Science and Technology* 39(11): 3972-80. ISSN: 0013 936X.

NAL Call Number: TD420.A1E5

Abstract: Effects of dietary methylmercury (MeHg) on gene expression were examined in three organs (liver, skeletal muscle, and brain) of the zebrafish (*Danio rerio*). Adult male fish were fed over 7, 21, and 63 days on three different diets: one control diet (C0: 0.08 microg of Hg g(-1), dry wt) and two diets (C1 and C2) contaminated by MeHg at 5 and 13.5 microg of Hg g(-1), dry wt. Total Hg and MeHg concentrations were determined in the three organs after each exposure duration, and a demethylation process was evidenced only in the liver. Thirteen genes known to be involved in antioxidant defenses, metal chelation, active efflux of organic compounds, mitochondrial metabolism, DNA repair, and apoptosis were investigated by quantitative real-time RT-PCR and normalized according to actin gene expression. Surprisingly, no change in the expression levels of these genes was observed in contaminated brain samples, although this organ accumulated the highest mercury concentration (63.5 +/- 4.4 microg g(-1), dry wt after 63 days). This lack of genetic response could explain the high neurotoxicity of MeHg. coxI and cytoplasmic and mitochondrial sod gene expressions were induced early in skeletal muscle and later in liver, indicating an impact on the mitochondrial metabolism and production of reactive oxygen species. Results demonstrated that skeletal muscle was not only an important storage reservoir but was also affected by MeHg contamination. The expression of the metallothionein mt2 and the DNA repair rad51 genes was up-regulated in liver between 21 and 63 days, whereas in skeletal muscle, mt2 remained uninduced, and gadd and rad51 were found to be repressed.

Descriptors: zebrafish dietary methylmercury, brain and liver drug effects, methylmercury compounds toxicity, skeletal muscle drug effects, antioxidants metabolism, apoptosis drug effects and physiology, base sequence, brain andchelating agents metabolism, DNA repair genetics and physiology, gene expression, liver metabolism, metallothionein genetics, metallothionein metabolism, methylmercury compounds metabolism, mitochondria metabolism, skeletal muscle metabolism, organic chemicals metabolism, reactive oxygen species metabolism, reverse transcriptase PCR, time factors.

Notes:

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Griffitt, R.J., R. Weil, K.A. Hyndman, N.D. Denslow, K. Powers, D. Taylor, and D.S. Barber (2007). **Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (*Danio rerio*).** *Environmental Science & Technology* 41(23): 8178-8186. ISSN: 0013-936X.

NAL Call Number: TD420.A1E5

Descriptors: zebrafish toxicity model, copper particles, nanoparticulates, copper, acute toxicity, soluble copper, 80 nm copper, pollutant in suspensions.

Gustavsson, L., H. Hollert, S. Jonsson, B. van Bavel, and M. Engwall (2007). **Reed beds receiving industrial sludge containing nitroaromatic compounds. effects of outgoing water and bed material extracts in the umu-c genotoxicity assay, DR-CALUX assay and on early life stage development in zebrafish (*Danio rerio*).** *Environmental Science and Pollution Research International* 14(3): 202-11. ISSN: 0944 1344.

NAL Call Number: GE 1

Abstract: GOAL, SCOPE AND BACKGROUND: Sweden has prohibited the deposition of organic waste since January, 2005. Since 1 million tons of sludge is produced every year in Sweden and the capacity for incineration does not fill the demands, other methods of sludge management have to be introduced to a larger degree. One common method in the USA and parts of Europe is the use of wetlands to treat wastewater and sewage sludge. The capacity of reed beds to affect the toxicity of a complex mixture of nitroaromatics in sludge, however, is not fully elucidated. In this study, an industrial sludge containing explosives and pharmaceutical residues was therefore treated in artificial reed beds and the change in toxicity was studied. Nitroaromatic compounds, which are the main ingredients of many pharmaceuticals and explosives, are well known to cause cytotoxicity and genotoxicity. Recently performed studies have also showed that embryos of zebrafish (Danio rerio) are sensitive to nitroaromatic compounds. Therefore, we tested the sludge passing through constructed wetlands in order to detect any changes in levels of embryotoxicity, genotoxicity and dioxin-like activity (AhR-agonists). We also compared unplanted and planted systems in order to examine the impact of the root system on the fate of the toxicants. METHODS: An industrial sludge containing a complex mixture of nitroaromatics was added daily to small-scale constructed wetlands (vertical flow), both unplanted and planted with Phragmites australis. Sludge with an average dry weight of 1.25%, was added with an average hydraulic loading rate of 1.2 L/day. Outgoing water was collected daily and stored at -20 degrees C. The artificial wetland sediment was Soxhlet extracted, followed by clean-up with multi-layer silica, or extracted by ultrasonic treatment, yielding one organic extract and one water extract of the same sample. Genotoxicity of the extracts was measured according to the ISO protocol for the umu-C genotoxicity assay (ISO/TC 147/SC 5/ WG9 N8), using Salmonella typhimurium TA1535/pSK1002 as test organism. Embryotoxicity and teratogenicity were studied using the fish egg assay with zebrafish (Danio rerio) and the dioxin-like activity was measured using the DR-CALUX assay. Chemical analyses of nitroaromatic compounds were performed using Solid Phase Micro Extraction (SPME) and GC-MS. RESULTS: Organic extracts of the bed material showed toxic potential in all three toxicity tests after two years of sludge loading. There was a difference between the planted and the unplanted beds, where the toxicity of organic extracts overall was higher in the bed material from the planted beds. The higher toxicity of the planted beds could have been caused by the higher levels of total carbon in the planted beds, which binds organic toxicants, and by enrichment caused by lower volumes of outgoing water from the planted beds. DISCUSSION: Developmental disorders were observed in zebrafish exposed directly in contact to bed material from unplanted beds, but not in fish exposed to bed material from planted beds. Hatching rates were slightly lower in zebrafish exposed to outgoing water from unplanted beds than in embryos exposed to outgoing water from planted beds. Genotoxicity in the outgoing water was below detection limit for both planted and unplanted beds. Most of the added toxicants via the sludge were unaccounted for in the outgoing water, suggesting that the beds had toxicant removal potential, although the mechanisms behind this remain unknown. CONCLUSIONS: During the experimental period, the beds received a sludge volume (dry weight) of around three times their own volume. In spite of this, the toxicity in the bed material was lower than in the sludge. Thus, the beds were probably able to actually decrease the toxicity of the added, sludge-associated

toxicants. When testing the acetone extracts of the bed material, the planted bed showed a higher toxicity than the unplanted beds in all three toxicity tests. The toxicity of water extracts from the unplanted beds, detected by the fish egg assay, were higher than the water extracts from the planted beds. No genotoxicity was detected in outgoing water from either planted or unplanted beds. All this together indicates that the planted reed beds retained semi-lipophilic acetone-soluble toxic compounds from the sludge better than the unplanted beds, which tended to leak out more of the water soluble toxic compounds in the outgoing water. The compounds identified by SPME/GC in the outgoing water were not in sufficient concentrations to have caused induction in the genotoxicity test. RECOMMENDATIONS AND PERSPECTIVES: This study has pointed out the benefits of using constructed wetlands receiving an industrial sludge containing a complex mixture of nitroaromatics to reduce toxicity in the outgoing water. The water from planted, constructed wetlands could therefore be directed to a recipient without further cleaning. The bed material should be investigated over a longer period of time in order to evaluate potential accumulation and leakage prior to proper usage or storage. The plants should be investigated in order to examine uptake and possible release when the plant biomass is degraded.

Descriptors: zebrafish embryos toxicity model, industrial waste, nitrogen compounds toxicity, sewage chemistry, chemical water pollutants toxicity, wetlands , environmental biodegradation, gas chromatography mass spectrometry, mutagenicity tests, nitrogen compounds chemistry and metabolism, ovum drug effects, ovum growth and development, plants metabolism, *Salmonella typhimurium* drug effects and genetics, Sweden, water movements, chemical water pollutants chemistry and metabolism, water purification.

Haendel, M.A., F. Tilton, G.S. Bailey, and R.L. Tanguay (2004). **Developmental toxicity of the dithiocarbamate pesticide sodium metam in zebrafish.** *Toxicological Sciences* 81(2): 390-400. ISSN: 1096-6080.

NAL Call Number: RA1190.F8

Abstract: Sodium metam (NaM), a dithiocarbamate, is a general agricultural biocide applied prior to planting for the elimination of nematodes, soil pathogens, and weeds. There is a remarkable paucity of information about the mechanism of action and the risk that dithiocarbamates may pose to developing vertebrates. We have characterized NaM toxicity during early life stage exposure in zebrafish. Zebrafish embryos are most sensitive to NaM exposure during gastrulation and early segmentation (4-14 hours post fertilization, hpf). For mortality, the dose response curve is steep with an LC(50) estimate of 1.95 microM (248 ppb) at 48 hpf. The most notable malformation among surviving embryos was a severely twisted notochord, which became evident by 24 hpf. Surprisingly, this notochord defect was not immediately lethal and the animals continued to grow despite delays in hatching, apparent paralysis, and an inability to feed. We have characterized the notochord malformation using histological and in situ hybridization techniques. collagen 2a1 mRNA expression is normally localized to the notochord sheath cells at 24 hpf, whereas in NaM-exposed embryos it is misexpressed in the notochord cells. Histological staining and myoD expression indicate that the myotomes of the NaM-exposed embryos are less defined, compacted and block-shaped compared to controls. The degradation product of NaM, methyl isothiocyanate (MITC), causes similar malformations at similar concentrations as NaM, suggesting that MITC or another common product may be the active toxicant. Our results indicate that developing zebrafish are sensitive to NaM and MITC and we believe that this model is ideal to elucidate the molecular mechanism(s) and etiology of NaM toxicity in vertebrates.

Descriptors: zebrafish embryos, pesticides toxicity, thiocarbamates toxicity, cell differentiation, drug effects, in situ hybridization, isothiocyanates pharmacokinetics and toxicity ,

notochord abnormalities, drug effects, growth and development, pesticides and thiocarbamates pharmacokinetics, tissue fixation.

Hallare, A.V., H.R. Kohler, and R. Triebskorn (2004). **Developmental toxicity and stress protein responses in zebrafish embryos after exposure to diclofenac and its solvent, DMSO**. *Chemosphere* 56(7): 659-666. ISSN: 0045 6535.
NAL Call Number: TD172.C54
Abstract: One of the most frequently detected pharmaceuticals in environmental water samples is the anti-rheumatic drug, diclofenac. Despite its increasing environmental significance, investigations concerning the effects of this drug on the early developmental stages of aquatic species are lacking up to now. To determine the developmental toxicity and proteotoxicity of this drug on the growing fish embryos, eggs of zebrafish were exposed to six concentrations of diclofenac (0, 1, 20, 100, 500, 1000, and 2000 micro g l-1) using DMSO as solvent. Early life stage parameters such as egg and embryo mortality, gastrulation, somite formation, movement and tail detachment, pigmentation, heart beat, and hatching success were noted and described within 48- and 96-h of exposure. After the 96-h exposure, the levels of stress proteins (hsp 70) were determined in both the diclofenac-treated and respective DMSO controls. Results showed no significant inhibition in the normal development until the end of 96 h for all exposure groups. However, there was a delay in the hatching time among embryos exposed to 1000 and 2000 micro g l-1. Late-hatched embryos (108 h) did not differ morphologically from normally hatched embryos. The mortality and average heart rate data did not show significant differences for all embryos in both diclofenac-treated and DMSO control groups. No significant malformations were likewise noted among all developing embryos throughout the exposure period. The levels of heat shock proteins in diclofenac-treated and control embryos did not differ significantly. DMSO control embryos, on the other hand, showed a concentration-dependent increase in hsp 70 levels. We suggest possible modulating effect of diclofenac in DMSO-triggered expression of stress proteins and this might have a possible repercussion on the use of DMSO as solvent in any toxicity assay. Since the present data indicate no significant embryotoxicity and proteotoxicity induced by diclofenac and due to the fact that the concentrations of diclofenac used in the present study is up to 2000-fold higher than the concentrations detected in the environment, it is unlikely that this drug would pose a hazard to early-life stages of zebrafish. Copyright © 2008 CABI
Descriptors: zebrafish embryos drug toxicity, dimethyl sulfoxide , drug residues, embryo mortality, embryonic development, fish eggs, fry, hatching, larva, heart rate, heat shock proteins, larvae, non steroidal antiinflammatory agents, poisoning, water pollution, *Danio rerio.*

Hallare, A.V., R. Pagulayan, N. Lacdan, H.R. Kohler, and R. Triebskorn (2005). **Assessing water quality in a tropical lake using biomarkers in zebrafish embryos: developmental toxicity and stress protein responses.** *Environmental Monitoring and Assessment* 104(1-3): 171-87. ISSN: 0167 6369.
NAL Call Number: TD194.E5
Abstract: In order to achieve a more substantial appraisal of lake water quality, the assessment must not be based only on chemical measurements and analyses of the water itself, but even more so on the impact of existing conditions on aquatic biota. This is possible with the use of biotests or biomarkers, e.g. investigations of the developmental parameters (96-h embryotoxicity evaluate) or of the induction of heat shock proteins (proteotoxicity evaluate). To evaluate the suitability of these tests for environmental screening, fertilized zebrafish eggs were exposed to water samples collected from five sites of varying levels of stress from Laguna Lake, Philippines. Reconstituted water was used as laboratory control while water samples

from a highly polluted freshwater source was used as positive control. Developmental parameters were noted and described within 48 and 96 h of exposure. Dilution experiments of the positive control were also done to further assess and compare toxicity potentials of Laguna Lake waters with those originating from a polluted freshwater. After the 96-h exposure, the levels of stress proteins (hsp 70) were determined in embryos from all exposure groups. Results showed 100% mortality in embryos exposed to undiluted positive control (PC) within only 12 h. Increasing dilution levels, however, resulted in lower mortality and lower abnormality rates. No detectable developmental differences were noted among embryos exposed to either the laboratory control or Laguna Lake waters at the end of 96 h, regardless of the source. Very high survival rates and high hatching success rates were observed in embryos exposed to lake waters as well as laboratory control, and the data did not differ significantly among the groups. Likewise, no significant malformations were noted among all developing embryos throughout the exposure period. However, the levels of heat shock proteins in the two sites located closest to Manila, the Philippine capital (Northern West Bay and Central West Bay), showed a pronounced elevation relative to the control, indicating that these stress proteins protect the embryos from the detrimental effects of pollutants in the water. Based on the 96-h early life stage (ELS) test, the water quality of the lake is good for fishery propagation despite the current levels of pollutants in the water. This finding is in accordance with the Class C status (i.e. suitable for fish growth and propagation) as given to the lake by the local environmental agency. On the other hand, data on proteotoxicity showed that the fish are under stress, presumably deriving from pollutants. This calls for a continuous monitoring and improvement of the lake water. The present study indicates that the two biomarker methods are very easy to use, practical, rapid, and sensitive for assessing water quality in a tropical lake and recommends for their incorporation into the future monitoring program of Laguna Lake.

Descriptors: zebrafish embryos, biological markers analysis, environmental monitoring methods, fresh water analysis, HSP70 heat shock proteins biosynthesis, tropical climate, teratogens, metabolism, Philippines.

Hallare A.V. , M. Schirling, T. Luckenbach, H. R. Koehler , and R. Triebskorn (2005). **Combined effects of temperature and cadmium on developmental parameters and biomarker responses in zebrafish (*Danio rerio*) embryos.** *Journal of Thermal Biology* 30(1): 7-17. ISSN: 0306 4565.

NAL Call Number: QP82.2.T4J6

Abstract: To determine the interactions between temperature and cadmium on zebrafish (*Danio rerio*) development.. fertilized eggs were exposed to combinations of three temperature levels (21 degreeC. 26 degreeC. and 33 degreeC) and six cadmium concentrations (0, 0.25 0.5 2.0. 5.0. and 10.0mg/L). Endpoints used included LC50 value (48h), developmental rate. mortality, heart rate. hatching success. liver histopathology, embryo abnormalities, and heat shock protein (hsp) induction. Results showed a significant acceleration in the developmental rate with increasing temperature and irrespective of the presence of cadmium. Data on LC50 and ELS-test revealed that simultaneous exposure to both cadmium ions and cold stress (21 degreeC) was highly detrimental to growing ernbryos, causing a pronounced mortality and a significant reduction in average heart rate and embryo hatchability. In contrast. no similar reactions to cadmium were observed in pre-hatched embryos exposed to both control (26degreeC) and high temperature (33degreeC), and this can be explained by the significantly higher expression of hsp (hsp70) in embryos at these temperatures. Upon hatching, however, the larvae showed increased sensitivity to cadmium. The severity of malformations in the post-hatched larvae was in the order: hot cadmium stress>cold cadmium

stress>cadmium stress alone>no stress at all. Liver histopathology as well as depletion in glycogen reserves exhibited greater severity with increasing cadmium concentration, irrespective of temperature. The present study confirms that temperature effectively confounds cadmium toxicity and needs to be considered-for the accurate prediction and assessment of cadmium-induced toxicity in fish. Copyright 2004 Elsevier Ltd. All rights reserved. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, development, marine ecology, environmental sciences, toxicology, temperature variations, LD50 values, cadmium, cold and heat stress.

Handley Goldstone, H.M., M.W. Grow, and J.J. Stegeman (2005). **Cardiovascular gene expression profiles of dioxin exposure in zebrafish embryos.** *Toxicological Sciences* 85(1): 683-93. ISSN: 1096-6080.
NAL Call Number: RA1190.F8
Abstract: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a widespread environmental contaminant that causes altered heart morphology, circulatory impairment, edema, hemorrhage, and early life stage mortality in fish. TCDD toxicity is dependent, in large part, on the aryl hydrocarbon receptor (AHR), but understanding of the molecular mechanism of cardiovascular embryotoxicity remains incomplete. To identify genes potentially involved in cardiovascular effects, we constructed custom cDNA microarrays consisting of 4896 zebrafish adult heart cDNA clones and over 200 genes with known developmental, toxicological and housekeeping roles. Gene expression profiles were obtained for 3-day-old zebrafish after early embryonic exposure to either 0.5 or 5.0 nM TCDD. In all, 516 clones were significantly differentially expressed ($p < 0.005$) under at least one treatment condition; 123 high-priority clones were selected for further investigation. Cytochromes P450 1A and 1B1, and other members of the AHR gene battery, were strongly and dose-dependently induced by TCDD. Importantly, altered expression of cardiac sarcomere components, including cardiac troponin T2 and multiple myosin isoforms, was consistent with the hypothesis that TCDD causes dilated cardiomyopathy. Observed increases in expression levels of mitochondrial energy transfer genes also may be related to cardiomyopathy. Other TCDD-responsive genes included fatty acid and steroid metabolism enzymes, ribosomal and signal-transduction proteins, and 18 expressed sequence tags (ESTs) with no known protein homologs. As the first broad-scale study of TCDD-modulated gene expression in a non-mammalian system, this work provides an important perspective on mechanisms of TCDD toxicity.
Descriptors: zebrafish embryo dioxin exposure, environmental pollutants toxicity, gene expression drug effects, heart embryology, tetrachlorodibenzodioxin toxicity, drug effects, metabolism, gene expression profiling, heart drug effects, oligonucleotide array sequence analysis.
Notes: Comments: Erratum In:Toxicol Sci. 2005 Jun; 85(2):1066
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Hassoun, E., C. Kariya, and F.E. Williams (2005). **Dichloroacetate-induced developmental toxicity and production of reactive oxygen species in zebrafish embryos.** *Journal of Biochemical and Molecular Toxicology* 19(1): 52-8. ISSN: 1095-6670.
Descriptors: zebrafish embryo model for dichloroacetate toxicity, reactive oxygen species metabolism, behavior drug effects, blood circulation drug effects, chemically induced craniofacial abnormalities, chemically induced edema and pathology, abnormalities and drug effects, embryology, metabolism, heart rate drug effects, nitric oxide biosynthesis and metabolism, ovum drug effects, pathology and physiology, superoxides metabolism, water toxins.

Heiden, T.C., E. Dengler, W.J. Kao, W. Heideman, and R.E. Peterson (2007). **Developmental toxicity of low generation PAMAM dendrimers in zebrafish.** *Toxicology and Applied Pharmacology* 225(1): 70-9. ISSN: 0041-008X.

NAL Call Number: 391.8T662

Abstract: Biological molecules and intracellular structures operate at the nanoscale; therefore, development of nanomedicines shows great promise for the treatment of disease by using targeted drug delivery and gene therapies. PAMAM dendrimers, which are highly branched polymers with low polydispersity and high functionality, provide an ideal architecture for construction of effective drug carriers, gene transfer devices and imaging of biological systems. For example, dendrimers bioconjugated with selective ligands such as Arg-Gly-Asp (RGD) would theoretically target cells that contain integrin receptors and show potential for use as drug delivery devices. While RGD-conjugated dendrimers are generally considered not to be cytotoxic, there currently exists little information on the risks that such materials pose to human health. In an effort to compliment and extend the knowledge gleaned from cell culture assays, we have used the zebrafish embryo as a rapid, medium throughput, cost-effective whole-animal model to provide a more comprehensive and predictive developmental toxicity screen for nanomaterials such as PAMAM dendrimers. Using the zebrafish embryo, we have assessed the developmental toxicity of low generation (G3.5 and G4) PAMAM dendrimers, as well as RGD-conjugated forms for comparison. Our results demonstrate that G4 dendrimers, which have amino functional groups, are toxic and attenuate growth and development of zebrafish embryos at sublethal concentrations; however, G3.5 dendrimers, with carboxylic acid terminal functional groups, are not toxic to zebrafish embryos. Furthermore, RGD-conjugated G4 dendrimers are less potent in causing embryo toxicity than G4 dendrimers. RGD-conjugated G3.5 dendrimers do not elicit toxicity at the highest concentrations tested and warrant further study for use as a drug delivery device.

Descriptors: zebrafish embryos, drug carriers toxicity, drug effects on embryonic development, models, polyamines toxicity, drug dose response relationship, drug carriers administration and dosage, drug carriers chemistry, drug delivery systems, integrins metabolism, ligands, oligopeptides administration and dosage, oligopeptides toxicity, polyamines administration and dosage, polyamines chemistry, structure activity relationship, time factors, toxicity tests.

Heiden, T.K., M.J.3. Carvan, and R.J. Hutz (2006). **Inhibition of follicular development, vitellogenesis, and serum 17beta-estradiol concentrations in zebrafish following chronic, sublethal dietary exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin.** *Toxicological Sciences* 90(2): 490-9. ISSN: 1096-6080.

NAL Call Number: RA1190.F8

Abstract: The environmental toxicant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent endocrine disruptor with the ability to affect several biologic processes, including reproduction. In fish, sublethal exposure to TCDD is known to modulate overall reproductive capacity, but impacts on follicular development and vitellogenesis are unknown. Here we show that chronic, dietary exposure to 0.08, 0.32, or 0.80 ng TCDD female(-1) day(-1) decreased egg production by more than 50% and that spawning success was reduced by as much as 96%. Serum estradiol concentrations were decreased more than twofold, accounting, in part, for observed decreases in serum vitellogenin concentrations by as much as 29%. Our data suggest that decreased egg production is likely the result of TCDD-mediated inhibition of the transition from pre-vitellogenic stage follicles to vitellogenic stage follicles, as well as the induction of follicular atresia. The majority of reproductive toxicity of TCDD is likely due to direct impacts on the ovary, yet histopathologic observations suggest liver

toxicity could also contribute to observed impacts on vitellogenesis. Importantly, even when overall egg production is not significantly affected, our data show that subtle physiologic changes induced by TCDD can lead to altered gonadogenesis. This suggests that long-term exposure to very low concentrations of TCDD could greatly affect fecundity and reproductive success in fishes.

Descriptors: zebrafish endocrine disruptors model, environmental pollutants toxicity, estradiol blood, ovarian follicle drug effects, tetrachlorodibenzodioxin toxicity, vitellogenins blood, body burden, diet, endocrine disruptors pharmacokinetics and toxicity, environmental pollutants pharmacokinetics, fertility drug effects, liver drug effects, liver pathology, ovarian follicle growth and development, tetrachlorodibenzodioxin pharmacokinetics.

Heiden, T.K., R.J. Hutz, and M.J.3. Carvan (2005). **Accumulation, tissue distribution, and maternal transfer of dietary 2,3,7,8,-tetrachlorodibenzo-p-dioxin: impacts on reproductive success of zebrafish.** *Toxicological Sciences* 87(2): 497-507. ISSN: 1096-6080.

NAL Call Number: RA1190.F8

Abstract: TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is a reproductive toxicant and endocrine disruptor in nearly all vertebrates; however, the mechanisms by which TCDD alters the reproductive system is not well understood. The zebrafish provides a powerful vertebrate model system to investigate molecular mechanisms by which TCDD affects the reproductive system, but little is known regarding reproductive toxic response of zebrafish following chronic, sublethal exposure to TCDD. Here we investigate the accumulation of TCDD in selected tissues of adult female zebrafish and maternal transfer to offspring following dietary exposure to TCDD (0.08-2.16 ng TCDD/fish/day). TCDD accumulated in tissues of zebrafish in a dose- and time-dependent manner, except for brain. Chronic dietary exposure resulting in the accumulation of 1.1-36 ng/g fish did not induce an overt toxic response or suppress spawning activity. The ovosomatic index was impacted with an accumulation of as little as 0.6 ng/g fish, and 10% of the females showed signs of ovarian necrosis following accumulation of approximately 3 ng/g TCDD. Offspring health was impacted with an accumulation of as little as 1.1 ng/g female; thus the lowest observed effect level (LOEL) for reproductive toxicity in female zebrafish is approximately 0.6-1.1 ng/g fish. Maternal transfer resulted in the accumulation of 0.094-1.2 ng/g, TCDD, which was sufficient to induce the typical endpoints of larval TCDD toxicity, commonly referred to as blue sac syndrome. This study provides the necessary framework to utilize the zebrafish model system for further investigations into the molecular mechanisms by which TCDD exerts its reproductive toxic responses.

Descriptors: dioxin effects on zebrafish, environmental pollutants pharmacokinetics and toxicity, reproduction drug effects, tetrachlorodibenzodioxin pharmacokinetics and toxicity, zygote metabolism, body burden, diet, drug dose response relationship, endpoint determination, ovary pathology, tissue distribution, zygote chemistry.

Hen Chow, E.S. and S.H. Cheng (2003). **Cadmium affects muscle type development and axon growth in zebrafish embryonic somitogenesis.** *Toxicological Sciences* 73(1): 149-59. ISSN: 1096-6080.

NAL Call Number: RA1190.F8

Abstract: We have previously reported that exposure to cadmium during zebrafish embryonic development caused morphological malformations of organs and ectopic expression of genes involved in regulating developmental process. One of the most common developmental defects observed was altered axial curvature resulting from defects in the myotomes of the somites. In this study, we investigated the mechanisms of cadmium-induced toxicity in

zebrafish somitogenesis. We showed that the critical period of exposure was the gastrulation period, which actually preceded the formation of the first morphologically distinct somites. The somites thus formed lost the typical chevron V-shape and are packed disorderly. The myogenic lineage commitment of the axial mesodermal cells was not affected, as the myogenic regulatory transcription factors were expressed normally. There were, however, losses of fast and slow muscle fibers in the myotomes. The innervation of the muscle blocks by spinal motoneurons is an important process of the somitogenesis. Both primary and secondary motoneurons appear to form normally while the axon growth is affected in cadmium-treated embryos. The notochord, which is essential in the patterning of the somites and the central nervous system, showed abnormal morphological features and failed to extend to the tail region. Taken together, it appears that cadmium exposure led to abnormal somite patterning of the muscle fibers and defects in axonogenesis.

Descriptors: zebrafish embryos, axons drug effects, cadmium toxicity, drug effects, embryonic development, skeletal muscle drug effects and embryology, nervous system drug effects and embryology, somites drug effects, activated leukocyte cell adhesion molecule metabolism, immunohistochemistry, in situ hybridization, membrane proteins metabolism, motor neurons drug effects, myod protein genetics metabolism, nervous system cytology, neural pathways drug effects, growth and development, notochord cytology, drug effects, growth and development.

Hernandez, P.P., V. Moreno, F.A. Olivari, and M.L. Allende (2006). **Sub-lethal concentrations of waterborne copper are toxic to lateral line neuromasts in zebrafish (*Danio rerio*).** *Hearing Research* 213(1/2): 1-10. ISSN: 0378 5955.

Abstract: In teleosts, the lateral line system is composed of neuromasts containing hair cells that are analogous to those present in the inner ear of all vertebrates. In the embryo and early larva of zebrafish (*Danio rerio*), this system is composed of the anterior lateral line (ALL), which covers the head, and the posterior lateral line (PLL), present in the trunk and tail. The mechanosensory hair cells found in neuromasts can be labelled in vivo using fluorescent dyes such as 4-di-2-Asp (DiAsp) or FM1-43. We have studied the effects of water-borne copper exposure on the function of the lateral line system in zebrafish larvae. Our results show that transient incubation of post-hatching larvae for 2 h with non-lethal concentrations of copper (1-50 micro M CuSO4) induces cellular damage localized to neuromasts, apoptosis, and loss of hair cell markers. This effect is specific to copper, as other metals did not show these effects. Since hair cells in fish can regenerate, we followed the reappearance of viable hair cells in neuromasts after copper removal. In the PLL, we determined that there is a threshold concentration of copper above which regeneration does not occur, whereas, at lower concentrations, the length of time it takes for viable hair cells to reappear is dependent on the amount of copper used during the treatment. The ALL behaves differently though, as regeneration can occur even after treatments with concentrations of copper an order of magnitude higher than the one that irreversibly affects the PLL. Regeneration of hair cells is dependent on cell division within the neuromasts as damage that precludes proliferation prevents reappearance of this cell type. Copyright © 2008 CABI

Descriptors: zebrafish, apoptosis, cell division, cell lines, copper exposure, immunohistochemistry, larvae, neurons, polluted water, sublethal effects, toxicity, water pollution, water quality, *Danio rerio.*

Hill, A., C.V. Howard, U. Strahle, and A. Cossins (2003). **Neurodevelopmental defects in zebrafish (*Danio rerio*) at environmentally relevant dioxin (TCDD) concentrations.** *Toxicological Sciences* 76(2): 392-9. ISSN: 1096-6080.

NAL Call Number: RA1190.F8
Abstract: Persistent ecotoxicants, such as dioxin and PCBs, are thought to pose one of the greatest threats to public and ecological health in the industrial world. These compounds cause a range of macroscopic malformations, particularly to the craniofacial apparatus and cardiovascular system during vertebrate development. However, little is known about microscopic effects, especially on the sensitive early life stages or on the molecular basis of developmental neurotoxicity. Using zebrafish (*Danio rerio)*, we have explored neurological deficits caused by early-life exposure to environmentally relevant concentrations of dioxin. We show, using a quantitative stereological technique, that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) substantially reduces the capacity for embryonic brain development, causing a 30% reduction in total neuronal number in the 168-h larval brain. Using transgenic GFP-expressing zebrafish lines, we link this to decreased expression of key developmentally regulated genes, namely neurogenin and sonic hedgehog. This disruption of neuronal development provides the basis for understanding the neurotoxic effects of these compounds.
Descriptors: zebrafish, brain drug effects, drug effects, environmental pollutants toxicity, tetrachlorodibenzodioxin toxicity, genetically modified, apoptosis drug effects, basic helix loop helix transcription factors, brain abnormalities, drug dose response relationship, abnormalities, metabolism, developmental gene expression regulation drug effects, Hedgehog proteins, in situ hybridization, nerve tissue trans activators genetics, transcription factors genetics, proteins genetics and metabolism.

Hill, A., V. Howard, and A. Cossins (2004). **Characterization of TCDD-induced craniofacial malformations and retardation of zebrafish growth**. *Journal of Fish Biology* 64(4): 911-922. ISSN: 0022 1112.
NAL Call Number: QL614.J68
Abstract: The documented 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced effects on zebrafish *Danio rerio* including craniofacial malformations and a general retardation of growth, were further characterized in the present study. A significant decrease in total body length and the length of each bone in the upper and lower jaw was identified in exposed larvae from an exposure concentration of 30ngl-1 TCDD. This study is the first quantitative evidence for the effects of TCDD on the upper jaw and also demonstrates that TCDD-induced craniofacial malformations and retardation of growth are very sensitive endpoints of dioxin toxicity. Copyright 2004 The Fisheries Society of the British Isles. Copyright©Thomson Reuters 2009
Descriptors: zebrafish dioxin toxicity model, pollution assessment control and management, toxicology, body length, characterization of craniofacial malformations, ecotoxicology, growth retardation, dioxin, toxicity, endpoints.

Hill, A.J., S.M. Bello, A.L. Prasch, R.E. Peterson, and W. Heideman (2004). **Water permeability and TCDD-induced edema in zebrafish early-life stages.** *Toxicological Sciences* 78(1): 78-87. ISSN: 1096-6080.
NAL Call Number: RA1190.F8
Abstract: A common response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure in teleost embryos is blue-sac disease, characterized by pericardial and yolk-sac edema. The cellular and extracellular fluids of freshwater fish are hyperosmotic compared to the surrounding water. In order to be in osmotic balance, freshwater fish must maintain a barrier to minimize water entry and excrete excess water that passes the barrier. We hypothesized that edema observed in TCDD-exposed zebrafish was caused by a failure of a barrier to incoming water. As a test of this hypothesis, we removed the osmotic gradient that drives water

entry by increasing the osmolarity of the surrounding water with mannitol. Abolishing the osmotic gradient between the interior body fluids and the water environment of the developing zebrafish significantly reduced both pericardial and yolk-sac edema. When added after edema formation had already started, mannitol only partially reversed pre-existing edema. An alternate hypothesis is that TCDD impairs water excretion, allowing water to accumulate as edema fluid. However, we were unable to demonstrate an alteration in kidney function: expression of early markers for kidney development appeared normal, and we did not observe TCDD-induced changes in kidney filtration. An alteration in the overall shape of the kidney was observed, but this may be a consequence of compression by edema. In conclusion, TCDD exposure may inhibit the function of a permeability barrier to water, which is critical for maintaining osmotic balance in early development.
Descriptors: zebrafish embryo dioxin exposure, aging physiology, chemically induced edema, environmental pollutants toxicity, tetrachlorodibenzodioxin toxicity, zebrafish growth and development, diuretics pharmacology, edema pathology and physiopathology feedback, in situ hybridization, kidney embryology, growth, development and physiology, kidney glomerulus embryology, growth and development and physiology, mannitol pharmacology, Na+ K+ exchanging ATPase metabolism, osmolar concentration, permeability drug effects, water metabolism.

Hill, A.J., H. Teraoka, W. Heideman, and R.E. Peterson (2005). **Zebrafish as a model vertebrate for investigating chemical toxicity.** *Toxicological Sciences* 86(1): 6-19. ISSN: 1096-6080.
NAL Call Number: RA1190.F8
Abstract: Zebrafish (*Danio rerio*) has been a prominent model vertebrate in a variety of biological disciplines. Substantial information gathered from developmental and genetic research, together with near-completion of the zebrafish genome project, has placed zebrafish in an attractive position for use as a toxicological model. Although still in its infancy, there is a clear potential for zebrafish to provide valuable new insights into chemical toxicity, drug discovery, and human disease using recent advances in forward and reverse genetic techniques coupled with large-scale, high-throughput screening. Here we present an overview of the rapidly increasing use of zebrafish in toxicology. Advantages of the zebrafish both in identifying endpoints of toxicity and in elucidating mechanisms of toxicity are highlighted.
Descriptors: zebrafish embryo toxicology, models, toxicity tests, cultured cells, oligonucleotide array sequence analysis.

Hill, R.L.J. and D.M. Janz (2003). **Developmental estrogenic exposure in zebrafish (*Danio rerio*): I. effects on sexratio and breeding success.** *Aquatic Toxicology* 63(4): 417-29. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: In vivo studies of fishes exposed to xenoestrogens have reported vitellogenin (Vtg) induction, ovatestes, altered sex ratios, and impaired reproductive capacity. The objective of this study was to determine concentration dependent effects of a weak estrogen receptor agonist, 4-nonylphenol (NP) and a potent estrogen receptor agonist, 17alpha-ethinylestradiol (EE) on sex ratios, gonad morphology, Vtg induction and breeding success in zebrafish (*Danio rerio*). Fish were exposed from 2 to 60 days post-hatch (dph) to NP (10, 30, or 100 microg/l nominal), EE (1, 10, or 100 ng/l nominal), or solvent control (acetone; 0.2% v/v) in a static-renewal system with replacement every 48 h. At 60 dph, 20 fish from each treatment were euthanized for histological examination of gonads and Western blotting for Vtg using pooled heart homogenates. Remaining fish were reared in clean water until adulthood (120 dph) for breeding studies. Due to high mortality in the 100 ng/l EE group, insufficient

fish were available for analyses. The percentage of males at 60 dph changed from 45% (9/20) in solvent controls to 0% at 10 ng/l EE and 10% at 100 microg/l NP. A concentration dependent increase in the number of fish with undeveloped gonads at 60 dph was observed in the EE exposure group. Two fish with ovatestes were observed at 100 microg/l NP, while one was observed at 30 microg/l NP. Western blotting showed induction of Vtg at 30 and 100 microg/l NP and 10 ng/l EE. The sex ratios of adults determined at 160 dph revealed no significant departure from 1 male:1 female, suggesting that exposure of zebrafish to estrogenic chemicals during sexual differentiation and early gametogenesis did not irreversibly alter phenotypic sex. The condition factor of adult fish and ovo-somatic index of adult female fish were also unaffected by developmental exposure to NP or EE. Despite this, breeding trials conducted in adult fish from 120 to 160 dph revealed significant reductions in the percent of viable eggs, hatchability, and swim-up success at 10 ng/l EE and 100 microg/l NP. Our results suggest that functional reproductive capacity (breeding success) may be more sensitive than gross morphological endpoints (condition, ovo-somatic index, sex ratio) in adult zebrafish exposed to xenoestrogens during sexual differentiation and early gametogenesis.
Descriptors: zebrafish, ethinyl estradiol analogs and derivatives, ethinyl estradiol toxicity, phenols toxicity, sex differentiation drug effects, drug dose response relationship, environmental exposure adverse effects, estradiol congeners toxicity, fertility drug effects, gametogenesis drug effects, chemically induced hermaphroditism, larva anatomy and histology, drug effects, growth and development, ovary anatomy and histology, drug effects, growth and development, reproduction drug effects, sex ratio, testis anatomy and histology, drug effects, growth and development, time factors, zebrafish anatomy and histology.

Hillegass, J.M., C.M. Villano, K.R. Cooper, and L.A. White (2007). **Matrix metalloproteinase-13 is required for zebra fish (*Danio rerio*) development and is a target for glucocorticoids.** *Toxicological Sciences* 100(1): 168-79. ISSN: 1096-6080.
NAL Call Number: RA1190.F8
Abstract: Matrix metalloproteinases (MMPs) are endopeptidases that degrade the proteins of the extracellular matrix (ECM). Expression and activity of the MMPs are essential for embryogenesis, where MMPs participate in the normal ECM remodeling that occurs during tissue morphogenesis and development. Studies have demonstrated that MMP gene expression is inhibited by glucocorticoids in mammalian cell culture systems and that exposure to glucocorticoids causes developmental abnormalities in several species. Therefore, we proposed that glucocorticoids impede normal development through alteration of MMP expression. Zebra fish *(Danio rerio*) were used as a model to study MMP-13 expression both during normal embryogenesis and following acute exposure to two glucocorticoids, dexamethasone, and hydrocortisone. MMP-13 is one of three collagenases identified in vertebrates that catalyzes the degradation of type I collagens at neutral pH. MMP-13 expression varied during zebra fish development, with peak expression at 48 h post-fertilization (hpf). Morpholino knockdown studies showed that MMP-13 expression is necessary for normal zebra fish embryogenesis. Acute exposure to dexamethasone and hydrocortisone resulted in abnormal zebra fish development including craniofacial abnormalities, altered somitogenesis, blood pooling and pericardial and yolk sac edema as well as increased MMP-13 mRNA and activity at 72 hpf. In situ hybridization experiments were used to confirm the increase in MMP-13 expression following glucocorticoid treatment and showed elevated MMP-13 expression in the rostral trunk, brain, eye, heart, and anterior kidney of treated embryos. These data demonstrate that normal zebra fish embryogenesis requires MMP-13 and that dexamethasone and hydrocortisone modulate the expression of this gene, leading to increased activity and potentially contributing to subsequent dysmorphogenesis.

Descriptors: zebrafish embryos toxicity model, dexamethasone toxicity, developmental gene expression regulation drug effects, glucocorticoids toxicity, hydrocortisone toxicity, matrix metalloproteinase 13 biosynthesis, metabolism, amino acid sequence, collagen metabolism, chemically induced craniofacial abnormalities and metabolism, chemically induced edema, edema metabolism, abnormalities, drug effects, enzymology, embryonic development drug effects, enzyme induction drug effects, mice, molecular sequence data, messenger RNA biosynthesis, sequence alignment, time factors.

Hillegass, J.M., C.M. Villano, K.R. Cooper, and L.A. White (2008). **Glucocorticoids alter craniofacial development and Increase expression and activity of matrix metalloproteinases in developing zebrafish (*Danio rerio*)**. *Toxicological Sciences* 102(2): 413-424. ISSN: 1096-6080.
NAL Call Number: RA1190.F8
Descriptors: zebrafish embryos, cell migration, dexamethasone, embryogenesis, extracellular matrix, fetuses, gelatinase, gelatinase A and B, gene expression, glucocorticoid receptors, hemorrhage, hydrocortisone, infants, matrix metalloproteinase, morphogenesis, mortality, respiratory distress syndrome, teratogenesis, teratogenicity, craniofacial growth, *Danio rerio*.

Hinfray, N., O. Palluel, C. Turies, C. Cousin, J.M. Porcher, and F. Brion (2006). **Brain and gonadal aromatase as potential targets of endocrine disrupting chemicals in a model species, the zebrafish (*Danio rerio*).** *Environmental Toxicology* 21(4): 332-7. ISSN: 1520 4081.
NAL Call Number: RA1221.T69
Descriptors: zebrafish, aromatase metabolism, brain enzymology, endocrine disruptors pharmacology, ovary enzymology, androstatrienes pharmacology, aromatase genetics, inhibitors pharmacology, brain drug effects, estradiol pharmacology, gene expression regulation drug effects, toxicity models, ovary drug effects, messenger RNA metabolism.

Hinton, D.E., S.W. Kullman, R.o.n.C. Hardman, D.C. Volz, P.-J. Chen, M. Carney, and D.C. Bencic (2005). **Resolving mechanisms of toxicity while pursuing ecotoxicological relevance?** *Marine Pollution Bulletin* 51(8-12): 635-648. ISSN: 0025-326X.
Descriptors: zebrafish, Accidents, aquatic organisms, biocomplexity, bioindicators, biomarkers, contaminants, contamination, cytotoxicity, disasters, ecotoxicology, exposure, fish, freshwater fish, genotoxicity, histochemistry, Laboratories, marine pollution, model studies, oil Spills, oil spills, organizations, pollutants, reviews, synergism, test organisms, toxicants, toxicity, toxicity tests, toxicology, water pollution effects, biomarkers, cyprinodon variegatus, Danio rerio, Oryzias latipes, Pimephales promelas, Xiphophorus, fathead minnow, medaka, sheepshead minnow, p 1000 marine pollution, X 24250 reviews, Q5 01504 effects on organisms, SW 3030 effects of pollution, AQ 00008 effects of pollution, EE 40 water pollution: monitoring, control & remediation.

Hoffmann, J.L. and J.T. Oris (2006). **Altered gene expression: a mechanism for reproductive toxicity in zebrafish exposed to benzo[a]pyrene.** *Aquatic Toxicology* 78(4): 332-40. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: Exposure of fish to polycyclic aromatic hydrocarbons (PAHs) has consistently been shown to have a negative impact on reproduction (e.g. decreased spawning success, decreased ovarian somatic index (OSI) and lower circulating levels of 17beta-estradiol and vitellogenin). While an understanding of the mechanism behind these changes has yet to be fully elucidated, it has been proposed that PAHs can alter the expression of genes important

in regulating reproduction. The objectives of this study were to: (1) determine the effect of exposure to waterborne benzo[a]pyrene (B[a]P) (0, 1.5 and 3.0 microg/L) for 56 days on egg production and OSI in female zebrafish (*Danio rerio*) and (2) determine the effect of B[a]P on transcription of genes involved in reproduction including gonadotropins (follicle stimulating hormone (FSH) and luteinizing hormone (LH)), steroidogenic enzymes (CYP11A1, CYP17, CYP19A1, CYP19A2 and 20beta-HSD), estrogen receptor beta (ERbeta) and vitellogenin. Cytochrome P4501A1 (CYP1A1) was also measured in the liver and heads as an indicator of exposure to B[a]P. A reduction in total egg output was observed in B[a] P exposed fish as well as a decrease in OSI in fish exposed to 3.0 microg/L B[a]P. A significant increase in CYP1A1 expression in the heads as compared to the control was observed whereas no significant difference in CYP1A1 was detected in livers. A significant increase in 20beta-HSD mRNA occurred in heads and pre-vitellogenic oocytes from fish exposed to 1.5 and 3.0 microg/L as compared to the controls. CYP19A2 and vitellogenin were significantly increased following exposure to 3.0 microg/L in the heads and liver, respectively. No effects on the expression of FSH, LH, CYP19A1 or ERbeta were observed. Results from this study demonstrate that reproduction in zebrafish is affected by waterborne exposure to B[a] P and that altered transcription of genes important in regulating reproduction (20beta-HSD, CYP19A2 and vitellogenin) may be one of the underlying mechanisms of B[a]P-induced reproductive toxicity.

Descriptors: zebrafish, benzoapyrene toxicity, ovary physiology, oviposition physiology, 20 hydroxysteroid dehydrogenases biosynthesis, 20 hydroxysteroid dehydrogenases genetics, cytochrome P450 enzyme system biosynthesis and genetics, estrogen receptor beta biosynthesis and genetics, follicle stimulating hormone biosynthesis and genetics, developmental gene expression regulation drug effects, enzymologic gene expression regulation drug effects, liver enzymology, luteinizing hormone biosynthesis and genetics, oocytes enzymology, ovary drug effects, oviposition drug effects, messenger RNA biosynthesis and genetics, reverse transcriptase PCR, vitellogenins biosynthesis and genetics.

Hoffmann, J.L., S.P. Torontali, R.G. Thomason, D.M. Lee, J.L. Brill, B.B. Price, G.J. Carr, and D.J. Versteeg (2006). **Hepatic gene expression profiling using genechips in zebrafish exposed to 17(Sa(B-ethynylestradiol).** *Aquatic Toxicology* 79(3): 233-46. ISSN: 0166 445X.

NAL Call Number: QH541.5.W3A6

Abstract: Genomic, proteomic, and metabolomic technologies continue to receive increasing interest from environmental toxicologists. This interest is due to the great potential of these technologies to identify detailed modes of action and to provide assistance in the evaluation of a contaminant's risk to aquatic organisms. Our experimental model is the zebrafish (*Danio rerio)* exposed to reference endocrine disrupting compounds in order to investigate compound-induced changes in gene transcript profiles. Adult, female zebrafish were exposed to 0, 15, 40, and 100 ng/L of 17(Sa(B-ethynylestradiol (EE2) and concentration and time-dependent changes in hepatic gene expression were examined using Affymetrix GeneChip Zebrafish Genome Microarrays. At 24, 48, and 168 h, fish were sacrificed and liver mRNA was extracted for gene expression analysis (24 and 168 h only). In an effort to link gene expression changes to effects on higher levels of biological organization, body and ovary weights were measured and blood was collected for measurement of plasma steroid hormones (17(Sb(B-estradiol (E2), testosterone (T)) and vitellogenin (VTG) using ELISA. EE2 exposure significantly affected gene expression, GSI, E2, T, and VTG. We observed 1622 genes that were significantly affected (p <or= 0.001) in a concentration-dependent manner by EE2 exposure at either 24 or 168 h. Gene ontology (GO) analysis revealed that EE2 exposure affected genes involved in hormone metabolism, vitamin A metabolism,

steroid binding, sterol metabolism, and cell growth. Plasma VTG was significantly increased at 24, 48, and 168 h (p <or= 0.05) at 40 and 100 ng/L and at 15 ng/L at 168 h. E2 and T were significantly reduced following EE2 exposure at 48 and 168 h. GSI was decreased in a concentration-dependent manner at 168 h. In this study, we identified genes involved in a variety of biological processes that have the potential to be used as markers of exposure to estrogenic substances. Future work will evaluate the use of these genes in zebrafish exposed to weak estrogens to determine if these genes are indicative of exposure to estrogens with varying potencies.

Descriptors: zebrafish endocrine disrupters model, *Danio rerio*, water pollution, estradiol, endocrine disrupting chemicals, genotoxicity, liver, gene expression, vitellogenin, testosterone, biomarkers, microarray technology , reverse transcriptase PCR, gonadosomatic index, internet resource.

Hoyt, P.R., M.J. Doktycz, K.L. Beattie, and M.S. Greeley (2003). **DNA microarrays detect 4-nonylphenol-induced alterations in gene expression during zebrafish early development**. *Ecotoxicology* 12(6): 469-474. ISSN: 0963 9292.

NAL Call Number: RA565.A1E27

Abstract: Technological advances in the biological sciences have led to a growing realization of the inherent complexity of the toxic actions of man-made chemicals and industrial compounds. An organism's response to toxic exposure is often a complex summation of the individual responses of various different cell types, tissues, and organs within an individual. Furthermore, within a population, various factors including gender, age, fitness, exposure history, genetic variation, and developmental stage significantly affect how each individual will react following exposure. Because of this complexity, characterizing the responses of organisms to environmental toxin exposure is an area of research well suited to the utilization of the gene-expression profiling capability of DNA microarrays. Microarrays are capable of screening large numbers of genes for response to environmental exposure, with the resulting genesets comprising de facto biomarkers for such exposures. In many cases, the genesets described contain response transcripts anticipated from known mechanistic pathways, but in other cases, equally indicative biomarkers may be found that are unexpected. We investigated the response of zebrafish embryos exposed in vitro to the environmental contaminant 4-nonylphenol (4NP). Nonylphenol is one of several alkylphenol ethoxylate compounds widely used in agricultural and industrial processes that have become ubiquitous environmental contaminants. By combining data from differing levels of exposure, we have identified a group of genes that appear indicative of embryo exposure to 4NP at concentrations ranging from high near-lethal levels to lower, more environmentally relevant levels. These biomarker sets can be further expanded and adapted for use in environmental monitoring as well as in mechanistic studies of complex toxicological mechanisms during both early and adult developmental stages. Copyright © 2008 CABI

Descriptors: zebrafish toxicology model, DNA microarrays, embryos, environment, gene expression, genes, *Danio rerio.*

Hu Hai, Y., L. Xu Fu, G. Li Ben, Cao Jun, R. Dawson, and Tao Shu (2005). **Prediction of the bioconcentration factor of PCBs in fish using the molecular connectivity index and fragment constant models**. *Water Environment Research* 77(1): 87-97. ISSN: 1061 4303.

NAL Call Number: TD419.R47

Abstract: Models based on molecular connectivity index (MCI) and fragment constant (FC) method were developed for prediction of the bioconcentration factor (BCF) for polychlorobiphenyls (PCBs) in fish. The mean residuals for the MCI and FC models were 0.195 and

0.223 log units, respectively. The two models were then compared in terms of their mean residuals. In addition to the chlorine atom substitution number, other important structural features exhibiting a significant influence on the BCFs of PCB congeners were discussed and incorporated to the models. These features include the degree of the ortho-substitution, the presence of chlorine pairs in the three- and five- positions, and the crowding of chlorine atoms on the phenyl ring. Copyright © 2008 CABI
Descriptors: zebrafish chlorine toxicity model, aquatic environment, chlorine, equations, hydrophobicity, indexes, models, polychlorinated biphenyls, prediction, regression analysis, toxicity, water pollution, *Danio rerio*, fishes, goldfish, *Oryzias latipes*, *Poecilia reticulata*, rainbow trout.

Huang, C.C., P.C. Chen, C.W. Huang, and J. Yu (2007). **Aristolochic acid induces heart failure in zebrafish embryos that is mediated by inflammation.** *Toxicological Sciences* 100(2): 486-94. ISSN: 1096-6080.
NAL Call Number: RA1190.F8
Abstract: Aristolochic Acid (AA) is a component of Chinese herbs that has been found to be toxic to multiple organs in adults. Its toxicity to developing embryos has not been reported. Here, we describe that AA specifically causes heart defects in developing zebrafish embryos in a dosage-dependent manner. The treated embryos are able to develop their hearts normally up to 24 h postfertilization, when cardiac contraction initiates, but begin to show deformation and reduction of the hearts followed by gradual contractility loss and eventually lethality, suggesting that AA is primarily affecting cardiac physiology rather than cardiogenesis. Histological analyses reveal that the AA-treated hearts develop hypertrophy and disorganization of cardiomyocytes and loss of endocardium. By transmission electron microscopy, we observed broken and disorganized cardiac fibers in the AA-treated hearts. AA induces the expression of proinflammation genes, including cox-2, IL-1beta, and others. The AA-induced cardiac defects can be attenuated by the cox-2 antagonist NS398 via reducing the expression of the inflammatory genes. This attenuation could be further enhanced by known heart failure drugs, such as angiotensin-converting enzyme inhibitor and beta-adrenergic receptor antagonist. In contrast, the heart defects are enhanced by a beta-adrenergic receptor agonist. In summary, AA causes profound toxicity to zebrafish embryos that exhibit pathophysiological and pharmacological features resembling those of heart failure in humans and other model organisms, and thus, zebrafish could be a new model for studies on heart failure.
Descriptors: zebrafish embryos herb toxicity, aristolochic acids toxicity, drug effects, heart drug effects and chemically induced failure , inflammation metabolism, mutagens toxicity, angiotensin converting enzyme inhibitors pharmacology, cyclooxygenase 2 genetics, metabolism, inhibitors pharmacology, drug dose response relationship, doxorubicin toxicity, drug synergism, physiopathology, endocardium drug effects, embryology, ultrastructure, gene expression drug effects, developmental gene expression regulation drug effects, heart failure embryology and metabolism, inflammation genetics, isoproterenol pharmacology, metoprolol pharmacology, myocardial contraction drug effects, myocardium metabolism and ultrastructure, cardiac myocytes drug effects, metabolism and ultrastructure, nitrobenzenes pharmacology, sulfonamides pharmacology.

Huang Yi, X., P. Sun Le, B. Hong Qing, Wu Feng, N. Chen Jian, X. Cao Guo, L. Yang Chang, and S. Li Yong (2004). **Evaluation of molluscicidal effectiveness and acute toxicity to freshwater fish of magnesium borate ore powder.** *Chinese Journal of Schistosomiasis Control* 16(2): 122-125. ISSN: 1005 6661.
Abstract: A study was conducted to examine the molluscicidal effect and acute toxicity

to freshwater fish of magnesium borate ore powder. Dusting with magnesium borate ore powder was carried out in the laboratory and in different field situations. The acute toxicity of magnesium borate powder was determined in *Brachydanio rerio* [*Danio rerio*]. The LC50s of exposed snails [*Oncomelania*] were 228.19, 34.89, 12.47 g/m2 at 3, 5 and 7 days, respectively, in the laboratory. The corrected mortalities and the reduced rates for snail density were all 100.0% at 180 days after dusting 200 g/m2 of the powder in marshland. The reduced rate for density of snails was 96.1% at 210 days after dusting 200 g/m2 of the powder in hilly regions. However, the molluscicidal effect was not up to standard in coastal regions of sandy soil; the powder had no effect after dusting 80 g/m2 and the corrected mortalities of snails were lower after dusting 200 g/m2. The toxicity of magnesium borate ore powder against plants was shown in the field, and the toxicity increased with the dosage. The basicity of the soil was increased after dusting magnesium borate ore powder; the basicity increased with the dosage. The LC50s of magnesium borate powder in *B. rerio* exposed for 48, 72 and 96 h were 964.32, 633.66 and 523.74 mg/litre, respectively. Copyright © 2008 CABI
Descriptors: zebrafish, *Oncomelania*, disease vectors, dosage, freshwater fishes, marshes, molluscicidal properties, mortality, population density, soil alkalinity, toxicity, intermediate hosts, freshwater molluscs, *Danio rerio*, fishes, snails.
Language of Text: Chinese.

Ibabe, A., A. Herrero, and M.P. Cajaraville (2005). **Modulation of peroxisome proliferator-activated receptors (PPARs) by PPAR(alpha)- and PPAR(gamma)-specific ligands and by 17beta-estradiol in isolated zebrafish hepatocytes.** *Toxicology in Vitro* 19(6): 725-35. ISSN: 0887-2333.
NAL Call Number: RA1190.T692
Abstract: Peroxisome proliferation is a phenomenon occurring when responsive animals are exposed to certain compounds so-called peroxisome proliferators and is regulated through a nuclear receptor named peroxisome proliferator-activated receptor (PPAR). PPAR family members exhibit a strong binding affinity for both saturated and unsaturated fatty acids. Activators of PPAR(alpha) include a variety of endogenously present fatty acids, leukotrienes and hydroxyeicosatetraenoic acids (HETEs) and clinically used drugs, such as fibrates. PPAR(beta) activators include fatty acids, prostaglandin A2 (PGA2) and prostacyclin (PGI2). PPAR(gamma) is the most selective receptor and, among others, 15-deoxy-Delta(12,14) prostaglandin J2 (PGJ2) has been described to be a PPAR(gamma)-specific ligand. The aim of the present study was to determine if known PPAR(alpha) and PPAR(gamma) ligands were able to alter the expression of these subtypes in an in vitro model of zebrafish primary hepatocyte culture. With this purpose, a PPAR(alpha) specific ligand (8S-HETE), a PPAR-gamma specific ligand (PGJ) and a peroxisome proliferator of the fibrate class (clofibrate) were selected. In addition, the female hormone 17beta-estradiol was also used as it is known to interact with PPARs. After cell exposure for 24 h, cells were immunohistochemically stained for both PPARs and immunolabeling was quantified as percentage of positive nuclei and cells. Levels of expression of PPARs were also measured by image analysis as grey level per cell. Expression was induced for both PPAR(alpha) and PPAR(gamma) by clofibrate (at 0.5 mM for PPAR(alpha) and at 1 and 2 mM for PPAR(gamma)), by HETE (1 microM), and by PGJ2 (0.3 and 1 microM for PPAR(alpha) and 0.3 microM for PPAR(gamma)). Expression of PPARgamma was also induced at 10 microM by 17beta-estradiol. The percentage of PPAR(alpha) positive nuclei increased significantly at 1 microM HETE and the percentage of PPAR(gamma) positive cells decreased at 10 microM 17beta-estradiol. As a conclusion, clofibrate, HETE and PGJ2 are able to induce expression of both PPAR(alpha) and PPAR(gamma) in zebrafish primary hepatocyte cultures. Further studies are needed

to identify how the expression of different PPAR subtypes is regulated and to elucidate the implication of PPAR subtypes in zebrafish cell functions.
Descriptors: zebrafish hepatocyte culture, estradiol pharmacology, hepatocytes drug effects, PPAR alpha drug effects, antilipemic agents pharmacology, cell nucleus drug effects and metabolism, cell proliferation drug effects, cell separation and survival drug effects, clofibrate pharmacology, cytoplasm drug effects and metabolism, hepatocytes ultrastructure, hydroxyeicosatetraenoic acids pharmacology, immunohistochemistry, ligands, prostaglandin D2 analogs and derivatives and pharmacology.

Incardona, J.P., H.L. Day, T.K. Collier, and N.L. Scholz (2006). **Developmental toxicity of 4-ring polycyclic aromatic hydrocarbons in zebrafish is differentially dependent on AH receptor isoforms and hepatic cytochrome P4501A metabolism.** *Toxicology and Applied Pharmacology* 217(3): 308-21. ISSN: 0041-008X.
NAL Call Number: 391.8T662
Abstract: Polycyclic aromatic hydrocarbons (PAHs) derived from fossil fuels are ubiquitous contaminants and occur in aquatic habitats as highly variable and complex mixtures of compounds containing 2 to 6 rings. For aquatic species, PAHs are generally accepted as acting through either of two modes of action: (1) "dioxin-like" toxicity mediated by activation of the aryl hydrocarbon receptor (AHR), which controls a battery of genes involved in PAH metabolism, such as cytochrome P4501A (CYP1A) and (2) "nonpolar narcosis", in which tissue uptake is dependent solely on hydrophobicity and toxicity is mediated through nonspecific partitioning into lipid bilayers. As part of a systematic analysis of mechanisms of PAH developmental toxicity in zebrafish, we show here that three tetracyclic PAHs (pyrene, chrysene, and benz[a]anthracene) activate the AHR pathway tissue-specifically to induce distinct patterns of CYP1A expression. Using morpholino knockdown of ahr1a, ahr2, and cyp1a, we show that distinct embryolarval syndromes induced by exposure to two of these compounds are differentially dependent on tissue-specific activation of AHR isoforms or metabolism by CYP1A. Exposure of embryos with and without circulation (silent heart morphants) resulted in dramatically different patterns of CYP1A induction, with circulation required to deliver some compounds to internal tissues. Therefore, biological effects of PAHs cannot be predicted simply by quantitative measures of AHR activity or a compound's hydrophobicity. These results indicate that current models of PAH toxicity in fish are greatly oversimplified and that individual PAHs are pharmacologically active compounds with distinct and specific cellular targets.
Descriptors: zebrafish PAH toxicity model, cytochrome P450 CYP1 A1 biosynthesis, drug effects, liver drug effects, aromatic polycyclic hydrocarbons toxicity, aryl hydrocarbon receptors metabolism, chemical water pollutants toxicity, drug induced abnormalities, benzaanthracenes toxicity , chrysenes toxicity, embryology, metabolism, enzyme induction, developmental gene expression regulation drug effects and genetics , chemically induced congenital heart defects and pathology, larva drug effects, growth, development and metabolism, liver enzymology, morpholines pharmacology, antisense oligonucleotides pharmacology, protein isoforms, pyrenes toxicity, aryl hydrocarbon receptors genetics.

Incardona, J., C. Stehr, T. Linbo, and N. Scholz (2006). **Fipronil causes locomotor defects in developing zebrafish**. *Marine Environmental Research* 62(Suppl. S): S74-S75. ISSN: 0141 1136.
NAL Call Number: QH545.W3M36
Descriptors: zebrafish toxicology models, molecular genetics, fipronil, reproductive system, marine ecology, environmental sciences, notochord degeneration, reproductive system disease, phenotyping, genetic techniques.

Incardona, J.P., T.K. Collier, and N.L. Scholz (2004). **Mechanisms of polycyclic aromatic hydrocarbon toxicity in early life history stages of fish** . *Marine Environmental Research* 58(2-5): 363. ISSN: 0141 1136.
NAL Call Number: QH545.W3M36
Descriptors: zebrafish, cardiovascular system, development, nervous system, toxicology, anemia, blood and lymphatic disease, peripheral vascular defects, atrioventricular conduction block, cardiac morphogenesis, craniofacial skeleton , heart rate, neuronal cell death, polycylic aromatic hydrocarbon.

Intorre, L., V. Meucci, D. Di Bello, G. Monni, G. Soldani, and C. Pretti (2007). **Tolerance of benzalkonium chloride, formalin, malachite green, and potassium permanganate in goldfish and zebrafish.** *Journal of the American Veterinary Medical Association* 231(4): 590-95. ISSN: 0003-1488.
NAL Call Number: 41.8 Am3
Abstract: OBJECTIVE: To determine tolerance of goldfish and zebrafish to benzalkonium chloride, formalin, malachite green, and potassium permanganate. DESIGN: Tolerance study. ANIMALS: Adult goldfish (*Carassius auratus*) and zebrafish (*Danio rerio*). PROCEDURES: Groups of fish (n = 10/group) were exposed to each disinfectant at the therapeutic dosage; at 0.25, 0.5, 3, and 5 times the concentration used for the therapeutic dosage; and at the concentration used for the therapeutic dosage but for 3 or 5 times the recommended exposure time. RESULTS: In both species, exposure to malachite green at the therapeutic dosage resulted in toxic effects, including death. Exposure to formalin at the therapeutic dosage resulted in toxic effects in goldfish, but not zebrafish, and exposure to potassium permanganate resulted in toxic effects in zebrafish, but not goldfish. On the basis of the ratio of therapeutic dosage to median lethal dosage, in goldfish, formalin was more toxic than benzalkonium chloride, which was more toxic than malachite green, which was more toxic than potassium permanganate. In zebrafish, potassium permanganate was more toxic than formalin and benzalkonium chloride, which were approximately equally toxic and more toxic than malachite green. Extending treatment time increased the toxicity of potassium permanganate in zebrafish and the toxicity of formalin and malachite green in goldfish, but did not alter the toxicity of the other disinfectants. CONCLUSIONS AND CLINICAL RELEVANCE: Results indicated that there was no consistency between zebrafish and goldfish in their tolerance to disinfectants, and that therapeutic dosages reported in the literature for these disinfectants were not always safe.
Descriptors: zebrafish and goldfish, disinfectants toxicity, goldfish growth and development, benzalkonium compounds toxicity, drug dose response relationship, environmental exposure, formaldehyde toxicity, potassium permanganate toxicity, random allocation, rosaniline dyes toxicity, species specificity, time factors, toxicity tests.

Islinger, M., D. Willimski, A. Volkl, and T. Braunbeck (2003). **Effects of 17a-ethinylestradiol on the expression of three estrogen-responsive genes and cellular ultrastructure of liver and testes in male zebrafish.** *Aquatic Toxicology* 62(2): 85-103. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: In order to monitor the influence of estrogenic compounds on the reproductive physiology of fish, molecular markers for zebrafish vitellogenin, estrogen receptor and ZP2 were developed. For this purpose, sequence information about the zebrafish estrogen receptor and vitellogenin had to be obtained. By means of RT-PCR, a sequence fragment of the zebrafish estrogen receptor alpha was cloned and sequenced. Continuous cDNAs of two zebrafish vitellogenin-like gene products (zfvg1 and zfvg3) were constructed by the

help of expressed sequence tags of zebrafish and completely sequenced. The sequences of the estrogen receptor and of the vitellogenins showed significant similarities to corresponding cDNAs of other fish species. Expression of these gene products was measured following exposure to 17alpha-ethinylestradiol and compared with histological endpoints. RT-PCR was used as a semiquantitative technique to record gene expression in adult male zebrafish, which were exposed to 17alpha-ethinylestradiol in time-and dose-response experiments. As for time-dependent expression, all hepatic genes investigated were expressed at considerable amounts from 24 h after onset of exposure to 50 ng/l 17alpha-ethinylestradiol to the end of experiment (17 days). In testes, expression of the estrogen receptor- as well as ZP2-mRNA remained unchanged for the entire experiment, except for the individuals exposed for 17 days, which displayed elevated expression levels of ZP2. In the dose-response experiment, male zebrafish were exposed to 17alpha-ethinylestradiol in concentrations from 0.25-85 ng/l for 4 and 21 days. LOECs for vitellogenin as well as estrogen receptor alpha expression were found to be 2.5 ng/l already after 4 d of exposure. Extension of the exposure time to 21 days resulted in enhanced transcription of vitellogenin-mRNAs at 2.5 ng/l 17alpha-ethinylestradiol, whereas the detection limit could not be lowered. In contrast, in testes no induction of both ZP2 as well as estrogen receptor expression was detected at any concentration tested. To examine estrogen-caused alterations at the ultrastructural level, liver and testes of males exposed to 25 ng/l 17alpha-ethinylestradiol were analysed. Male livers responded with a feminisation reflected by the proliferation of rough endoplasmatic reticulum and Golgi apparatus typical of female hepatocytes during vitellogenesis. However, in testes no signs of feminisation were detectable; rather, destructive phenomena like phagocytosis of sperm cells by Sertoli cells were observed. Thus, in sexually differentiated males no reorganisation of the gonadal tissue towards an ovary could be definitely detected at any level investigated.
Descriptors: zebrafish, egG proteins biosynthesis, estradiol congeners adverse effects, ethinyl estradiol adverse effects, gene expression regulation drug effects, membrane glycoproteins biosynthesis, cell surface receptors, estrogen receptorsbiosynthesis, vitellogenins biosynthesis, chemical water pollutants adverse effects, base sequence, DNA primers, complementary DNA, liver physiology, molecular sequence data, reproduction, reverse transcriptase PCR, testis physiology.

Johnson, A., E. Carew, and K.A. Sloman (2007). **The effects of copper on the morphological and functional development of zebrafish embryos.** *Aquatic Toxicology* 84(4): 431-8. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: Waterborne copper exposure can exert a variety of physiological effects in fish, including the disruption of sensory system function, which has wide-reaching implications for fish behaviour. In developing fish larvae, copper is known to affect key parameters, such as survival and growth and more recently has been shown to interfere with the octavolateral system. The present study aimed to take a combined view of morphological (e.g. length, yolk sac area) and functional (e.g. heart beat, behaviour) processes to understand the complex effect of copper on fish development. In the first of two experiments, zebrafish embryos were exposed to a range of copper concentrations (11-1000 microg l(-1)) from fertilisation for a 72 h period. The greatest mortality was seen between 5 and 24h post-fertilisation (hpf) and was more pronounced at the higher copper concentrations. Copper also had an inhibitory effect on hatching. Length and yolk sac area of individuals were recorded across treatments at 72 hpf and elevated copper was found to slow development. Individuals from the higher copper treatments had the fastest heart rates at 28 hpf suggesting that stress responses were induced in the embryos during copper exposure. In the second experiment, embryos were

exposed in a similar manner to two copper concentrations, based on those from Experiment 1 that resulted in <50% mortality. At 120 hpf, embryos exposed to both copper concentrations possessed significantly fewer functional neuromasts, an effect which was associated with a reduced ability to orientate in a current. Therefore, although mortality at these copper concentrations was low initially, and then almost non-existent after 24 hpf, the inability of copper-exposed larvae to orientate in a water current as a result of lateral line dysfunction is likely to seriously compromise survival.

Descriptors: zebrafish embryos copper exposure model, copper pharmacology, drug effects, behavior drug effects, body size drug effects, copper toxicity, metabolism, embryonic development drug effects, heart rate drug effects, nonparametric statistics, swimming physiology.

Jonsson, M.E., M.J. Jenny, B.R. Woodin, M.E. Hahn, and J.J. Stegeman (2007). **Role of AHR2 in the expression of novel cytochrome P450 1 family genes, cell cycle genes, and morphological defects in developing zebrafish exposed to 3,3',4,4',5-pentachlorobiphenyl or 2,3,7,8-tetrachlorodibenzo-p-dioxin.** *Toxicological Sciences* 100(1): 180-93. ISSN: 1096-6080.

NAL Call Number: RA1190.F8

Abstract: Halogenated agonists for the aryl hydrocarbon receptor (AHR), such as 3,3',4,4',5-pentachlorobiphenyl (PCB126) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), cause developmental toxicity in fish. AHR dependence of these effects is known for TCDD but only presumed for PCB126, and the AHR-regulated genes involved are known only in part. We defined the role of AHR in regulation of four cytochrome P450 1 (CYP1) genes and the effect of PCB126 on cell cycle genes (i.e., PCNA and cyclin E) in zebra fish *(Danio rerio*) embryos. Basal and PCB126-induced expression of CYP1A, CYP1B1, CYP1C1, and CYP1C2 was examined over time as well as in relation to cell cycle gene expression and morphological effects of PCB126 in developing zebra fish. The four CYP1 genes differed in the time for maximal basal and induced expression, i.e., CYP1B1 peaked within 2 days postfertilization (dpf), the CYP1Cs around hatching (3 dpf), and CYP1A after hatching (14-21 dpf). These results indicate developmental periods when the CYP1s may play physiological roles. PCB126 (0.3-100nM) caused concentration-dependent CYP1 gene induction (EC50: 1.4-2.7nM, Lowest observed effect concentration [LOEC]: 0.3-1nM) and pericardial edema (EC50: 4.4nM, LOEC: 3nM) in 3-dpf embryos. Blockage of AHR2 translation significantly inhibited these effects of PCB126 and TCDD. PCNA gene expression was reduced by PCB126 in a concentration-dependent manner, suggesting that PCB126 could suppress cell proliferation. Our results indicate that the four CYP1 genes examined are regulated by AHR2 and that the effect of PCB126 on morphology in zebra fish embryos is AHR2 dependent. Moreover, the developmental patterns of expression and induction suggest that CYP1 enzymes could function in normal development and in developmental toxicity of PCB126 in fish embryos.

Descriptors: zebrafish embryo toxicology model, cell cycle proteins metabolism, cytochrome P450 enzyme system metabolism, environmental pollutants toxicity, developmental gene expression regulation drug effects, enzymologic gene expression regulation drug effects, polychlorinated biphenyls toxicity, aryl hydrocarbon receptors agonists, tetrachlorodibenzodioxin toxicity, metabolism, proteins agonists, aryl hydrocarbon hydroxylases metabolism, cell cycle cell proliferation drug effects, chemically induced craniofacial abnormalities and metabolism, cyclin e metabolism, cytochrome P450 enzyme system genetics, drug dose response relationship, chemically induced edema and metabolism, abnormalities, drug effects and enzymology, metabolism, embryonic development drug effects, chemically induced congenital heart defects and metabolism, isoenzymes metabolism, antisense oligonucleotides

metabolism, proliferating cell nuclear antigen metabolism, aryl hydrocarbon receptors genetics and metabolism, time factors.

Jonsson, M.E., R. Orrego, B.R. Woodin, J.V. Goldstone, and J.J. Stegeman (2007). **Basal and 3,3',4,4',5-pentachlorobiphenyl-induced expression of cytochrome P450 1A, 1B and 1C genes in zebrafish.** *Toxicology and Applied Pharmacology* 221(1): 29-41. ISSN: 0041-008X.
NAL Call Number: 391.8T662
Abstract: The cytochrome P4501C (CYP1C) gene subfamily was recently discovered in fish, and zebrafish (*Danio rerio*) CYP1C1 transcript has been cloned. Here we cloned the paralogous CYP1C2, showing that the amino acid sequence is 78% identical to CYP1C1, and examined gene structure and expression of CYP1A, CYP1B1, CYP1C1, and CYP1C2. Xenobiotic response elements were observed upstream of the coding regions in all four genes. Zebrafish adults and embryos were exposed (24 h) to 100 nM 3,3',4,4',5-polychlorinated biphenyl (PCB126) or 20 ppm acetone and subsequently held in clean water for 24 h (adults) or 48 h (embryos). All adult organs examined (eye, gill, heart, liver, kidney, brain, gut, and gonads) and embryos showed basal expression of the four genes. CYP1A was most strongly expressed in liver, whereas CYP1B1, CYP1C1, and CYP1C2 were most strongly expressed in heart and eye. CYP1B1 and the CYP1C genes showed an expression pattern similar to one another and to mammalian CYP1B1. In embryos CYP1C1 and CYP1C2 tended to have a higher basal expression than CYP1A and CYP1B1. PCB126 induced CYP1A in all organs, and CYP1B1 and CYP1C1 in all organs except gonads, or gonads and brain, respectively. CYP1C2 induction was significant only in the liver. However, in embryos all four genes were induced strongly by PCB126. The results are consistent with CYP1C1 and CYP1C2, as well as CYP1A and CYP1B1, being regulated by the aryl hydrocarbon receptor. While CYP1A may have a protective role against AHR agonists in liver and gut, CYP1B1, CYP1C1, and CYP1C2 may also play endogenous roles in eye and heart and possibly other organs, as well as during development.
Descriptors: zebrafish toxicology model, cytochrome P450 enzyme system genetics, gene expression profiling, polychlorinated biphenyls pharmacology, amino acid sequence, aryl hydrocarbon hydroxylases genetics, molecular cloning, cytochrome P450 CYPA1A1 genetics, drug dose response relationship, drug effects, estrogen antagonists administration and dosage and pharmacology, developmental gene expression regulation drug effects, enzymologic gene expression regulation drug effects, isoenzymes genetics, molecular sequence data, polychlorinated biphenyls administration and dosage, amino acid sequence homology, beta naphthoflavone pharmacology.

Jorgensen, A., O. Andersen, P. Bjerregaard, and L.J. Rasmussen (2007). **Identification and characterisation of an androgen receptor from zebrafish *Danio rerio*.** *Comparative Biochemistry and Physiology. Part C: Part C: Toxicology and Pharmacology* 146(4): 561-8. ISSN: 1532 0456.
NAL Call Number: QP901.C6
Abstract: Androgens play key roles in vertebrate sex differentiation, gonadal differentiation and sexual behaviour. The action of androgens is primarily mediated through androgen receptors (ARs). The present study describes the isolation, sequencing and initial characterisation of an androgen receptor from zebrafish Danio rerio. The predicted protein of 868 residues has an estimated calculated molecular weight of 96 kDa. The amino acid sequence of the zebrafish AR (zfRA) shows high homology with other vertebrate ARs. The highest overall similarity was 82% to ARs from fathead minnow (*Pimephales promelas*) and goldfish (*Carassius auratus*). Binding assays with zfAR demonstrated high affinity, saturable, single class

binding site, with the characteristics of an androgen receptor. Saturation experiments along with subsequent Scatchard analysis determined that the Kd of the zfAR for 3H-testosterone was 2 nM. Androgen binding affinities in competition with 3H-testosterone were: 5alpha-dihydrotestosterone>11-ketotestosterone>testosterone>androstenedione. The deletion of 12 amino acids (zfARd12) in the ligand binding domain of zfAR resulted in impaired binding to the receptor. Therefore, it was not possible to determine Kd for the zfARd12. The characterisation of this zfAR provides a new perspective for understanding the mechanisms underlying androgen actions in a model vertebrate species commonly used for studies investigating potential endocrine disrupters.
Descriptors: zebrafish, androgen receptors genetics, amino acid sequence, binding sites, binding, competitive, molecular cloning, cyprinidae physiology, goldfish physiology, molecular sequence data, androgen receptors chemistry and metabolism, amino acid sequence homology, testosterone metabolism.

Jurcikova, J., P. Mikula, R. Dobsikova, D. Nemethova, and Z. Svobodova (2007). **Effects of Metazachlor on vitellogenin Induction in zebrafish (*Danio rerio*)**. *Acta Veterinaria (Brno)* 76(Supp 8): S61-S66. ISSN: 0001-7213.
NAL Call Number: SF604.B7
Descriptors: zebrafish, metazachlor, vitellogenin, induction, 17 beta -oestradiol, Butisan 400 SC.

Kammann, U., S. Biselli, H. Huhnerfuss, N. Reineke, N. Theobald, M. Vobach, and W. Wosniok (2004). **Genotoxic and teratogenic potential of marine sediment extracts investigated with comet assay and zebrafish test.** *Environmental Pollution* 132(2): 279-87. ISSN: 0269 7491.
NAL Call Number: QH545.A1E52
Descriptors: zebrafish, environmental monitoring methods, environmental pollution, geologic sediments, seawater, North Sea and Baltic Sea, comet assay, lethal dose 50, mutagenicity tests, toxicity tests.

Kammann, U., M. Vobach, and W. Wosniok (2006). **Toxic effects of brominated indoles and phenols on zebrafish embryos.** *Archives of Environmental Contamination and Toxicology* 51(1): 97-102. ISSN: 0090 4341.
NAL Call Number: TD.172.A7
Abstract: Organobromine compounds in the marine environment have been the focus of growing attention in past years. In contrast to anthropogenic brominated flame retardants, other brominated compounds are produced naturally, e.g., by common polychaete worms and algae. Brominated phenols and indoles assumed to be of biogenic origin have been detected in water and sediment extracts from the German Bight. These substances as well as some of their isomers have been tested with the zebrafish embryo test and were found to cause lethal as well as nonlethal malformations. The zebrafish test was able to detect a log K(OW)-related toxicity for bromophenols, suggesting nonpolar narcosis as a major mode of action. Different effect patterns could be observed for brominated indoles and bromophenols. The comparison of effective concentrations in the zebrafish embryo test with the concentrations determined in water samples suggests the possibility that brominated indoles may affect early life stages of marine fish species in the North Sea.
Descriptors: zebrafish embryo bromophenols toxicity model, embryonic development drug effects, indoles and phenols toxicity, lethal dose 50, pigmentation drug effects, quantitative structure activity relationship, spine drug effects, spine embryology, yolk sac drug effects.

Kammann, U., S. Biselli, N. Reineke, W. Wosniok, D. Danischewski, H. Huehnerfuss, A. Kinder, A. Sierts-Herrmann, N. Theobald, H.H. Vahl, M. Vobach, J. Westendorf, and H. Steinhart (2005). **Bioassay-directed fractionation of organic extracts of marine surface sediments from the north and baltic sea - part II: results of the biotest battery and development of a biotest index**. *Journal of Soils and Sediments* 5(4): 225-232. ISSN: 1439 0108.

NAL Call Number: S622

Abstract: Goal, Scope and Background. The ecological relevance of contaminants in mixtures is difficult to assess, because of possible interactions and due to lacking toxicity data for many substances present in environmental samples. Marine sediment extracts, which contain a mixture of environmental contaminants in low concentrations, were the object of this study. The extracts were investigated with a set of different biotests in order to identify the compound or the substance class responsible for the toxicity. For this goal, a combination of biotests, biotest-directed fractionation and chemical analysis has been applied. Further on, a strategy for the development of a biotest index to describe the toxicity of the fractions without a prior ranking of the test results is proposed. This article (Part II) focuses on the biological results of the approach.Methods. The toxicological potential of organic extracts of sediments from the North Sea and the Baltic Sea was analyzed in a bioassay-directed fractionation procedure with a set of biotests: luciferase reporter gene assays on hormone receptor and Ah receptor, arabinose resistance test, fish embryo test (*Danio rerio*), comet assay, acetylcholinesterase inhibition test, heat-shock protein 70 induction, oxidative stress and luminescence inhibition test (*Vibrio fischeri*). The test results provided the basis for the calculation of a biotest index by factor analysis to compare the toxicity of the samples and fractions. Results and Discussion.Results of 11 biotests on different fractionation levels of the samples were described and discussed with regard to the occurrence of contaminants and their toxic potentials. Polychlorinated biphenyls, polycyclic aromatic hydrocarbons, quinones, brominated indoles and brominated phenols were in the focus of interest. A biotest index was constructed to compare the toxic responses in the samples and to group the biotest results.Conclusion. The procedure presented in this study is well suited for bioassay-directed fractionation of marine sediment extracts. However, in relatively low contaminated samples, high enrichment factors and sufficient fractionation is necessary to allow identification of low concentrations of contaminants which is required to link effects and possible causes. In the present case, the relation between substances and effects was difficult to uncover due to relatively low concentrations of pollutants compared to the biogenic matrix and to the remaining complexity of the fractions. The results, with respect to the brominated phenols and indoles in the samples, highlight the successful use of bioassay directed fractionation in the case of high concentrations and high toxicity.Recommendation and Outlook. In general, it has been shown that a marine risk assessment requires focusing on the input of diffuse sources and taking into account the fact of mixture toxicity. Effects resulting from biogenic substances will make the assessment of the influence of anthropogenic substances even more difficult.
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Descriptors: zebrafish embryos marine sediment toxicity testing, toxicology, pollution assessment control and management, marine ecology, environmental sciences, chemical toxicity, comet assay, electrophoretic techniques, genetic techniques, bioassay directed fractionation, luciferase reporter gene assay, arabinose resistance test, acetylcholinesterase inhibition test , luminescence inhibition test, oxidative stress, marine surface sediment, *Danio rerio*.

Kao, T. and N. Scholz (2004). **Effects of copper on mechanosensory structures in developing fish embryos and Larvae**. *2003 Georgia basin/Puget sound research conference,March 3, 1931-April 3, 2003, Vancouver, BC (Canada),* Puget Sound Action Team: Olympia WA 98504 USA,
Descriptors: zebrafish embryo and larvae copper toxicity testing, copper, drainage systems, embryonic development, exposure, fish larvae, habitats, larval development, lateral line, model studies, neurophysiology, orientation behavior, pesticides, pollutants, pollution indicators, salmon, storm runoff, stormwater runoff, streams, surface water, survival, toxicity tests, urban runoff and watersheds, water pollution effects, *Danio rerio*, Georgia basin,Puget sound , Olympia, WA, USA .

Kapp, T., U. Kammann, M. Vobach, and W. Vetter (2006). **Synthesis of low and high chlorinated toxaphene and comparison of their toxicity by zebrafish (*Danio rerio*) embryo test**. *Environmental Toxicology and Chemistry* 25(11): 2884-2889. ISSN: 0730 7268.
NAL Call Number: QH545A1E58
Abstract: Toxaphene, also known as camphechlor, is a persistent organochlorine pesticide of complex composition. It is technically produced by photochlorination of camphene with elemental chlorine gas under ultraviolet irradiation. In the present work, a novel, laboratory-scale synthesis using sulfuryl chloride as a chlorinating reagent is described. This approach allowed the degree of chlorination of the resulting mixtures to be arbitrarily adjusted by varying the reaction conditions. Both the compositions and the chlorine contents of the low- and high-chlorinated mixtures acquired using this method were similar to those of environmentally altered toxaphene and technical toxaphene, respectively. For comparison of these mixtures regarding toxicity, they were subjected to the zebrafish (*Danio rerio*) embryo test. Median effective concentrations (EC50s) were calculated based on the presence of lethal and nonlethal embryonic malformations. Surprisingly, low-chlorinated toxaphene, comprising compounds that also are present in environmentally transformed toxaphene, exhibited a twofold-higher toxicity (according to the EC50 for nonlethal effects) toward the test organisms compared with high-chlorinated toxaphene, the composition of which resembled that of the technical product. Although the effective concentrations in the embryo test were much higher than those in aquatic ecosystems burdened with toxaphene, the present results lead to the assumption that toxaphene is becoming more toxic during transformation in the environment. A decrease in the total amount of toxaphene during environmental breakdown would then be compensated for, at least in part, by the higher toxicity of weathered toxaphene in sediments, soils, and biota of contaminated ecosystems. Copyright © 2008 CABI
Descriptors: zebrafish embryos, aquatic animals, camphechlor, embryos, insecticides, lethal dose, malformations, polluted water, synthesis, toxicity testing, water pollution, *Danio rerio.*

Kazeto, Y., A.R. Place, and J.M. Trant (2004). **Effects of endocrine disrupting chemicals on the expression of CYP19 genes in zebrafish (*Danio rerio*) juveniles.** *Aquatic Toxicology* 69(1): 25-34. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Descriptors: zebrafish endocrine disrution model, aromatase biosynthesis, atrazine toxicity, benzoapyrene toxicity, clofibrate toxicity, ethinyl estradiol toxicity, phenols toxicity, metabolism, drug dose response relationship, enzyme induction drug effects, reverse transcriptase PCR.

Keiter, S., A. Rastall, T. Kosmehl, K. Wurm, L. Erdinger, T. Braunbeck, and H. Hollert (2006). **Ecotoxicological assessment of sediment, suspended matter and water samples in the upper Danube River. A pilot study in search for the causes for the decline of fish catches**. *Envi-*

ronmental Science and Pollution Research 13(5): 308-319. ISSN: 0944 1344.
NAL Call Number: GE 1
Abstract: Fish populations, especially those of the grayling (*Thymallus thymallus*), have declined over the last two decades in the upper Danube River between Sigmaringen and Ulm, despite intensive and continuous stocking and improvement of water quality since the 1970s. Similar problems have been reported for other rivers, e.g. in Switzerland, Great Britain, the United States and Canada. In order to assess if ecotoxicological effects might be related to the decline in fish catch at the upper Danube River, sediment, suspended matter and waste water samples from sewage treatment plants were collected at selected locations and analysed in a bioanalytical approach using a battery of bioassays. Freeze-dried sediments and suspended particulate matters were extracted with acetone in a Soxhlet apparatus. Organic pollutants from sewage water were concentrated using XAD-resins. To investigate the ecotoxicological burden, the following bioassays were used: (1) neutral red assay with RTL-W1 cells (cytotoxicity), (2) comet assay with RTL-W1 cells (genotoxicity), (3) *Arthrobacter globiformis* dehydrogenase assay (toxicity to bacteria), (4) yeast oestrogen screen assay (endocrine disruption), (5) fish egg assay with the zebrafish (*Danio rerio;* embryo toxicity) and (6) Ames test with TA98 (mutagenicity). The results of the in vitro tests elucidated a considerable genotoxic, cytotoxic, mutagenic, bacteriotoxic, embryotoxic and estrogenic burden in the upper Danube River, although with a very inhomogeneous distribution of effects. The samples taken from Riedlingen, for example, induced low embryo toxicity, but the second highest 17 beta -estradiol equivalent concentration (1.8 ng/litre). Using the fish egg assay with native sediments, a broad range of embryotoxic effects could be elucidated, with clear-cut dose-response relationships for the embryotoxic effects of contaminated sediments. With native sediments, embryotoxicity was clearly higher than with corresponding pore waters, thus corroborating the view that - at least for fish eggs - the bioavailability of particle-bound lipophilic substances in native sediments is higher than generally assumed. The effect observed most frequently in the fish egg assay was a developmental delay. A comparison of our own results with locations along the rivers Rhine and Neckar demonstrated similar or even higher ranges of ecotoxicological burdens in the Danube River. The complex pattern of ecotoxicological effects caused by environmental samples from the Danube River, when assessed in an in vitro biotest battery using both acute and more specific endpoints, showed that integration of different endpoints is essential for appropriate hazard assessment. Overall, the ecotoxicological hazard potential shown has indeed to be considered as one potential reason for the decline in fish catches at the upper Danube River. However, based on the results of this pilot study, it is not possible to elucidate that chemically induced alterations are responsible for the fish decline. For linking the weight of evidence obtained by in vitro assays and fish population investigations, the application of additional, more specific biomarkers (e.g. vitellogenin induction, EROD and micronucleus assay) has been initiated in fish taken from the field as well as in situ investigations. Copyright © 2008 CABI
Descriptors: zebrafish, bioassays, cytotoxicity, genotoxicity, mortality, mutagenesis, organic compounds, oxidoreductases, pollutants, river water, rivers, sediment, sewage effluent, suspended solids, toxicity, waste water, water pollution, water quality, *Arthrobacter globiformis*, *Danio rerio*, fishes, *Thymallus thymallus.*

Kent, M.L., C.M. Whipps, J.L. Matthews, D. Florio, V. Watral, J.K. Bishop Stewart, M. Poort, and L. Bermudez (2004). **Mycobacteriosis in zebrafish (*Danio rerio*) research facilities.** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 138(3): 383-90. ISSN: 1532 0456.
NAL Call Number: QP901.C6

Abstract: The Zebrafish International Resource Center was established to support the zebrafish research community, and includes a diagnostic service. One of the most common diseases that we have diagnosed is mycobacteriosis, which represented 18% of the diagnostic cases submitted from November 1999 to June 2003. We describe here the severity of the disease and associated pathological changes of 24 diagnostic cases from 14 laboratories. Identifications of the bacteria are provided for seven of these cases. For two cases in which culture of the organism was not successful, these identifications were based on ribosomal DNA (rDNA) sequence analysis obtained directly from infected tissues. Biochemical characteristics and rDNA sequence analysis from cultures are reported for the other isolates. Two severe outbreaks from different facilities on different continents were associated with an organism identified as *Mycobacterium haemophilum* based on rDNA sequence from tissues. Another severe outbreak was associated with an organism most closely related to *Mycobacterium peregrinum.* These species are recognized pathogens of humans, but this is the first report of them from fish. Bacteria identified as *Mycobacterium chelonae* or *M. abscessus* were recovered from fish in cases categorized as moderate disease or as an incidental finding. These findings indicate that species of *Mycobacterium* previously undescribed from fish (i.e., *M. haemophilum and M. peregrinum*) may pose significant health problems in zebrafish research facilities, whereas species and strains that are already recognized as common in fish usually cause limited disease on a population basis in zebrafish.
Descriptors: laboratory zebrafish, laboratory and fish diseases microbiology, *Mycobacterium* infections microbiology, *Mycobacterium* infections veterinary, zebrafish microbiology, fish diseases diagnosis and pathology, *Mycobacterium* classification, genetics, isolation and purification, *Mycobacterium* infections diagnosis and pathology, ribosomal RNA 16s genetics, time factors.

Kinnberg, K., H. Holbech, G.I. Petersen, and P. Bjerregaard (2007). **Effects of the fungicide prochloraz on the sexual development of zebrafish (*Danio rerio*)**. *Comparative Biochemistry and Physiology C, Toxicology and Pharmacology* 145(2): 165-170. ISSN: 1532 0456.
NAL Call Number: QP901.C6
Abstract: Some chemicals have the potential to adversely affect sexual development through multiple endocrine actions. Prochloraz is an imidazole fungicide that displays diverse mechanisms of action, including inhibition of aromatase activity, inhibition of androgen synthesis, and antagonism of the androgen receptor. The objective of this study was to assess the effects of prochloraz on the sexual development of zebrafish (*Danio rerio*) in the Fish Sexual Development Test (FSDT) proposed as an OECD test guideline for detection of endocrine disruptors. Zebrafish were exposed to prochloraz (0, 16, 64 or 202 micro g/L) for 60 days from 24 h post fertilization. Fish exposed to 202 micro g/L prochloraz showed an increased proportion of males. Furthermore, the incidence of intersex and the stages of the gonads were altered in the treated fish compared to the control fish. A significant vitellogenin decrease was observed in both female and male zebrafish at an exposure concentration of 202 micro g/L prochloraz. However, in the male fish, significantly increased vitellogenin concentrations were observed in the groups exposed to 16 or 64 micro g/L prochloraz. This study serves as a part of the validation of the FSDT and indicates that the FSDT is suitable in detecting compounds with multiple endocrine actions. This is of importance in the assessment of the potential risk of existing and new chemicals. Copyright © 2008 CABI
Descriptors: zebrafish endocrine disrupters test, fungicides, gonads, intersexuality, nontarget organisms, prochloraz, sexual development, vitellogenins, *Danio rerio*, Fish Sexual Development Test (FSDT).

Konishi, T., S. Matsumoto, Y. Tsuruwaka, K. Shiraki, K. Hirata, Y. Tamaru, and M. Takagi (2006). **Enhancing the tolerance of zebrafish (*Danio rerio*) to heavy metal toxicity by the expression of plant phytochelatin synthase.** *Journal of Biotechnology* 122(3): 316-25. ISSN: 0168-1656.

NAL Call Number: QH442.J69

Abstract: Phytochelatin synthase (PC synthase) catalyzes a biosynthesis of phytochelatins (PCs), which are small molecules and glutathione (GSH)-derived metal-binding peptides that are essential for the detoxification of heavy metal ions in plants, fungi and worms. In order to enhance tolerance to heavy metal cytotoxicity, mRNA coding for PC synthase from *Arabidopsis thaliana* (AtPCS1) was introduced into the early embryos of zebrafish. As a result, the heterogeneous expression of PC synthase and the synthesis of PCs from GSH in embryos could be detected. The developing embryos expressing PC synthase (PC-embryos) became more tolerant to Cd toxicity (500 microM exposure). PC-embryos had significantly longer apparent lethal times for 50% of the population (LT50) of 8.17+/-1.08 days, although control embryos had apparent LT50 of 5.43+/-0.66 days. These data suggest that PC synthase can function in developmental zebrafish, and that PCs are highly effective in detoxifying Cd toxicity even in the whole body of a vertebrate species.

Descriptors: zebrafish embryos heavy metal toxicity, aminoacyltransferases genetics, arabidopsis enzymology, cadmium toxicity, chemical water pollutants toxicity, metabolism, aminoacyltransferases metabolism, drug tolerance, drug effects, metabolism, gene expression, glutathione metabolism, larva drug effects, larva metabolism, messenger RNA metabolism, survival rate.

Kosmehl, T., A.V. Hallare, G. Reifferscheid, W. Manz, T. Braunbeck, and H. Hollert (2006). **A novel contact assay for testing genotoxicity of chemicals and whole sediments in zebrafish embryos.** *Environmental Toxicology and Chemistry* 25(8): 2097-106. ISSN: 0730 7268.

NAL Call Number: QH545A1E58

Abstract: Broad consensus exists that whole-sediment exposure protocols represent the most realistic scenario to simulate in situ exposure conditions. So far, however, several endpoints including genotoxicity in vertebrate-based systems could be tested only after transfer of particle-bound substances into the aqueous phase. The present study was carried out to develop a protocol for generating a suspension of single cells from sediment-exposed zebrafish embryos that is suitable for detecting particle-bound genotoxicity in the alkaline single cell gel electrophoresis (comet assay). In this solid-phase genotoxicity assay, a whole-body cell suspension derived from zebrafish embryos exposed to native (whole) sediments is assayed in the comet assay. Several chemical and mechanical isolation procedures were compared to optimize cell yield and minimize DNA damage by the method itself. If compared to collagenase isolation, mechanical cell dissociation gave less DNA damage; trypsinization resulted in similarly low DNA damage but significantly lower cell yield. In order to test the optimized protocol, effects of well-known genotoxicants (4-nitroquinoline-N-oxide, nitrofurantoin, hydrogen peroxide, benzo[a]pyrene) and of two sediments from the upper Rhine River (Germany) on zebrafish embryos were investigated. Results documented clear-cut genotoxicity for all four substances and for one of the two whole-sediment samples. An ultraviolet (UV) light exposure of whole embryos and primary cultures from embryos elucidated only minor effects for the whole embryos compared to the primary cells. Consequently, UV irradiation cannot be suggested as a positive control in intact zebrafish embryos. In conclusion, the newly developed sediment contact assay can be recommended for the detection of both single substances but also the bioavailable fraction of the total hazard potential of sediments.

Descriptors: zebrafish, drug effects, geologic sediments chemistry, chemical water pollutants toxicity, cell line, comet assay, DNA damage, mutagenicity tests.

Kreiling, J.A., R. Creton, and C. Reinisch (2007). **Early embryonic exposure to polychlorinated biphenyls disrupts heat-shock protein 70 cognate expression in zebrafish.** *Journal of Toxicology and Environmental Health. Part A* 70(12): 1005-13. ISSN: 1528-7394.

Abstract: Polychlorinated biphenyls (PCBs) are persistent environmental contaminants that have documented neurological effects in children exposed in utero. To better define neuronally linked molecular targets during early development, zebrafish embryos were exposed to Aroclor 1254, a mixture of PCB congeners that are common environmental contaminants. Microarray analysis of the zebrafish genome revealed consistent significant changes in 38 genes. Of these genes, 55% (21) are neuronally related. One gene that showed a consistent 50% reduction in expression in PCB-treated embryos was heat-shock protein 70 cognate (Hsc70). The reduction in Hsc70 expression was confirmed by real-time polymerase chain reaction (PCR), revealing a consistent 30% reduction in expression in PCB-treated embryos. Early embryonic exposure to PCBs also induced structural changes in the ventro-rostral cluster as detected by immunocytochemistry. In addition, there was a significant reduction in dorso-rostral neurite outgrowth emanating from the RoL1 cell cluster following PCB exposure. The serotonergic neurons in the developing diencephalon showed a 34% reduction in fluorescence when labeled with a serotonin antibody following PCB exposure, corresponding to a reduction in serotonin concentration in the neurons. The total size of the labeled neurons was not significantly different between treated and control embryos, indicating that the development of the neurons was not affected, only the production of serotonin within the neurons. The structural and biochemical changes in the developing central nervous system following early embryonic exposure to Aroclor 1254 may lead to alterations in the function of the affected regions.

Descriptors: zebrafish embryos, Aroclor 1254 toxicity, HSP70 heat shock proteins metabolism, chemical water pollutants toxicity, drug effects, gene expression profiling , neurons drug effects, neurons metabolism, PCR, serotonin metabolism.

Krone, P.H., S.R. Blechinger, T.G. Evans, J.A. Ryan, E.J. Noonan, and L.E. Hightower (2005). **Use of fish liver PLHC-1 cells and zebrafish embryos in cytotoxicity assays.** *Methods* 35(2): 176-87. ISSN: 0882-4010.

NAL Call Number: QP519.7.M47

Abstract: Heat shock proteins (HSPs) indicate exposure to cellular stress and adverse cellular effects, thus serving as biomarkers of these effects. The highly conserved Hsp70 proteins are expressed under proteotoxic conditions, whereas small HSPs are expressed in response to stressors acting on the cytoskeleton and cell signaling pathways. *Poeciliopsis lucida* hepatocellular carcinoma line 1 (PLHC-1) cells have been used extensively for studying effects of cytotoxicity. A number of assays have been developed to examine DNA levels, protein levels, growth rate, morphological changes, and viability. The boundary between sub-lethal and lethal effects of particular stressors has been determined. The methodology and analytical framework for these techniques along with sample assays using cadmium stressed PLHC-1 cells are described. A range of methodologies have been developed in the past decade that allow the analysis and interpretation of gene expression and function in vivo in zebrafish embryos, and many of these are now being applied to the development of embryotoxicity assays. Here we provide the theoretical background and methodology for utilizing Hsp70 expression as an indicator of toxicity in the zebrafish embryo. Hsp70 expression is activated in a tissue-specific manner in zebrafish larvae following exposure to a number of different

toxicants, including cadmium. This has allowed the development of an hsp70/eGFP reporter gene system in stable transgenic zebrafish that serves as a reliable yet extremely quick indicator of cell-specific toxicity in the context of the multicellular, living embryo.
Descriptors: zebrafish embryos cytotoxicity, fish metabolism, liver metabolism, genetically modified, cadmium metabolism, fish liver cell line, tumor cell line, cell survival, cytoskeleton metabolism, DNA metabolism, drug dose response relationship, polyacrylamide gel electrophoresis, embryonic development, fishes, developmental gene expression regulation, reporter genes, green fluorescent proteins metabolism, HSP70 heat shock proteins genetics and metabolism, signal transduction, statistics.

Kuiper, R.V., A.J. Murk, P.E. Leonards, G.C. Grinwis, M. van den Berg, and J.G. Vos (2006). **In vivo and in vitro Ah-receptor activation by commercial and fractionated pentabromodiphenylether using zebrafish (*Danio rerio*) and the DR-CALUX assay.** *Aquatic Toxicology* 79(4): 366-75. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: The present study addresses the toxicity of a commercial pentabrominated diphenylether (PeBDE) flame retardant mixture, DE-71, in a model aquatic vertebrate. Four weeks' exposure of juvenile zebrafish (*Danio rerio*) to water-borne DE-71 resulted in dose-dependent induction of CYP1A immunoreactivity, predominantly in the endocardium and the endothelium of larger blood vessels, such as ventral aorta and branchial arteries, as well as the larger hepatic and pancreatic blood vessels. To investigate the impact of possible contaminating PBDD/Fs in the DE-71 product, the study was repeated after DE-71 had been fractionated into a non-planar (cleaned PBDEs) and a planar fraction (PBDD/Fs). Zebrafish were exposed under similar conditions to the planar and cleaned DE-71 fractions, and to uncleaned DE-71. In addition, the above fractions were chemically analyzed and tested in a reporter gene assay (DR-CALUX) for their aromatic hydrocarbon-receptor (AhR) stimulating potencies. A relatively strong CALUX response was detected from the planar DE-71 fraction (19.7ng TCDD equivalent (TEQ)/g DE-71), coinciding with a strong induction of CYP1A immunoreactivity in zebrafish. CYP1A immunoreactivity in zebrafish exposed to uncleaned DE-71 was intense, although the CALUX response was 10-fold less compared to the planar fraction. Only weak CYP1A immunoreactivity was found in fish exposed to cleaned DE-71, and none in control animals; no CALUX response was detected in cleaned DE-71. The present findings indicate that chemical impurities of the commercial PeBDE product account for AhR-mediated effects. Analytical isolation of a planar fraction from the commercial product increased the in vitro (DR-CALUX) signal 10 times. Immunohistochemistry showed a strong tissue specific reaction to DE-71 in vivo at these relatively low TEQ levels regardless of chemical pretreatment of the mix, reflecting the sensitivity of CYP1A induction in juvenile zebrafish to AhR agonists.
Descriptors: juvenile zebrafish toxicity model, cytochrome P450 enzyme system drug effects, flame retardants toxicity, phenyl ethers toxicity, polybrominated biphenyls toxicity, aryl hydrocarbon receptors drug effects, antibodies analysis and metabolism, cytochrome P450 enzyme system immunology, drug dose response relationship, endocardium drug effects, endothelium drug effects, reporter genes, gills and liver drug effects, phenyl ethers chemistry, polybrominated biphenyls chemistry, random allocation, aryl hydrocarbon receptors metabolism.

Kuiper, R.V., E.J. van den Brandhof, P.E. Leonards, L.T. van der Ven, P.W. Wester, and J.G. Vos (2007). **Toxicity of tetrabromobisphenol A (TBBPA) in zebrafish (*Danio rerio*) in a partial life-cycle test.** *Archives of Toxicology* 81(1): 1-9. ISSN: 0340 5761.

Abstract: Toxicological effects of the widely used flame retardant, tetrabromobisphenol A (TBBPA) were assessed in a partial life-cycle test with zebrafish (*Danio rerio*). Exposure of adult fish during 30 days to water-borne TBBPA in nominal concentrations ranging from 0 (control) to 1.5 microM was followed by exposure of the offspring in early life stages up to 47 days posthatching (dph) to the same concentrations. Adults exposed to 3 and 6 microM showed severe disorientation and lethargy shortly after beginning of exposure and were euthanized. Because semistatic exposure resulted in fluctuating water concentrations, pooled fish samples were chemically analyzed for internal dose assessment. Egg production was decreased in fish exposed to TBBPA concentrations of 0.047 microM and higher, and a critical effect level of 7.2 microg/g lipid with a lower 5% confidence limit of 3.9 microg/g lipid for 50% decreased egg production was calculated. Histology of adult ovaries indicated a relative increase of premature oocytes in two surviving females exposed to 1.5 microM. Hatching of TBBPA-exposed larvae was decreased except in animals exposed to 0.375 microM. In the highest exposure concentration, early posthatching mortality was high (81%) in larvae and the surviving juveniles showed a significant predominance of the female phenotype. Exposure of eggs from control parents up to 6 microM TBBPA resulted in increasing malformation and pericardial fluid accumulation from 1.5 microM; at higher concentrations, all embryos failed to hatch. The presented results indicate decreased reproductive success in zebrafish at environmentally relevant TBBPA concentrations.
Descriptors: zebrafish flame retardant toxicity model, life cycle stages drug effects, polybrominated biphenyls toxicity, behavior drug effects, drug dose response relationship, drug effects, embryology and metabolism, environmental exposure analysis, flame retardants metabolism and toxicity, ovary drug effects, metabolism and pathology, polybrominated biphenyls metabolism, reproduction drug effects, respiration drug effects, swimming, thyroid gland drug effects, metabolism and pathology, time factors, vitellogenesis drug effects, chemical water pollutants analysis and chemistry , metabolism.

Kuster, E. (2005). **Cholin- and carboxylesterase activities in developing zebrafish embryos (*Danio rerio*) and their potential use for insecticide hazard assessment.** *Aquatic Toxicology* 75(1): 76-85. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: Insecticides are a potential hazard for non-target organisms like fish particularly at run off events. The study of effects to embryos of the zebra fish *Danio* rerio is already an accepted tool in waste water monitoring, but effects of various groups of substances (like most pesticides) to the zebrafish embryo remain to be studied. Enzymes are often taken as biomarkers of exposure and effect. Therefore cholinesterase isozymes and carboxylesterase were examined for their suitability as biomarkers of insecticide exposure. The activities of cholinesterase and of carboxylesterase were monitored in the first 48 h post-fertilization (hpf) of zebrafish development. Significant specific activities in the range of 0.5-25 U could be measured from the sixth somite stage (12 h) up to the Long Pec stage (48 h) for different cholinesterases using acetyl-, acetyl-beta-methyl-, butyryl- and propionylthiocholin as substrates. The specific activity of carboxylesterase ranged from 4 to 16 Umg(-1) protein in the respective developmental stages. Substrate specificity was analysed using specific inhibitors (eserine sulphate, DPDA, BW284c51). The results showed that the observed cholinesterase activities in the whole embryo may be attributed mainly to acetylcholinesterase with a partial capability to use propionylthiocholine as a second substrate. The potential use of cholin- and carboxylesterase as biomarkers was investigated using the organophosphate paraoxon-methyl. A 40% inhibition of enzyme activities was reached by 0.4 microM paraoxon-methyl indicating the possible use of these enzymes as biomarkers of exposure.

Descriptors: zebrafish embryo toxicity model, carboxylesterase metabolism, cholinesterases metabolism, paraoxon toxicity, pesticides toxicity, metabolism, benzenaminium, 4,4' 3 oxo 1,5 pentanediylbisn,n dimethyl n 2 propenyl , dibromide pharmacology, carboxylesterase antagonists and inhibitors, cholinesterase inhibitors pharmacology, colorimetry, dopamine analogs and derivatives, dopamine pharmacology, drug effects, enzymology, substrate specificity.

Kuster, E. and R. Altenburger (2007). **Suborganismic and organismic effects of aldicarb and its metabolite aldicarb-sulfoxide to the zebrafish embryo (*Danio rerio*).** *Chemosphere* 68(4): 751-60. ISSN: 0045 6535.
NAL Call Number: TD172.C54
Abstract: The new European chemical regulation (REACH) requires a short-term fish test for chemicals where the level of production exceeds 10tons per year. For ethical reasons (3R-concept), an alternative to the acute fish test should be introduced to decrease the number of animal testing with fish. The zebrafish embryo (*Danio rerio)* test became a valuable tool in ecotoxicology and already replaces the acute fish test for the evaluation of wastewater in Germany. Recent efforts are targeted to use this and other fish embryo tests for the effect assessment of chemicals. The toxic effects of the carbamate insecticide aldicarb and its metabolite aldicarb-sulfoxide to zebrafish embryos were analysed using two approaches with different endpoints. Organismic tests were conducted with zebrafish embryos exposed to the pesticides for 48h. In addition, suborganismic effects were examined analysing the enzyme inhibition of cholinesterases and carboxylesterases. On the organismic level, the only sublethal effect seen was the increase of heart rate at low and decrease at higher concentration with the use of aldicarb-sulfoxide but not with aldicarb (concentration range 0.2-300microM). In contrast, analysis of enzyme inhibitions showed high to very high effects caused by the two carbamates. The enzyme inhibition analysis of whole homogenates of exposed embryos may be advantageous for toxicant screening (biomarker of exposure) and might be used to bridge the gap of sensitivity of the (48h old) zebrafish embryos to adult fish when exposed to anti-cholinesterase substances (biomarker of prospective effect).
Descriptors: zebrafish embryos ecotoxicology testing model, aldicarb analogs, derivatives and toxicity, carboxylesterase antagonists and inhibitors, cholinesterase inhibitors toxicity, insecticides toxicity, carboxylesterase metabolism, cholinesterases metabolism.

Lance, S.L., A.S. Peterson, and M. Hagedorn (2004). **Developmental expression of aquaporin-3 in zebrafish embryos (*Danio rerio*).** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 138(3): 251-8. ISSN: 1532 0456.
NAL Call Number: QP901.C6
Abstract: Fish embryos have never been successfully cryopreserved because of the low permeability of cryoprotectants into the yolk. Recently, we used aquaporin-3 fused with a green fluorescent protein (AQP3GFP) to modify the zebrafish embryo, and demonstrated that the pores functioned physiologically. This increased the water and cryoprotectant permeability of the membranes. We have continued our work on AQP3-modified embryos and here we report their developmental expression of AQP3, the success of various culture media on their survival and development, and their reproductive success. The AQP3GFP expression begins within 30 m after the mRNA AQP3GFP injection into the yolk of the 1- to 4-cell embryo. This expression is distributed in the membranes throughout the blastoderm and the yolk syncytial layer within 24 h. It diminishes after 96 h. We found no difference in the survival or normal development of embryos from AQP3GFP or wild-type adults. Additionally, zebrafish embryos did not require special culture medium to survive after AQP3GFP modification.

In fact, they survived best in embryo medium (ca. 40 mOsm). Embryos reared entirely in embryo medium had a higher percent survival and a higher percent normal development than those exposed to a high osmolality sucrose culture medium (ca. 330 mOsm). The mechanism whereby these embryos can maintain their internal osmolality in a hypoosmotic solution with water channels in their membranes is unknown.

Descriptors: zebrafish embryology, aquaporins metabolism, developmental gene expression regulation drug effects, metabolism, aquaporin 3, culture media, drug effects, embryology, metabolism, confocal microscopy, osmolar concentration, sodium chloride pharmacology, sucrose pharmacology, survival rate.

Lancieri, M., F. Aniello, D. Esposito, L. Migliore, and L. Gaudio (2004). **Embryotoxicity of flumequine on *Danio rerio*: mortality, morphological/histological alterations and changes in calmodulin gene expression.** *Fresenius Environmental Bulletin* 13(12a): 1415-1419. ISSN: 1018-4619.

Descriptors: zebrafish embryo flumequine toxicity model, antibiotics, aquaculture, biological stress, calcium-binding protein, calmodulin, cartilage, contamination, DNA probes, defects, disease control, embryonic growth stage, embryonic development, embryos, environmental factors, fins, fish, flumequine, freshwater fish, gene expression, histology, model studies, mortality, mortality causes, muscle, muscles, pesticides, pollution effects, spinal nerves, stress, tissues, toxicity, mRNA, *Danio rerio.*

Leaver, M.J., J. Wright, P. Hodgson, E. Boukouvala, and S.G. George (2007). **Piscine UDP-glucuronosyltransferase 1B**. *Aquatic Toxicology* 84(3): 356-365. ISSN: 0166-445X.

NAL Call Number: QH541.5.W3A6

Descriptors: zebrafish, 3-methylcholanthrene, acids, adipose tissue, aglycones, alternative splicing, amino acids, benzo(a)pyrene, bile acids, bilirubin, brain, cardiac muscle, detoxification, development, eggs, exons, flounders, gametes, gastrulation, genomes, gills, hepatocytes, intestine, introns, kidney, lindane, agrochemicals, liver, muscles, phylogeny, pollutants, steroid hormones, structure, UDP-glucuronosyltransferase, water pollution effects, adipose tissues, introns, mRNA, polyaromatic hydrocarbons, steroids, *Danio rerio*, *Platichthys flesus*, *Pleuronectes platessa*, *Tetraodon nigroviridis.*

Lee, K.C., W.W.W. Lee, T.P. Loh, and H.Y. Song (2005). **Total synthesis of antillatoxin and biological evaluation of antillatoxin and fragments using zebrafish embryo.** *Abstracts of Papers American Chemical Society* 229(Part 2): U385. ISSN: 0065 7727.

NAL Call Number: 381Am33Pa

Descriptors: zebrafish embryo antillatoxin model, development, biochemistry and molecular biophysics, embryo screening and development.

Lefebvre, K.A., V.L. Trainer, and N.L. Scholz (2004). **Morphological abnormalities and sensorimotor deficits in larval fish exposed to dissolved saxitoxin.** *Aquatic Toxicology* 66(2): 159-170. ISSN: 0166 445X.

NAL Call Number: QH541.5.W3A6

Abstract: The dietary uptake of one suite of dinoflagellate-produced neurotoxins, that are commonly called paralytic shellfish poisoning (PSP) toxins, is known to cause acute fish kills. However, little is known about the effects of dissolved phase exposure and the potential sublethal effects of this route of exposure on early developmental stages of fish. Toxin exposure during early development is of particular concern because the embryos and larvae of some marine fish species may be unable to actively avoid the dissolved toxins that algal

cells release into the water column during harmful algal blooms. Here we use the zebrafish (*Danio rerio*) as a model experimental system to explore the sublethal effects of a dissolved PSP toxin, saxitoxin (STX), on early development in fish, including sensorimotor function, morphology, and long-term growth and survival. Aqueous phase exposures of 229+-7 mug STX eq.l-1 caused reductions in sensorimotor function as early as 48 h postfertilization (hpf) and paralysis in all larvae by 4 days postfertilization (dpf). Rohon-Beard mechanosensory neurons appeared to be more sensitive to STX than dorsal root ganglion neurons at this dose. Additionally, exposure to 481+-40 mug STX eq.l-1 resulted in severe edema of the eye, pericardium, and yolk sac in all exposed larvae by 6 dpf. The onset of paralysis in STX-exposed larvae was stage-specific, with older larvae becoming paralyzed more quickly than younger larvae (5 h at 6 dpf as compared to 8 and 46 h for 4 and 2 dpf larvae, respectively). When transferred to clean water, many larvae recovered from the morphological and sensorimotor effects of STX. Thus, the sublethal effects of the toxin on larval morphology and behavior were reversible. However, zebrafish exposed to STX transiently during larval development (from 2 to 4 dpf) had significantly reduced growth and survival at 18 and 30 days of age. Collectively, these data show that (1) dissolved phase STX is bioavailable to fish embryos and larvae, (2) the toxin is a paralytic with potencies that are stage-specific for fish larvae, (3) the observed toxicological effects of STX exposure are reversible, and (4) a short-term toxin exposure can negatively impact the survival of fish several weeks later. Dissolved algal toxins may therefore have important sublethal effects on vulnerable species of fish. Copyright©Thomson Reuters 2009

Descriptors: zebrafish PSP toxicity model, development, nervous system, neural coordination, toxicology, chemically induced paralysis, nervous system disease, toxicity, chemically induced, paralytic shellfish poisoning (PSP), toxicity, sensorimotor deficit, chemically induced growth, harmful algal bloom, larval development, morphology, survival.

Lema, S.C., I.R. Schultz, N.L. Scholz, J.P. Incardona, and P. Swanson (2007). **Neural defects and cardiac arrhythmia in fish larvae following embryonic exposure to 2,2',4,4'-tetrabromodiphenyl ether (PBDE 47)**. *Aquatic Toxicology* 82(4): 296-307. ISSN: 0166-445X.

NAL Call Number: QH541.5.W3A6

Descriptors: zebrafish fire retardant toxicity model, arrhythmia, bioavailability, brain, cardiovascular system, cerebrospinal fluid, congeners , contaminants, embryogenesis, embryos, ethers, etiology, exposure, fire retardant chemicals, fire retardants, fires, fluid flow, fluorescent indicators, foams, hatching, heart, hindbrain, larvae, lipids, microinjection, model studies, models, molecular modelling, neural tube, ontogeny, plasticity, plastics, polybrominated diphenyl ethers, retardants, tachycardia, textiles, toxicity, ventricles (cerebral), water pollution effects, wildlife, marine mammals, polybrominated diphenyl ethers, polyurethane, *Danio rerio.*

Levin, E.D. (2005). **Special issue: marine and freshwater toxin impacts on neurobehavioral function**. *Neurotoxicology and Teratology* 27(5): 770 pp. ISSN: 0892 0362.

NAL Call Number: RA1224

Abstract: Papers on the following topics are presented: prospects on behavioural studies of marine and freshwater toxins; behavioural effects and drug vulnerability in rats exposed to *Pfiesteria* toxin; developmental toxicity of domoic acid in zebrafish (*Danio rerio*); persisting behavioural consequences of prenatal domoic acid exposure in rats; effects of marine algal toxins on thermoregulation in mice; symptoms and treatment of chronic biotoxin-associated illness; neurohistochemical biomarkers of the marine neurotoxicant domoic acid; and mapping and reconstruction of domoic-acid-induced neurodegeneration in the mouse brain.

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Descriptors: zebrafish, aquatic plants, aquatic weeds, behavior, biochemical markers, brain, illness, neurotoxicity, symptoms, thermoregulation, toxic substances, toxicity, toxins, weeds, algae, *Danio rerio*, mice, rats.

Levin, E.D. and E. Chen (2004). **Nicotinic involvement in memory function in zebrafish.** *Neurotoxicology and Teratology* 26(6): 731-5. ISSN: 0892-0362.
NAL Call Number: RA1224
Abstract: Zebrafish are an emerging model for the study of the molecular mechanisms of brain function. To conduct studies of the neural bases of behavior in zebrafish, we must understand the behavioral function of zebrafish and how it is altered by perturbations of brain function. This study determined nicotine actions on memory function in zebrafish. With the methods that we have developed to assess memory in zebrafish using delayed spatial alternation (DSA), we determined the dose effect function of acute nicotine on memory function in zebrafish. As in rodents and primates, low nicotine doses improve memory in zebrafish, while high nicotine doses have diminished effect and can impair memory. This study shows that nicotine affects memory function in zebrafish much like in rats, mice, monkeys and humans. Now, zebrafish can be used to help understand the molecular mechanisms crucial to nicotine effects on memory.
Descriptors: zebrafish embryos nicotine effects on memory , brain drug effects, memory drug effects, memory disorders chemically induced, nicotine pharmacology, acetylcholine metabolism, brain physiology, drug dose response relationship, memory physiology, memory disorders physiopathology, models, nicotinic agonists pharmacology, nicotinic drug receptors effects, nicotinic receptors, metabolism, spatial behavior drug effects, spatial behavior physiology.

Levin, E.D., E. Chrysanthis, K. Yacisin, and E. Linney (2003). **Chlorpyrifos exposure of developing zebrafish: effects on survival and long-term effects on response latency and spatial discrimination.** *Neurotoxicology and Teratology* 25(1): 51-7. ISSN: 0892-0362.
NAL Call Number: RA1224
Abstract: Chlorpyrifos (CPF) is a widely used insecticide, which has been shown to interfere with neurobehavioral development. Rat models have been key in demonstrating that prenatal CPF exposure causes choice accuracy deficits and motor alterations, which persist into adulthood. Complementary nonmammalian models can be useful in determining the molecular mechanisms underlying the persisting behavioral effects of developmental CPF exposure. Zebrafish with their clear chorion and extensive developmental information base provide an excellent model for assessment of molecular processes of toxicant impacted neurodevelopment. To facilitate the use of the zebrafish model and to compare it to the more typical rodent models, the behavioral phenotype of CPF toxicity in zebrafish must be well characterized. Our laboratory has developed methods for assessing spatial discrimination learning in zebrafish, which can differentiate response latency from choice accuracy in a three chambered fish tank. Low and high doses of CPF (10 and 100 ng/ml on days 1-5 postfertilization) both had significant persisting effects on both spatial discrimination and response latency over 18 weeks of testing. The high, but not the low dose, significantly accelerated mortality rates of the fish during the study from 20-38 weeks of age. Developmental exposure to either 10 or 100 ng/ml of CPF caused significant spatial discrimination impairments in zebrafish when they were adults. The impairment caused by 10 ng/ml was seen during early but not later testing, while the impairment caused by 100 ng/ml became more pronounced with continued testing. The higher dose caused a more pervasive impairment. The 10 and 100 ng/ml

doses had opposite effects on response latency. The low 10 ng/ml dose significantly slowed response latency, while the high 100 ng/ml dose significant increased response latency. Both of these effects diminished with continued testing. CPF exposure during early development caused clear behavioral impairments, which lasted throughout adulthood in zebrafish. The molecular mechanisms by which early developmental CPF exposure produces these behavioral impairments expressed in adulthood can now be studied in the zebrafish model.
Descriptors: zebrafish embryos and larvae, chlorpyrifos toxicity model, drug effects, insecticides toxicity, larva drug effects, prenatal exposure delayed effects, behavior drug effects and physiology, central nervous system drug effects and physiopathology, discrimination learning drug effects and physiology, drug dose response relationship, embryology, embryonic development, larva growth and development, reaction time drug effects and physiology, space perception drug effects and physiology, survival rate.

Levin, E.D., H.A. Swain, S. Donerly, and E. Linney (2004). **Developmental chlorpyrifos effects on hatchling zebrafish swimming behavior.** *Neurotoxicology and Teratology* 26(6): 719-23. ISSN: 0892-0362.
NAL Call Number: RA1224
Abstract: Chlorpyrifos (CPF), a widely used organophosphate insecticide and potent acetylcholinesterase inhibitor, interferes with neurobehavioral development. Rat models have been key in demonstrating that developmental CPF exposure causes learning deficits and locomotor activity alterations, which persist into adulthood. Complementary nonmammalian models can be useful in determining the neurodevelopmental mechanisms underlying these persisting behavioral effects. Zebrafish (*Danio rerio*) with their clear chorion and extensive developmental information base provide an excellent model for assessment of molecular processes of toxicant-impacted neurodevelopment. We have developed methods for assessing spatial discrimination learning in adult zebrafish and have documented persisting effects of developmental CPF exposure on swimming activity and learning after low and high doses of CPF (10 and 100 ng/ml) administered to zebrafish embryos on Days 1-5 postfertilization (pf). In the current study, we developed methods for behavioral assessment of CPF exposure on swimming activity in newly hatched zebrafish. An equal area segmented annular grid (concentric circles divided into quadrants through the diameter) was made in a 16-mm diameter cylinder. The test area was placed on a heating device secured to an Olympus SZH10 dissecting scope stage. Zebrafish embryos were exposed to 10 ng/ml CPF, 100 ng/ml CPF, or vehicle control (25 microl/ml DMSO) (n=8-10/treatment group). Each treatment group was kept in a total volume of 25 ml of egg water (60 mg/ml Instant Ocean) including DMSO with or without CPF mixed to above dilutions in an incubator set at 28.5 degrees C. CPF dilutions or vehicle were changed daily with exposure ending on Day 5 pf. Testing of larval zebrafish was performed on Days 6 and 9 pf. The fish were placed in the test cylinder with 1.5 ml of egg H(2)O (28.5 degrees C). After a 2-min acclimation period, the swimming activity of the fish was measured for a 3-min testing session. The 100 ng/ml CPF dose caused significant slowing of swimming activity on Days 6 and 9 pf and had persisting effects of impairing spatial discrimination and decreasing response latency in adulthood. Developmental exposure to 10 ng/ml of CPF did not cause a significant change in locomotor activity during the period soon after hatching. CPF exposure during early development caused clear behavioral impairments detectable during the posthatching period. In a previous study, we found that early developmental CPF exposure caused behavioral alterations in zebrafish, which lasted throughout adulthood. The molecular mechanisms by which early developmental CPF exposure produces these behavioral impairments expressed in adulthood can now be studied in the zebrafish model.

Descriptors: zebrafish model chlorpyrifos toxicity, drug effects, larva drug effects, motor activity drug effects, behavior drug effects, cholinesterase inhibitors toxicity, disease models, drug dose response relationship, embryology, physiology, habituation, psychophysiologic drug effects and physiology, larva growth and development, motor activity physiology, physical stimulation methods, reaction time drug effects, reaction time physiology, swimming physiology, drug effects on behavior.

Li, W.H., P.C. Chan, and K.M. Chan (2004). **Metal uptake in zebrafish embryo-larvae exposed to metal-contaminated sediments.** *Marine Environmental Research* 58(2-5): 829-32. ISSN: 0141-1136.
NAL Call Number: QH545.W3M36
Abstract: Heavy metal contamination in coastal and estuarine regions of inner Tolo Harbour, as well as some river and nullah areas, is one of the major water-pollution issues in Hong Kong. Zebrafish *(Danio rerio*) embryo-larvae was used to study metal uptake from coastal sediments collected from Fo Tan River (industrial area, Sha Tin), Tai Po River (residential area, Tai Po) and Wu Kai Sa (rural area, Tolo Harbour). Exposure experiments (7-days) were carried out using different concentrations (0.1%, 0.5%, 1% and 0% as control) of sediments that were added to aquaria containing fertilized zebrafish eggs until they hatched to become larvae. Uptake of heavy metals (Cd, Cu, Pb and Zn) was determined in whole embryo-larvae following exposure. Significant levels of Cd, Cu, Pb and Zn were detected in the embryo-larvae exposed to sediments from Tai Po River. However, significant levels of only Cd and Cu were found in embryo-larvae exposed to sediments from Fo Tan River.
Descriptors: zebrafish metal contamination exposure model, environmental pollutants pharmacokinetics, geologic sediments analysis, heavy metals pharmacokinetics, environmental pollutants analysis and toxicity, enzyme induction drug effects, hong kong, larva metabolism, metallothionein biosynthesis, heavy metals analysis and toxicity, reproduction drug effects and physiology, reverse transcriptase PCR, atomic spectrophotometry, Fo Tan River, Tai Po River, Tolo Harbour, Hong Kong.

Li, Y., Q. Zhang, and S. Dai (2003). **Impact of aldicarb and its complex pollution on DNA of zebrafish embryo**. *Journal of Applied Ecology* 14(6): 982-4. ISSN: 0021-8901.
NAL Call Number: 410 J828
Abstract: This paper deals with the impact of a complex pollution system composed of a very toxic carbamate pesticide, aldicarb and the widely used anionic surfactant, sodium dodecylbenzenesulfonate(SDBS), on DNA of zebrafish (*Brachydanio rerio*) embryo. The results indicated that DNA damage caused by aldicarb became more serious with its increasing concentration, but single-strand break caused by aldicarb in low concentration and short time could be repaired, and high concentration led to double-strand break which was difficult to repaire. SDBS in certain concentration (20 mg.L-1) could reduce the toxicity of aldicarb in the complex pollution system.
Descriptors: zebrafish pesticide toxicity model, aldicarb toxicity, benzenesulfonates toxicity, DNA damage, insecticides toxicity and surface active agents, drug dose response relationship, drug effects.
Language of Text: Chinese.

Li Yan, Zhao QingShun, and Yin DaQiang (2005). **Nested PCR-RFLP analyses of zebrafish p53 gene point mutation induced by pentachlorophenol**. *China Environmental Science* 25(3): 357-360. ISSN: 1000 6923.

Abstract: The hepatic genomic DNA of zebrafish [*Danio rerio*] was extracted after contamination exposure to 50 micro g pentachlorophenol (PCP)/litre for 10 days in vivo. A fragment containing exons 2, 3 and 4, and introns 2 and 3 of zebrafish p53 gene was amplified through nested PCR-RFLP analysis of the amplified products using restriction enzymes Nco I, Sac I, Sca I, Bcl I, Nsi I and Rsa I to detect the occurrence of point mutation of the objective fragment on the enzyme site. 680 bp of the 6 enzyme sites of 20 zebrafishes were detected, but no point mutation was detected. If p53 gene point mutation could be induced by PCP in zebrafish, the mutation rate of its bp was less than 1/680. Copyright © 2008 CABI

Descriptors: zebrafish pentachlorophenol exposure, genetic mapping and markers, genome analysis, mutagens, mutants, mutations, PCR, restriction fragment length polymorphism, toxic substances, *Danio rerio.*

Language of Text: Chinese.

Liao, T., Y. Xu, X.P. Zhong, Y. Liang, and J.W. Wang (2005). **Comparative vitellogenic responses in zebrafish (*brachydanio rerio*) and rare minnow (*Gobiocypris rarus*) exposed to 17[alpha]-ethinylestradiol.** *Acta Hydrobiologica Sinica* 29(5): 513-517. ISSN: 1000 3207.

Abstract: In this study, the response of zebrafish and rare minnow to 17[alpha]-ethinylestradiol (EE2) was studied using vitellogenin (Vtg) as a biomarker. Juvenile rare minnow and zebrafish were exposed to waterborne EE2 at 5 ng/L, 20ng/L and 100ng/L EE2 in laboratory aquaria. After the exposure of 5, 10, 15 days, the fish were sampled to measure Vtg in whole body homogenates (WBH) The results showed that Vtg levels in juvenile rare minnow increased significantly at 5ng/L,20ng/L and 100ng/L, EE2 after 5 days exposure and reached a maximal level on the day 15th. Although Vtg concentration in juvenile zebrafish increased significantly in 100 ng/L EE2 exposure group after 5 days, a significant increase of Vtg in 5ng/L. and 20ng/L treatment was just found on the day 15th and on the day 10th respectively. These indicate that rare minnow is more sensitive than zebrafish in Vtg induction to 17[alpha]-ethinylestradiol. Zebrafish Vtg was identified in this study. After being separated by SDS-PAGE, zebrafish Vtg produced two protein hands with molecular weights of 140 kDa and 170kDa. Because the development of species-specific methods for its quantification has been hampered due to problems such as the small blood volume and the lack of sufficient pure antigen and specific antibodies,a polyclonal antibody raised against carp Vtg was used as first antibody to measure Vtg in whole body homogenates of zebrafish and rare minnow in this study. The results of western blotting showed that the carp Vtg antibodies cross-reacted with zebrafish Vtg and could be used to measure zebrafish Vtg.

Descriptors: zebrafish, *Danio rerio, Gobiocypris rarus*, proteins, vitellogenin concentration, response to 17alpha ethinyloestradiol exposure, hormones, 17alpha ethinyloestradiol, oogenesis, laboratory aquarium exposure.

Language of Text: Chinese.

Lin, C.C., M.N. Hui, and S.H. Cheng (2007). **Toxicity and cardiac effects of carbaryl in early developing zebrafish (*Danio rerio*) embryos.** *Toxicology and Applied Pharmacology* 222(2): 159-68. ISSN: 0041-008X.

NAL Call Number: 391.8T662

Abstract: Carbaryl, an acetylcholinesterase inhibitor, is known to be moderately toxic to adult zebrafish and has been reported to cause heart malformations and irregular heartbeat in medaka. We performed experiments to study the toxicity of carbaryl, specifically its effects on the heart, in early developing zebrafish embryos. LC50 and EC50 values for carbaryl at 28 h post-fertilization were 44.66 microg/ml and 7.52 microg/ml, respectively, and 10 microg/

ml carbaryl was used in subsequent experiments. After confirming acetylcholinesterase inhibition by carbaryl using an enzymatic method, we observed red blood cell accumulation, delayed hatching and pericardial edema, but not heart malformation as described in some previous reports. Our chronic exposure data also demonstrated carbaryl-induced bradycardia, which is a common effect of acetylcholinesterase inhibitors due to the accumulation of acetylcholine, in embryos from 1 day post-fertilization (dpf) to 5 dpf. The distance between the sinus venosus, the point where blood enters the atrium, and the bulbus arteriosus, the point where blood leaves the ventricle, indicated normal looping of the heart tube. Immunostaining of myosin heavy chains with the ventricle-specific antibody MF20 and the atrium-specific antibody S46 showed normal development of heart chambers. At the same time, acute exposure resulted in carbaryl-induced bradycardia. Heart rate dropped significantly after a 10-min exposure to 100 microg/ml carbaryl but recovered when carbaryl was removed. The novel observation of carbaryl-induced bradycardia in 1- and 2-dpf embryos suggested that carbaryl affected cardiac function possibly through an alternative mechanism other than acetylcholinesterase inhibition such as inhibition of calcium ion channels, since acetylcholine receptors in zebrafish are not functional until 3 dpf. However, the exact nature of this mechanism is currently unknown, and thus further studies are required.
Descriptors: zebrafish, carbaryl toxicity, drug effects, heart drug effects, chemically induced bradycardia, bradycardia physiopathology, cholinesterase inhibitors toxicity, drug dose response relationship, embryology, physiology, heart embryology and physiology, heart rate drug effects, immunohistochemistry, myosin heavy chains analysis, pericardium abnormalities, drug effects and metabolism, time factors, toxicity tests methods.

Lin, L.L. and D.M. Janz (2006). **Effects of binary mixtures of xenoestrogens on gonadal development and reproduction in zebrafish.** *Aquatic Toxicology* 80(4): 382-95. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: Previous studies exposing fish to xenoestrogens have demonstrated vitellogenin (VTG) induction, delayed gametogenesis, altered sex ratio, and decreased reproductive performance, with a majority of those studies focusing on exposure to single chemicals. The objective of this study was to determine the effects of binary mixtures of a weak estrogen receptor agonist, nonylphenol (NP) and a potent estrogen receptor agonist, 17alpha-ethinylestradiol (EE) on sex ratios, gametogenesis, VTG induction, heat shock protein 70 (HSP70) expression and reproductive capacity in zebrafish (*Danio rerio*). Fish were exposed from 2 to 60 days post-hatch (dph) to nominal concentrations of 10 or 100 microg/l NP (NP10 or NP100, respectively), 1 or 10 ng/l EE (EE1 or EE10, respectively), 1 ng/l EE+10 or 100 microg/l NP (EE1+NP10 or EE1+NP100, respectively), 10 ng/l EE+10 or 100 microg/l NP (EE10+NP10 or EE10+NP100, respectively) or solvent control (0.01% acetone, v/v) in a static-renewal system with replacement every 48 h. At 60 dph, fish from each treatment were euthanized for histological examination of gonads, and whole body VTG and HSP70 levels. Remaining fish were reared in clean water until adulthood (240 dph) for breeding studies. In all EE10 exposure groups (EE10, EE10+NP10 and EE10+NP100), increasing NP concentration acted antagonistically to the action of EE in terms of VTG induction at 60 dph. Similarly, non-additivity was observed with egg production, where EE1+NP100 exposure resulted in significantly more eggs produced per breeding trial than EE1 alone. Histological staging of oogenesis revealed suppressed gametogenesis in an additive fashion in females at 60 dph. There were no differences among treatment groups in whole body HSP70 expression in 60 dph fish or in gonadal HSP70 expression in adult fish. Although there was no statistical evidence of non-additivity, breeding trials in adults revealed significant reductions in egg viability, egg hatchability and/or F1 swim-up success,

suggesting that developmental exposures to xenoestrogens may cause irreversible effects on egg quality and progeny even after periods of depuration. In conclusion, these results suggest that environmentally relevant mixtures of NP and EE can produce additive or non-additive effects that depend on the particular response being determined and the respective exposure concentrations of each chemical.

Descriptors: zebrafish xenoestrogens model, estrogens toxicity, ethinyl estradiol and phenols toxicity, reproduction drug effects, Western blotting, body size drug effects, gametogenesis drug effects, gene expression drug effects, gonads chemistry, drug effects, growth and development, HSP70 heat shock proteins analysis and biosynthesis, larva drug effects, estrogen receptors agonists, sex ratio, vitellogenins analysis and biosynthesis.

Linbo, T.L., C.M. Stehr, J.P. Incardona, and N.L. Scholz (2006). **Dissolved copper triggers cell death in the peripheral mechanosensory system of larval fish**. *Environmental Toxicology and Chemistry* 25(2): 597-603. ISSN: 0730-7268.

NAL Call Number: QH545A1E58

Descriptors: zebrafish larva copper toxicity model, confocal microscopy, fish larvae, fluorescence, freshwater fish, histology, larvae, lateral line, microscopy, mortality, neuroimaging, neurons, neurotoxicity, nonpoint pollution sources, nonpoint pollution, pollution effects, predators, rheotaxis, runoff, scanning electron microscopy, toxicity tests, water pollution effects, water pollution, watersheds, neuromasts, *Danio rerio.*

Linderoth, M., M. Ledesma, Y. Zebuhr, and L. Balk (2006). **Sex steroids in the female zebrafish (*Danio rerio*). effects of cyproterone acetate and leachate-contaminated sediment extract.** *Aquatic Toxicology* 79(2): 192-200. ISSN: 0166 445X.

NAL Call Number: QH541.5.W3A6

Abstract: In Molnbyggen, a leachate-contaminated lake in Sweden, effects on the reproductive system of perch included a decreased frequency of sexually mature female perch, reduced gonadosomatic index and decreased plasma levels of androstenedione and testosterone, but the contaminants responsible for these effects have not yet been identified. A biomarker-directed fractionation approach could be used to narrow the search for the compound(s) responsible for the adverse effects in Molnbyggen female perch. Thus, it is important to develop and test a suitable biomarker, which can be used in this type of approach. We evaluated if decreased concentrations of sex steroids could work as an in vivo end-point in female zebrafish fed an organic extract of bottom sediments from Molnbyggen. The responsiveness of zebrafish to endocrine alteration was evaluated using a positive control substance, cyproterone acetate (CA), which is known to inhibit steroid biosynthesis. After the 21-day exposure period the concentrations of testosterone (T) and estradiol (E2) were successfully determined by HRGC/HRMS in whole-body homogenates of individual fish. Median T concentrations were 1.7pmol/g fish in controls and were decreased after exposure both to CA (0.55pmol/g fish) and Molnbyggen sediment extract (1.2-1.3pmol/g fish). Median E2 concentrations were 5.3pmol/g fish in controls and were decreased after CA exposure (2.3pmol/g fish) and after exposure to Molnbyggen sediment extract (4.0-4.5pmol/g fish). This suggests that sex steroid concentrations in zebrafish are responsive to endocrine alteration and indicates that compounds with endocrine altering potency are present in Molnbyggen sediments.

Descriptors: female zebrafish, endocrine disruptors screening test, androgen antagonists toxicity, cyproterone acetate toxicity, endocrine system drug effects, geologic sediments chemistry, chemical water pollutants toxicity, androgen antagonists administration and dosage, cyproterone acetate administration and dosage, environmental exposure, gonadal steroid hormones analysis, reproduction drug effects, Lake Molnbyggen, Sweden.

Lindholst, C., P.M. Wynne, P. Marriott, S.N. Pedersen, and P. Bjerregaard (2003). **Metabolism of bisphenol A in zebrafish (*Danio rerio*) and rainbow trout (*Oncorhynchus mykiss*) in relation to estrogenic response.** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 135(2): 169-77. ISSN: 1532 0456.

NAL Call Number: QP901.C6

Abstract: The kinetics of bisphenol A (BPA) were investigated in zebrafish (*Danio rerio*) exposed to 100 microg BPA/l. BPA uptake was measured during a 7-day period followed by an elimination phase of similar duration. After 2, 6, 12, 24, 48, 72, 120 and 168 h of uptake/elimination, fish were analysed for their content of BPA, bisphenol A glucuronic acid (BPAGA) and bisphenol A sulfate (BPAS). Within the first 24 h steady state levels of BPA, BPAGA and BPAS were reached and the total body concentrations were calculated to be 569, 12,600 and 39.9 ng/g fish, respectively. Elimination rates of the three compounds in zebrafish were estimated by fitting the data to a compartment model. An initial rapid elimination phase was observed for BPA and BPAS with total body half lives (T(1/2)) of <1.1 h and 30 min, followed by a slower second elimination phase with T(1/2) values of 139 and 71 h, respectively. Excretion of BPAGA occurred from a single compartment with a T(1/2) of 35 h. The steady state concentration of BPA and its metabolites were investigated in rainbow trout *(Oncorhynchus mykiss*) exposed to 100 microg BPA/l. The toxicokinetic parameters from zebrafish and rainbow trout were compared; including previously published data on the rainbow trout. The data indicate that the smaller estrogenic sensitivity observed for the zebrafish may be caused by a more rapid metabolism of BPA in the zebrafish liver.

Descriptors: zebrafish and rainbow trout bisphenol A (BPA) model, estrogens metabolism, *Oncorhynchus mykiss* metabolism, phenols pharmacokinetics, metabolism, bile metabolism, half life, liver metabolism, phenols blood, phenols metabolism, phenols toxicity.

Linney, E., B. Dobbs McAuliffe, H. Sajadi, and R.L. Malek (2004). **Microarray gene expression profiling during the segmentation phase of zebrafish development.** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 138(3): 351-62. ISSN: 1532 0456.

NAL Call Number: QP901.C6

Abstract: We analyzed 15,512 unique transcripts from wild-type *Danio rerio* using a long oligonucleotide microarray containing >16,000 65-mers probes. Total RNA was isolated from staged embryos at 2 h intervals over a 24-h period. On average, at any given time point, 27% of the probe set detected corresponding transcripts in embryonic RNA. There were two predominant patterns in the nearly 4000 genes that changed expression in at least one time point during the first 24 hpf. At 12 hpf, we detected 420 up-regulated and 386 down-regulated genes. By 24 hpf, the number of up- and down-regulated genes had increased to 954 and 766, respectively. While the majority of these genes maintained their new level of expression for the duration of the time course, we identified five genes with phasic regulation over the 24-h time course. Two of these genes, germ cell nuclear factor and mesogenin, have been identified as being expressed during gastrulation (5 1/4 to 10 h postfertilization) and subsequently repressed. A cluster containing 36 distinct ribosomal proteins was up-regulated at 12 h, indicating a capability for de novo protein synthesis during and after this stage. Twenty-three muscle-specific genes were up-regulated late during the initial 24 hpf, corresponding to the development and differentiation of the somites.

Descriptors: zebrafish embryos, gene expression profiling standards , developmental gene expression regulation , oligonucleotide array sequence analysis standards, cluster analysis, cytochrome P450 enzyme system metabolism, embryology, metabolism, in situ hybridization, nucleic acid hybridization, quality control, messenger RNA analysis, reproducibility of results, time factors.

Linney, E., L. Upchurch, and S. Donerly (2004). **Zebrafish as a neurotoxicological model.** *Neurotoxicology and Teratology* 26(6): 709-18. ISSN: 0892-0362.

NAL Call Number: RA1224

Abstract: At a time when common regulatory pathways are being identified in several different species and genomics is beginning to allow comparisons of genes, how they are arranged on chromosomes and how they are regulated, zebrafish has emerged as a valuable and complementary vertebrate model. Some of the characteristics that prove of value are described and illustrated. Fluorescent transgenic lines of zebrafish embryos are presented for time-line studies with neurotoxicants. While genetic knockout technology has yet to be developed for the model, the anti-sense, morpholino approach allows for knockdown of expression of genes for the 3 day, embryonic period. This can provide for phenocopies of mutant genes for those genes essential to embryonic development or it can provide for a limited inhibition of gene expression that allows subsequent development of the fish. With the zebrafish genomic sequencing effort, microarray technology is now developing for the model system. These resources and technologies allow one to challenge the system with toxicants, and to view the immediate effects of the toxicants with transgenic embryos that fluoresce in part or all of the nervous system. Behavioral and learning protocols have been developed for the organism so that early exposures can be assayed for effects upon adult fish. Microarray technology should allow for one to identify specific genes and pathways affected by a neurotoxicant. In the future, these approaches should provide a working protocol for exploring molecular mechanisms of neurotoxicants. This type of complementary approach should then allow for more efficient examination and testing of mechanisms in mammalian models.

Descriptors: zebrafish trangenic embryo, neurotoxicoligy disease model, drug effects, neurotoxins toxicity, genetically modified embryology, antisense elements genetics, bacterial proteins, behavior drug effects and physiology, chlorpyrifos toxicity, embryology, physiology, environmental exposure, developmental gene expression regulation drug effects and genetics, genomic library, green fluorescent proteins, learning drug effects and physiology, luminescent proteins, mutation drug effects and genetics, oligonucleotide array sequence analysis methods, time factors.

Notes: Comments: Erratum In: Neurotoxicol Teratol.2005-Feb;27(1):175

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Liu, F., R.J. Kendall, and C.W. Theodorakis (2005). **Joint toxicity of sodium arsenate and sodium perchlorate to zebrafish *Danio rerio* larvae.** *Environmental Toxicology and Chemistry* 24(6): 1505-7. ISSN: 0730 7268.

NAL Call Number: QH545A1E58

Abstract: Joint toxicity of arsenate and perchlorate was tested in larvae of *Danio rerio*. Results indicated that the 96-h median lethal concentrations of sodium arsenate and sodium perchlorate were 258.8 and 1,401.2 mg/L, respectively, and that arsenate and perchlorate generally showed a concentration-additive effect.

Descriptors: zebrafish larvae toxicity model, arsenates toxicity, chemically induced fish diseases, perchloric acid toxicity, sodium compounds toxicity, drug dose response relationship, larva, lethal dose 50.

Liu, F.J., G.P. Cobb, T.A. Anderson, Q.Q. Cheng, and C.W. Theodorakis (2006). **Uptake, accumulation and depuration of sodium perchlorate and sodium arsenate in zebrafish (*Danio rerio*).** *Chemosphere* 65(10): 1679-89. ISSN: 0045 6535.

NAL Call Number: TD172.C54

Abstract: In toxicokinetics studies, interactions between chemicals in mixtures has been

largely neglected. This study examines a mixture of perchlorate and arsenate because (1) they have the potential to co-occur in contaminated aquatic habitats, and (2) a previous study by the authors found possible toxicological interactive effects. In the present study, zebrafish *(Danio rerio*) were exposed to two concentrations of sodium perchlorate (10 and 100 mg l(-1)), sodium arsenate (1 and 10 mg l(-1)), and the mixture-sodium perchlorate+sodium arsenate (10+1 mg l(-1) and 100+10 mg l(-1) Na(2)HAsO(4)-high mixture) for 90 d. Their uptake and accumulation by zebrafish was evaluated at 10, 30, 60, and 90 d. In addition, depuration was examined at 1, 3, and 5d after cessation of the exposure. The uptake of either chemical was concentration-dependent, with significantly higher uptake at high concentrations at either exposure interval. In contrast, there was no significant difference in whole body residue between single chemicals and the corresponding mixture except for 100 mg l(-1) sodium arsenate at 90 d. However, there was increasing accumulation over time at the high concentration of either chemical alone and their mixture, and this increasing trend was more pronounced in the single chemical exposures than in the mixture. At the concentrations tested in the current study, both chemicals reduced the uptake but enhanced the depuration of the other chemical from the zebrafish. This study represents the first examination of the interaction of two anions-perchlorate and arsenate with respect to toxicokinetics.
Descriptors: zebrafish chemical toxicants model, arsenates, perchloric acid, sodium compounds and chemical water pollutants pharmacokinetics, arsenates chemistry and metabolism, drug dose response relationship, drug interactions, mathematics, metabolic detoxication, drug, perchloric acid chemistry and metabolism, sodium compounds chemistry and metabolism, chemical water pollutants analysis.

Liu, F.J., J.S. Wang, and C.W. Theodorakis (2006). **Thyrotoxicity of sodium arsenate, sodium perchlorate, and their mixture in zebrafish *Danio rerio*.** *Environmental Science and Technology* 40(10): 3429-36. ISSN: 0013 936X.
NAL Call Number: TD420.A1E5
Abstract: Both perchlorate and arsenate are environmental contaminants. Perchlorate is a definitive thyroid disruptor, and arsenic may disrupt thyroid homeostasis via multiple pathways. To evaluate the effects of sodium perchlorate and sodium arsenate on thyroid function and possible interactions between them, zebrafish (*Danio rerio*) were exposed to sodium perchlorate (10 and 100 mg/L), sodium arsenate (1 and 10 mg/L), and the mixture sodium perchlorate + sodium arsenate (10 + 1 and 100 + 10 mg/ L) for up to 90 days. At day 10, 30, 60, and 90, fish were sampled and analyzed forthyroid histopathological end points including follicular cell height, follicle size, colloid size, colloid depletion, hyperplasia, and angiogenesis. Effects on epithelial cell height (hypertrophy) were seen as early as 10 days after exposure. Perchlorate induced changes in all parameters staring at 30 days of exposure. Prolonged perchlorate exposure induced angiogenesis, a relatively new marker of thyroid disruption. Sodium arsenate was less effective than sodium perchlorate in causing thyroid histopathologies, but transient responses were seen for hypertrophy, hyperplasia, and colloid depletion (% colloid). This is the first report of arsenate-induced effects on thyroid histopathology. However, because statistically significant effects were not consistently seen in all end points, evidence for arsenate as a thyroid disruptor remains equivocal. In general, the sensitivity of the following histopathological indicators for indicating thyroid perturbations is, in descending order: follicular cell height > percent of colloid area/follicle area > colloid area/ follicular cell height > hyperplasia > angiogenesis > colloid area >follicle area = fish growth.
Descriptors: zebrafish toxicology model, arsenates toxicity, endocrine disruptors toxicity, epithelial cells drug effects, perchloric acid toxicity, sodium compounds toxicity, thyroid gland drug effects, chemical water pollutants toxicity, angiogenesis inducing agents, envi-

ronmental exposure, epithelial cells pathology, hyperplasia pathology, lethal dose 50, thyroid function tests, thyroid gland pathology, time factors.

Liu, H., H. Yu, J.P. Giesy, Y. Sun, and X. Wang (2007). **Toxicity of HC Orange No. 1 to Daphnia magna, zebrafish (*brachydanio rerio*) embryos, and goldfish (*Carassius auratus*).** *Chemosphere* 66(11): 2159-65. ISSN: 0045 6535.
NAL Call Number: TD172.C54
Abstract: HC Orange No. 1 (HCO1; 2-nitro-4'-hydroxydiphenylamine) (CAS No. 54381-08-7) is used as a color additive in hair dyes and can be released into aquatic environments in wastewater. In this paper, the effects of HCO1 on aquatic organisms were studied using a battery of toxicological tests. These included measuring immobilization of , inhibition of zebrafish embryo development, and acute lethality in zebrafish and goldfish, which are different species belonging to different trophic levels. HCO1 was toxic to all of the organisms studied. In our experiments, HCO1 remarkably restrained the mobility of *D. magna*, which may cause subsequent death. The EC50 value for restrained the mobility of *D. magna* at 48 h was 1.54 mg HCO1 l(-1). In addition, HCO1 showed toxicity in zebrafish and goldfish, where LC50 values at 96 h were 4.04 and 5.37 mg l(-1), respectively. The results also indicated that HCO1 remarkably retarded the development of zebrafish embryos, which may cause embryo abnormality and even lethality. The most sensitive toxicological endpoint in the development of the embryos was failure to hatch, which had an EC50 of 0.19 mg HCO1 l(-1). These results indicated that HCO1 is a potential teratogen to zebrafish embryos. In addition, as HCO1 concentrations increased, the outcomes of each of these toxicity tests changed in a concentration-dependent manner. Together, the results revealed that HCO1 appears to be toxic to multiple different species of aquatic organisms. The EC50 (LC50) values contain sufficient discriminatory power for risk assessment of HCO1 in aquatic environments. Based on the present results, more efficient risk assessment procedures for HCO1 will be designed in the future, integrating more flexible testing methods into the testing schemes that employ only the necessary tools for each case.
Descriptors: zebrafish, goldfish, *Daphnia magna*, azo compounds toxicity, coloring agents toxicity, drug effects, embryonic development drug effects, drug dose response relationship, lethal dose 50, motor activity drug effects, toxicity tests, China.

Liu, H.L., X.H. Liu, X.Y. Wang, X.R. Wang, and H.X. Yu (2007). **Toxicity of BPA and TBBPA to *Daphnia magna* and zebrafish *brachydanio rerio*.** *Huan Jing Ke Xue Huanjing Kexue* 28(8): 1784-7. ISSN: 0250-3301.
Abstract: Acute toxicity of BPA and TBBPA to *Daphnia magna*, zebrafish (*Brachydanio rerio*) and development effects of zebrafish embryo and zebrafish. The tested chemicals remarkably restrained the were studied, and safety assessment was also made to *Daphnia magna* mobilization of *Daphnia magna*, which may cause them death. 48 h EC50 values for *D. magna* were 8.91 and 0.69 mg x L(-1) respectively. In addition, the chemicals showed the toxicity effects on zebrafish, and 96 h LC50 values were 9.06 and 1.78 mg x L(-1) respectively. BPA and TBBPA remarkably retarded the development of zebrafish embryos, which may cause embryo abnormality and death. And the most sensitive toxicological endpoints to BPA and TBBPA were found, 72 h EC50 (malformation and embryo do not hatch) values were 2.90 and 0.14 mg x L(-1) respectively. The results showed that zebrafish embryo should be the most sensitive to the tested chemicals. The results indicated that BPA and TBBPA were the pollutants with high toxicity, in which TBBPA had the higher toxicity. BPA is a potential teratogen to zebrafish embryos, while TBBPA restrains embryo from hatch.
Descriptors: zebrafish embryo teratogen model, toxicity, BPA, TBBPA, *Daphnia magna*,

abnormalities and mortalities, pollutants.
Language of Text: Chinese.

Loschau, M. and R. Kratke (2005). **Efficacy and toxicity of self-polishing biocide-free antifouling paints**. *Environmental Pollution* 138(2): 260-267. ISSN: 0269 7491.
NAL Call Number: QH545.A1E52
Abstract: The ban on harmful substances in antifouling paints requires the development of new antifouling strategies. Alternatives should be as effective as conventional paints but of lower toxicity. In the present study two commercially available, self-polishing antifouling paints were examined in order to get information on their antifouling properties and toxicological potential. Efficacy was shown in settlement assays with the marine barnacle species Balanus amphitrite, however, efficacy was related to toxic effects observed on target and non-target organisms. Toxicity of the paint extracts was concentration-dependent and differed according to the paint and the species investigated. Toxicity could at least partially be attributed to zinc leached from the paints. Effects of a water-soluble paint were more pronounced in larvae of *B. amphitrite, Artemia salina* and in the green algae *Dunaliella tertiolecta*. Embryos of the freshwater species *Danio rerio* and *Vibrio fisheri* were more affected by a paint based on organic solvents. Copyright © 2008 CABI
Descriptors: zebrafish and Vibrio embyo toxicity model, antifouling paint and agents, cytotoxicity , larvae, nontarget effects, nontarget organisms, polluted water, water pollution, water quality, zinc, *Artemia salina*, *Balanus* , *Danio rerio*, *Dunaliella tertiolecta*, *Vibrio.*

Loucks, E. and M.J.3. Carvan (2004). **Strain-dependent effects of developmental ethanol exposure in zebrafish.** *Neurotoxicology and Teratology* 26(6): 745-55. ISSN: 0892-0362.
NAL Call Number: RA1224
Abstract: Developmental ethanol exposure from maternal consumption of alcoholic beverages and many other consumer products has been linked to developmental abnormalities in humans and animal models. The sensitivity of an individual to ethanol-induced perturbation of developmental processes is strongly influenced by genetic factors. In this study, we show that there are strain- and dose-dependent differences in sensitivity to developmental ethanol exposure in zebrafish *(Danio rerio*), suggesting that genetic variation within regulatory factors, influencing critical developmental pathways, is responsible for these differences. Embryos/larvae from genetically distinct strains of zebrafish [Ekkwill (EK), AB, and Tuebingen (TU)] were treated with different concentrations of ethanol. Embryo/larval survival, neurocranial and craniofacial skeletal development, and CNS cell death were analyzed. EK was the most resistant strain to the embryolethal effects of ethanol exposure but had the greatest increase in ethanol-induced cell death. AB survival was affected moderately, as were the neurocranial and craniofacial skeletal structures and ethanol-induced cell death. TU had the lowest survival rate but was the most resistant to alterations in neurocranial and craniofacial skeletal elements. No single strain is the most sensitive or the most resistant to any of the phenotypes examined, suggesting that alcohol influences each of these pathways independently. Further analysis of the molecular and biochemical pathways underlying the strain-dependent differences reported herein could lead to a significant advancement in our mechanistic understanding of the teratogenic effects of ethanol in humans.
Descriptors: zebrafish ethanol toxicity model, drug effects, ethanol toxicity, larva drug effects, zebrafish abnormalities, nervous system alcohol induced disorders genetics and pathology, cell death genetics, chemically induced craniofacial abnormalities and genetics, disease models, drug dose response relationship, drug resistance genetics, abnormalities, larva

growth and development, phenotype, species specificity, survival rate, variation genetics drug effects, .

Maack, G. and H. Segner (2004). **Life-stage-dependent sensitivity of zebrafish (*Danio rerio*) to estrogen exposure.** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 139(1-3): 47-55. ISSN: 1532 0456.
NAL Call Number: QP901.C6
Abstract: The aim of this study was to identify periods in zebrafish *(Danio rerio*) development when estrogen exposure has long-term consequences on reproductive capabilities at the adult stage. To this end, zebrafish were exposed to 10 ng/L ethynylestradiol (EE(2)) during three stages of gonadal differentiation: (i) the juvenile hermaphroditic stage when gonads display the morphology of an immature ovary (in our zebrafish colony this lasted from 15 to 42 days post-fertilization [dpf]), (ii) the gonad transition stage when the hermaphroditic gonad differentiates into either testes or ovary (from 43 to day 71 dpf), and (iii) the premature stage of testicular and ovarian development (from 72 to 99 dpf). The consequences of stage-specific exposure to EE(2) were assessed by determining time to first spawning, fecundity (number of eggs per female per day), fertilization success (percentage of fertilized eggs) and sex ratio of the adults. Exposure during the gonad transition period induced a delay in the onset of spawning and a significant reduction of fecundity and fertilization success, whereas exposure during the hermaphroditic stage or during the premature stage had no significant impact on the reproductive parameters of adult fish. The results from this experiment pointed to the gonad transition stage as being most susceptible to persistent effects of developmental estrogen exposure. In a second experiment, the concentration dependency of the EE(2)effects was evaluated by exposing zebrafish during the gonad transition stage (43-71 dpf) to 1.67, 3 or 10 ng EE(2)/L. Significant effects of EE(2) on adult reproduction were found with 3 and 10 ng EE(2)/L, but not with 1.67 ng/L. Histological examination of the gonads revealed that at termination of EE(2) exposure (71 dpf), all individuals in the 3 and 10 ng EE(2)/L treatment possessed ovaries. However, this feminising effect appeared to be reversible since at the adult stage (190 dpf), both fish with ovaries and with testes were found. Thus, EE(2) exposure during the gonad transition stage seems to have no persistent effect on gonad histology but on reproductive capabilities.
Descriptors: zebrafish effects of estrogen exposure, estrogens pharmacology, ethinyl estradiol pharmacology, life cycle stages, sexual development drug effects, drug dose response relationship, ovary drug effects, reproduction drug effects, sex ratio, testis drug effects.

Magalhaes, D.P., R. Armando da Cunha, J.A. Albuquerque dos Santos, D.F. Buss, and D.F. Baptista (2007). **Behavioral response of zebrafish *Danio rerio* Hamilton 1822 to sublethal stress by sodium hypochlorite: ecotoxicological assay using an image analysis biomonitoring system.** *Ecotoxicology* 16(5): 417-22. ISSN: 0963 9292.
NAL Call Number: RA565.A1E27
Abstract: We evaluated behavioral responses of zebrafish *Danio rerio* exposed to sublethal concentrations of sodium hypochlorite using an image analysis biomonitoring system (IABS). First, the limits of normal variation in swimming activity of zebrafish were determined by monitoring traveled distance of 40 control fishes using the IABS. An acute toxicity test was performed to determine the LC(50(24 h)) for *D. rerio* to NaOCl. To evaluate the toxic effects in swimming activity, 32 fishes were exposed to 40%, 30%, 20%, 10% of the LC(50)and 32 were used as control using the IABS. We considered toxic concentrations where more than 10% intervals of the treated group were below the limits of normal variation and were significantly different from the control group. Two main responses were

observed: an escape response (increased swimming activity) at 10% treated group, a gradual decrease in swimming activity from the 20% of the LC(50) on, and an avoidance response at higher concentrations. The response of the 20% treated group were considered as a NOAEL and responses of the 30% and 40% treated groups indicated significant hypoactivity (adverse effect). This behavioral biomonitoring system has proven to be a useful tool to detect sub-lethal toxicity that could be incorporated in biomonitoring protocols in Brazil.
Descriptors: zebrafish behavior drug effects, sodium hypochlorite and chemical water pollutants toxicity, environmental monitoring, lethal dose 50.

Maier, S.E., J.Y. Bradfield, and J.R. West (2004). **Time course of developmental changes in zebrafish *(danio rerio*) embryos resulting from brief exposure to alcohol.** *Birth Defects Research* 70(5): 314. ISSN: 1542 0752.
NAL Call Number: QL991.T4
Descriptors: zebrafish embryos ethanol toxicity, development, toxicology, developmental changes, alcohol.

Malek, R.L., H. Sajadi, J. Abraham, M.A. Grundy, and G.S. Gerhard (2004). **The effects of temperature reduction on gene expression and oxidative stress in skeletal muscle from adult zebrafish.** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 138(3): 363-73. ISSN: 1532 0456.
NAL Call Number: QP901.C6
Abstract: Longevity is inversely proportional to ambient temperature in ectothermic organisms such as fish. However, the mechanism by which reducing temperature over a physiological range increases life span is not known and available data are derived primarily from invertebrates. With a rodent-like longevity and abundant biological resources, the zebrafish is an ideal vertebrate ectothermic model in which to investigate this phenomenon. As an initial approach, the effects of a year-long 10 degrees C reduction in water temperature on global gene expression in tail skeletal muscle from adult zebrafish were determined using an oligonucleotide microarray representing 15,512 genes. Expression levels for approximately 600 genes were up-regulated by 1.7-fold or greater by the reduction in temperature, while a similar number of transcripts were down regulated by more than 1.7-fold. Using gene ontology (GO) classifications for molecular function, two functional groups, "oxygen and reactive oxygen species metabolism" and "response to oxidative stress," were found to be overrepresented among up-regulated genes. Transcripts levels for the genes in these two categories were increased by temperature reduction (TR). However, temperature reduction did not suppress lipid peroxidation potential, protein carbonyl content, or 8-oxoguanine level. Additional studies will be required to further delineate the role of altered gene expression and oxidative stress on the longevity-promoting effects of temperature reduction.
Descriptors: zebrafish embryos, reduced ambient temperature effects, aging physiology, gene expression regulation, skeletal muscle metabolism, oxidative stress, temperature, down regulation genetics, feeding behavior, gene expression profiling standards, oligonucleotide array sequence analysis standards, quality control, proteins genetics.

Marguerie, A., C. Buckley, and A. Fleming (2006). **Validation of an embryotoxicology screen using zebrafish**. *Toxicology Letters* 164(Sp. Iss.): S220. ISSN: 0378 4274.
NAL Call Number: RA1190.T62
Descriptors: zebrafish, toxicology, methods and techniques, embryotoxicity, toxicity, depth behavioral analysis.

Martins, J., L. Oliva Teles, and V. Vasconcelos (2007). **Assays with *Daphnia magna* and *Danio rerio* as alert systems in aquatic toxicology.** *Environment International* 33(3): 414-25. ISSN: 0160 4120.

NAL Call Number: TD169.E54

Abstract: For the evaluation and monitoring of the water quality, a series of methodologies, which have as basis an ample variety of bioindicators, may be applied. The aim of this research was to evaluate the use of ecotoxicity assays with *Daphnia magna* and *Danio rerio* as alert systems in water contaminated with toxic substances. Using two toxicity databases, the sensibility of those aquatic organisms to a wide variety of chemical products and elements and to some chemical categories was investigated. The relation between the reference dose for human oral chronic exposure (RfD) of all chemical products and the acute toxicity values for both bioindicators was also studied. Acute toxicity tests with *D. magna* respond to a larger variety of chemicals with a higher sensitivity than those with *D. rerio*. Although mammals, crustaceans and fish have different routes of exposure, target organs and toxic mechanisms, acute toxicity essays with fish and *Daphnia* may be used as an initial screening before mammal models are used.

Descriptors: zebrafish, *Daphnia* drug effects, chemical water pollutants toxicity, *Daphnia* physiology, environmental monitoring , movement drug effects, toxicity tests.

Martyniuk, C.J., E.R. Gerrie, J.T. Popesku, M. Ekker, and V.L. Trudeau (2007). **Microarray analysis in the zebrafish (*Danio rerio*) liver and telencephalon after exposure to low concentration of 17alpha-ethinylestradiol.** *Aquatic Toxicology* 84(1): 38-49. ISSN: 0166 445X.

NAL Call Number: QH541.5.W3A6

Abstract: 17alpha-ethinylestradiol (EE2) is detected in sewage effluent at concentrations that can disrupt normal reproductive function in fish. The objectives of this study were to identify novel genomic responses to EE2 exposure using microarray and real-time RT-PCR analysis in the liver and telencephalon of male zebrafish. Zebrafish were exposed to an environmentally relevant nominal concentration of 10ng/L EE2 for a 21-day period. In the liver, common biomarkers for estrogenic exposure such as vitellogenin 1 and 3 (vtg1; vtg3), estrogen receptor alpha (esr1), and apolipoprotein A1 (apoA1) mRNA were identified by microarray analysis as being differentially regulated. Real-time RT-PCR confirmed that vtg1 was induced approximately 700-fold, vtg3 was induced approximately 100-fold and esr1 was induced approximately 20-fold. As determined by microarray analysis, ATPase Na+/K+ alpha 1a.4 (atp1a1a.4) and ATPase Na+/K+ beta 1a (atp1b1a) mRNA were down-regulated in the liver. Gene ontology (GO) analysis revealed that there were common biological processes and molecular functions regulated by EE2 in both tissues (e.g. electron transport and cell communication) but there were tissue specific changes in gene categories. For example, genes involved in protein metabolism, carbohydrate metabolism were down-regulated in the liver but were induced in the telencephalon. This study demonstrates that (1) tissues exhibit different gene responses to low EE2 exposure; (2) there are pronounced genomic effects in the liver and (3) multi-tissue gene profiling is needed to improve understanding of the effects of human pharmaceuticals on aquatic organisms.

Descriptors: zebrafish endocrine disruptors, ethinyl estradiol toxicity, liver drug effects, telencephalon drug effects, chemical water pollutants toxicity, apolipoprotein A 1 biosynthesis and genetics, estrogen receptor alpha biosynthesis and genetics, gene expression profiling and regulation drug effects, liver metabolism and physiology, Na+ K+ exchanging ATPase biosynthesis and genetics, oligonucleotide array sequence analysis, messenger RNA biosynthesisand genetics, reverse transcriptase PCR, telencephalon metabolism and physiology, vitellogenins biosynthesis and genetics.

Matsui, J.I., A.L. Egana, T.R. Sponholtz, A.R. Adolph, and J.E. Dowling (2006). **Effects of ethanol on photoreceptors and visual function in developing zebrafish.** *Investigative Ophthalmology and Visual Science* 47(10): 4589-97 . ISSN: 0146-0404.

Abstract: PURPOSE: Children born to mothers who have consumed alcohol during pregnancy have an array of retinal abnormalities and visual dysfunctions. In the past, rodent systems have been used to study the teratogenic effects of ethanol on vertebrate embryonic development. The exact developmental windows in which ethanol causes specific developmental defects have been difficult to determine because rodents and other mammals develop in utero. In this study, we characterized how ethanol affects the function and development of the visual system in an ex utero embryonic system, the zebrafish. METHODS: Zebrafish embryos were raised in fish water containing various concentrations of ethanol from 2 to 5 days after fertilization. The effects of ethanol on retinal morphology were assessed by histologic and immunohistochemical analyses and those on retinal function were analyzed by optokinetic response (OKR) and electroretinography (ERG). RESULTS: Zebrafish embryos exposed to moderate and high levels of ethanol during early embryonic development had morphological abnormalities of the eye characterized by hypoplasia of the optic nerve and inhibition of photoreceptor outer segment growth. Ethanol treatment also caused an increased visual threshold as measured by the OKR. Analysis with the ERG indicated that there was a severe reduction of both the a- and b-waves, suggesting that ethanol affects the function of the photoreceptors. Indeed, low levels of ethanol that did not cause obvious morphologic changes in either the body or retina did affect both the OKR visual threshold and the a- and b-wave amplitudes. CONCLUSIONS: Ethanol affects photoreceptor function at low concentrations that do not disturb retinal morphology. Higher levels of ethanol inhibit photoreceptor development and cause hypoplasia of the optic nerve.

Descriptors: zebrafish embryology, central nervous system depressants toxicity, drug effects, ethanol toxicity, vertebrate photoreceptors drug effects, visual acuity drug effects, drug induced abnormalities, electroretinography, pathology, embryonic development drug effects, immunoenzyme techniques, optokinetic nystagmus drug effects and physiology, optic nerve abnormalities, vertebrate photoreceptors physiology, retina embryology and physiopathology, sensory thresholds.

Matz, C.J. and P.H. Krone (2007). **Cell death, stress-responsive transgene activation, and deficits in the olfactory system of larval zebrafish following cadmium exposure.** *Environmental Science and Technology* 41(14): 5143-8. ISSN: 0013 936X.

NAL Call Number: TD420.A1E5

Abstract: Cadmium (Cd) is a well-described environmental pollutant known to have adverse effects in fish, including behavioral deficits. We have previously reported the development of an in vivo system that utilizes hsp70 gene activation as a measure of acute 3 h cadmium toxicity in whole living transgenic zebrafish larvae carrying a stably integrated hsp70-enhanced green fluorescent protein (eGFP) reporter gene. Here, we report that activation of this transgene in olfactory epithelium of zebrafish larvae during 96 h sublethal Cd exposure is predictive of cadmium-induced cell death, altered histological and surface organization of the epithelium, and changes in olfactory dependent behavior. The transgene is first activated in the olfactory epithelium at concentrations below those giving rise to significant defects, but exhibits a more robust response following exposure to Cd at concentrations that begin to cause significant cell death, morphological alterations, and behavioral deficits. Further, the data show that Cd-induced olfactory deficits reported previously in juvenile and adult fish can also occur during larval stages of fish development, and that such behavioral deficits are closely associated with cell death and structural alterations in the olfactory epithe-

lium.
Descriptors: transgenic zebrafish embryos, cadmium toxicity, cell death drug effects, gene expression drug effects, olfactory pathways drug effects, transgenes, chemical water pollutants toxicity, behavior, green fluorescent HSP70 heat shock in situ nick end labeling.

McAllister, B.G. and D.E. Kime (2003). **Early life exposure to environmental levels of the aromatase inhibitor tributyltin causes masculinisation and irreversible sperm damage in zebrafish (*Danio rerio*).** *Aquatic Toxicology* 65(3): 309-16. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: To determine whether early life exposure to tributyltin (TBT), an aromatase inhibitor, impaired reproductive function in fish, *Danio rerio* were exposed to environmentally realistic levels (0.01-100 ng l(-1)) of TBT from 0 to 30, 30 to 60, and 0 to 70 days post-hatch, and the sex ratio and sperm motility of the adults examined 3-5 months after cessation of exposure. Fish exposed for 70 days to 0.1 ng l(-1) of TBT, a concentration presently below the detection limit in water, showed a male biased population which produced a high incidence of sperm lacking flagella. At 1 ng l(-1), the motility of sperm was significantly lower than that of control fish, while at 10 ng l(-1), all sperm lacked flagella and, at 100 ng l(-1), milt volume had increased. The effect of exposure on sex ratio was similar after exposure from 0 to 70 and 0 to 30 days, but even 100 ng l(-1) gave only 65% males after exposure from 30 to 60 days. Effects on sperm motility and morphology and on milt volume were less pronounced after 30 day than 70 day exposure. Our data suggest that screening for aromatase inhibiting activity and assessment of its risks in early life to human and wildlife fertility needs to be urgently addressed, and that the reproductive toxicity of TBT may presently be underestimated.
Descriptors: zebrafish aromatase inhibitor model, environmental exposure, reproduction drug effects, spermatozoa drug effects, trialkyltin compounds toxicity, chemical water pollutants toxicity, sex ratio.

Mebs, D. and S. Kauferstein (2005). **Ichthyotoxicity caused by marine cone snail venoms?** *Toxicon* 46(3): 355-356. ISSN: 0041-0101.
NAL Call Number: 391.8T66
Descriptors: zebrafish toxicity model for venom, food organisms, hypnotics, lethal effects, prey, sedatives, symptoms, toxicity tests, venom, *Conus episcopatus*, *Danio rerio*.

Meinelt, T., K.B. Burnison, A. Stuber, M. Pietrock, and C.E.W. Steinberg (2004). **Antagonistic effects of calcium and natural organic matter on the toxicity of metals and xenobiotics to fish - a synopsis**. *Fresenius Environmental Bulletin* 13(1): 47-51. ISSN: 1018 4619.
Abstract: Natural Organic Matter (NOM) is part of the biogeochemical matrix and interacts with biota. It is capable to modify the toxicity of numerous substances. NOM e.g. can increase or decrease the toxicity of metals or xenobiotics. The interactions depend on the characteristics of the NOM, the physicochemical water parameters and the characteristics of the toxic substances. We carried out investigations to detect interactions between NOM and calcium (Ca2+) on the toxicity of cadmium (Cd), malachite green (MAG), acriflavine, formalin, FMC and trichlorfon. NOM decrease the toxicity of some substances, whereas high Ca2+-concentrations increase the toxicity of MAG, acriflavine and formalin. The Ca2+-induced increase in toxicity is explained by the stimulation of Ca2+-triggered proteins (calmodulin) which causes an increased uptake of the respective substances. However, NOM also increases the toxicity of toxic substances to fish embryos, as found for trichlorfon. The advantage of the NOM detoxication at low Ca2+-conditions is reversed at high Ca2+-condi-

tions. Since NOM as well as Ca2+ modify the toxicity of substances, the therapeutical index of these substances is altered if they are used for prophylaxis and therapy of fish diseases. This should be taken into consideration when applying these chemicals to fish. Copyright © 2008 CABI

Descriptors: zebrafish embryos toxicity testing, acriflavine, cadmium, calcium, calmodulin, detoxification, embryos, formaldehyde, malachite green, organic matter, physicochemical properties, poisoning, toxicity, trichlorfon, *Danio rerio.*

Meinelt, T., O. Korner, A. Stuber, and C.E.W. Steinberg (2004). **Influence of calcium and natural organic matter on the toxicity of an organophosphorus insecticide**. *Fresenius Environmental Bulletin* 13(3b): 262-267. ISSN: 1018 4619.

Abstract: Natural organic matter (NOM) and calcium (Ca2+) are capable to alter the resorptivity and toxicity of organic chemicals in aquatic systems. We exemplarily studied the impact of NOM and Ca2+ on the toxicity of the organophosphorous insecticide trichlorfon (TCF) to early life stages of zebrafish (*Danio rerio*). Embryo and larvae were exposed to different TCF concentrations for 144 h. Combinations of different Ca2+ and NOM concentrations were used during the exposure. The results show an enhanced survival of the fish in high Ca2+ concentrations, but an increased mortality at low Ca2+ conditions. We suggest that TCF acts as a complexing agent, which is able to bind Ca2+ ions to its functional groups. There appear to be 2 potential modes of action. First, the positive charge of the TCF-Ca2+ complex avert from uptake, or, additionally, TCF is protected from breakdown to the most toxic compound dichlorvos (DDVP). If NOM is added to the test solution the toxicity is enhanced, even if Ca2+ is present in the water. This effect is significant in low Ca2+ water due to the absorption of Ca2+ ions by NOM that are not longer available for detoxification. Copyright © 2008 CABI

Descriptors: zebrafish embryo and larva, calcium, calcium ions, detoxification, dichlorvos, nontarget effects, nontarget organisms, organic matter, pesticide residues, toxicity, trichlorfon (TCF), *Danio rerio*.

Meinelt, T., M. Pietrock, K. Burnison, and C. Steinberg (2005). **Formaldehyde toxicity is altered by calcium and organic matter**. *Journal of Applied Ichthyology* 21(2): 121-124. ISSN: 0175 8659.

NAL Call Number: QL614.244

Abstract: Formaldehyde is one of the shortlist substances permitted for use in aquaculture. Toxicity of formaldehyde and therapeutical success is influenced by water parameters. However, there is a lack of information on the interaction of organic carbon and calcium (Ca2+) on the toxicity of formaldehyde. Laboratory experiments were conducted to test the combined effects of Ca2+ and natural organic matter (NOM) on formaldehyde toxicity. Zebrafish embryos were exposed to five concentrations of formaldehyde for 144 h. Juveniles exposed to high concentrations died; the embryos exposed to low concentrations were unable to hatch, particularly visible in the groups without NOM. The toxicity-reducing effects of NOM are not attributable to the presence of Ca2+. Copyright©Thomson Reuters 2009

Descriptors: zebrafish toxicity testing, toxicology, aquaculture, applied and field techniques.

Meinelt, T., M. Pietrock, A. Wienke, and F. Volker (2003). **Humic substances and the water calcium content change the toxicity of malachite green**. *Journal of Applied Ichthyology* 19(6): 380-382. ISSN: 0175 8659.

NAL Call Number: QL614.244

Abstract: Laboratory experiments were conducted to test interactive effects of calcium

(Ca2+) content and the presence of humic substance (HS) on malachite green (MAG)-induced toxicity in fish embryos and larvae by means of a semistatic 144-h-embryo-larval-test with zebrafish (*Danio rerio*). Two kinds of reconstituted water samples were used to produce the test media by mixing salts into deionized water resulting in either hard water (Ca-HS), or soft water (Ca-HS). By adding HS two additional test media were produced (Ca+HS, Ca+HS). MAG was tested in concentrations of 0.05, 0.10, 0.15, 0.20, 0.25 mg L-1. The toxicity ranking of MAG (mg L-1) to embryos based on 96-h-LC50 in the different test water samples is: Ca-HS (0.061) > Ca+HS (0.123)=Ca-HS (0.12) <more or => Ca+HS (0.134) and on 144-h-LC50 to larvae is: Ca-HS (0.038) > Ca+HS (0.06) > Ca-HS (0.077)=Ca+HS (0.077). Mortality of all the groups was significantly different (P<0.05). Increased Ca2+ concentrations did not protect zebrafish embryos and larvae from MAG-induced toxicity. At high Ca2+ conditions, the mortality of the embryos as well as of the larvae is reduced in the Ca+HS group relative to the Ca-HS group. Thus, at high Ca2+ conditions the HS does affect the MAG-induced mortality. The mechanism which causes the higher toxicity of MAG in the presence of higher Ca2+ concentrations is poorly understood. A probable explanation could be the stimulation of the calcium-binding protein calmodulin as well as the calmodulin kinase II in cell membranes in the presence of high Ca2+ concentrations. Copyright © 2008 CABI

Descriptors: zebrafish malachite green toxicity, calcium, embryos, mortality, water, *Danio rerio*.

Min, K.J., J.W. Park, and C.K. Cha (2003). **Effects of co-existence of dichlorvos and phosalone on the bioconcentration in zebrafish (*Brachydanio rerio*).** *Journal of Food Hygiene and Safety* 8(4): 251-256. ISSN: 1229 1153.

Abstract: This study was performed to investigate the effect of co-existence of dichlorvos and phosalone on the bioconcentration in zebrafish . The bioconcentration of the pesticides was reached an equilibrium more rapidly in an exposure of the binary mixture than that in an individual exposure. The BCF values and depuration rate constants for dichlorvos and phosalone in the binary mixture in the zebrafish were not significantly different from that of single pesticide. The results suggest that the effect of co-existence of pesticides on bioconcentration and depuration in zebrafish can be evaluated with single pesticide datum.

Descriptors: zebrafish pesticide toxicity, dichlorvos , phosalone, agricultural chemicals.

Language of Text: Korean.

Miranda, CL., W.G. Chung, J.L. Wang Buhler, T. Musafia Jeknic, W.M. Baird, and D.R. Buhler (2006). **Comparative in vitro metabolism of benzo[a]pyrene by recombinant zebrafish CYP1A and liver microsomes from (Sb(B-naphthoflavone-treated rainbow trout.** *Aquatic Toxicology* 80(2): 101-08. ISSN: 0166 445X.

NAL Call Number: QH541.5.W3A6

Abstract: The zebrafish *(Danio rerio*) is a sensitive non-mammalian model used for studying polycyclic aromatic hydrocarbon (PAH)-induced chemical carcinogenesis. The susceptibility of zebrafish to PAH-induced carcinogenesis may be related to the ability of the zebrafish P450s to bioactivate these procarcinogens. As a part of our overall effort to identify the various P450 enzymes that are involved in the activation and detoxification of PAHs in zebrafish, therefore, we have examined the ability of recombinant zebrafish CYP1A (zCYP1A) expressed in yeast to metabolize BaP in vitro. Comparison studies also were conducted with liver microsomes from (Sb(B-naphthoflavone (BNF)-treated rainbow trout (*Oncorhynchus mykiss*). Results demonstrated that the trout liver microsomes were almost twice as active as zCYP1A in oxidizing BaP, with Vmax values of 1.7 and 0.94 nmol/min/

nmol P450 for trout and zebrafish preparations, respectively. Like trout CYP1A1, cDNA-expressed zCYP1A was found to oxidize BaP to phenols, quinones and diols (BaP-7,8-diol and BaP-9,10-diol) in the presence of exogenous human microsomal epoxide hydrolase (hEH). BaP-7,8-diol is the precursor of the ultimate carcinogen, BaP-7,8-diol-9,10-epoxide (BaPDE). The ability of zCYP1A to bioactivate BaP was confirmed by the formation of DNA adducts when calf thymus DNA was added to the incubation mixture. BaP-DNA binding was enhanced by the addition of hEH to the incubation mixture. HPLC analysis of the [33P]-postlabeled DNA adducts showed the formation of at least four adducts mediated by both zCYP1A and trout liver microsomes, and one of these adducts co-migrated with BaPDE-dG in HPLC analysis. The addition of hEH to the incubation mixture decreased the formation of BaPDE-dG by zCYP1A and by trout liver microsomes while increasing the formation of an unidentified DNA adduct in the case of zCYP1A. zCYP1A also mediated the binding of BaP to protein, providing further evidence that this enzyme is capable of oxidizing BaP to reactive metabolites that bind to macromolecules. It thus appears that zCYP1A may play an important role in BaP-induced carcinogenesis in the zebrafish model by catalyzing the sequential formation of the ultimate diol epoxide carcinogenic metabolite of BaP.
Descriptors: zebrafish and rainbow trout microsomes carcinogenesis model, in vitro culture, benzopyrene, biotransformation, oxidation, *Danio rerio*, *Oncorhynchus mykiss*, cytochrome P450, liver microsomes, DNA adducts, DNA binding proteins genetics, proteins, metabolites, kinetics, flavones.

Moreels, D., K. Van Cauwenberghe, B. Debaere, E. Rurangwa, N. Vromant, L. Bastiaens, L. Diels, D. Springael, R. Merckx, and F. Ollevier (2006). **Long-term exposure to environmentally relevant doses of methyl-tert-butyl ether causes significant reproductive dysfunction in the zebrafish (*Danio rerio*).** *Environmental Toxicology and Chemistry* 25(9): 2388-93. ISSN: 0730 7268.
NAL Call Number: QH545A1E58
Abstract: Methyl-tert-butyl ether (MTBE), an anthropogenic chemical used as a gasoline additive, is being detected at an increasing frequency in the environment. The acute lethal concentration that kills 50% of the fish test population and the chronic effects of exposure to MTBE were investigated in the zebrafish (*Danio rerio*). Chronic exposure over three weeks to effective MTBE conceritrations as low as 0.11 mg/L induced a significant increase in the vitellogenin concentration of male fish. The impact of a chronic, eight-week exposure at effective concentrations ranging from 0.44 to 220 mg/L had no significant effect on fecundity, fertilization, or hatch rate but highly significant impacts on sperm motility. Spermatozoa of all MTBE-exposure groups showed a significantly lower straight-line velocity and lower average path velocity compared to those of the nonexposed group. These results suggest that chronic exposure to MTBE negatively affects fish sperm motility at concentrations that are environmentally relevant and several orders of magnitude lower than concentrations inducing acute effects.
Descriptors: zebrafish reproductive toxicity, methyl ethers administration and dosage, methyl ethers toxicity, reproduction drug effects, drug dose response relationship, drug administration schedule, lethal dose 50, methyl ethers metabolism, ovum drug effects, spermatozoa drug effects.

Mukhi, S., J.A. Carr, T.A. Anderson, and R. Patino (2005). **Novel biomarkers of perchlorate exposure in zebrafish.** *Environmental Toxicology and Chemistry* 24(5): 1107-15. ISSN: 0730 7268.
NAL Call Number: QH545A1E58

Abstract: Perchlorate inhibits iodide uptake by thyroid follicles and lowers thyroid hormone production. Although several effects of perchlorate on the thyroid system have been reported, the utility of these pathologies as markers of environmental perchlorate exposures has not been adequately assessed. The present study examined time-course and concentration-dependent effects of perchlorate on thyroid follicle hypertrophy, colloid depletion, and angiogenesis; alterations in whole-body thyroxine (T4) levels; and somatic growth and condition factor of subadult and adult zebrafish. Changes in the intensity of the colloidal T4 ring previously observed in zebrafish also were examined immunohistochemically. Three-month-old zebrafish were exposed to ammonium perchlorate at measured perchlorate concentrations of 0, 11, 90, 1,131, and 11,480 ppb for 12 weeks and allowed to recover in clean water for 12 weeks. At two weeks of exposure, the lowest-observed-effective concentrations (LOECs) of perchlorate that induced angiogenesis and depressed the intensity of colloidal T4 ring were 90 and 1,131 ppb, respectively; other parameters were not affected (whole-body T4 was not determined at this time). At 12 weeks of exposure, LOECs for colloid depletion, hypertrophy, angiogenesis, and colloidal T4 ring were 11,480, 1,131, 90, and 11 ppb, respectively. All changes were reversible, but residual effects on angiogenesis and colloidal T4 ring intensity were still present after 12 weeks of recovery (LOEC, 11,480 ppb). Whole-body T4 concentration, body growth (length and weight), and condition factor were not affected by perchlorate. The sensitivity and longevity of changes in colloidal T4 ring intensity and angiogenesis suggest their usefulness as novel markers of perchlorate exposure. The 12-week LOEC for colloidal T4 ring is the lowest reported for any perchlorate biomarker in aquatic vertebrates.
Descriptors: zebrafish, environmental exposure adverse effects, perchloric acid toxicity, sodium compounds toxicity, thyroid gland drug effects, chemical water pollutants toxicity, angiogenesis inducing agents metabolism, biological markers metabolism, colloids metabolism, hypertrophy metabolism, somatoform disorders metabolism, thyroid gland pathology, thyroxine metabolism, time factors.

Mukhi, S., X. Pan, G.P. Cobb, and R. Patino (2005). **Toxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine to larval zebrafish (*Danio rerio*).** *Chemosphere* 61(2): 178-85. ISSN: 0045 6535.
NAL Call Number: TD172.C54
Abstract: Hexahydro-1,3,5-trinitro-1,3,5-triazine, a cyclonitramine commonly known as RDX, is used in the production of military munitions. Contamination of soil, sediment, and ground and surface waters with RDX has been reported in different places around the world. Acute and subacute toxicities of RDX have been relatively well documented in terrestrial vertebrates, but among aquatic vertebrates the information available is limited. The objective of this study was to characterize the acute toxicity of RDX to larval zebrafish. Mortality (LC50) and incidence of vertebral column deformities (EC50) were two of the end points measured in this study. The 96-h LC50 was estimated at 22.98 and 25.64 mgl(-1) in two different tests. The estimated no-observed-effective-concentration (NOEC) values of RDX on lethality were 13.27+/-0.05 and 15.32+/-0.30 mgl(-1); and the lowest-observed-effective-concentration (LOEC) values were 16.52+/-0.05 and 19.09+/-0.23 mgl(-1) in these two tests, respectively. The 96-h EC50 for vertebral deformities on survivors from one of the acute lethality tests was estimated at 20.84 mgl(-1), with NOEC and LOEC of 9.75+/-0.34 and 12.84+/-0.34 mgl(-1), respectively. Behavioral aberrations were also noted in this acute toxicity study, including the occurrence of whirling movement and lethargic behavior. The acute effects of RDX on survival, incidence of deformities, and behavior of larval zebrafish occurred at the high end of the most frequently reported concentrations of RDX in aquatic environments. The chronic effects of RDX in aquatic vertebrates need to be determined for

an adequate assessment of the ecological risk of environmental RDX.
Descriptors: zebrafish larva, rodenticides toxicity, triazines toxicity, larva growth and development, lethal dose 50, locomotion drug effects, spine abnormalities, spine embryology.

Mukhi, S. and R. Patino (2007). **Effects of prolonged exposure to perchlorate on thyroid and reproductive function in zebrafish.** *Toxicological Sciences* 96(2): 246-54. ISSN: 1096-6080.
NAL Call Number: RA1190.F8
Abstract: The objectives of this study were to determine the effects of prolonged exposure to perchlorate on (1) thyroid status and reproductive performance of adult zebrafish (*Danio rerio*) and (2) F1 embryo survival and early larval development. Using a static-renewal procedure, mixed sex populations of adult zebrafish were exposed to 0, 10, and 100 mg/l nominal concentrations of waterborne perchlorate for 10 weeks. Thyroid histology was qualitatively assessed, and females and males were separated and further exposed to their respective treatments for six additional weeks. Eight females in each tank replicate (n = 3) were paired weekly with four males from the same respective treatment, and packed-egg (spawn) volume (PEV) was measured each of the last five weeks. At least once during weeks 14-16 of exposure, other end points measured included fertilization rate, fertilized egg diameter, hatching rate, standard length, and craniofacial development of 4-day-postfertilization larvae and thyroid hormone content of 3.5-h embryos and of exposed mothers. At 10 weeks of exposure, perchlorate at both concentrations caused thyroidal hypertrophy and colloid depletion. A marked reduction in PEV was observed toward the end of the 6-week spawning period, but fertilization and embryo hatching rates were unaffected. Fertilized egg diameter and larval length were increased by parental exposure to perchlorate. Larval head depth was unaffected but the forward protrusion of the lower jaw-associated cartilage complexes, Meckel's and ceratohyal, was decreased. Exposure to both concentrations of perchlorate inhibited whole-body thyroxine content in mothers and embryos, but triiodothyronine content was unchanged. In conclusion, prolonged exposure of adult zebrafish to perchlorate not only disrupts their thyroid endocrine system but also impairs reproduction and influences early F1 development.
Descriptors: zebrafish larva, perchloric acid toxicity, reproduction drug effects, sodium compounds toxicity, thyroid gland drug effects, analysis of variance, body weight drug effects, drug dose response relationship, drug effects, embryology, larva drug effects, growth and development, ovum drug effects, growth and development, perchloric acid chemistry, reproduction physiology, sodium compounds chemistry, thyroid gland physiology, thyroxine analysis, time factors, triiodothyronine analysis, chemical water pollutants chemistry and toxicity, metabolism.

Muncke, J. and R.I. Eggen (2006). **Vitellogenin 1 mRNA as an early molecular biomarker for endocrine disruption in developing zebrafish (*Danio rerio*).** *Environmental Toxicology and Chemistry* 25(10): 2734-41. ISSN: 0730 7268.
NAL Call Number: QH545A1E58
Abstract: Contemporary ecotoxicology is faced with the challenge of mechanistic understanding, a prerequisite for advanced risk assessment where acute toxicity is not the main issue. To achieve this, bioassay systems that are fast and biologically integrating and that detect a multitude of effects on a molecular level are needed. We present here the concept of such a novel test system that is built on the *Danio rerio* teratogenicity (DarT) assay but is extended in time and is based on testing molecular effects in the subacute toxicity range, named MolDarT. As proof of principle, we show the use of measuring vitellogenin 1 gene (vtg1) mRNA levels as a molecular marker for estrogenicity in developing zebrafish, a first

module of MolDarT. Fertilized zebrafish eggs were exposed to 100, 1,000, and 2,000 ng/L (6.75 nM) 17 alpha-ethinylestradiol (EE2), and total RNA was isolated every 24 h up to 120 h postfertilization (hpf). Abundance of vtg1 mRNA was detected using reverse transcription real-time polymerase chain reaction and normalized to beta-actin mRNA abundance. Between 48 and 120 hpf, beta-actin mRNA levels were constant, making this gene a suitable reference gene for normalization. A significant up-regulation of vtg1 expression was detected at 48 hpf for 1,000 and 2,000 ng/L EE2. At 72, 96, and 120 hpf, vtg1 was significantly induced for all EE2 concentrations. Expression of vtg1 was also measured in unexposed developing zebrafish. At 24 hpf and at all later time points, zebrafish embryos contained vtg1 transcripts. These findings show that vtg1 is regularly expressed in developing zebrafish and that it is inducible by EE2. We propose the use of vtg1 as molecular target for estrogenicity in the MolDarT.

Descriptors: zebrafish model for endocrine disruptors toxicity, ethinyl estradiol toxicity, messenger RNA genetics, vitellogenins genetics, chemical water pollutants toxicity, actins metabolism, biological markers metabolism, drug dose response relationship, kinetics, mass spectrometry, reverse transcriptase PCR.

Muncke, J., M. Junghans, and R.I.L. Eggen (2007). **Testing estrogenicity of known and novel (xeno-)estrogens in the MolDarT using developing zebrafish (*Danio rerio*)**. *Environmental Toxicology* 22(2): 185-193. ISSN: 1520 4081.

NAL Call Number: RA1221.T69

Abstract: The MolDarT is a novel short-term assay for testing mechanism-based molecular effects in developing zebrafish embryos. The objective of this study was to evaluate the inducibility of vitellogenin1 mRNA (Vtg1) by the estrogenically active compounds 17 beta -Estradiol (E2), 17 alpha -Ethinylestradiol (EE2), Nonylphenol (NP), Bisphenol A (BPA), Cyproconazol, and the suspected xeno-estrogen Atrazin in the MolDarT. Freshly fertilized zebrafish eggs were exposed semistatically for 120 h. Using reverse transcription real-time PCR, the relative abundance of Vtg1 was measured. For EE2 a dose-response relationship was established with EC50=60.7 ng/L (205 pM). Induction of Vtg1 was significant at concentrations of 84 pM EE2 (25 ng EE2/L) and above, 10 nM E2 (2.7 micro g E2/L), 100 nM E2 (27 micro g E2/L), 10 micro M BPA (2280 micro g BPA/L), and 15 micro M BPA (3420 micro g BPA/L). At NP concentrations of 0.75 micro M (165 micro g NP/L) and 1.5 micro M (330 micro g NP/L) Vtg1 was significantly down-regulated. Both atrazine and cyproconazol showed no effect on relative Vtg1 abundance. With this study we further characterize the MolDarT assay and show its applicability for effect screening of compounds. Copyright © 2008 CABI

Descriptors: zebrafish embryos, assays, atrazine, cyproconazole, ethinylestradiol, fish eggs, messenger RNA, estrogenic properties, estrogens, toxicology, vitellogenins, *Danio rerio.*

Nakari, T. (2004). **Estrogenicity of municipal effluents assessed in vivo and in vitro**. *Environmental Toxicology* 19(3): 207-215. ISSN: 1520 4081.

NAL Call Number: RA1221.T69

Abstract: The estrogenic potency of municipal sewage treatment plant effluents was studied by comparing in vitro and in vivo test methods. The egg yolk protein precursor vitellogenin was used as a biological indicator of estrogenicity. The in vitro screening technique, based on the synthesis and secretion of vitellogenin from isolated rainbow trout liver cells and performed in the presence of estrogenic effluents, produced a dose-response curve. Three weeks' exposure of juvenile rainbow trout to undiluted estrogenic effluents caused a very pronounced increase in their plasma vitellogenin concentration. The response was very spe-

cific, because no effect was observed in the control fish. Moreover, the multigeneration test of zebrafish provided clear evidence of the estrogenicity of one of the effluents. The sex ratio of the offspring generations was changed to favour females. Also, the diluted effluent was sufficient to induce vitellogenin production in the exposed zebrafish males. The effective concentrations of the effluents that were tested were lower in the in vitro screening test than in the zebrafish multigeneration test. Based on the results of this study, the in vitro screening approach seems to be as least as sensitive as the in vivo approach to mixed effluents. Thus, using the in vitro approach affords advantages of both practicality and sensitivity for an initial screening of effluents for estrogenicity, but to ascertain significant effects, using the multigeneration in vivo test is recommended. Copyright © 2008 CABI
Descriptors: zebrafish and rainbow estrogrnic effects, trout biological indicators, estrogens, sewage, sex ratio, vitellogenins.

Nakari, T. and K. Erkomaa (2003). **Effects of phytosterols on zebrafish reproduction in multigeneration test.** *Environmental Pollution* 123(2): 267-73. ISSN: 0269 7491.
NAL Call Number: QH545.A1E52
Descriptors: zebrafish phytosterol toxicity, reproductive toxicology , beta sitosterol, wood, soybeans, vitellogenesis, *Danio rerio*, water pollution.

Nash, J. (2003). **Gender bent but not in the mind of fish.** *Journal of Fish Biology* 63(Supplement A): 238-239. ISSN: 0022 1112.
NAL Call Number: QL614.J68
Descriptors: zebrafish, development, endocrine system, reproductive system, reproduction, toxicology, histology and cytology, reproductive success, spawning.

Nayak, A.S., C.R. Lage, and C.H. Kim (2007). **Effects of low concentrations of arsenic on the innate immune system of the zebrafish (*Danio rerio*).** *Toxicological Sciences* 98(1): 118-24. ISSN: 1096-6080.
NAL Call Number: RA1190.F8
Abstract: Arsenic has been associated with a multitude of human health problems; however, its impact on host resistance to infection has not been extensively researched. In vertebrates, the innate immune response is vital for potentiating the adaptive immune response. Therefore, dampening of the innate immune response results in an immunocompromised host. In this present study, effects of low concentrations of arsenic on zebrafish resistance to infection are evaluated. Exposure to 2 and 10 ppb arsenic, both considered safe levels in drinking water, resulted in a greater than 50-fold increase in viral load and at least a 17-fold increase in bacterial load in embryos. To determine the cause of this amplified pathogen load, important components of the innate immune system were analyzed. Presence of arsenic dampened the overall innate immune health of the fish as evidenced by reductions in respiratory burst activity. Viral infection, after arsenic exposure, showed decreases of up to 13- and 1.5-fold changes in interferon and Mx mRNA expression, respectively. Bacterial infection, post arsenic exposure, demonstrated at least 2.5- and 4-fold declines in interleukin-1beta and tumor necrosis factor-alpha mRNA levels, respectively. Maximum expression of these essential cytokines was also delayed upon arsenic exposure. Our data indicate that arsenic exposure, at concentrations deemed safe in drinking water, suppresses the overall innate immune function in zebrafish and present the zebrafish as a unique model for studying immunotoxicity of environmental toxicants. To our knowledge, this is the first report describing the effects of such low levels of arsenic on host resistance to infection.
Descriptors: zebrafish, arsenic toxicity, natural immunity drug effects , zebrafish immu-

nology, blood bactericidal activity drug effects, colony forming units assay, cytokines biosynthesis, complementary DNA biosynthesis, RNA biosynthesis, messenger RNA biosynthesis, respiratory burst drug effects, reverse transcriptase PCR, tumor necrosis factor alpha biosynthesis.

Notch, E.G., D.M. Miniutti, and G.D. Mayer (2007). **17alpha-Ethinylestradiol decreases expression of multiple hepatic nucleotide excision repair genes in zebrafish (*Danio rerio*).** *Aquatic Toxicology* 84(3): 301-9. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: Waterborne 17alpha-ethinylestradiol (EE(2)) alters hormone-mediated biological indicators in fish. These alterations include increased plasma vitellogenin, increased intersex individuals, decreased egg and sperm production, reduced gamete quality, and complete feminization of male fish. Together, these observations implicate aquatic estrogens in a broad range of detrimental effects on fish reproduction and fitness. In addition to impairing reproductive processes, EE(2) is also a strong promoter of hepatic tumor formation. Since many ubiquitous, aquatic hepatocarcinogens form DNA adducts that are preferentially repaired by nucleotide excision repair (NER) processes, we hypothesized that EE(2) may exert co-carcinogenic effects by reducing an organisms ability to repair DNA adducts via this mechanism. The present study used fluorescence-based quantitative RT-PCR to examine effects of environmentally relevant concentrations of the semisynthetic estrogen, EE(2), on hepatic nucleotide excision repair (NER) gene expression. Adult male and female zebrafish (*Danio rerio*) were exposed to 1ng/L, 10ng/L or 100ng/L concentrations of EE(2), or to a solvent control (0.05%, v/v ethanol), for 7 days with static water renewal every 24h. Effectiveness of EE(2) exposure in the liver was confirmed by examining hepatic expression of two estrogen-responsive biomarkers, vitellogenin-1 and cytochrome P450-1A1 (CYP1A1). Quantitative analysis confirmed that exposure to 100ng/L EE(2) caused significant decreases in transcript abundance of several hepatic NER genes in male zebrafish, including XPC (>17-fold), XPA (>7-fold), XPD (>8-fold), and XPF (>8-fold). Adult female zebrafish exhibited a four-fold decreased in XPC mRNA abundance at all exposure concentrations. Decreased mRNA abundance of NER genes was also seen to a lesser degree at lower concentrations of EE(2). Adult male zebrafish showed greater reduction of hepatic NER transcript levels than their female counterparts, which is consistent with the sexually dimorphic incidence of hepatocellular carcinoma in many species. Decreased transcript levels of NER genes have been shown to be an important epidemiological marker for increased cancer risk and decreased repair capacity in humans.
Descriptors: zebrafish, DNA repair drug effects and genetics, ethinyl estradiol toxicity, liver drug effects, chemical water pollutants toxicity, cytochrome P450 CYPA1A1 biosynthesis, cytochrome P450 CYPA1A1 genetics, DNA damage, estrogens toxicity, liver enzymology and metabolism, messenger RNA biosynthesis and genetics, reverse transcriptase PCR, sex factors, vitellogenins biosynthesis, vitellogenins genetics, .

Noury, P., O. Geffard, R. Tutundjian, and J. Garric (2006). **Non destructive in vivo measurement of ethoxyresorufin biotransformation by zebrafish prolarva: development and application.** *Environmental Toxicology* 21(4): 324-31. ISSN: 1520 4081.
NAL Call Number: RA1221.T69
Descriptors: zebrafish prolarva, environmental monitoring methods, oxazines metabolism, benzoapyrene pharmacology, biological assay methods, biotransformation, cytochrome P450 CYPA1A1 analysis, cytochrome P450 CYPA1A1 metabolism, kinetics, larva drug effects and

metabolism, water pollution, chemical analysis, proteins analysis and metabolism, beta naphthoflavone pharmacology.

Novillo, A., M. Kishida, M. Toledo, I.P. Callard, and G.V. Callard (2006). **Environmental chemicals induce a novel splice variant of the brain form of cytochrome p450 aromatase in zebrafish embryos**. *Journal of Experimental Zoology* 305A(2): 163. ISSN: 1548 8969.
NAL Call Number: QL1.J854
Descriptors: zebrafish cytochrome p450 aromatase toxicology, biochemistry and molecular biophysics, impacted pond.

Ohkimoto, K., M.Y. Liu, M. Suiko, Y. Sakakibara, and M.C. Liu (2004). **Characterization of a zebrafish estrogen-sulfating cytosolic sulfotransferase: inhibitory effects and mechanism of action of phytoestrogens.** *Chemico Biological Interactions* 147(1): 1-7. ISSN: 0009 2797.
NAL Call Number: QH345.C5
Abstract: Cytosolic sulfotransferases (STs) are generally thought to be involved in detoxification of xenobiotics, as well as homeostasis of endogenous compounds such as thyroid/steroid hormones and catecholamine hormones/neurotransmitters. We report here the identification and characterization of a zebrafish estrogen-sulfating cytosolic ST. The zebrafish ST was bacterially expressed, purified, and examined for enzymatic activities using a variety of endogenous compounds as substrates. Results showed that the enzyme displayed much higher activities toward two endogenous estrogens, estrone (E(1)) and 17beta-estradiol (E(2)), in comparison with thyroid hormones, 3,3',5-triiodothyronine (T(3)) and thyroxine (T(4)), dopamine, dihydroxyphenylalanine (Dopa), and dehydroepiandrosterone (DHEA). The kinetic parameters, K(m), and V(max), with estrogens and thyroid hormones as substrates were determined. The calculated V(max)/K(m) for E(1), E(2), T(3), and T(4) were, respectively, 31.6, 16.7, 1.5, and 0.8 nmol min(-1) mg(-1) microM(-1), indicating clearly the estrogens being preferred physiological substrates for the enzyme. The inhibitory effects of isoflavone phytoestrogens on the sulfation of E(2) by this zebrafish ST were examined. The IC(50) determined for quercetin, genistein, and daidzein were 0.7, 2.5, and 8 microM, respectively. Kinetic analyses revealed that the mechanism underlying the inhibition by these isoflavones to be of the competitive type.
Descriptors: zebrafish, cytosol enzymology, estrogens metabolism, isoflavones pharmacology, plant preparations pharmacology, sulfotransferases metabolism, cytosol metabolism, drug dose response relationship, genistein metabolism and pharmacology, glutathione transferase metabolism, isoflavones antagonists, inhibitors and metabolism, phytestrogens, plant preparations antagonists, inhibitors and metabolism, quercetin metabolism and pharmacology, recombinant fusion proteins metabolism, substrate specificity, sulfotransferases classification, genetics, isolation and purification, thyroid hormones metabolism, proteins genetics, isolation and purification.

Orn, S., H. Holbech, T.H. Madsen, L. Norrgren, and G.I. Petersen (2003). **Gonad development and vitellogenin production in zebrafish (*Danio rerio*) exposed to ethinylestradiol and methyltestosterone.** *Aquatic Toxicology* 65(4): 397-411. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: In a partial life-cycle test, the impact of 17alpha-ethinylestradiol (EE2) and 17alpha-methyltestosterone (MT) on juvenile zebrafish was evaluated by use of vitellogenin measurements and gonadal development. Exposure to EE2 (1-25 ng/l) resulted in a dose-dependent increase in vitellogenin production starting at 2 ng/l. Significant changes in sex ratios in female direction were detected at 1 ng/l, with complete sex reversal taking place

after exposure to 2 ng/l. No intersex fish were observed after exposure to EE2. Exposure to MT resulted in decreased vitellogenin concentrations. Complete sex reversal was detected in all MT concentrations used (26-1000 ng/l). A large proportion of intersex fish was observed after exposure to 1000 ng MT/l. The period of gonadal sex reversal in non-exposed zebrafish was also studied. The main morphological features of the transformation of ovaries into testis were observed 4-5 weeks after hatching.
Descriptors: juvenile zebrafish, ethinyl estradiol pharmacology, gonads drug effects, life cycle stages, methyltestosterone pharmacology, vitellogenins drug effects, growth, development and metabolism, drug dose response relationship, gonads anatomy, histology and gonads growth and development, sex ratio and reversal, gonadal, vitellogenins biosynthesis.

Orn, S., A. Svenson, T. Viktor, H. Holbech, and L. Norrgren (2006). **Male-biased sexratios and vitellogenin induction in zebrafish exposed to effluent water from a Swedish pulp mill.** *Archives of Environmental Contamination and Toxicology* 51(3): 445-51. ISSN: 0090 4341.
NAL Call Number: TD.172.A7
Abstract: Juvenile zebrafish (*Danio rerio*) were exposed to different dilutions (0, 0.67, 2.5, 10, and 50%) of effluent water from a Swedish pulp mill that previously has been reported to be androgenic to fish. Exposure was performed between days 10-38 days post-hatch. Fish were sampled for whole-body vitellogenin concentrations at day 38 post-hatch and for histological examination of gonads at day 60 post-hatch. In fish exposed to the highest concentration of pulp mill effluent, elevated concentrations of vitellogenin were measured. The androgenicity of the pulp mill water was confirmed by the increased number of males recorded at 60 days post-hatch. Image analysis of testes indicated stimulation of spennato genesis. Intersex fish were observed in all exposure groups. An androgenic activity equivalent to 5.6 ng/L dihydroxytestosterone was measured using the yeast androgen screen (YAS) assay. The present study demonstrates that both androgenic and estrogenic effects can be detected when exposing zebrafish during the juvenile period to complex mixtures of chemicals.
Descriptors: juvenile zebrafish, environmental monitoring methods, gonads drug effects, paper, vitellogenins metabolism, chemical water pollutants toxicity, gonads metabolism, sex ratio, Sweden.

Orn, S., S. Yamani, and L. Norrgren (2006). **Comparison of vitellogenin induction, sexratio, and gonad morphology between zebrafish and Japanese *medaka* after exposure to 17 alpha -ethinylestradiol and 17 beta -trenbolone**. *Archives of Environmental Contamination and Toxicology* 51(2): 237-243. ISSN: 0090 4341.
NAL Call Number: TD172.A7
Abstract: The pharmaceutical estrogen 17 alpha -ethinylestradiol (EE2) and the anabolic androgen 17 beta -trenbolone (Tb) can interfere with the endocrine and reproductive systems of fish. The potency of these chemicals in zebrafish *(Danio rerio*) and Japanese *medaka* (*Oryzias latipes*) was assessed using the core end points vitellogenin (Vtg) concentration at 38 days post-hatch and sex ratio and gonad morphology at 60 days post-hatch. Vtg concentrations were measured in fish whole-body homogenate samples using enzyme-linked immunosorbent assay. Increased Vtg concentration and feminization of fish after exposure to 10 ng/L EE2, as well as masculinization after exposure to 50 ng/L of Tb, were observed in zebrafish. Intersex was observed in medaka exposed to EE2. A decrease in Vtg production after Tb exposure (50 ng/L) was measured in both zebrafish and medaka. Analyses of gonad morphology revealed increased testicular area and sperm percentage in Tb-exposed zebrafish, whereas increased sperm percentage was measured in Tb-exposed medaka. The higher sensitivity of zebrafish compared with medaka to both EE2 and Tb was revealed in the study.

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Descriptors: zebrafish environmental estrogens models, medaka, androgens, gonads, morphology, estrogens, sex ratio, spermatozoa, steroid hormones, synthetic androgens, testes, toxicity, vitellogenins, *Danio rerio*, fishes, *Oryzias latipes.*

Ortiz Zarragoitia, M. and M.P. Cajaraville (2005). **Effects of selected xenoestrogens on liver peroxisomes, vitellogenin levels and spermatogenic cell proliferation in male zebrafish.** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 141(2): 133-44. ISSN: 1532 0456.
NAL Call Number: QP901.C6
Abstract: Environmental estrogenic compounds or xenoestrogens can mimic natural estrogens and cause a variety of adverse effects on aquatic wildlife. The purpose of the present work was to investigate if xenoestrogens are able to cause proliferation of liver peroxisomes using zebrafish (*Danio rerio*) as a model. Adult male zebrafish were exposed for 15 days to 17beta-estradiol (E2) and the xenoestrogens dibutylphthalate (DBP), methoxychlor (MXC), 4-tert-octylphenol (OP) and 17alpha-ethynylestradiol (EE2). All five tested compounds caused significant proliferation of liver peroxisomes ($p < 0.05$) as indicated by increased peroxisomal surface and numerical densities and elevated activities of the peroxisomal beta-oxidation enzyme acyl-CoA oxidase (AOX). In the case of DBP, MXC and E2, positive significant correlations between peroxisomal density parameters and AOX were found. The treatments did not produce gross alterations in testis histology, but spermatogenic cell proliferation was disturbed in E2 and EE2-treated groups and vitellogenin levels increased significantly in fish exposed to MXC, OP, EE2 and E2 with respect to controls. Furthermore, a significant correlation between vitellogenin levels and AOX activity was found for MXC, OP and EE2 treatments, suggesting that for the latter xenoestrogens early estrogenic effects are associated with liver peroxisome proliferation. No such association occurred with typical peroxisome proliferators such as DBP.
Descriptors: zebrafish environmental estrogens model, dibutyl phthalate toxicity, estradiol toxicity, ethinyl estradiol toxicity, liver drug effects, methoxychlor toxicity, peroxisomes drug effects, phenols toxicity, spermatogenesis drug effects, vitellogenins analysis, acyl Co A oxidase metabolism, cell proliferation drug effects, liver ultrastructure, proliferating cell nuclear antigen analysis.

Ortiz Zarragoitia, M., J.M. Trant, and M.P. Cajaravillet (2006). **Effects of dibutylphthalate and ethynylestradiol on liver peroxisomes, reproduction, and development of zebrafish (*Danio rerio*).** *Environmental Toxicology and Chemistry* 25(9): 2394-404. ISSN: 0730 7268.
NAL Call Number: QH545A1E58
Abstract: The aim of the present work was to study the effects of the peroxisome proliferator dibutylphthalate (DBP) and the xenoestrogen 17alpha-ethynylestradiol (EE2) on liver peroxisomes, reproduction, and development of zebrafish (*Danio rerio*). In experiment 1, newly fertilized zebrafish eggs were exposed for five weeks, covering the entire period of sexual determination, to nominal concentrations of 25 and 100 microg/L of DBP and 5 microg/L of EE2. In experiment 2, adult female zebrafish were exposed for 15 d to 100 and 500 microg/L of DBP and 5 microg/L of EE2, and afterward, they were paired with untreated males to study the effects in the resultant offspring. Ethynylestradiol provoked marked mortality (approximately 50%) and delayed development of larvae as well as sterility of adult females, possibly related to alterations in aromatase gene expression. Ethynylestradiol up-regulated vitellogenin expression in the early life stages and increased vitellogenin synthesis and accumulation in adult females. Ethynylestradiol caused liver peroxisome proliferation

in early life stages but not in adult females. Dibutylphthalate caused teratogenic effects in early life stages and mortality of the larvae obtained from exposed females. Dibutylphthalate provoked liver peroxisome proliferation and up-regulation of cytochrome P450A1 in early life stages at the end of the exposure and in adult females. Dibutylphthalate also up-regulated the expression of aromatase genes. In conclusion, the xenoestrogen EE2 caused liver peroxisome proliferation in early life stages of zebrafish, but the peroxisome proliferator DBP did not behave as a typical xenoestrogen. Overall, changes in gene expression were more marked during early life stages than in adult female zebrafish.
Descriptors: female zebrafish, dibutyl phthalate toxicity, ethinyl estradiol toxicity, liver drug effects, peroxisomes drug effects, reproduction drug effects, developmental gene expression regulation, larva drug effects, growth and development, ovum drug effects, peroxisomes metabolism, vitellogenins metabolism.

Osterauer, R. and H.R. Kohler (2008). **Temperature-dependent effects of the pesticides thiacloprid and diazinon on the embryonic development of zebrafish (*Danio rerio*)**. *Aquatic Toxicology* 86(4): 485-494. ISSN: 0166-445X.
NAL Call Number: QH541.5.W3A6
Descriptors: zebrafish embryos, developmental stages, diazinon, edema, embryogenesis, hatching, heart rate, insecticides, metabolism, mortality, pesticides, pollutants, spine, temperature effects, yolk sac, thiacloprid, *Danio rerio*.

Paris Palacios, S. and S. Biagianti Risbourg (2006). **Hepatocyte nuclear structure and subcellular distribution of copper in zebrafish *brachydanio rerio* and roach Rutilus rutilus (Teleostei, Cyprinidae) exposed to copper sulphate.** *Aquatic Toxicology* 77(3): 306-13. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: Copper is a trace element essential to life, but also a heavy metal with toxic effect clearly demonstrated. Cu induced perturbations in fish liver are well documented but the variability of the reported results is large. In this study two cyprinids, zebrafish and roach, were exposed to copper. Reported histocytological changes are either adaptative or degenerative depending on fish species, concentration of metal, and duration of exposure. Hepatic subcellular distribution of copper was determined by X-ray microanalysis in control and Cu-exposed roach and zebrafish. Sublethal copper sulphate contamination induced the development of a particular nucleolar alteration forming a network or honeycomb like structure in liver. This perturbation is observable in almost all the hepatocytes of zebrafish and roach exposed to copper for a minimum of 4 days of exposure. It seemed to concern more precisely the pars fibrosa. X-ray microanalysis showed that the appearance of network nucleolus was in relation to a Cu accumulation. Cu deposit was well located in the network as pars granulosa and nucloplasm showed very lower metal concentrations. The origin and consequence of network structure in nucleolus was discussed.
Descriptors: zebrafish , copper sulfate toxicity, cyprinidae physiology, hepatocytes drug effects, liver drug effects, cell nucleolus drug effects, cell nucleus drug effects and metabolism, cell nucleus ultrastructure, copper sulfate analysis and metabolism, electron probe microanalysis veterinary, hepatocytes metabolism and ultrastructure, liver pathology, electron transmission microscopy veterinary.

Parng, C. (2005). **In vivo zebrafish assays for toxicity testing.** *Current Opinion in Drug Discovery and Development* 8(1): 100-6. ISSN: 1367 6733.
Abstract: Toxicity, due to complications of in vivo adsorption, distribution, metabolism

and excretion (ADME), is a major cause of failure during drug development; many drugs shown to be safe in cell culture prove toxic in animal studies. Effective in vivo toxicity screening early in the development process can reduce the number of compounds that progress to laborious and costly late-stage animal testing. The transparent zebrafish provides accessibility to internal organs, tissues and even cells, and has emerged as an invaluable model organism for toxicity testing and drug discovery. Straightforward in vivo zebrafish assays can serve as an intermediate step between cell-based and mammalian testing, thus streamlining the drug development time-line.

Descriptors: zebrafish, toxicology methods, carcinogens toxicity, chemically induced cardiovascular diseases , immune system drug effects, chemically induced labyrinth diseases, neurotoxicity syndromes pathology, toxicogenetics, xenobiotics metabolism.

Parng, C., N.M. Roy, C. Ton, Y. Lin, and P. McGrath (2007). **Neurotoxicity assessment using zebrafish.** *Journal of Pharmacological and Toxicological Methods* 55(1): 103-12. ISSN: 1056-8719.

NAL Call Number: QP901.J6

Abstract: INTRODUCTION: Transparency is a unique attribute of zebrafish that permits direct assessment of drug effects on the nervous system using whole mount antibody immunostaining and histochemistry. METHODS: To assess pharmacological effects of drugs on the optic nerves, motor neurons, and dopaminergic neurons, we performed whole mount immunostaining and visualized different neuronal cell types in vivo. In addition, we assessed neuronal apoptosis, proliferation, oxidation and the integrity of the myelin sheath using TUNEL staining, immunostaining and in situ hybridization. The number of dopaminergic neurons was examined and morphometric analysis was performed to quantify the staining signals for myelin basic protein and apoptosis. RESULTS: We showed that compounds that induce neurotoxicity in humans caused similar neurotoxicity in zebrafish. For example, ethanol induced defects in optic nerves and motor neurons and affected neuronal proliferation; 6-hydroxydopamine caused neuronal oxidation and dopaminergic neuron loss; acrylamide induced demyelination; taxol, neomycin, TCDD and retinoic acid induced neuronal apoptosis. DISCUSSION: Effects of drug treatment on different neurons can easily be visually assessed and quantified in intact animals. These results support the use of zebrafish as a predictive model for assessing neurotoxicity.

Descriptors: zebrafish drug effects model, neurotoxins toxicity, toxicity tests methods, acrylamide toxicity, anthracenes toxicity, apoptosis drug effects, cell proliferation drug effects, drug effects, ethanol toxicity, glutarates toxicity, in situ hybridization, in situ nick end labeling, motor neurons drug effects, myelin sheath drug effects, neomycin toxicity, neurons cytology, neurons drug effects, optic nerve drug effects, oxidopamine toxicity, paclitaxel toxicity, tetrachlorodibenzodioxin toxicity, tretinoin toxicity.

Parng, C., C. Ton, Y.X. Lin, N.M. Roy, and P. McGrath (2006). **A zebrafish assay for identifying neuroprotectants in vivo.** *Neurotoxicology and Teratology* 28(4): 509-16. ISSN: 0892-0362.

NAL Call Number: RA1224

Abstract: In this study, we developed an in vivo method to determine drug effects on oxidation-induced apoptosis in the zebrafish brain caused by treatment with L-hydroxyglutaric acid (LGA). We confirmed that LGA-induced apoptosis was caused by oxidation by examining the presence of an oxidative product, nitrotyrosine. Next, we examined the effects of 14 characterized neuroprotectants on LGA-treated zebrafish, including: D-methionine (D-Met), Indole-3-carbinol, deferoxamine (DFO), dihydroxybenzoate (DHB), deprenyl, L-NAME (N(G)-nitro-L-arginine methyl ester), n-acetyl L-cysteine (L-NAC), 2-oxothi-

azolidine-4-carboxylate (OTC), lipoic acid, minocycline, isatin, cortisone, ascorbic acid and alpha-tocopherol. Eleven of 14 neuroprotectants and 7 of 7 synthetic anti-oxidants exhibit significant protection in zebrafish. Buthionine sulfoximine (BSO), used as a negative control, exhibited no significant protective effects. In addition, three blood-brain barrier (BBB) impermeable compounds exhibited no significant effects. Our results in zebrafish were similar to results reported in mammals supporting the utility of this in vivo method for identifying potential neuroprotective anti-oxidants.
Descriptors: zebrafish drug effects model, neuroprotective agents pharmacology, acridines toxicity, antibodies diagnostic use, antioxidants pharmacology, apoptosis drug effects, biological markers, brain cytology, brain drug effects, drug dose response relationship, preclinical drug evaluation, glutarates pharmacology, image interpretation, computer assisted, fluorescence microscopy, nitric oxide biosynthesis, oxidation reduction, tyrosine analogs and derivatives, tyrosine immunology.

Patino, R., M.R. Wainscott, E.I. Cruz Li, S. Balakrishnan, C. McMurry, V.S. Blazer, and T.A. Anderson (2003). **Effects of ammonium perchlorate on the reproductive performance and thyroid follicle histology of zebrafish.** *Environmental Toxicology and Chemistry* 22(5): 1115-21. ISSN: 0730 7268.
NAL Call Number: QH545A1E58
Abstract: Adult zebrafish were reared up to eight weeks in control water or in water containing ammonium perchlorate (AP) at measured perchlorate concentrations of 18 (environmentally relevant, high) and 677 ppm. Groups of eight females were paired with four males on a weekly basis to assess AP effects on spawned egg volume, an index of reproductive performance. All treatments were applied to four to five spawning replicates. At 677 ppm, spawn volume was reduced within one week and became negligible after four weeks. At 18 ppm, spawn volume was unaffected even after eight weeks. Also, perchlorate at 18 ppm did not affect percentage egg fertilization. Fish were collected at the end of the exposures (677 ppm, four weeks; control and 18 ppm, eight weeks) for whole-body perchlorate content and thyroid histopathological analysis. Fish perchlorate levels were about one-hundredth of those of treatment water levels, indicating that waterborne perchlorate does not accumulate in whole fish. At 677 ppm for four weeks, perchlorate caused thyroid follicle cell (nuclear) hypertrophy and angiogenesis, whereas at 18 ppm for eight weeks, its effects were more pronounced and included hypertrophy, angiogenesis, hyperplasia, and colloid depletion. In conclusion, an eight-week exposure of adult zebrafish to 18 ppm perchlorate (high environmentally relevant concentrations) affected the histological condition of their thyroid follicles but not their reproductive performance. The effect of 677 ppm perchlorate on reproduction may be due to extrathyroidal toxicity. Further research is needed to determine if AP at lower environmentally relevant concentrations also affects the thyroid follicles of zebrafish.
Descriptors: zebrafish perchloric acid toxicity model, quaternary ammonium compounds toxicity, reproduction drug effects, thyroid gland drug effects, chemical water pollutants toxicity, environmental exposure adverse effects, fertilization drug effects, ovum drug effects and physiology, thyroid gland pathology and ultrastructure, time factors.

Piersma, A.H. (2006). **Alternative methods for developmental toxicity testing**. *Basic and Clinical Pharmacology and Toxicology* 98(5): 427-431 . ISSN: 1742 7835.
Abstract: Alternatives to animal testing in developmental toxicology have been the Subject of three decades of research. The aims of these investigations have been to reduce animal experimentation, to refine effect assessment and mechanistic studies, and to accelerate and simplify safety testing in an area of toxicology that uses relatively many animals. Many

alternatives have been developed over the years with different compexities, using biologic material ranging from continuous cell lines to complete embryos. The validation of alternatives and their application in testing strategies is still in its infancy, although significant steps towards these aims are currently being made. The introduction of the genomics technology is a promising emerging area in developmental toxicity testing in vitro. Future application of alternatives in testing strategies for developmental toxicity may significantly gain from the inclusion of gene expression studies. given the unique programme of gene expression changes in embryonic and foetal development. Copyright©Thomson Reuters 2009
Descriptors: zebrafish, toxicology, development, molecular genetics, reproduction, animal experimentation, developmental toxicology, genomic technology, cell line, safety testing.

Pieterse, B., A.L. Boxtel, K. Krab, G. Marsh, P.H. Cenijn, J.H. Kamstra, M. Antink, A. Brouwer, J. Legler, B.v.d. Burg, and M.J. Wagner (2008). **Microarray analysis Reveals a Mechanism of Phenolic Polybrominated Diphenylether Toxicity in zebrafish**. *Environmental Science & Technology* 42(5): 1773-1779. ISSN: 0013-936X.
NAL Call Number: TD420.A1E5
Descriptors: adult zebrafish, polybrominated diphenylether, (pBdE), 2,2',4,4'- tetrabromodiphenylether, (BdE47), prevalent, marine organism, algae, human, fish, in vivo, toxicity, toxic.

Pistekova, V., E. Voslarova, and Z. Svobodova (2005). **Nitrite toxicity to *Danio rerio*: effects of chloride concentrations during acclimatization and in toxicity tests**. *Acta Veterinaria (Brno)* 74(3): 435-440. ISSN: 0001-7213.
NAL Call Number: SF604.B7
Descriptors: zebrafish, acclimatization, aquariums, bioaccumulation, chlorides, chlorophylls, fish (cyprinid), freshwater fish, Nitrites, ornamental fish, pollution indicators, testing procedures, toxicity, toxicity tests, water pollution effects, *Danio rerio*, Czech Republic.

Plachinta, M., T. Zhang, and D.M. Rawson (2004). **Studies on cryoprotectant toxicity to zebrafish (*Danio rerio*) oocytes.** *Cryo Letters* 25(6): 415-24. ISSN: 0143 2044.
NAL Call Number: QC278.C72
Abstract: Cryopreservation of fish germ cells is an important measure in conservation of fish genetic material. Although investigations on cryopreservation of fish sperm and embryos have been carried out extensively, cryopreservation of fish oocytes has not been studied systematically. In the present study the toxicity of cryoprotectants to zebrafish (*Danio rerio*) oocytes was investigated. Commonly used cryoprotectants dimethyl sulfoxide (DMSO), methanol, ethylene glycol (EG), propylene glycol (PG), sucrose and glucose were studied. Stage III (vitellogenic), stage IV (maturation) and stage V (mature egg) zebrafish oocytes were incubated in Hank's medium containing different concentrations of cryoprotectants (0.25-4M) for 30 min at room temperature. Three different tests were used to assess oocyte viability: trypan blue (TB) staining, thiazolyl blue (MTT) staining and in vitro maturation followed by observation of germinal vesicle breakdown (GVBD). Results showed that the toxic effect of cryoprotectant on oocytes generally increased with increasing concentration. MTT test was shown to be the least sensitive testing method and gave poor correlation to subsequent GVBD results. Sensitivity of vital tests increases in the order of MTT, TB and GVBD. GVBD test showed that cryoprotectant toxicity to stage III zebrafish oocytes increased in the order of methanol, PG, DMSO, EG, glucose and sucrose. No Observed Effect Concentrations (NOECs) for stage III oocytes were 2M, 1M, 1M, 0.5M, less than 0.25M and less than 0.25M for methanol, PG, DMSO, EG, glucose and sucrose respec-

tively. TB test also showed that the toxicity of tested cryoprotectants increased in the same order. The sensitivity of oocytes to cryoprotectants appeared to increase with development stage with stage V oocytes being the most sensitive.
Descriptors: zebrafish oocytes toxicity, cryoprotective agents toxicity, oocytes drug effects, alcohols toxicity, cell survival drug effects, cryopreservation, dimethyl sulfoxide toxicity, glucose toxicity, sucrose toxicity.

Pomati, F., C.J. Cotsapas, S. Castiglioni, E. Zuccato, and D. Calamari (2007). **Gene expression profiles in zebrafish (*Danio rerio*) liver cells exposed to a mixture of pharmaceuticals at environmentally relevant concentrations.** *Chemosphere* 70(1): 65-73. ISSN: 0045 6535.
NAL Call Number: TD172.C54
Abstract: The effects of a mixture of pharmaceuticals at environmentally relevant concentrations were studied on growth and transcriptional regulation in zebrafish liver (ZFL) cells. The mixture of 13 ingredients was assembled to mimic the association and low concentration (ng/l) of drugs as detected in the environment, and decidedly inhibited ZFL cells proliferation in vitro over a 72h exposure time. Using an oligonucleotide DNA-microarray targeting 14000 zebrafish transcripts, we profiled gene expression in ZFL cells treated with ecologically relevant levels of the drug mixture. Compared to unexposed controls, ZFL cells challenged with pharmaceuticals were characterised by transcriptional repression involving primary metabolism and regulation of the cell cycle. On the other hand, we observed upregulation of genes identifying protein kinase signalling pathways and DNA-repair mechanisms. Part of the identified transcripts could be associated with general toxicity, while others were possibly linked to the effects of specific pharmaceuticals. Drugs also caused overexpression of oestrogen receptor beta and the oestrogen responsive protein GREB1. The context drawn by our data highlights a similarity in the response to the drug mixture in fish and previously employed human cells, and further prioritise studies targeting the potential risks associated with the presence of pharmaceuticals in the environment.
Descriptors: zebrafish, gene expression, liver cells, pharmaceuticals, environmental, concentrations.

Pomati, F., C. Orlandi, M. Clerici, F. Luciani, and E. Zuccato (2008). **Effects and interactions in an environmentally relevant mixture of pharmaceuticals**. *Toxicological Sciences* 102(1): 129-137. ISSN: 1096-6080.
NAL Call Number: RA1190.F8
Descriptors: zebrafish embryos, atenolol, bezafibrate, ciprofloxacin, cytotoxicity, dose dependency, drugs, embryos, lincomycin, pharmaceuticals, risk assessment, statistical analysis, toxicity, tumor cells, *Danio rerio*, *Escherichia coli*.

Prasch, A.L., E.A. Andreasen, R.E. Peterson, and W. Heideman (2004). **Interactions between 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and hypoxia signaling pathways in zebrafish: hypoxia decreases responses to TCDD in zebrafish embryos.** *Toxicological Sciences* 78(1): 68-77. ISSN: 1096-6080.
NAL Call Number: RA1190.F8
Abstract: The aryl hydrocarbon receptor (AHR) interacts with the aryl hydrocarbon receptor nuclear translocator (ARNT) to form a heterodimer that binds to promoters in target genes to alter transcription in response to xenobiotics such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The ARNT protein also forms heterodimers with other proteins such as HIF-1alpha and HIF-2alpha to alter gene expression in response to low oxygen conditions. Because ARNT is shared between multiple signaling pathways it is possible that activation

of one ARNT-requiring pathway could inhibit the activation of other pathways that depend on ARNT. One hypothesis to explain TCDD toxicity in early life stage fish is that TCDD activation of zfAHR2 sequesters zfARNT2 away from the hypoxia signaling pathway. To test this hypothesis we measured the ability of TCDD to prevent induction of heme oxygenase by hypoxia (40% saturation), as well as the ability of hypoxia to increase the sensitivity of zebrafish to the effects of TCDD during the first week of life. As a further test of the model we examined mutant zebrafish that lack zfARNT2 for phenotypes that resemble the effects of TCDD exposure. Our results demonstrate that sequestration of zfARNT2 is not causing TCDD toxicity. TCDD did not inhibit hypoxia induction of heme oxygenase, hypoxia and TCDD exposures were not additive in causing developmental toxicity, and mutant embryos that lack zfARNT2 do not develop defects mimicking TCDD toxicity. However, our results demonstrate some level of cross talk between the two pathways in the zebrafish embryo. Hypoxia decreased TCDD induction of zfCYP1A mRNA, and decreased the potency of TCDD in causing edema. It is not clear whether this is mediated through competition for zfARNT2, or through other mechanisms.

Descriptors: zebrafish larva hypoxia, anoxia physiopathology, physiology, environmental pollutants pharmacology, signal transduction drug effects, tetrachlorodibenzodioxin pharmacology, Northern blotting, cytochrome P450 enzyme system biosynthesis and genetics, drug dose response relationship, fertilization, kinetics, larva, receptor cross talk physiology, aryl hydrocarbon receptors physiology, teratogens toxicity, water.

Prasch, A.L., W. Heideman, and R.E. Peterson (2004). **ARNT2 is not required for TCDD developmental toxicity in zebrafish.** *Toxicological Sciences* 82(1): 250-8. ISSN: 1096-6080.

NAL Call Number: RA1190.F8

Abstract: ZfAHR2 has been identified as the receptor that is essential for mediating the developmental toxicity caused by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in zebrafish. One form of zfARNT2, zfARNT2b, forms a functional heterodimer with zfAHR2 that specifically recognizes XREs in gel shift experiments and induces XRE-driven transcription in COS-7 cells treated with TCDD. However, it has not been demonstrated that zfARNT2b acts as the physiological dimerization partner for zfAHR2 to mediate TCDD toxicity in developing zebrafish. An antisense morpholino targeted against zfARNT2 (zfarnt2-MO) along with a line of mutant zebrafish lacking expression of the zfarnt2 gene have been used to test the hypothesis that zfARNT2 mediates the developmental toxicity of TCDD. Injection of the zfarnt2-MO decreased expression of the zfARNT2 protein but did not provide any protection against the formation of pericardial edema at 72 hpf. In addition, in TCDD dose response studies the zfarnt2(-/-) embryos showed no protection against three endpoints of TCDD toxicity observed at 96 hpf: pericardial edema, reduced trunk blood flow, and shortened lower jaw. Finally, immunostaining results at 96 hpf demonstrate that the zfarnt2(-/-) embryos show a similar pattern of TCDD-induced zfCYP1A expression as WT embryos. These results demonstrate that zfARNT2 is not essential for mediating TCDD developmental toxicity in zebrafish and suggest that alternate dimerization partner(s) exist for zfAHR2 in vivo.

Descriptors: zebrafish embryos TDCC toxicity, drug induced abnormalities genetics, developmental gene expression regulation drug effects, aryl hydrocarbon receptorsbiosynthesis, teratogens toxicity, tetrachlorodibenzodioxin toxicity, transcription factors biosynthesis, proteins biosynthesis, genetically modified, aryl hydrocarbon receptor nuclear translocator, drug dose response relationship, embryo drug effects, metabolism and pathology, morpholines pharmacology, oligodeoxyribonucleotides, antisense pharmacology, aryl hydrocarbon receptors genetics, toxicity tests, transcription factors genetics.

Prasch, A.L., H. Teraoka, S.A. Carney, Dong Wu, T. Hiraga, J.J. Stegeman, W. Heideman, and R.E. Peterson (2003). **Aryl hydrocarbon receptor 2 mediates 2,3,7,8-Tetrachlorodibenzo-p-dioxin developmental toxicity in zebrafish**. *Toxicological Sciences* 76(1): 138-150. ISSN: 1096 6080.

NAL Call Number: RA1190.F8

Abstract: In order to use the zebrafish *[Danio rerio]* as a model vertebrate to investigate the developmental toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), it is essential to know whether one or both forms of the zebrafish aryl hydrocarbon receptor (AHR), zfAHR1 or zfAHR2, mediate toxicity. To determine the role of zfAHR2, an antisense morpholino approach was used to knock down translation of the protein. No effect of the zfahr2 morpholino (zfahr2-MO) was seen on normal development in embryos not treated with TCDD. Injection of embryos at the 1-2 cell stage with zfahr2-MO decreased TCDD-induced transcription of zfCYP1A mRNA until 96 h post fertilization (hpf), and immuno-histochemical detection of zfCYP1A protein in embryos at 72 hpf revealed a dramatic decrease in expression. The zfahr2-MO completely protected embryos from TCDD-induced oedema and anemia and provided protection against TCDD-induced reductions in peripheral blood flow initially; however, a slight reduction in blood flow was observed at later times when the morpholino was no longer effective. Due to persistence of TCDD and decreasing effectiveness of the zfahr2-MO over time, the morpholino provided only transient protection against TCDD-induced inhibition of chondrogenesis of the lower jaw, and no protection against an effect of TCDD that was initiated late in development, blockade of swimbladder inflation. The zfahr2-MO did not protect embryos from TCDD-induced mortality but did produce a 48 h delay in its onset. Endpoints of TCDD developmental toxicity manifested in zfahr2 morphants at late stages of development, beyond 144 hpf, were clearly different from TCDD-exposed embryos injected with a control morpholino. Most strikingly, zfahr2 morphants exposed to TCDD never developed oedema. Taken together, these results demonstrate that zfAHR2 mediates several endpoints of TCDD developmental toxicity in zebrafish.
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Descriptors: zebrafish TCDD toxicity model, biochemical receptors, biological development, cytotoxicity, hydrocarbons, messenger RNA, mortality, transcription, translation, *Danio rerio*.

Raldua, D. and P.J. Babin (2007). **BLT-1, a specific inhibitor of the HDL receptor SR-BI, induces a copper-dependent phenotype during zebrafish development.** *Toxicology Letters* 175(1-3): 1-7. ISSN: 0378-4274.

NAL Call Number: RA1190.T62

Abstract: Block lipid transport-1 (BLT-1) is a small chemical widely used to inhibit the transfer of lipids between high-density lipoproteins (HDL) and cells mediated by scavenger receptor B, type 1 (SR-BI). This study demonstrated that BLT-1 induced in zebrafish (*Danio rerio*) embryos a copper-dependent phenotype with a twisted notochord, brain ventricle enlargement, and absence of melanisation, phenocopying neocuproine-treated, or calamity mutants. This finding supports an unexpected link between copper availability and SR-BI activity. The copper-chelating activity of BLT-1, revealed by its dramatic effect during embryo development, should be considered in any evaluation of the pharmacological effect of this thiosemicarbazone derivative on SR-BI activity and the potential therapeutic value of this molecule.

Descriptors: zebrafish copperchelating model, HDL, lipid, blood lipid transport , copper.

Ramirez, O.A. and F.P. Garcia (2005). **Genotoxic damage in zebra fish (*Danio rerio*) by arsenic in waters from Zimapan, Hidalgo, Mexico.** *Mutagenesis* 20(4): 291-95. ISSN: 0267-8357.
NAL Call Number: QH465.A1M873
Abstract: The induction of micronuclei in gill cells of zebra fish (Danio rerio) maintained in calcium-magnesium bicarbonated waters from a reference well and 'Zimapan 5' well, the latter with an arsenic (As) content ranging from 0.395 to 0.630 p.p.m., was studied. The specimens were studied during 180 days in three separated lots: in reference well-water (negative control), in reference water to which was added 5 mg/l As(5+) (positive control); and in water from 'Zimapan 5' well, with 65 specimens/lot. In waters an As concentration diminution was observed with time, whereas in fish there was an increase. After 30 days there was an As diminution in water from positive control of 1092.65 p.p.b. (36.42 p.p.b./day), whereas in fish it had increased to 523.81 p.p.b. (17.46 p.p.b./day). For the water from 'Zimapan 5' well, there was a diminution of 211.40 p.p.b. (7.04 p.p.b./day), and in fish there was an increase of 74.73 p.p.b. (2.49 p.p.b./day). In relation to micronucleus frequency in gill cells, at the end of 180 days in the negative control there was a spontaneous generation of 0.8 micronuclei/1000 cells, in the positive control there was a micronucleus frequency 163.5 times greater than in the negative control, whereas for the fish exposed to 'Zimapan 5' well-water the micronucleus frequency was 56.25 times greater than in the negative control. Taken together these results demonstrate the genotoxicity to Danio rerio of As in the well water.
Descriptors: zebrafish, arsenic toxicity, chemical water pollutants toxicity, well water, environmental monitoring, fresh water, micronucleus tests, mutagenicity tests, chemical water pollutants analysis, Mexico.

Ramsdell, J.S., J.A. Tiedeken, and A.F. Ramsdell (2005). **Characterization of developmental toxicity of algal toxins after microinjection of fish embryos**. *Neurotoxicology and Teratology* 27(6): 906. ISSN: 0892 0362.
NAL Call Number: RA1224
Descriptors: zebrafish embryos algal toxin effects, nervous system, toxicology, development, microinjection, hatching success, touch response, developmental toxicity, bioaccumulation potential, oviparous species, internal dose calibration.

Reimers, M.J., A.R. Flockton, and R.L. Tanguay (2004). **Ethanol and acetaldehyde mediated developmental toxicity in zebrafish.** *Neurotoxicology and Teratology* 26(6): 769-81. ISSN: 0892-0362.
NAL Call Number: RA1224
Abstract: Ethanol is a well-established developmental toxicant; however, the mechanism(s) of this toxicity remains unclear. Zebrafish are becoming an important model system for the evaluation of chemical and drug toxicity. In this study, zebrafish embryos were utilized to compare the developmental toxicity resulting from either ethanol or acetaldehyde exposure. Embryos were exposed to waterborne ethanol concentrations for various lengths of time but encompassed the earliest stages of embryogenesis. The waterborne ethanol concentration that causes 50% mortality (LC(50)) following a 45-h ethanol exposure was approximately 340 mM (1.98% v/v). A number of reproducible endpoints resulted from ethanol exposure and included pericardial edema, yolk sac edema, axial malformations, otolith defects, delayed development, and axial blistering. When the exposure period was reduced, similar signs of toxicity were produced at nearly identical ethanol concentrations. To estimate the embryonic dose following a given waterborne ethanol concentration, a kinetic alcohol dehydrogenase (ADH) assay was adapted. The average embryonic ethanol dose was calculated to be a frac-

tion of the waterborne concentration. Embryos exposed to waterborne acetaldehyde resulted in similar, but not identical, endpoints as those induced by ethanol. Embryos were however, almost three orders of magnitude more sensitive to acetaldehyde than to ethanol. Ethanol and acetaldehyde both negatively impact embryonic development; however, ethanol is more teratogenic based on teratogenic indices (TIs). These results demonstrate that the zebrafish model will provide an opportunity to further evaluate the mechanism of action of ethanol on vertebrate development.

Descriptors: zebrafish, multiple chemically induced abnormalities, acetaldehyde toxicity, drug effects, ethanol toxicity, zebrafish abnormalities, multiple pathology and multiple physiopathology abnormalities, alcohol dehydrogenase analysis and metabolism, biological assay methods, bone and bones abnormalities and drug effects, disease models, drug dose response relationship, inner ear abnormalities and drug effects, chemically induced edema, pericardium drug effects and physiopathology, survival rate, teratogens toxicity, yolk sac drug effects and physiopathology.

Reimers, M.J., J.K. La Du, C.B. Periera, J. Giovanini, and R.L. Tanguay (2006). **Ethanol-dependent toxicity in zebrafish is partially attenuated by antioxidants.** *Neurotoxicology and Teratology* 28(4): 497-508. ISSN: 0892-0362.

NAL Call Number: RA1224

Abstract: Ethanol is a well-established developmental toxicant; however, the molecular and cellular mechanism(s) of toxicity remains unclear. It has been suggested that ethanol metabolism leads to oxidative stress resulting in an increase in cell death. Alcohol developmental toxicity has not been well studied in zebrafish; however, zebrafish represent an excellent vertebrate model for investigating and understanding normal and aberrant development. To evaluate ethanol metabolism dependent toxicity, chemical inhibitors of the ethanol metabolizing enzymes were utilized. Embryos co-exposed to ethanol and a combination of ethanol metabolism inhibitors led to a significant increase in the occurrence of pericardial edema. Further, in the presence of the inhibitor mixture there was an increase in developmental malformations at lower ethanol concentrations. Cell death has been implicated as a potential explanation for ethanol-dependent toxicity. Using cell death assays, ethanol significantly increased embryonic cell death. To determine if oxidative stress underlies cardiovascular dysfunction, embryos were co-exposed to ethanol and several antioxidants. The antioxidants, glutathione and lipoic acid, partially attenuated the incidence of pericardial edema. The effectiveness of the antioxidants to protect the embryos from ethanol-induced cell death was also evaluated. The antioxidants provided no protection against cell death. Thus, ethanol-mediated pericardial edema and cell death appear to be mechanistically distinct.

Descriptors: zebrafish thanol toxicity model, antioxidants pharmacology, central nervous system depressants antagonists, inhibitors and depressants toxicity, ethanol antagonists, inhibitors and toxicity, apoptosis drug effects, cell death drug effects, drug dose response relationship, chemically induced edema, edema prevention and control embryonic development drug effects, in situ nick end labeling, oxidative stress drug effects, pericardium drug effects.

Reineke, N., S. Biselli, S. Franke, W. Francke, N. Heinzel, H. Huhnerfuss, H. Iznaguen, U. Kammann, N. Theobald, M. Vobach, and W. Wosniok (2006). **Brominated indoles and phenols in marine sediment and water extracts from the North and Baltic Seas - concentrations and effects**. *Archives of Environmental Contamination and Toxicology* 51(2): 186-196. ISSN: 0090 4341.

NAL Call Number: TD172.A7

Abstract: This work presents results from analytical as well as ecotoxicologic investigations of

sediment and water samples from the North and Baltic Seas. A bioassay-directed procedure was used to investigate cause-effect relationships between observed effects in acute laboratory bioassays (luminescent bacteria assay with *Vibrio fischeri* and embryo test with *Danio rerio*) and analyte concentrations in extracted samples. Brominated phenols and indoles - including 4-bromophenol; 2,4-dibromophenol; 4- and 6-bromoindole; 3,4-, 4,6-, and 3,6-dibromoindole; and tribrominated compounds - were identified in partly remarkable concentrations (up to 40 000 ng g-1 total organic carbon TOC for 4-bromophenol) in North Sea sediment samples and water samples (913 ng L-1 3,6-dibromoindole) from the German Bight. The toxicity of some of the identified brominated substances was low, with median effect concentration levels (EC50) ranging from 0.08 to 21.7 mg/L for *V. fischeri* and 4.3 to 46.3 mg/L for *D. rerio.* Comparison of the concentrations of analytes with ECs showed a toxicity contribution of brominated phenols and indoles to overall toxicity of the fraction. In the case of one water sample from the German Bight, brominated phenols and indoles accounted for the observed toxicity. Brominated phenols and indoles, which are assumed to be of biogenic origin, have rarely been discussed so far in the context of ecotoxicologic effects in marine ecosystems. Copyright © 2008 CABI

Descriptors: zebrafish embryos, aquatic organisms, bioassays, indoles, marine environment, marine fishes, marine sediments, organic carbon, phenols, pollutants, toxicity, water pollution, water quality, *Danio rerio*, fishes, *Vibrio fischeri.*

Reis, J.L.R., M. Dezotti, and G.L.J. Sant' Anna (2007). **Toxicity evaluation of the process effluent streams of a petrochemical industry**. *Environmental Technology* 28(2): 147-155. ISSN: 0959 3330.

Abstract: The physico-chemical characteristics and the acute toxicity of several wastewater streams, generated in the industrial production of synthetic rubber, were determined. The acute toxicity was evaluated in bioassays using different organisms: *Danio rerio* (fish), *Lactuca sativa* (lettuce) and *Brachianus calyciflorus* (rotifer). The removal of toxicity attained in the industrial wastewater treatment plant was also determined upstream and downstream of the activated sludge process. The results obtained indicate that the critical streams in terms of acute toxicity are the effluents from the liquid polymer unit and the spent caustic butadiene washing stage. The biological treatment was able to partially remove the toxicity of the industrial wastewater. However, a residual toxicity level persisted in the biotreated wastewater. The results obtained with *Lactuca sativa* showed a high degree of reproducibility, using root length or germination index as evaluation parameters. The effect of volatile pollutants on the toxicity results obtained with lettuce seeds was assessed, using ethanol as a model compound. Modifications on the assay procedure were proposed. A strong correlation between the toxic responses of Lactuca sativa and Danio rerio was observed for most industrial effluent streams. Copyright © 2008 CABI

Descriptors: zebrafish, activated sludge, bioassays, biological treatment, ethanol, factory effluents, industrial effluents, lettuces, physicochemical properties, pollutants, roots, seed germination, seeds, streams, toxicity, volatile compounds, waste water, waste water treatment, waste water treatment plants, Danio rerio, Lactuca sativa, rotifera.

Rico, E.P., D.B. Rosemberg, R.D. Dias, M.R. Bogo, and C.D. Bonan (2007). **Ethanol alters acetylcholinesterase activity and gene expression in zebrafish brain.** *Toxicology Letters* 174(1-3): 25-30. ISSN: 0378-4274.

NAL Call Number: RA1190.T62

Abstract: Alcohol abuse is a health problem throughout the world and alcohol consumption is linked to the occurrence of several pathological conditions. Acute ethanol administration

exerts a variety of actions on the central nervous system (CNS). Zebrafish has been used as an attractive model system to investigate behavioral and neurochemical changes promoted by alcohol intoxication. Here we investigated the in vitro and in vivo effects promoted by ethanol and its metabolites on zebrafish brain acetylcholinesterase (AChE). There was a significant increase of AChE (33%) activity after acute 1% ethanol exposure. However, ethanol in vitro did not alter AChE activity. Acetaldehyde, the first metabolite of alcohol metabolism, promoted a dose-dependent decrease (15%, 27.5% and 46.5%) at 0.25%, 0.5% and 1%, respectively. Acetate, a product of acetaldehyde degradation, did not change AChE activity. Furthermore, the acute ethanol exposure was able to inhibit AChE transcripts at 0.5% and 1%. These findings suggest that the alterations on zebrafish AChE could reveal molecular mechanisms related to cholinergic signaling in alcoholism.

Descriptors: zebrafish, acetylcholinesterase metabolism, brain drug effects, central nervous system depressants toxicity, ethanol toxicity, acetaldehyde toxicity, acetates toxicity, acetylcholinesterase genetics, brain enzymology, enzymologic gene expression regulation drug effects, messenger RNA metabolism, zebrafish.

Rico, E.P., D.B. Rosemberg, M.R. Senger, M.B. Arizi, G.F. Bernardi, R.D. Dias, M.R. Bogo, and C.D. Bonan (2006). **Methanol alters ecto-nucleotidases and acetylcholinesterase in zebrafish brain.** *Neurotoxicology and Teratology* 28(4): 489-96. ISSN: 0892-0362 .

NAL Call Number: RA1224

Abstract: Methanol is a neurotoxic compound that is responsible for serious damage on CNS. Besides being found as an environmental contaminant, this alcohol is also employed as a component of cryoprotector solutions for zebrafish embryos. Here we tested the acute effect of methanol on ecto-nucleotidase (NTPDase, ecto-5'-nucleotidase) and acetylcholinesterase (AChE) activities in zebrafish brain. After acute treatment, there were significant decreases on ATP (26% and 45%) and ADP hydrolysis (26% and 30%) at 0.5% and 1.0%, respectively. However, no significant alteration on ecto-5'-nucleotidase activity was verified in zebrafish brain. A significant inhibition on AChE activity (39%, 33% and 30%) was observed at the range of 0.25% to 1.0% methanol exposure. Four NTPDase sequences were identified from phylogenetic analyses, which one is similar to NTPDase1 and the others to NTPDase2. Methanol was able to inhibit NTPDase1, two isoforms of NTPDase2 and AChE transcripts. To evaluate if methanol affects directly these enzymes activities, we have performed in vitro assays. ATP hydrolysis presented a significant inhibition (19% and 34%) at 1.5% and 3.0%, respectively, and ADP hydrolysis decreased only at 3.0% (29.2%). Nevertheless, AMP hydrolysis and AChE were not altered after in vitro exposure. The inhibitory effect observed on these enzymes could contribute to the neurodegenerative events promoted by methanol in zebrafish brain.

Descriptors: zebrafish, 5' nucleotidase metabolism, acetylcholinesterase metabolism, brain enzymology, methanol pharmacology, 5' nucleotidase biosynthesis, acetylcholinesterase biosynthesis, adenosine triphosphate metabolism, brain drug effects, enzyme induction, nerve tissue proteins biosynthesis, phylogeny, reverse transcriptase PCR.

Ridel, C.D., K.S. Warren, and S.K. O'shea (2006). **Quantification of cadmium bioaccumulation in early zebrafish (*danio rerio*) embryos**. *Abstracts of Papers American Chemical Society* 231: 852. ISSN: 0065 7727.

NAL Call Number: 381Am33Pa

Descriptors: zebrafish, toxicology, development, reproductive system, cardiac arrhythmia, heart disease, chemically induced, inductively coupled plasma mass spectrometry, spectrum analysis techniques, embryonic development, bioconcentration factor, cadmium, zebrafish.

Riggio, M., S. Filosa, E. Parisi, and R. Scudiero (2003). **Changes in zinc, copper and metallothionein contents during oocyte growth and early development of the teleost *Danio rerio* (zebrafish).** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 135(2): 191-6. ISSN: 1532 0456.
NAL Call Number: QP901.C6
Abstract: In the present report, we investigated zinc, copper and metallothionein (MT) contents in zebrafish oocytes and embryos. Our results demonstrate that the metal content increases during oocytes maturation. Zinc increases from 30 ng/oocyte (stage-1 oocytes) to 100 ng/oocyte (stage-3 oocytes); copper varied from 1 ng/oocyte (stage-1 oocytes) to 3.5 ng/oocyte (stage-3 oocytes). During embryogenesis, zinc and copper contents dramatically increase after fertilisation around the 512-cells stage, then slowly decrease until the mid-gastrula stage. During oocyte growth, the changes in the MT level are proportional to metal content, whereas during embryogenesis the pattern of MT accumulation does not parallel that of the two metals. Indeed, the maternal pool of MT decreases steadily during the early stages of the development until the gastrula stage. We have examined the effect of cadmium on the expression of MT during zebrafish development. After cadmium exposure, MT content increases in embryos at the blastula stage, whereas no induction occurs in embryos at the gastrula stage. However, pre-treatment of embryos at the gastrula stage with 5-aza-2'-deoxycytidine induces MT synthesis following exposure to cadmium. These observations show that changes in metal levels are not correlated to MT content in the embryo, whereas DNA methylation is one of the factors regulating MT expression.
Descriptors: zebrafish, copper analysis, metallothionein analysis, oocytes growth and development, oocytes metabolism, zinc analysis, cadmium pharmacology, drug effects, metabolism, embryonic development, metallothionein biosynthesis.

Robu, M., M. Pickart, C. Mielke, S. Ekker, and S. Beiraghi (2004). **Study of teratogenic effects of nicotine in zebrafish.** *Birth Defects Research* 70(5): 316. ISSN: 1542 0752.
NAL Call Number: QL991.T4
Descriptors: zebrafish, biochemistry and molecular biophysics, development, toxicology, differential display, genetic techniques, microarray , genetic techniques, teratogenic effects, zebrafish.

Roex, E.W., R. Keijzers, and C.A. van Gestel (2003). **Acetylcholinesterase inhibition and increased food consumption rate in the zebrafish, *Danio rerio*, after chronic exposure to parathion.** *Aquatic Toxicology* 64(4): 451-60. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: Acetylcholinesterase (AChE) inhibition is widely regarded as a good biomarker of exposure to organophosphorus pesticides (OP). However, less is known about the relationship between AChE inhibition and consequences for growth, reproduction and survival on organisms. Acute toxicity tests with fish have shown that high percentages of AChE inhibition are needed to cause detrimental effects, but not much is known about the consequences of chronic exposure to this group of chemicals for both AChE activity and higher levels of biological organisation. In this study, zebrafish (Danio rerio) were exposed to sublethal concentrations of the OP parathion for 250 days in a flow-through system. Besides AChE activity, a variety of other parameters were measured: whole-body protein and lactate content, consumption rate, survival, growth and reproduction. AChE inhibition was correlated with exposure concentration, but not with exposure time, and was significant above 0.9 microg/l after 144 days and above 4.3 microg/l after 250 days of exposure. Both parathion and the cosolvent dimethylsulfoxide (DMSO) significantly increased food consumption

rate of the fish. Survival, growth, reproduction and lactate content were not affected, while protein concentrations showed only minor effects. These findings support the hypothesis that AChE is a very sensitive biomarker for exposure, but not accurately predict higher level adverse effects following long-term exposure to OPs.

Descriptors: zebrafish, acetylcholinesterase drug effects, cholinesterase inhibitors toxicity, eating drug effects, insecticides toxicity, parathion toxicity, body composition drug effects, reproduction drug effects, toxicity tests, chronic, zebrafish growth and development.

Rosemberg, D.B., E.P. Rico, M.R. Senger, M.B. Arizi, R.D. Dias, M.R. Bogo, and C.D. Bonan (2007). **Acute and subchronic copper treatments alter extracellular nucleotide hydrolysis in zebrafish brain membranes.** *Toxicology* 236(1-2): 132-9. ISSN: 0300-483X.

NAL Call Number: RA1190.T69

Abstract: Copper is a divalent cation with physiological importance since deficiency of copper homeostasis can cause serious neurological diseases. ATP is an important signalling molecule stored at nerve endings and its inactivation is promoted by ecto-nucleotidases. In this study, we verified the effect of acute and subchronic copper treatments on ecto-nucleotidase activities in zebrafish brain membranes. Treatment with copper sulfate (15 microg/L) during 24h inhibited ATP hydrolysis (16%), whereas ADP and AMP hydrolysis were not altered. Nevertheless, a 96-h exposure with the copper concentration mentioned above inhibited NTPDase (31% and 42% for ATP and ADP hydrolysis, respectively) and ecto-5'-nucleotidase (40%) activities. NTPDase1, NTPDase2_mg and NTPDase2_mv transcripts were decreased after copper exposures during 24 and 96 h. Subchronic copper treatment also reduced the NTPDase2_mq and ecto-5'-nucleotidase expression. In vitro assays demonstrated that NTPDase activities were reduced after copper exposure during 40 min. ATP hydrolysis was inhibited at 0.25, 0.5 and 1mM (13%, 31% and 48%, respectively) and ADP hydrolysis also had a significant decrease at these same copper concentrations (41%, 63% and 68%, respectively). In contrast to the subchronic exposure, no significant changes on ecto-5'-nucleotidase were observed after in vitro assays. Lineweaver-Burk plots suggested that both inhibitory effects on nucleotide hydrolysis may occur in a non-competitive manner. Altogether, these findings indicate that copper is able to promote distinct changes on ecto-nucleotidases after in vivo and in vitro treatments and, consequently, it could control the nucleotide and nucleoside levels, modulating the purinergic signalling.

Descriptors: zebrafish, adenosine diphosphate metabolism , adenosine triphosphatases antagonists and inhibitors, adenosine triphosphate metabolism, brain drug effects, copper toxicity, adenosine monophosphate metabolism, adenosine triphosphatases genetics, adenosine triphosphatases metabolism, brain enzymology, brain metabolism, enzymologic gene expression regulation drug effects, hydrolysis, membranes drug effects, membranes enzymology, membranes metabolism, messenger RNA metabolism, zebrafish.

Rubinstein, A.L. (2006). **Zebrafish assays for drug toxicity screening.** *Expert Opinion on Drug Metabolism and Toxicology* 2(2): 231-40. ISSN: 1742 5255.

Abstract: Zebrafish are vertebrate organisms that are of growing interest for preclinical drug discovery applications. Zebrafish embryos develop most of the major organ systems present in mammals, including the cardiovascular, nervous and digestive systems, in < 1 week. Additional characteristics that make them advantageous for compound screening are their small size, transparency and ability to absorb compounds through the water. Furthermore, gene function analysis with antisense technology is now routine procedure. Thus, it is relatively simple to assess whether compounds or gene knockdowns cause toxic effects in zebrafish. Assays are being developed to exploit the unique characteristics of zebrafish for pharmaco-

logical toxicology. This review discusses assays that may be used to assess in vivo toxicity and provides examples of compounds known to be toxic to humans that have been demonstrated to function similarly in zebrafish.

Descriptors: zebrafish, preclinical drug evaluation methods, models, toxicity tests methods, genetically modified, preclinical drug evaluation trends, forecasting, toxicity tests trends , zebrafish growth and development.

Santos, E.M., G.C. Paull, K.J. Van Look, V.L. Workman, W.V. Holt, R. van Aerle, P. Kille, and C.R. Tyler (2007). **Gonadal transcriptome responses and physiological consequences of exposure to oestrogen in breeding zebrafish (*Danio rerio*).** *Aquatic Toxicology* 83(2): 134-42. ISSN: 0166 445X.

NAL Call Number: QH541.5.W3A6

Abstract: Environmental oestrogens are widespread in the aquatic environment and cause alterations in sexual development and function in vertebrates. The molecular pathways underpinning these effects, however, remain poorly understood. In this study, we aimed at generating a mechanistic understanding of the disruptive effects of exposure to environmentally relevant concentrations of 17 alpha-ethinyloestradiol (EE(2)) on reproduction in zebrafish, by anchoring the transcriptomic alterations induced with the physiological consequences of exposure. Breeding colonies of zebrafish were exposed for a 21-day period to three concentrations of EE(2) (0.05, 0.5 and 5 ng/L) and the gonadal transcriptomic alterations induced (determined using a 17,000 oligonucleotide microarray) were analysed together with physiological effects seen on reproductive output of both males and females. Exposure to 5 ng EE(2)/L resulted in reproductive impairment characterised by a decrease in egg production, alterations in sperm quality and reduced fertilisation success. The effects seen were associated with altered expression of 114 and 131 genes in the gonads of males and females, respectively. The biological processes most affected by the exposure were protein metabolism in males and mitochondria organisation and biogenesis in females. Genes involved in the regulation of cell cycle progression, the ubiquitin system and glutathione peroxidase were affected by the EE(2) exposure and associated with the changes observed in gamete quality in both genders. In summary, we demonstrated that EE(2) exposure compromised the reproductive health of breeding zebrafish at environmentally relevant concentrations. The molecular mechanisms mediating some of these effects were identified and included those impacting processes central to gametogenesis in both males and females.

Descriptors: zebrafish, ethinyl estradiol toxicity, gene expression drug effects, gonads drug effects, chemical water pollutants toxicity, cluster analysis , environmental exposure, fertilization drug effects, genes drug effects, genes physiology, oogenesis drug effects, phenotype, principal component analysis, spermatozoa drug effects, water analysis.

Santos, M.M., J. Micael, A.P. Carvalho, R. Morabito, P. Booy, P. Massanisso, M. Lamoree, and M.A. Reis Henriques (2006). **Estrogens counteract the masculinizing effect of tributyltin in zebrafish**. *Comparative Biochemistry and Physiology C, Toxicology and Pharmacology* 142(1/2): 151-155. ISSN: 1532 0456.

NAL Call Number: QP901.C6

Abstract: Recently, it has been demonstrated that the biocide tributyltin (TBT) can interfere with fish sex differentiation, leading to a bias of sex toward males. On the contrary, it is well known that estrogenic compounds can induce fish feminization. Yet, the combined effects of mixtures of androgenic and estrogenic compounds on fish sex differentiation have never been investigated before, even though in the environment animals are frequently exposed to both groups of xenobiotics. Therefore, in order to investigate whether exposure to estrogenic com-

pounds can block the masculinizing effect of TBT, 5 days post-fertilization zebrafish (Danio rerio) larvae were exposed for a four month period to TBT and to the synthetic estrogen-ethinylestradiol (EE2). The fish were fed a diet containing TBT at nominal concentrations of 25 and 100 ng TBT/g, and two groups of animals were also dosed with TBT plus EE2 at nominal water concentration of 3.5 ng/L, using a flow-through design. As expected, fish exposed to TBT showed a bias of sex toward males (62.5% males in control tanks and 86% and 82% in TBT 25 and TBT 100 ng TBT/g, respectively). Co-exposure to EE2 completely blocked the masculinizing effect of TBT, with 7% males in the TBT 25 ng/g+EE2 treatment and 0% in the EE2 alone and in the TBT 100 ng/g+EE2 exposed groups. These results clearly indicate that EE2, at environmentally relevant concentrations, can block the TBT masculinizing effects in zebrafish, which suggests that in the aquatic environment the presence of estrogens may neutralize the fish masculinizing effect of TBT. Our findings highlight the need of testing the combined effects of contaminants, as single exposure studies may not be sufficient to predict the effects of mixtures of xenobiotics with antagonistic properties. Copyright © 2008 CABI
Descriptors: zebrafish, antifouling agents, biocides, nontarget effects, nontarget organisms, estrogens, sex differentiation, Danio rerio, zebrafish.

Sawant, M.S., S. Zhang, and Q. Wang (2005). **Effects of base analogues 5-bromouracil and 6-aminopurine on development of zebrafish *danio rerio*.** *Chinese Journal of Oceanology and Limnology* 23(1): 54-59. ISSN: 0254 4059.
Abstract: Zebrafish *(Danio rerio*) genetic screens allow isolation of a wide array of problems in vertebrate biology. The effects of base analogues 5-bromouracil and 6-aminopurine on the development of zebrafish embryos are reported for the first time in this study. The early development of the zebrafish embryos was little affected by 5-bromouracil and 6-aminopurine, while the late development (organogenesis) was significantly impaired. Embryos exposed to 5-bromouracil mainly showed curled tail, wavy body, golden pigmentation and the mouth with protruding lower jaw. 6-aminopurine-treated embryos had defective anterior structures, curled tails and wavy body. RAPD analysis showed that the majority of 5-bromouracil- and 6-aminopurine-treated larvae and fish shared banding patterns in common with the control, suggesting that most mutagenesis induced by these agents are point mutations. However, some fish derived from 5-bromouracil-treated embryos had golden (gol) pigmentation; and RAPID analysis revealed that their band patterns differed from those of the control. Possibly, 5-bromouracil can occasionally cause relatively extensive changes in the fish genome. The results of this study may provide valuable help for detailed studies of mutagenesis. Copyright©Thomson Reuters 2009
Descriptors: zebrafish embryos, development, toxicology, curled tail, genetic disease, chemically induced toxicity, defective anterior structure, golden pigmentation, congenital disease, protruding lower jaw, wavy body, rapd analysis, random amplified polymorphic DNA analysis, genetic techniques, late development, mutagenesis, organogenesis.

Scalzo, F.M. and E.D. Levin (2004). **The use of zebrafish (*Danio rerio*) as a model system in neurobehavioral toxicology.** *Neurotoxicology and Teratology* 26(6): 707-8. ISSN: 0892-0362.
NAL Call Number: RA1224
Descriptors: zebrafish neurobehavioral toxicology model, central nervous system drug effects , disease models, toxicology standards, behavior drug effects, behavior physiology, central nervous system growth, development and physiology, neurotoxins toxicity, toxicology trends, .

Scalzo, F.M. and P.S. Page-McCaw (2005). **Neurobehavioral effects of stressors in embryonic zebrafish**. *Neurotoxicology and Teratology* 27(6): 906. ISSN: 0892 0362.
NAL Call Number: RA1224
Descriptors: zebrafish embryos, nervous system, neural coordination, toxicology, development, model, mathematical and computer techniques, stress response, neurobehavioral effects, neurotoxic effect.

Schafers, C., M. Teigeler, A. Wenzel, G. Maack, M. Fenske, and H. Segner (2007). **Concentration- and time-dependent effects of the synthetic estrogen, 17alpha-ethinylestradiol, on reproductive capabilities of the zebrafish, *Danio rerio*.** *Journal of Toxicology and Environmental Health. Part A* 70(9): 768-79. ISSN: 1528-7394.
Abstract: Partial or full life-cycle tests are needed to assess the potential of endocrine-disrupting compounds (EDCs) to adversely affect development and reproduction of fish. Small fish species such as zebrafish, *Danio rerio*, are under consideration as model organisms for appropriate test protocols. The present study examines how reproductive effects resulting from exposure of zebrafish to the synthetic estrogen 17alpha-ethinylestradiol (EE2) vary with concentration (0.05 to 10 ng EE2 L(-1), nominal), and with timing/duration of exposure (partial life-cycle, full life-cycle, and two-generation exposure). Partial life-cycle exposure of the parental (F1) generation until completion of gonad differentiation (0-75 d postfertilization, dpf) impaired juvenile growth, time to sexual maturity, adult fecundity (egg production/female/day), and adult fertilization success at 1.1 ng EE2 L(-1) and higher. Lifelong exposure of the F1 generation until 177 dpf resulted in lowest observed effect concentrations (LOECs) for time to sexual maturity, fecundity, and fertilization success identical to those of the developmental test (0-75 dpf), but the slope of the concentration-response curve was steeper. Reproduction of zebrafish was completely inhibited at 9.3 ng EE2 L(-1), and this was essentially irreversible as a 3-mo depuration restored fertilization success to only a very low rate. Accordingly, elevated endogenous vitellogenin (VTG) synthesis and degenerative changes in gonad morphology persisted in depurated zebrafish. Full life-cycle exposure of the filial (F2) generation until 162 dpf impaired growth, delayed onset of spawning and reduced fecundity and fertilization success at 2.0 ng EE2 L(-1). In conclusion, results show that the impact of estrogenic agents on zebrafish sexual development and reproductive functions as well as the reversibility of effects, varies with exposure concentration (reversibility at < or = 1.1 ng EE2 L(-1) and irreversibility at 9.3 ng EE2 L(-1)), and between partial and full life-cycle exposure (exposure to 10 ng EE2 L(-1) during critical period exerted no permanent effect on sexual differentiation, but life-cycle exposure did).
Descriptors: zebrafish endocrine disruptors model, ethinyl estradiol administration and dosage, growth drug effects, reproduction drug effects, biometry, environmental exposure adverse effects and analysis, ethinyl estradiol toxicity, fertilization drug effects, ovary drug effects and pathology, sexual development drug effects, zebrafish anatomy and histology.

Scheerbaum, D. and U. Noack (2003). **Sensitivity of the zebra fish embryo test to industrial effluents compared to the golden orfe toxicity test**. *Fresenius Environmental Bulletin* 12(6): 663-664. ISSN: 1018-4619.
Descriptors: zebrafish, acute toxicity, bioassays, ecotoxicology, effluents, embryonic growth stage, embryos, fish, freshwater fish, industrial wastewater, industrial effluents, industrial wastes, lethal effects, monitoring, sublethal effects, testing procedures, toxicity testing, toxicity tests, wastewater disposal, water pollution effects, *Danio rerio*, *Leuciscus idus*, comparative studies, Ide, Orfe.

Schmale, M.C. (2004). **Toxicology and Pharmacology**. *Comparative Biochemistry and Physiology. Part C:* 138(3): 229-409. ISSN: 1532 0456.
NAL Call Number: QP901.C6
Abstract: This issue of the Comparative Biochemistry and Physiology journal contains 18 articles presented in a conference on Aquatic Animal Models of Human Disease held from September 29 to October 2, 2003 at the American Type Culture Collection in Manassas, Virginia, USA. It represents the use of different aquatic animal species and approaches to study human diseases. Of the 18 papers presented, 7 focuses on the use of the zebrafish as a model system. Copyright © 2008 CABI
Descriptors: zebrafish as a research model, aquatic animals, human diseases, *Danio rerio*, humans.

Schreurs, R.H., J. Legler, E. Artola Garicano, T.L. Sinnige, P.H. Lanser, W. Seinen, and B. Van der Burg (2004). **In vitro and in vivo antiestrogenic effects of polycyclic musks in zebrafish.** *Environmental Science and Technology* 38(4): 997-1002. ISSN: 0013 936X.
NAL Call Number: TD420.A1E5
Abstract: The polycyclic musks 6-acetyl-1,1,2,4,4,7-hexamethyltetraline (AHTN) and 1,2,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-2-benzopyran (HHCB) are used as fragrance ingredients in perfumes, soaps, and household cleaning products. They are known to be ubiquitously present in the aquatic environment, and because of their lipophilic nature, they tend to bioaccumulate in aquatic biota. In surface waters, concentrations between 1 ng/L and 5 microg/L have been found, depending mainly on the proportion of sewage effluents in the water. In fish, under normal environmental conditions, concentrations in the microgram per kilogram fresh weight (fw) range are found. In a previous study we showed that AHTN and HHCB exert mainly antiestrogenic effects on the human estrogen receptor alpha (ERalpha) and ERbeta in an in vitro reporter gene assay. In the current study, we assessed the in vitro antiestrogenic effects of both musks on zebrafish ERs. Antagonism was observed on zfERbeta, and more pronounced on the newly cloned zfERgamma. Using a transgenic zebrafish assay, we studied antiestrogenicity of the musks in vivo. Dose-dependent antagonistic effects were observed at concentrations of 0.1 and 1 microM AHTN and HHCB. GC-MS analysis showed that the musks bioaccumulated in the fish, with internal concentrations (15-150 mg/kg fw) which were roughly 600 times higher than the nominal test doses. To our knowledge, this is the first time that environmental contaminants are shown to be antiestrogenic in an in vivo fish assay that focuses solely on ER-mediated effects. This makes the transgenic zebrafish assay a promising tool for the rapid detection of both estrogenic and antiestrogenic effects of chemicals in fish.
Descriptors: zebrafish polycyclic musk effects, benzopyrans pharmacokinetics and toxicity, estrogen receptor modulators toxicity and drug effects, tetrahydronaphthalenes toxicity, chemical water pollutants toxicity, genetically modified, body weight, cell culture techniques, drug dose response relationship, endocrine system drug effects, perfume, tetrahydronaphthalenes pharmacokinetics, chemical water pollutants pharmacokinetics, .

Schreurs, R.H.M.M., E. Sonneveld, J.H.J. Jansen, W. Seinen, and B. Van der Burg (2005). **Interaction of polycyclic musks and UV filters with the estrogen receptor (ER), androgen receptor (AR), and progesterone receptor (PR) in reporter gene Bioassays**. *Toxicological Sciences* 83(2): 264-272. ISSN: 1096-6080.
NAL Call Number: RA1190.F8
Descriptors: zebrafish, androgen receptors, antagonists, aromatic hydrocarbons, bioassays, endocrinology, estrogen receptors, filters, fragrances, genes, odor, progesterone receptors,

receptors, reporter gene, sex hormones, ultraviolet radiation, xenobiotics, camphor, *Danio rerio.*

Seiler, T.B., A.C. Rastall, E. Leist, L. Erdinger, T. Braunbeck, and H. Hollert (2006). **Membrane Dialysis Extraction (MDE): a novel approach for extracting toxicologically relevant hydrophobic organic compounds from soils and sediments for assessment in biotests**. *Journal of Soils and Sediments* 6(1): 20-29. ISSN: 1439 0108.

NAL Call Number: S622

Abstract: Organic solvents are routinely used to extract toxicants from polluted soils and sediments prior to chemical analysis or bioassay. Conventional extraction methods often require the use of heated organic solvents, in some cases under high pressure. These conditions can result in loss of volatile compounds from the sample and the degradation of thermally labile target analytes. Moreover, extracts of soils and sediments also frequently contain substantial quantities of organic macromolecules which can act as sorbing phases for target analytes and in doing so interfere with both chemical analysis and bioassays. Membrane dialysis extraction (MDE) is described as a simple, passive extraction method for selectively extracting toxicologically relevant hydrophobic organic compounds (HOCs) from polluted soils and sediments, and was analysed for its applicability in ecotoxicological investigations. Toxicologically relevant hydrophobic organic compounds were extracted from wet and dry sediments obtained from the Upper Rhine at Iffezheim (Germany) by sealing replicate samples in individual lengths of pre-cleaned low-density polyethylene (LD-PE) tubing and then dialysing in n-hexane. The efficacy of the MDE method in ecotoxicological investigations was assessed by testing the concentrated extracts in the neutral red assay for acute cytotoxicity, in the EROD assay for the presence of dioxin-like compounds and in the Danio rerio fish egg assay for embryotoxic and teratogenic effects. The sediment sample condition (with or without water content), length of dialysis membrane and duration of dialysis were analysed with respect to their impact on 3 endpoints. The results of the MDE investigations were compared to the data obtained in samples prepared using conventional Soxhlet extraction. The membrane dialysis extraction was found to be at least as efficient as the Soxhlet methodology to extract toxicologically relevant HOCs from sediment samples. In most cases, MDE-derived extracts showed a higher toxicological potential than the Soxhlet extracts. The lack of any significant effects in any of the MDE controls indicated these differences were not caused by contamination of the LD-PE membrane used. The elevated toxicological potential of MDE extracts is most likely the result of enhanced bioavailability of toxic compounds in consequence of lower amounts of organic macromolecules (i.e. sorbing phases) in the MDE extracts. This effect is probably the result of a size-selective restriction by the LD-PE membrane. The MDE method was found to be a simple, efficient and cost-effective method for the extraction of sediment samples. It can be used to extract toxicologically relevant hydrophobic organic compounds from both wet and dry sediments without the risk of loosing volatile and thermally labile target analytes. The size-selectivity of the LD-PE membrane also appears to have the capacity to increase the bioavailability of potential target analytes in the resulting extracts by retaining much of the organic macromolecules present in the sample. Thus, results suggest that MDE may be particularly useful for the extraction of toxicologically relevant hydrophobic organic compounds from soils and sediments for bioassays and other ecotoxicological investigations. Further validation of MDE has been initiated and the applicability of the methodology to other sample types will be investigated. Of particular interest is the potential application of MDE in the recovery of hydrophobic target analytes from biological samples such as muscles, other soft tissues and blood. Copyright © 2008 CABI

Descriptors: zebrafish, bioassays, contaminants, cytotoxicity, dialysis, dioxins, extraction, extracts, fish eggs, membranes, methodology, organic compounds, polluted soils, sediment, soil pollution, soil toxicity, soil types, teratogens, toxic substances, toxicology, *Danio rerio.*

Seki, M., S. Fujishima, T. Nozaka, M. Maeda, and K. Kobayashi (2006). **Comparison of response to 17 beta -estradiol and 17 beta -trenbolone among three small fish species**. *Environmental Toxicology and Chemistry* 25(10): 2742-2752. ISSN: 0730-7268.
NAL Call Number: QH545A1E58
Descriptors: zebrafish, fathead minnow, medaka, 17 beta -estradiol, androgens, assay, blood, environmental factors, estrogens, exposure, fathead minnows, freshwater fish, geochemistry, gonadosomatic index, liver, morphology, secondary sexual characters, sex hormones, toxicology, vitellogenin, water pollution effects, estrogenic activity, *Danio rerio*, *Oryzias latipes*, *Pimephales promelas*.

Senger, M.R., E.P. Rico, M.d.B. Arizi, D.B. Rosemberg, R.D. Dias, M.R. Bogo, and C.D. Bonan (2005). **Carbofuran and malathion inhibit nucleotide hydrolysis in zebrafish (*Danio rerio*) brain membranes**. *Toxicology* 212(2/3): 107-115. ISSN: 0300 483X.
NAL Call Number: RA1190.T69
Abstract: Carbofuran and malathion are broad spectrum pesticides widely used in agricultural practice throughout the world. Toxicity of these pesticides has been correlated with their inhibitory effects on acetylcholinesterase activity. Nucleotides are extracellular signaling molecules, which trigger multiple biological effects. Studies have demonstrated the co-transmission of acetylcholine and ATP at the nerve endings. The control of neurotransmitter ATP levels is promoted by enzymes named ectonucleotidases, which include nucleoside triphosphate diphosphohydrolase (NTPDase) family and ecto-5'-nucleotidase. Since acetylcholine and ATP are co-released at the synapse and the acetylcholinesterase inhibition is an important target for pesticide action, here we verified the effect of exposure in vitro and in vivo to carbofuran and malathion on ectonucleotidase activities from brain membranes of zebrafish. To verify if carbofuran and malathion have a direct inhibitory effect on NTPDase and 5'-nucleotidase activities in brain membranes of zebrafish, we have tested in vitro concentrations of pesticides varying from 0.25 to 5 mM. Carbofuran, in vitro, inhibited ATP and ADP hydrolysis in an uncompetitive manner, but no effect was observed on AMP hydrolysis. Malathion decreased ATP and ADP hydrolysis in competitive and an uncompetitive manner, respectively, but not altered AMP hydrolysis. After exposure to carbofuran (50 and 500 micro g/L) during 7 days, ADP hydrolysis was significantly decreased in both concentrations tested (by 19 and 24.5%, respectively). Malathion, at 500 micro g/L, was able to inhibit ADP and AMP hydrolysis (by 28 and 58.5%, respectively). This study has shown that ectonucleotidases from brain membranes of zebrafish can be a potential target for pesticide neurotoxicity. Copyright © 2008 CABI
Descriptors: zebrafish pesticide model, acetylcholine, acetylcholinesterase, ATP, brain, carbofuran, enzyme activity, enzyme inhibitors, enzymes, hydrolysis, insecticide residues, insecticides, malathion, neurotoxicity, neurotransmitters, nontarget effects, nontarget organisms, nucleotides, toxicity, *Danio rerio.*

Senger, M.R., E.P. Rico, M. de Bem Arizi, A.P. Frazzon, R.D. Dias, M.R. Bogo, and C.D. Bonan (2006). **Exposure to Hg2+ and Pb2+ changes NTPDase and ecto-5'-nucleotidase activities in central nervous system of zebrafish (*Danio rerio*).** *Toxicology* 226(2-3): 229-37. ISSN: 0300-483X.
NAL Call Number: RA1190.T69

Abstract: Neurotransmission can be affected by exposure to heavy metals, such as mercury and lead. ATP is a signaling molecule that can be metabolized by a group of enzymes called ecto-nucleotidases. Here we investigated the effects of mercury chloride (HgCl(2)) and lead acetate (Pb(CH(3)COO)(2)) on NTPDase (nucleoside triphosphate diphosphohydrolase) and ecto-5'-nucleotidase activities in zebrafish brain membranes. In vitro exposure to HgCl(2) decreased ATP and ADP hydrolysis in an uncompetitive mechanism and AMP hydrolysis in a non-competitive manner. Pb(CH(3)COO)(2) inhibited ATP hydrolysis in an uncompetitive manner, but not ADP and AMP hydrolysis. In vivo exposure of zebrafish to HgCl(2) or Pb(CH(3)COO)(2) (20mug/L, during 24, 96h and 30 days) caused differential effects on nucleotide hydrolysis. HgCl(2), during 96h, inhibited the hydrolysis of ATP, ADP and AMP. After 30 days of exposure to HgCl(2), ATP hydrolysis returned to the control levels, ADP hydrolysis was strongly increased and AMP hydrolysis remained inhibited. Exposure to Pb(CH(3)COO)(2) during 96h caused a significant decrease only on ATP hydrolysis. After 30 days, Pb(CH(3)COO)(2) promoted the inhibition of ATP, ADP and AMP hydrolysis. Semi-quantitative RT-PCR analysis showed no changes in the expression of NTPDase1 and 5'-nucleotidase, following 30 days of exposure to both metals. This study demonstrated that Hg(2+) and Pb(2+) affect the ecto-nucleotidase activities, an important enzymatic pathway for the control of purinergic signaling.
Descriptors: zebrafish, 5' nucleotidase metabolism, adenosine triphosphatases metabolism, central nervous system drug effects and enzymology, lead and mercury toxicity , 5' nucleotidase biosynthesis, adenosine diphosphate metabolism, adenosine triphosphate metabolism, brain enzymology, brain chemistry drug effects, membranes drug effects and metabolism, nerve tissue proteins analysis and biosynthesis, receptors, purinergic drug effects, reverse transcriptase PCR.

Senger, M.R., D.B. Rosemberg, E.P. Rico, M. de Bem Arizi, R.D. Dias, M.R. Bogo, and C.D. Bonan (2006). **In vitro effect of zinc and cadmium on acetylcholinesterase and ectonucleotidase activities in zebrafish (*Danio rerio)* brain.** *Toxicology in Vitro* 20(6): 954-8. ISSN: 0887-2333.

NAL Call Number: RA1190.T692

Abstract: Zinc and cadmium are environmental contaminants that induce a wide range of effects on CNS. Here we tested the in vitro effect of these metals on acetylcholinesterase (AChE) and ectonucleotidase (NTPDase and ecto-5'-nucleotidase) activities in zebrafish brain. Both zinc and cadmium treatments did not alter significantly the zebrafish brain AChE activity. ATP hydrolysis presented a significant increase at 1 mM zinc (17%) and the AMPase activity had a dose-dependent increase at 0.5 and 1 mM zinc exposure (188% and 199%). After cadmium treatment, ATPase activity was significantly increased (53% and 48%) at 0.5 and 1 mM, respectively. Cadmium, in the range 0.25-1 mM, inhibited ADP hydrolysis in a dose-dependent manner (13.4-69%). Ecto-5'-nucleotidase activity was only inhibited (38%) in the presence of 1 mM cadmium. It is possible to suggest that changes on NTPDase and ecto-5'-nucleotidase activities can be an important mechanism involved in neurotoxic effects promoted by zinc and cadmium.
Descriptors: zebrafish neurotoxins model, 5' nucleotidase metabolism, acetylcholinesterase metabolism, brain drug effects, cadmium and zinc toxicity, adenosine triphosphatases metabolism, brain enzymology, nucleotidases metabolism.

Seok, S.H., M.W. Baek, H.Y. Lee, D.J. Kim, Y.R. Na, K.J. Noh, S.H. Park, H.K. Lee, B.H. Lee, D.Y. Ryu, and J.H. Park (2007). **Arsenite-induced apoptosis is prevented by antioxidants in zebrafish liver cell line.** *Toxicology In Vitro* 21(5): 870-7. ISSN: 0887-2333.

NAL Call Number: RA1190.T692

Abstract: This study evaluated oxidative stress-induced apoptosis as a possible mechanism of arsenite toxicity in zebrafish liver cell line (ZFL cells). The heat shock protein 70 (HSP70), a chaperone protein, appears to provide protection against oxidative stress and apoptosis. Using the MTT assay, we demonstrated that survival of ZFL cells treated with arsenite for 24h decreased in a dose-dependent manner. The possible mechanisms that promote the cytotoxicity of arsenite were addressed. Cell viability assays revealed that arsenite caused a dose-dependent increase in cell death, and pretreatment of the ZFL cells with antioxidants blunted these effects. Antioxidants such as N-acetyl-cysteine (NAC, 5 mM) and dithiothreitol (DTT, 80 microM) significantly prevented ZFL cells from arsenite-induced death. Nuclear staining was performed using 1 microg/ml Hoechst, and cells were analyzed with a fluorescent microscope. Arsenite (30 microM) induced massive apoptosis that was identified by morphology and condensation and fragmentation of the nuclei of the ZFL cells. Pretreatment with NAC or DTT before arsenite insult effectively protected the cells against oxidative stress-induced apoptosis from the arsenite. Using a transfected human hsp 70 promoter-enhanced green fluorescent protein (EGFP) reporter, pHhsp70-EGFP, the induction of HSP70 against oxidative stress-induced apoptosis by arsenite was observed. The induction of HSP70 by arsenite increased in a dose-dependent manner, and pretreatment of transfected ZFL cells with NAC or DTT before arsenite insult reduced EGFP expression. Taken together, our results provide evidence that stimulation of the heat shock response is a sensitive biomarker of arsenic exposure and that arsenite causes oxidative stress-induced apoptosis in ZFL cells.

Descriptors: zebrafish liver cell line, antioxidants pharmacology, apoptosis drug effects, arsenites antagonists and inhibitors, arsenites toxicity, hepatitis, toxic pathology , prevention and control, liver pathology, acetylcysteine pharmacology, cell line, cell survival drug effects, dithiothreitol pharmacology, drug dose response relationship, reporter genes drug effects, green fluorescent proteins metabolism, HSP70 heat shock proteins biosynthesis, genetics and physiology, oxidative stress drug effects, plasmids genetics, transfection, trypan blue.

Seok, S.H., M.W. Baek, H.Y. Lee, D.J. Kim, Y.R. Na, K.J. Noh, S.H. Park, H.K. Lee, B.H. Lee, D.Y. Ryu, and J.H. Park (2007). **Quantitative GFP fluorescence as an indicator of arsenite developmental toxicity in mosaic heat shock protein 70 transgenic zebrafish.** *Toxicology and Applied Pharmacology* 225(2): 154-61. ISSN: 0041-008X.

NAL Call Number: 391.8T662

Abstract: In transgenic zebrafish (*Danio rerio*), green fluorescent protein (GFP) is a promising marker for environmental pollutants. In using GFP, one of the obstacles which we faced was how to compare toxicity among different toxicants or among a specific toxicant in different model species with the intensity of GFP expression. Using a fluorescence detection method, we first validated our method for estimating the amount of GFP fluorescence present in transgenic fish, which we used as an indicator of developmental toxicity caused by the well-known toxicant, arsenite. To this end, we developed mosaic transgenic zebrafish with the human heat shock response element (HSE) fused to the enhanced GFP (EGFP) reporter gene to indicate exposure to arsenite. We confirmed that EGFP expression sites correlate with gross morphological disruption caused by arsenite exposure. Arsenite (300.0 microM) caused stronger EGFP fluorescence intensity and quantity than 50.0 microM and 10.0 microM arsenite in our transgenic zebrafish. Furthermore, arsenite-induced apoptosis was demonstrated by TUNEL assay. Apoptosis was inhibited by the antioxidant, N-acetyl-cystein (NAC) in this transgenic zebrafish. The distribution of TUNEL-positive cells in embryonic tissues was correlated with the sites of arsenite toxicity and EGFP expression. The EGFP

values quantified using the standard curve equation from the known GFP quantity were consistent with the arsenite-induced EGFP expression pattern and arsenite concentration, indicating that this technique can be a reliable and applicable measurement. In conclusion, we propose that fluorescence-based EGFP quantification in transgenic fish containing the hsp70 promoter-EGFP reporter-gene construct is a useful indicator of development toxicity caused by arsenite.

Descriptors: zebrafish, arsenites toxicity, environmental pollutants toxicity, fluorescent dyes metabolism, green fluorescent proteins metabolism, acetylcysteine pharmacology, genetically modified, antioxidants pharmacology, apoptosis drug effects, arsenites administration and dosage, biological markers, drug dose response relationship, drug effects, metabolism, environmental pollutants administration and dosage, developmental gene expression regulation drug effects, reporter genes drug effects, HSP70 heat shock HSP70 heat shock proteins metabolism, in situ nick end labeling, mosaicism.

Shafizadeh, E., R.T. Peterson, and S. Lin (2004). **Induction of reversible hemolytic anemia in living zebrafish using a novel small molecule.** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 138(3): 245-9. ISSN: 1532 0456.

NAL Call Number: QP901.C6

Abstract: We used zebrafish to screen and identify small molecules that affect the process of vertebrate hematopoietic development. Zebrafish embryos were exposed to a library of 5000 synthetic compounds and screened for defects in primitive erythropoiesis. Here, we present the characterization of hemolytic anemia induced in zebrafish by the small molecule 5115318 (3-[5-methyl-furan 2-yl]-propionic acid N'-phenyl-hydrazide). This compound is capable of generating hemoglobin aggregates and Heinz bodies in red cells in vivo only. The induced anemia is reversible and treated fish recover in about 4 days. This study shows the feasibility of using zebrafish to screen for small molecules that can modulate the specific process of erythropoiesis.

Descriptors: zebrafish embryos screening synthetic compounds, chemically induced hemolytic anemia, hemolytic anemia pathology, preclinical drug evaluation methods, furans pharmacology, propionic acids pharmacology, hemolytic anemia drug therapy, disease models, drug effects, embryology, pathology, erythroid cells drug effects and pathology, erythropoiesis drug effects, furans chemistry, molecular structure, propionic acids chemistry.

Shen, A. and X. Shen (2005). **Effects of diesel fuel on the superoxide dismutase and catalase of zebra fish**. *Marine Fisheries/Haiyang Yuye* 27(4): 314-318. ISSN: 1004-2490.

Descriptors: zebrafish , comparative studies, fuels, oil pollution, pollution effects, toxicity tests.

Shen, X. and Q. Yuan (2006). **Study on acute toxicity effect of styrene on aquatic organism**. *Marine Environmental Science/Haiyang Huanjing Kexue* 25(4): 33-35. ISSN: 1007-6336.

Descriptors: zebrafish and Daphnia models for acute toxicity, aquatic organisms, styrene, pollution, toxicity, *Danio rerio*, *Daphnia magna.*

Shrader, E.A., T.R. Henry, M.S.J. Greeley, and B.P. Bradley (2003). **Proteomics in zebrafish exposed to endocrine disrupting chemicals.** *Ecotoxicology* 12(6): 485-8. ISSN: 0963 9292.

NAL Call Number: RA565.A1E27

Abstract: Embryonic zebrafish were examined for changes in protein expression following exposure to sublethal concentrations of 17beta-estradiol (E2) and the estrogen mimic 4-nonylphenol (4-NP). Protein Expression Signatures were derived from embryo homogenates by

two-dimensional electrophoresis and digital imaging. In both experiments approximately 30% of the proteins sampled were specific to either E2 or 4-NP and about 33% were common to the control, 4-NP and E2. However, of the proteins induced by either E2 or 4-NP, 28% were common to both chemicals at 1 ppm but only 7% were common to both at 0.1 ppm. While there are many proteins that respond specifically to each chemical, relatively few are common to the two chemicals suggesting that the response pathways of the two chemicals are distinct.
Descriptors: zebrafish embryos, estradiol toxicity, gene expression profiling, phenols toxicity, protein array analysis, proteomics, two dimensional gel electrophoresis endocrine system drug effects, mutagenicity tests, pollution assessment control and management, toxicology, digital imaging, two dimensional electrophoresis, electrophoretic techniques, ecotoxicology, protein expression signatures, proteomics, response pathways.

Silva, A.C., M. Dezotti, and G.L. Sant' Anna Junior (2004). **Treatment and detoxification of a sanitary landfill leachate**. *Chemosphere* 55(2): 207-214. ISSN: 0045 6535.
NAL Call Number: TD172.C54
Abstract: The leachate from an old sanitary landfill (Gramacho Metropolitan Landfill, Rio de Janeiro) was characterized and submitted to coagulation and flocculation treatment followed by ozonation and ammonia stripping. The performance of the treatment was assessed by monitoring the removal of organic matter (COD and TOC), ammonium nitrogen and metals. Detoxification was assessed by determining acute toxicity, using the following organisms: *Vibrio fischeri, Daphnia similis, Artemia salina and Brachydanio rerio*. Membrane fractionation was employed to infer the range of molecular masses of the pollutants found in the effluent, as well as the toxicity associated to these fractions. Of the techniques under investigation, coagulation and flocculation followed by ammonia stripping were the most effective for toxicity and ammonium nitrogen removal. Membrane fractionation was effective for COD removal; however, acute toxicity was almost the same in all the fractionated samples. Ozonation was moderately effective for COD removal, but significant toxicity removal was only attained when high ozone doses were used. Copyright © 2008 CABI
Descriptors: zebrafish, aluminium sulfate, ammonia, ammonium nitrogen, aquatic organisms, bioassays, chemical oxygen demand, coagulation, detoxification, environmental impact, flocculation, fractionation, landfill leachates, landfills, ozone, pollutants, sewage effluent, toxic substances, toxicity, trophic levels, ultrafiltration, waste treatment, water pollution, *Artemia salina*, *Danio rerio*, *Daphnia*, *Vibrio*, Rio de Janeiro, Brazil .

Smith, D.G., C. Wilburn, and R.A. McCarthy (2003). **Methoprene photolytic compounds disrupt zebrafish development, producing phenocopies of mutants in the sonic Hedgehog signaling pathway.** *Marine Biotechnology* 5(2): 201-12. ISSN: 1436-2228.
NAL Call Number: TP248.27.M37M37
Abstract: Environmental chemicals have been proposed to impact endocrine or retinoid pathways, causing developmental abnormalities in humans and other vertebrates. Presented evidence shows that exposure of zebrafish embryos to sunlight-induced photolytic products of the pesticide methoprene results in developmental defects in the head, heart, pectoral fins, and somites, and in spinal motor and optic nerve axons. Exposed embryos are phenocopies of zebrafish you-type mutants and, as in the mutant sonic-you, show underexpression of the signaling protein sonic hedgehog. Reduced expression of sonic hedgehog is also displayed in embryos treated with the retinoic acid synthesis inhibitor citral. This study identifies citral-related compounds as embryonic signaling disruptors of potential environmental concern.
Descriptors: zebrafish embryos, methoprene toxicity, photolysis, signal transduction genet-

ics, trans activators genetics, zebrafish abnormalities, drug effects, embryonic development, embryonic induction genetics, developmental gene expression regulation drug effects, Hedgehog proteins, hormones radiation effects, hormones toxicity, juvenile hormones radiation effects and toxicity, methoprene radiation effects, mutation, pesticides radiation effects and toxicity, phenotype.

Smolders, R., G. De Boeck, and R. Blust (2003). **Changes in cellular energy budget as a measure of whole effluent toxicity in zebrafish (*Danio rerio*).** *Environmental Toxicology and Chemistry* 22(4): 890-9. ISSN: 0730 7268.
NAL Call Number: QH545A1E58
Abstract: Organisms exposed to suboptimal conditions face an a priori cost of combating stress in terms of metabolic resources. The energy available for maintenance, growth, and reproduction, based on the biochemical analysis of the energy budget rather than on the direct measurement of those endpoints, may therefore provide a sensitive measure of stress in an organism. The aim of this study was to validate changes in energy budget as an ecologically relevant sublethal parameter by comparing these responses with physiological, growth, and reproductive endpoints. A toxicity test with zebrafish (*Danio rerio*) was conducted where fish were exposed to a control and 50, 75, and 100% effluent for 28 d under flow-through conditions. Effects of effluent exposure were measured as changes in glycogen, lipid, and protein budgets. Furthermore, the observed effects were linked to different processes like growth, condition, respiration, and reproduction within the same populations. Our results indicate that changes in lipid budgets in exposed fish were the most sensitive endpoint. Excellent relations were found between cellular energy budgets, relative condition factor, and respiration (r2 = 0.795, p < 0.001; and r2 = 0.735, p < 0.001, respectively), but relations were poorer for reproductive output (r2 = 0.410, p < 0.034). As a whole, especially changes in lipid energy budgets provide a sensitive and fast indicator of altered condition in zebrafish under the given exposure regime and allow linking cellular effects to other endpoints within the same exposed populations.
Descriptors: zebrafish effluent toxicity model, energy metabolism drug effects, chemical water pollutants toxicity, drug dose response relationship, lipid metabolism, reproduction drug effects, time factors, toxicity tests, zebrafish growth and development and proteins metabolism.

Snape, J.R., S.J. Maund, D.B. Pickford, and T.H. Hutchinson (2004). **Ecotoxicogenomics: the challenge of integrating genomics into aquatic and terrestrial ecotoxicology**. *Aquatic Toxicology* 67(2): 143-154. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: Rapid progress in the field of genomics (the study of how an individual's entire genetic make-up, the genome, translates into biological functions) is beginning to provide tools that may assist our understanding of how chemicals can impact on human and ecosystem health. In many ways, if scientific and regulatory efforts in the 20th century have sought to establish which chemicals cause damage to ecosystems, then the challenge in ecotoxicology for the 21st century is to understand the mechanisms of toxicity to different wildlife species. In the human context, 'toxicogenomics' is the study of expression of genes important in adaptive responses to toxic exposures and a reflection of the toxic processes per se. Given the parallel implications for ecological (environmental) risk assessment, we propose the term 'ecotoxicogenomics' to describe the integration of genomics (transcriptomics, proteomics and metabolomics) into ecotoxicology. Ecotoxicogenomics is defined as the study of gene and protein expression in non-target organisms that is important in responses to

environmental toxicant exposures. The potential of ecotoxicogenomic tools in ecological risk assessment seems great. Many of the standardized methods used to assess potential impact of chemicals on aquatic organisms rely on measuring whole-organism responses (e.g. mortality, growth, reproduction) of generally sensitive indicator species at maintained concentrations, and deriving 'endpoints' based on these phenomena (e.g. median lethal concentrations, no observed effect concentrations, etc.). Whilst such phenomenological approaches are useful for identifying chemicals of potential concern they provide little understanding of the mechanism of chemical toxicity. Without this understanding, it will be difficult to address some of the key challenges that currently face aquatic ecotoxicology, e.g. predicting toxicant responses across the very broad diversity of the phylogenetic groups present in aquatic ecosystems; estimating how changes at one ecological level or organization will affect other levels (e.g. predicting population-level effects); predicting the influence of time-varying exposure on toxicant responses. Ecotoxicogenomic tools may provide us with a better mechanistic understanding of aquatic ecotoxicology. For ecotoxicogenomics to fulfil its potential, collaborative efforts are necessary through the parallel use of model microorganisms (e.g. *Saccharomyces cerevisiae*) together with aquatic (e.g. *Danio rerio, Daphnia magna, Lemna minor and Xenopus tropicalis*) and terrestrial (e.g. *Arabidopsis thaliana, Caenorhabdites elegans and Eisenia foetida*) plants, animals and microorganisms. Copyright © 2008 CABI
Descriptors: zebrafish and other species ecotoxicogenomics, gene expression, genomic imprinting, indicator species, risk assessment, toxicology, *Arabidopsis thaliana*, *Danio rerio*, *Daphnia magna*, *Eisenia fetida*, *Lemna minor*, *Saccharomyces cerevisiae*, *Xenopus.*

Stehr, C.M., T.L. Linbo, J.P. Incardona, and N.L. Scholz (2006). **The developmental neurotoxicity of fipronil: notochord degeneration and locomotor defects in zebrafish embryos and larvae** . *Toxicological Sciences* 92(1): 270-278. ISSN: 1096 6080.
NAL Call Number: RA1190.F8
Abstract: Fipronil is a phenylpyrazole insecticide designed to selectively inhibit insect gamma-aminobutyric acid (GABA) receptors. Although fipronil is often used in or near aquatic environments, few studies have assessed the effects of this neurotoxicant on aquatic vertebrates at sensitive life stages. We explored the toxicological effects of fipronil on embryos and larvae using the zebrafish *(Danio rerio*) experimental model system. Embryos exposed to fipronil at nominal concentrations at or above 0.7 micro M (333 micro g/l) displayed notochord degeneration, shortening along the rostral-caudal body axis, and ineffective tail flips and uncoordinated muscle contractions along the body axis in response to touch. This phenotype closely resembles zebrafish locomotor mutants of the accordion class and is consistent with loss of reciprocal inhibitory neurotransmission by glycinergic commissural interneurons in the spinal cord. Consistent with the hypothesis that notochord degeneration may be due to abnormal mechanical stress from muscle tetany, the expression patterns of gene and protein markers specific to notochord development were unaffected by fipronil. Moreover, the degenerative effects of fipronil (1.1 micro M) were reversed by coexposure to the sodium channel blocker MS-222 (0.6 mM). The notochord effects of fipronil were phenocopied by exposure to 70 micro M strychnine, a glycinergic receptor antagonist. In contrast, exposure to gabazine, a potent vertebrate GABAA antagonist, resulted in a hyperactive touch response but did not cause notochord degeneration. Although specifically developed to target insect GABA receptors with low vertebrate toxicity, our results suggest that fipronil impairs the development of spinal locomotor pathways in fish by inhibiting a structurally related glycine receptor subtype. This represents an unanticipated and potentially novel mechanism for fipronil toxicity in vertebrates. Copyright © 2008 CABI
Descriptors: zebrafish neurotoxin model , biological development, developmental stages,

embryos, fipronil, insecticide residues, larvae, locomotion, neurotoxicity, nontarget effects and organisms, polluted water, water pollution, water quality, *Danio rerio.*

Suiko, M., Y. Sakakibara, Y. Liu Ming, S. Yang Yuh, and M.C. Liu (2005). **Cytosolic sulfotransferases and environmental estrogenic chemicals**. *Journal of Pesticide Science* 30(4): 345-353. ISSN: 1348 589X.

Abstract: Over the past three decades, a substantial body of evidence has accumulated on the estrogenic activities of numerous environmental compounds. These "environmental estrogens," consisting of pesticides and a variety of industrial chemicals and their by-products, are becoming ubiquitous in the environment and are making their way into the food chain. An important issue is whether vertebrate animals are equipped with mechanisms for the inactivation and/or disposal of environmental estrogens. This review attempts to summarize the currently available data concerning the sulfation of environmental estrogenic compounds by the cytosolic sulfotransferases in vertebrate animals. Copyright © 2008 CABI

Descriptors: zebrafish environmental estrogenic chemicals, enzymes, nontarget effects, nontarget organisms, estrogens, pesticides, reviews, transferases, *Danio rerio*, humans, mice, vertebrates.

Notes: A review.

Sun, F. (2006). **Risk assessment of carbamate pesticides for aquatic organisms**. *Plant Protection Bulletin Taipei* 48(2): 153-162. ISSN: 0577 750X.

NAL Call Number: 464.9 C47

Abstract: The risks to aquatic organisms of 9 carbamate pesticides, which have high utility in Taiwan, were assessed. Water fleas (belonging to family Daphniidae) and fishes *(Brachydanio rerio [Danio rerio], Pimephales promelas, Cyprinus carpio [carp], Oryzias latipes, Poecilia reticulata, Lepomis macrochirus and Salmo gairdneri* [rainbow trout]) were used as indicators to represent invertebrate and vertebrate aquatic organisms, respectively. Thirteen formulations of the 9 active ingredients of carbamate pesticides were used in an aquarium environment according to the 'Plant Protection Manual': benfuracarb 5% GR and 20% EC, benomyl 50% WP, benthiocarb [thiobencarb] 10% GR and 50% EC, carbaryl 50% and 85% WP, carbofuran 40.64% SC, carbosulfan 40% WP and 48.34% EC, fenobucarb 50% EC, isoprocarb 20% EC, and oxamyl 24% SL. Based on the applied concentrations, only oxamyl 24% SL, as rice seed-dip treatment, had middle toxicity to aquatic organisms and was safe for use in rice fields under the applied concentration. Among the 13 pesticide formulations, only oxamyl 24% SL had low potential risk to aquatic organisms. There were high potential acute toxicity risks to aquatic organisms from the use of the other pesticides in aquatic areas. Potential risks to aquatic organisms presumed from the risk quotient (derived from the estimated environmental concentration and toxicity to organisms) according to the US Environmental Protection Agency are discussed. Copyright © 2008 CABI

Descriptors: carbamate pesticides effects on zebrafish and other species, aquatic invertebrates, aquatic organisms, benfuracarb, benomyl, carbamate pesticides, carbaryl, carbofuran, carbosulfan, fenobucarb, isoprocarb, oxamyl, rice, risk assessment, safety, thiobencarb, toxicity, carp, *Danio rerio*, *daphniidae*, *fishes*, *Lepomis macrochirus*, *Oryza*, *Oryza sativa*, *Oryzias latipes*, *Pimephales promelas*, *Poecilia reticulata*, rainbow trout, Taiwan.

Language of Text: Chinese.

Sun, L.W., M.M.L.Y.Q. Qu, Y. Wu, Y. Chen, Z. Kong, and Z. Liu (2004). **Toxic effects of aminophenols on aquatic life using the zebrafish embryo test and the comet assay.** *Bulletin of Environmental Contamination and Toxicology* 73(4): 628-34. ISSN: 0007 4861.

NAL Call Number: RA1270.P35A1
Descriptors: zebrafish embryos, pollutants, *Danio rerio*, embryotoxicity, *Cyprinus carpio*, kidney cells, genotoxicity.

Swain, H.A., C. Sigstad, and F.M. Scalzo (2004). **Effects of dizocilpine (MK-801) on circling behavior, swimming activity, and place preference in zebrafish *(Danio rerio)*.** *Neurotoxicology and Teratology* 26(6): 725-9. ISSN: 0892-0362.
NAL Call Number: RA1224
Abstract: Glutamate transmission plays an important role in many behavioral systems, including motor activity, learning, and memory. The noncompetitive NMDA receptor antagonist (+)MK-801 has been shown to increase motor activity and impair learning and memory in a variety of tasks in rats, mice, and other species. In an attempt to characterize the effects of MK-801 on motor activity and cognitive performance in an emerging neurobehavioral model, the zebrafish (*Danio rerio*), we examined the effects of MK-801 on circling behavior, swimming activity, and latency to enter, as well as preference for, an enriched chamber (EC). In Experiment 1, the effects of a 37-min acute exposure to (+)MK-801 (0, 2.0, and 20.0 microM) on circling behavior were measured in a round observation chamber. (+)MK-801 was observed to increase circling behavior in a dose-dependent manner. In the second experiment, fish were treated with 0, 2, 20, or 200 microM (+)MK-801 for 1 h, and swimming activity was measured in a rectangular observation chamber for 60 min following dosing. The lowest dose of (+)MK-801 decreased swimming activity. In the third experiment, fish were treated with either 0 or 20 microM (+)MK-801 for 1 h each day over four consecutive days. The fish were tested in a modified T-maze to assess both latency to enter, and preference for, an EC 24, 27, and 48 h after the last treatment. The results showed that untreated fish exhibited a preference for the EC at the 27- and 48-h trials, but (+)MK-801-treated fish did not exhibit a preference for the EC at any trial. No significant reduction in latency to enter the chamber was found for either treated or control fish. Together, the results of these experiments suggest that NMDA receptor antagonism (1) increases circling behavior, (2) alters swimming activity, and (3) impairs place preference. These findings lend further support for the usefulness of the zebrafish for assessing the acute and chronic exposure effects of water-soluble compounds on motor and cognitive functions.
Descriptors: zebrafish chemical toxicity model, drug effects on behavior, dizocilpine maleate adverse effects, motor activity drug effects, spatial behavior, behavior physiology, brain drug effects and metabolism, disease models, drug dose response relationship, excitatory amino acid antagonists adverse effects, maze learning drug effects, motor activity physiology, reaction time drug effects and physiology, sex factors, spatial behavior physiology, swimming physiology.

Teraoka, H., W. Dong, Y. Okuhara, H. Iwasa, A. Shindo, A.J. Hill, A. Kawakami, and T. Hiraga (2006). **Impairment of lower jaw growth in developing zebrafish exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin and reduced Hedgehog expression.** *Aquatic Toxicology* 78(2): 103-13. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) has been shown to cause a multitude of detrimental effects to developing zebrafish (*Danio rerio*). Previously, we demonstrated that jaw growth was impaired by TCDD exposure, but the exact mechanism underlying these malformations remained unknown. In the present study, we investigated the involvement of hedgehog genes and their downstream signaling in TCDD-mediated jaw malformation. We demonstrate that the developing lower jaw expresses sonic hedgehog a

(shha), sonic hedgehog b (shhb) and their receptors, patched1 (ptc1) and patched2 (ptc2), as well as the downstream transcription factors, gli1 and gli2a. Loss of Hh signaling in mutants (sonic you) and larvae treated with a Hh inhibitor (cyclopamine), resulted in similar effects as those caused by TCDD. Moreover, TCDD exposure caused downregulation of shha and shhb in a manner dependent on aryl hydrocarbon receptor 2 (ahr2). Although this suggested an involvement of Hh signaling in TCDD-mediated impairment of jaw growth, we did not observe downregulation of ptc1 and ptc2, receptors dependent on Hh signaling. Furthermore, while the overall occurrence of apoptosis in the developing jaw was minimal, it was significantly increased in larvae treated with cyclopamine. In contrast, both TCDD and cyclopamine markedly reduced immunoreactivity against phosphorylated histone 3, a cell proliferation marker in the developing jaw. Taken together, our data suggest that Ahr2-mediated downregulation of Hh signaling, leading to a failure of cell proliferation, contributes to TCDD induced inhibition of lower jaw growth. TCDD may impair jaw growth through other pathway(s) in addition to Hh signaling.

Descriptors: zebrafish embryos, developmental gene expression regulation drug effects, Hedgehog proteins drug effects, jaw embryology, tetrachlorodibenzodioxin toxicity, chemical water pollutants toxicity, apoptosis drug effects, down regulation, drug effects , environmental exposure, Hedgehog proteins antagonists, inhibitors, genetics and physiology, in situ nick end labeling, jaw drug effects, metabolism, chemically induced abnormalities, abnormalities pathology, kruppel like transcription factors analysis and drug effects, oncogene proteins analysis and drug effects, cell surface receptors analysis and drug effects, trans activators analysis and drug effects, veratrum alkaloids toxicity, .

Tiedeken, J.A., J.S. Ramsdell, and A.F. Ramsdell (2005). **Developmental toxicity of domoic acid in zebrafish (*Danio rerio*).** *Neurotoxicology and Teratology* 27(5): 711-7. ISSN: 0892-0362.

NAL Call Number: RA1224

Abstract: Domoic acid (DA) is a rigid analog of the excitatory amino acid glutamate. It is produced by the diatom genus *Pseudo-nitzschia* and is a potent neurotoxin in both adult and developing animals. We have used zebrafish (*Danio rerio*) as a model to investigate and characterize the developmental toxicity of DA. Domoic acid was administered by microinjection to fertilized eggs at the 128- to 512-cell stages in concentrations ranging from 0.12 to 17 mg/kg (DA/egg weight). DA reduced hatching success by 40% at 0.4 mg/kg and by more than 50% at doses of 1.2 mg/kg and higher. Fifty percent of embryos treated with 1.2 mg/kg DA showed marked tonic-clonic type convulsions at 2 days post fertilization. Four days post fertilization (dpf), all embryos treated with 4.0 mg/kg DA and higher showed a complete absence of touch response reflexes. Commencing 5 dpf, rapid and constant pectoral fin movements were observed, a response which may be related to the hallmark effect in rodents of stereotypic scratching. These data indicate that zebrafish show symptoms of developmental DA toxicity as well as a similar sensitivity comparable to the effects of DA characterized in laboratory rodents.

Descriptors: zebrafish neurotoxins model, kainic acid analogs and derivatives, neurotoxins toxicity, zebrafish growth and development, drug induced abnormalities pathology, drug effects on swimming behavior, cardiovascular system drug effects, drug dose response relationship, drug effects, kainic acid administration, dosage and toxicity, microinjections, reflex drug effects.

Tilton, F., J.K. La Du, M. Vue, N. Alzarban, and R.L. Tanguay (2006). **Dithiocarbamates have a common toxic effect on zebrafish body axis formation.** *Toxicology and Applied Pharmacology* 216(1): 55-68. ISSN: 0041-008X.

NAL Call Number: 391.8T662

Abstract: We previously determined that the dithiocarbamate pesticide sodium metam (NaM) and its active ingredient methylisothiocyanate (MITC) were developmentally toxic causing notochord distortions in the zebrafish. In this study, developing zebrafish were exposed to isothiocyanates (ITCs), dithiocarbamates (DTCs) and several degradation products to determine the teratogenic relationship of these chemical classes at the molecular level. All dithiocarbamates tested elicited notochord distortions with notochord NOELs from <4 to 40 ppb, while none of the ITCs caused notochord distortions with the exception of MITC. Carbon disulfide (CS(2)), a common DTC degradate, also caused distortions at concentrations >200 times the DTCs. Whole mount in situ hybridization of developmental markers for collagen (collagen2a1), muscle (myoD), and body axis formation (no tail) was perturbed well after cessation of treatment with pyrolidine-DTC (PDTC), dimethyl-DTC (DMDTC), NaM, MITC, and CS(2). Therefore, distinct albeit related chemical classes share a common toxic effect on zebrafish notochord development. To test the responsiveness of the distortion to metal perturbation, five metal chelators and 2 metals were studied. The membrane permeable copper chelator neocuproine (NCu) was found to cause notochord distortions similar to DTC-related molecules. DMDTC and NCu treated animals were protected with copper, and collagen 2a1 and no tail gene expression patterns were identical to controls in these animals. PDTC, NaM, MITC, and CS(2) were not responsive to copper indicating that the chelation of metals is not the primary means by which these molecules elicit their developmental toxicity. Embryos treated with DMDTC, NaM, and NCu were rescued by adding triciaine (MS-222) which abolishes the spontaneous muscle contractions that begin at 18 hpf. In these animals, only collagen 2a1 expression showed a similar pattern to the other notochord distorting molecules. This indicates that the perturbation of no tail expression is in response to the muscle contractions distorting the notochord, while collagen 2a1 is associated with the impact of these molecules on much earlier developmental processes.

Descriptors: zebrafish notochord distortions, body patterning drug effects, thiocarbamates toxicity, aminobenzoic acids toxicity, analysis of variance, body patterning genetics, body patterning physiology, carbon disulfide toxicity, chelating agents toxicity, collagen Type II genetics, copper toxicity, dimethyldithiocarbamate chemistry and toxicity, disulfiram toxicity, drug dose response relationship, abnormalities , developmental gene expression regulation drug effects, in situ hybridization, isothiocyanates chemistry and toxicity, molecular structure, notochord abnormalities and drug effects, phenanthrolines toxicity, pyrrolidines chemistry and toxicity, T-box domain thiocarbamates chemistry, toxicity tests methods, proteins genetics.

Timme Laragy, A.R., C.J. Cockman, C.W. Matson, and R.T. Di Giulio (2007). **Synergistic induction of AHR regulated genes in developmental toxicity from co-exposure to two model PAHs in zebrafish.** *Aquatic Toxicology* 85(4): 241-50. ISSN: 0166 445X.

NAL Call Number: QH541.5.W3A6

Abstract: Polycyclic aromatic hydrocarbons (PAHs) are pollutants created by the incomplete combustion of carbon, and are increasing in the environment largely due to the burning of fossil fuels. PAHs occur as complex mixtures, and some combinations have been shown to cause synergistic developmental toxicity in fish embryos, characterized by pericardial edema and craniofacial malformations. Previous studies have indicated that in the zebrafish model, this toxicity is mediated by the aryl hydrocarbon receptor 2 (AHR2), and enhanced by inhibition of CYP1A activity. In this study, we further examined this interaction of the model PAH and AHR agonist beta-naphthoflavone (BNF) with and without

the AHR partial agonist/antagonist and CYP1A inhibitor alpha-naphthoflavone (ANF) to determine (1) whether ANF was acting as an AHR antagonist, (2) what alterations BNF and ANF both alone and in combination had on mRNA expression of the AHR regulated genes cytochrome P450 (cyp) 1a, 1b1, and 1c1, and the AHR repressor (ahrr2) prior to versus during deformity onset, and (3) compare CYP1A enzyme activity with mRNA induction. Zebrafish embryos were exposed from 24-48 or 24-96hpf to BNF, 1-100mug/L, ANF, 1-150mug/L, a BNF+ANF co-exposure (1mug/L+100mug/L), or a DMSO solvent control. RNA was extracted and examined by quantitative real-time PCR. Both BNF and ANF each individually resulted in a dose dependent increase CYP1A, CYP1B1, CYP1C1, and AHRR2 mRNA, confirming their activities as AHR agonists. In the BNF+ANF co-exposures prior to deformity onset, expression of these genes was synergistic, and expression levels of the AHR regulated genes resembled the higher doses of BNF alone. Gene induction during deformities was also significantly increased in the co-exposure, but to a lesser magnitude than prior to deformity onset. EROD measurements of CYP1A activity showed ANF inhibited activity induction by BNF in the co-exposure group; this finding is not predicted by mRNA expression, which is synergistically induced in this treatment. This suggests that inhibition of CYP1A activity may alter metabolism and/or increase the half-life of the AHR agonist(s), allowing for increased AHR activation. This study furthers a mechanistic understanding of interactions underlying PAH synergistic toxicity.
Descriptors: zebrafish synergistic toxicity model, synergism, induction, AHR regulated, genes, developmental toxicity, PAH.

Tisler, T., J. Zagorc Koncan, M. Cotman, and A. Drolc (2004). **Toxicity potential of disinfection agent in tannery wastewater**. *Water Research* 38(16): 3503-3510. ISSN: 0043 1354.
NAL Call Number: TD420.W3
Abstract: Wastewater from a tannery was investigated using chemical-specific analyses and assessment of the acute toxicity of the whole effluent over a 2-year period. The wastewater samples were overloaded with organic and inorganic compounds, and measured concentrations of the chemical parameters as well as dilution factors estimating acute toxicity, frequently exceeded the permissible limits for the discharge of wastewater from a tannery into the receiving stream. In the later part of the monitoring programme, the toxicity of the samples was significantly increased in comparison to the previous samples. The agent for hide disinfection was assumed to be the reason for the increased toxicity of the wastewater samples, and the extremely high acute and chronic toxicity of the agent to bacteria, algae, daphnids, and fish confirmed this suspicion. The most sensitive species was Daphnia magna; the 48 h EC50 was 0.70x10-5 v/v% and the 21 d IC25 was 0.40x10-6 v/v% of the agent. After withdrawal of this highly toxic agent for hide disinfection from the technological process in the tannery, the toxicity of the wastewater declined to the previous level. Copyright © 2008 CABI
Descriptors: zebrafish and other species effects of tannery sludge, aluminium, ammonia, aquatic organisms, bactericides, bioassays, biochemical oxygen demand, biological indicators, chemical analysis, chemical oxygen demand, discharge, disinfectants, disinfection, factory effluents, hides and skins, indicator species, leather industry, tannery sludge, oils, pH, phosphorus, pollutants, sulfate, sulfides, toxicity, waste water, waste water treatment, water pollution, water quality, *Danio rerio*, *Daphnia magna*, *fishes*, *Scenedesmus*, *Vibrio.*

Tisler, T. and N. Kozuh Erzen (2006). **Abamectin in the aquatic environment**. *Ecotoxicology* 15(6): 495-502 . ISSN: 0963-9292.
NAL Call Number: RA565.A1E27

Descriptors: zebrafish, algae, aquatic environment, aquatic insects, avermectin, bioaccumulation, ecotoxicology, environmental factors, freshwater fish, insects, parasites, pesticides, pollutants, pollution indicators, reproduction, runoff, side effects, survival, toxicity, toxicity tests, varieties, water pollution effects, abamectin, insects, survival, *Danio rerio*, *Daphnia magna*, *Scenedesmus subspicatus.*

Tokumoto, T., M. Tokumoto, and P. Thomas (2007). **Interactions of diethylstilbestrol (DES) and DES analogs with membrane progestin receptor- alpha and the correlation with their nongenomic progestin activities**. *Endocrinology* 148(7): 3459-3467. ISSN: 0013-7227.
NAL Call Number: 448.8EN2
Descriptors: zebrafish, bioassays, endocrinology, hormones, sexual maturity, steroids, toxicity tests, *Carassius auratus*, *Danio rerio*, goldfish.

Tramujas, F.F., L.F. Favaro, L.M. Pauka, and H.C.S.d. Assis (2006). **Aspectos reprodutivos do peixe-zebra, *Danio rerio*, exposto a doses subletais de deltametrina [Reproductive aspects of zebrafish, *Danio rerio*, exposed to sublethal doses of deltamethrin]** . *Archives of Veterinary Science* 11(1): 48-53. ISSN: 1517 784X.
NAL Call Number: SF604
Abstract: The aim of this study was to evaluate the possible endocrine alterations in the reproduction of zebrafish (*Danio rerio*) following the protocol of USEPA (2002). Fish were exposed to sublethal concentrations of deltamethrin (6 and 10 micro g/litre of deltamethrin) and acetone, used as solvent, for 14 days. The number of eggs, hatching, size and histology of the gonads were evaluated. No significant difference in the evaluated parameters among the groups was observed. Therefore, deltamethrin did not alter the reproduction of zebrafish. However, in the present study, the progenies were not evaluated. Endocrine disruptor substances can affect the neuroendocrine and reproductive system of the animal and the alterations can appear in the progenitors or in the progeny at different stages of development. Copyright © 2008 CABI
Descriptors: zebrafish endocrine disruptors testing, adverse effects, deltamethrin, drug toxicity, gonads, reproduction, *Danio rerio*.
Language of Text: Portuguese.

Tsay, H.J., Y.H. Wang, W.L. Chen, M.Y. Huang, and Y.H. Chen (2007). **Treatment with sodium benzoate leads to malformation of zebrafish larvae.** *Neurotoxicology and Teratology* 29(5): 562-9. ISSN: 0892-0362.
NAL Call Number: RA1224
Abstract: Sodium benzoate (SB) is a commonly used food preservative and anti-microbial agent in many foods from soup to cereals. However, little is known about the SB-induced toxicity and teratogenicity during early embryonic development. Here, we used zebrafish as a model to test the toxicity and teratogenicity because of their transparent eggs; therefore, the organogenesis of zebrafish embryos is easy to observe. After low dosages of SB (1-1000 ppm) treatment, the zebrafish embryos exhibited a 100% survival rate. As the exposure dosages increased, the survival rates decreased. No embryos survived after treatment with 2000 ppm SB. The 50% lethal dose (LD(50)) of zebrafish is found to be in the range of 1400-1500 ppm. Gut abnormalities, malformation of pronephros, defective hatching gland and edema in pericardial sac were observed after treatment with SB. Compared to untreated littermates (vehicle-treated control), SB-treated embryos exhibited significantly reduced tactile sensitivity frequencies of touch-induced movement (vehicle-treated control: 27.60+/-1.98 v.s. 1000 ppm SB: 7.89+/-5.28; N=30). Subtle changes are easily observed by staining with specific

monoclonal antibodies F59, Znp1 and alpha6F to detect morphology changes in muscle fibers, motor axons and pronephros, respectively. Our data showed that the treatment of SB led to misalignment of muscle fibers, motor neuron innervations, excess acetyl-choline receptor cluster and defective pronephric tubes. On the basis of these observations, we suggest that sodium benzoate is able to induce neurotoxicity and nephrotoxicity of zebrafish larvae.
Descriptors: zebrafish larva, drug induced abnormalities pathology and psychology, larva anatomy and histology, sodium benzoate toxicity, teratogens, monoclonal antibodies diagnostic use, drug dose response relationship, drug effects, gastrointestinal tract abnormalities, kidney abnormalities, growth and development, motor activity drug effects, motor neurons drug effects and pathology, muscle fibers drug effects and pathology, neuromuscular junction abnormalities and pathology, phenotype, physical stimulation, cholinergic receptors drug effects and genetics, time factors.

Tseng, H.P., T.H. Hseu, D.R. Buhler, W.D. Wang, and C.H. Hu (2005). **Constitutive and xenobiotics-induced expression of a novel CYP3A gene from zebrafish larva.** *Toxicology and Applied Pharmacology* 205(3): 247-58. ISSN: 0041-008X.
NAL Call Number: 391.8T662
Abstract: In mammals, CYP3A isozymes collectively comprise the largest portion of the liver and small intestinal CYP protein. They are involved in the metabolism of an extensive range of endogenous substrates and xenobiotics and make a significant contribution to the termination of the action of steroid hormones. A full-length cDNA of CYP3A gene, named CYP3A65, was cloned from zebrafish by RT-PCR. The CYP3A65 mRNA was initially transcribed only in the liver and intestine upon hatching of the zebrafish embryos. Like the human CYP3A genes, CYP3A65 transcription in the foregut region was enhanced by treatment of the zebrafish larvae with the steroid dexamethasone and the macrocyclic antibiotic rifampicin. Differing from mammalian CYP3A genes, CYP3A65 transcription was also elicited by 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) during early larval stages. Repression of AHR2 translation by antisense morpholino oligonucleotides abrogated both of constitutive and TCDD-stimulated CYP3A65 transcription in larval intestine. These findings suggested that the AHR2 signaling pathway plays an essential role in CYP3A65 transcription.
Descriptors: zebrafish larva, aryl hydrocarbon hydroxylases genetics, developmental gene expression regulation drug effects, larva genetics, CYP3A gene, oxidoreductases, n demethylating genetics, xenobiotics pharmacology, aryl hydrocarbon hydroxylases drug effects, aryl hydrocarbon hydroxylases metabolism, dexamethasone pharmacology, developmental gene expression regulation genetics, in situ hybridization methods, intestines drug effects and embryology, larva drug effects, larva metabolism, antisense oligonucleotides pharmacology, oxidoreductases, n demethylating drug effects and metabolism, messenger RNA, aryl hydrocarbon receptors antagonists and inhibitors and drug effects, reverse transcriptase PCR methods, rifampin pharmacology, protein sequence analysis methods, signal transduction drug effects and physiology, tetrachlorodibenzodioxin antagonists, inhibitors and pharmacology, xenobiotics chemistry , zebrafish metabolism, proteins antagonists, inhibitors, drug effects and metabolism.

Van den Belt, K., P. Berckmans, C. Vangenechten, R. Verheyen, and H. Witters (2004). **Comparative study on the in vitro/in vivo estrogenic potencies of 17 beta -estradiol, estrone, 17 alpha -ethynylestradiol and nonylphenol**. *Aquatic Toxicology* 66(2): 183-195. ISSN: 0166-445X.
NAL Call Number: QH541.5.W3A6

Descriptors: zebrafish, pollution effects, sex hormones, vitellogenesis, *Danio rerio*, in vitro and in vivo testing.

Van der Ven, K., D. Keil, L.N. Moens, P.V. Hummelen, P. van Remortel, M. Maras, and W. De Coen (2006). **Effects of the antidepressant mianserin in zebrafish: molecular markers of endocrine disruption.** *Chemosphere* 65(10): 1836-45 . ISSN: 0045 6535.
NAL Call Number: TD172.C54
Abstract: Due to their environmental occurrence and intrinsic biological activity, human pharmaceuticals have received increasing attention from environmental and health agencies. Of particular, ecotoxicological concern are drugs that affect nervous- and endocrine-systems. Zebrafish genome-wide oligo arrays are used to collect mechanistic information on mianserin-induced changes in gene expression in zebrafish. Gene expression analysis in brain and gonad tissue clearly demonstrated the estrogenic activity of mianserin and its potency to disrupt normal endocrine (estrogenic) signaling, based on induction of molecular biomarkers of estrogenicity (e.g., vitellogenin1 and zona pellucida proteins). The possible mechanism underlying this estrogenic activity of mianserin is disturbance of the Hypothalamo-Pituitary-Gonadal (HPG) axis by direct interference of mianserin with the serotonergic and adrenergic systems in the brain of zebrafish. Taking into account the importance of the HPG-axis, and considering the concept of 'critical window of exposure', our results reveal the importance for more elaborate testing of endocrine disruptive effects of aquatic antidepressants at different lifestages and during longer exposure periods (e.g., life cycle studies). Although there is a low concordance between the gene expression results in this study and previous cDNA microarray hybridizations, the global mechanistic expression patterns are similar in both platforms. This argues in favor of pathway-driven analysis of gene expression results compared to gene-per-gene analysis.
Descriptors: zebrafish endocrine disruptor model, antidepressive agents adverse effects, endocrine disruptors adverse effects, gene expression regulation drug effects, mianserin adverse effects, biological markers analysis, brain drug effects, oligonucleotide array sequence analysis, ovary drug effects, PCR methods, testis drug effects, vitellogenins drug effects and genetics, proteins drug effects.

Van der Ven, K., D. Keil, L.N. Moens, K. Van Leemput, P. van Remortel, and W.M. De Coen (2006). **Neuropharmaceuticals in the environment: mianserin-induced neuroendocrine disruption in zebrafish (*Danio rerio*) using cDNA microarrays.** *Environmental Toxicology and Chemistry* 25(10): 2645-52. ISSN: 0730 7268.
NAL Call Number: QH545A1E58
Abstract: Because of their environmental occurrence and high biological activity, human pharmaceuticals have received increasing attention from environmental and health agencies. A major bottleneck in their risk assessment is the lack of relevant and specific effect data. We developed an approach using gene expression analysis in quantifying adverse effects of neuroendocrine pharmaceuticals in the environment. We studied effects of mianserin on zebrafish (*Danio rerio*) gene expression using a brain-specific, custom microarray, with real-time polymerase chain reaction as confirmation. After exposure (0, 25, and 250 microg/L) for 2, 4, and 14 d, RNA was extracted from brain tissue and used for microarray hybridization. In parallel, we investigated the impact of exposure on egg production, fertilization, and hatching. After 2 d of exposure, microarray analysis showed a clear effect of mianserin on important neuroendocrine-related genes (e.g., aromatase and estrogen receptor), indicating that antidepressants can modulate neuroendocrine processes. This initial neuroendocrine effect was followed by a "late gene expression effect" on neuronal plasticity, supporting the

current concept regarding the mode of action for antidepressants in mammals. Clear adverse effects on egg viability were seen after 14 d of exposure at the highest concentration tested. Based on the specific molecular impact and the effects on reproduction, we conclude that further investigation of the adverse effects on the brain-liver-gonad axis is needed for a correct ecological risk assessment of antidepressants.

Descriptors: zebrafish neuroendocrine toxicology model, brain drug effects, endocrine disruptors toxicity, mianserin toxicity, chemical water pollutants toxicity, base sequence, DNA primers, complementary DNA, gene expression drug effects, oligonucleotide array sequence analysis, PCR, reproduction drug effects.

Van der Ven, L.T., H. Holbech, M. Fenske, E.J. Van den Brandhof, F.K. Gielis Proper, and P.W. Wester (2003). **Vitellogenin expression in zebrafish *Danio rerio*: evaluation by histochemistry, immunohistochemistry, and in situ mRNA hybridisation.** *Aquatic Toxicology* 65(1): 1-11. ISSN: 0166 445X.

NAL Call Number: QH541.5.W3A6

Abstract: Several histological methods were tested for their potential to detect the in vivo induction of vitellogenin in zebrafish, after exposure to 17beta-oestradiol (E2), and validated by correlating semi-quantitative measurements on digital images to vitellogenin plasma values measured by ELISA and morphological criteria. All methods, except for vitellogenin-specific immunohistochemistry on liver, detected vitellogenin production in male zebrafish at the exposure level of 1 nM E2/l, and correlated well to each other and to ELISA results on plasma, thus indicating their specificity. The level of sensitivity is in the range of the induction of clinical (histopathological) effects, although slightly below the level of sensitivity of the plasma ELISA. Vitellogenin specific in situ mRNA hybridisation on liver appeared laborious and not applicable on routinely prepared material. Vitellogenin specific immunohistochemistry on plasma and basophilia of male liver are cost- and effort-effective detection methods of vitellogenin production, and can be applied routinely on standard histological sections. These methods are, therefore, suitable to evaluate vitellogenin production as an indicator of exposure to compounds with estrogenic activity, at the level of induction of clinical effects. They are a useful tool for hazard identification of endocrine disruption, especially when combined with routine histopathology.

Descriptors: zebrafish, gene expression genetics, messenger RNA genetics, vitellogenins genetics, enzyme linked immunosorbent assay, estradiol pharmacology, gene expression drug effects, immunohistochemistry, in situ hybridization, liver metabolism, myocardium metabolism, vitellogenins biosynthesis.

Van der Ven, L.T., E.J. Van den Brandhof, J.H. Vos, D.M. Power, and P.W. Wester (2006). **Effects of the antithyroid agent propylthiouracil in a partial life cycle assay with zebrafish.** *Environmental Science and Technology* 40(1): 74-81. ISSN: 0013 936X.

NAL Call Number: TD420.A1E5

Abstract: Some ubiquitous pollutants of the aquatic environment, such as PCBs or other polyhalogenated aromatic hydrocarbons, may disrupt the thyroid hormone system. In a partial life cycle assay with zebrafish (*Danio rerio*), we studied the effects of the reference compound propylthiouracil (PTU) on reproduction, growth and development, histopathology of some target tissues, and plasma thyroid hormone levels. PTU induced a concentration-dependent increase of egg production with a concomitant decrease of mature oocyte size but had no effect on fertilization rate or hatching. In F1, serious dysmorphogenesis was found in 4 dph larvae at the highest PTU level tested (100 mg/L), and there was a dose-dependent decrease in body length and weight at 42 dph (significant at 100 mg/L

PTU). At this time, there was also a decreased scale thickness, suggesting inhibited metamorphosis, detectable at 1 mg/L PTU and higher. PTU also induced activation of the thyroid follicles in a concentration-dependent way, in juveniles associated with hyperemia in the thyroid area, and depletion of liver glycogen. Effects in adults were associated with decreased circulating levels of the thyroid hormones T3 and T4. These observations indicate that disruption of the thyroid hormone system may affect the fitness of these aquatic organisms. The zebrafish model may contribute to the identification of thyroid hormone disrupting activity in water samples and also in the interpretation of histological observations in free-ranging fish species.

Descriptors: zebrafish larva, antithyroid agents toxicity, liver drug effects, propylthiouracil toxicity, thyroid gland drug effects, chemical water pollutants toxicity, biological assay, body size drug effects, body weight drug effects, larva drug effects on growth and development, life cycle stages, liver ultrastructure, liver glycogen blood, reproduction drug effects, thyroid gland ultrastructure, thyroid hormones blood.

Van der Ven, L.T., E.J. van den Brandhof, J.H. Vos, and P.W. Wester (2007). **Effects of the estrogen agonist 17beta-estradiol and antagonist tamoxifen in a partial life-cycle assay with zebrafish (*Danio rerio*).** *Environmental Toxicology and Chemistry* 26(1): 92-9. ISSN: 0730 7268.

NAL Call Number: QH545A1E58

Abstract: A partial life-cycle assay (PLC) with zebrafish (*Danio rerio*) was conducted to identify endocrine-disrupting effects of 17beta-estradiol (E2) and tamoxifen (TMX) as reference for estrogen agonist and antagonist activity. Adult zebrafish were exposed for 21 d and offspring for another 42 d, allowing differentiation of gonads in control animals. The assessed end points included reproductive variables (egg production, fertilization, and hatching), gonad differentiation of juveniles, histopathology, and vitellogenin (VTG) expression. With E2, the most sensitive end points were feminization of offspring (at 0.1 nM) and increased VTG production in males (at 0.32 nM). At 1 nM, decreased F, survival, increased F, body length and weight, VTG-related edema and kidney lesions, and inhibited spermatogenesis were observed. Oocyte atresia occurred at even higher concentrations. Exposure to TMX resulted in specific effects at an intermediate test concentration (87 nM), including oocyte atresia with granulosa cell transformation and disturbed spermatogenesis (asynchrony within cysts). In F1, decreased hatching, survival, and body weight and length as well as decreased feminization were observed. Decreased vitellogenesis and egg production in females and clustering of Leydig cells in males occurred at higher concentrations. Toxicological profiles of estrogen agonists and antagonists are complex and specific; a valid and refined characterization of endocrine activity of field samples therefore can be obtained only by using a varied set of end points, including histology, as applied in the presented PLC. Evaluation of only a single end point can easily produce under- or overestimation of the actual hazard.

Descriptors: zebrafish estrogen agonist and antagonist toxicology, estradiol pharmacology, tamoxifen pharmacology, immunohistochemistry, life cycle stages, vitellogenins metabolism.

Van der Ven, L.T., P.W. Wester, and J.G. Vos (2003). **Histopathology as a tool for the evaluation of endocrine disruption in zebrafish (*Danio rerio*).** *Environmental Toxicology and Chemistry* 22(4): 908-13. ISSN: 0730 7268.

NAL Call Number: QH545A1E58

Abstract: The importance of histology as a tool in the evaluation of endocrine disruption in fish depends on the choice and interpretation of appropriate endpoints, as is illustrated by the analysis of the effects of exposure to the estrogen 17beta-estradiol (E2) and the nonarom-

atizable androgen 17-methyldihydrotestosterone (MDHT). The E2 led to the disappearance of vitellogenic oocytes in the ovary and an increased area of relatively large, eosinophilic cells in the testis, which were identified as spermatogonia under high-power magnification; this was a relative increase, as was shown by histomorphometry, because of a decreased size of spermatogenic cysts and a relative decrease of spermatocyte cysts. The E2 also induced an accumulation of acidophilic fluid in vessels and interstitial spaces, confirmed by immunohistochemistry as vitellogenin, and basophilia in the liver also associated with the production of vitellogenin. The MDHT induced activation of Sertoli cells in the testis and a decreased presence of vitellogenic oocytes and a reduced growth of previtellogenic oocytes in the ovary. These observations indicate the advantages of examining multiple organ systems on whole-body sections and the application of adequate magnifications. Inclusion of additional techniques such as morphometry and immunohistochemistry is valuable to further uncover insidious effects of endocrine disruptors.

Descriptors: zebrafish endocrine disruptors, dihydrotestosterone analogs and derivatives, dihydrotestosterone toxicity, estradiol toxicity, chemically induced fish diseases , fish diseases pathology, air sacs drug effects and pathology, immunohistochemistry, liver drug effects and pathology, ovary drug effects and pathology, testis drug effects and pathology.

Versonnen, B.J. and C.R. Janssen (2004). **Xenoestrogenic effects of ethinylestradiol in zebrafish (*Danio rerio*).** *Environmental Toxicology* 19(3): 198-206. ISSN: 1520 4081.

NAL Call Number: RA1221.T69

Descriptors: zebrafish embryo and juvenile models for estrogens toxicity, ethinyl estradiol toxicity, vitellogenins analysis, xenobiotics toxicity, proteins analysis, age factors, biometry, body weight drug effects, drug dose response relationship, drug effects and pathology, physiology, ethinyl estradiol administration and dosage, larva anatomy, histology, drug effects and physiology, life cycle stages, molecular weight, mortality, sex factors, vitellogenins biosynthesis and blood, zebrafish anatomy, histology, proteins biosynthesis and blood proteins.

Versonnen, B.J., P. Roose, E.M. Monteyne, and C.R. Janssen (2004). **Estrogenic and toxic effects of methoxychlor on zebrafish (*Danio rerio*).** *Environmental Toxicology and Chemistry* 23(9): 2194-201. ISSN: 0730 7268.

NAL Call Number: QH545A1E58

Abstract: Although zebrafish *(Danio rerio)* have been suggested as a good candidate for screening potential endocrine disruptors, little information is available on the effects of weak estrogens on this species. We investigated the sensitivity of different life stages of zebrafish toward toxic and estrogenic properties of methoxychlor (MXC). Short-term tests with adults resulted in a sex-specific 96-h lethal concentration for 50% (LC50) of the test animals of 36 microg/L for males and 129 microg/L for females. To determine the estrogenic capacity of MXC, adult zebrafish were exposed to 0, 0.5, 5, and 50 microg MXC/L for 14 d. Induction of vitellogenin ([VTG] measured with protein electrophoresis and Western blot) in males was detected at 5 and 50 microg MXC/L. Females, however, did not exhibit higher blood VTG concentrations at the tested MXC concentrations. In a second series of experiments, juvenile zebrafish were exposed to 0, 0.05, 0.5, and 5 microg MXC/L for 33 d. Survival, length, weight, and condition of larvae were examined as indicators of toxic stress and the VTG content in whole body homogenates of juveniles was measured to determine xenoestrogenic effects. No effects of the tested concentrations of MXC were observed. Finally, the effect of MXC on zebrafish eggs, exposed to 0, 1, 10, and 32 microg MXC/L, was examined. Hatching and survival of hatched zebrafish were affected at 10 and 32 microg MXC/L. This study demonstrated that adult male zebrafish are sensitive toward the estrogenic effects of MXC.

However, the use of VTG induction to detect effects of (xeno)estrogens in early life stages has to be further investigated, as low concentrations of VTG were detectable in exposed as well as unexposed juvenile fish.
Descriptors: male zebrafish, estrogens toxicity, insecticides toxicity, methoxychlor toxicity, pesticide residues toxicity, chemical water pollutants toxicity, lethal dose 50, no observed adverse effect level, time factors, vitellogenins biosynthesis, vitellogenins blood , zebrafish embryology, growth and development.

Villano, C.M. and L.A. White (2006). **The aryl hydrocarbon receptor-signaling pathway and tissue remodeling: insights from the zebrafish (*Danio rerio*) model system.** *Toxicological Sciences* 92(1): 1-4. ISSN: 1096-6080.
NAL Call Number: RA1190.F8
Descriptors: zebrafish model, aryl hydrocarbon receptors metabolism, signal transduction, tetrachlorodibenzodioxin toxicity, .
Notes: Comment On: Toxicol Sci. 2006; 92(1):254-69
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Vobach, M. and U. Kammann (2003). **The fish egg assay, a test system for ecotoxicological assessment.** *Informationen Fur Die Fischwirtschaft Aus Der Fischereiforschung* 50(3): 126-130. ISSN: 1437-5842.
NAL Call Number: SH1.I 584
Descriptors: zebrafish egg assay, embryonic development, ecotoxicological assessment, toxicity tests, *Danio rerio.*
Language of Text: German.

Voelker, D., N. Stetefeld, K. Schirmer, and S. Scholz (2008). **The role of cyp1a and heme oxygenase 1 gene expression for the toxicity of 3,4-dichloroaniline in zebrafish (*Danio rerio*) embryos**. *Aquatic Toxicology* 86(1): 112-120. ISSN: 0166-445X.
NAL Call Number: QH541.5.W3A6
Descriptors: zebrafish embryos, 3,4-dichloroaniline, contaminants, cytochromes, gene expression, heme oxygenase (decyclizing), toxicity, transcription, siRNA, *Danio rerio.*

Voelker, D., C. Vess, M. Tillmann, R. Nagel, G.W. Otto, R. Geisler, K. Schirmer, and S. Scholz (2007). **Differential gene expression as a toxicant-sensitive endpoint in zebrafish embryos and larvae.** *Aquatic Toxicology* 81(4): 355-64. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: The zebrafish (*Danio rerio*) embryo toxicity test (DarT) is under consideration as an alternative to the acute fish toxicity test. Microscopically visible developmental disorders or death are the endpoints used to report on toxicity in DarT. These endpoints are easily observed. They, however, rarely reveal mechanisms leading to a toxic effect and are relatively insensitive compared to chronic toxic effects. We hypothesized that, by using gene expression profiles as an additional endpoint, it may be possible to increase the sensitivity and predictive value of DarT. Therefore, as a proof of principle, we exposed zebrafish embryos to the reference compound 3,4-dichloroaniline (3,4-DCA) and analyzed gene expression patterns with a 14k oligonucleotide array. Important stress response genes not included in the microarray were additionally quantified by reverse transcriptase polymerase chain reaction. Six genes involved in biotransformation (cyp1a, ahr2), stress response (nfe212, maft, hmox1) and cell cycle control (fzr1) were significantly regulated. With the exception of fzr1, these genes proved to be differentially expressed in post hatch life stages as well. The identi-

fied genes point toward an aryl hydrocarbon receptor-mediated response. Differential gene expression in embryos exposed for 48 h was observed at 3,4-DCA concentrations as low as 0.78 microM, which is more than 10-fold below the concentrations that elicited visible toxic effects. Upon exposure for 5 days, differential expression was detected at concentrations as low as 0.22 microM of 3,4-DCA, which was close to the lowest observed effect concentration (0.11 microM) in the 30-day early life stage test. This study therefore indicates that gene expression analysis in DarT is able to reveal mechanistic information and may also be exploited for the development of replacement methods for chronic fish tests.
Descriptors: zebrafish embryos and larva, endpoint determination methods, DarT, gene expression profiling methods, gene expression regulation drug effects, toxicity tests methods, metabolism, aniline compounds toxicity, DNA primers, drug effects, metabolism, larva drug effects and metabolism, oligonucleotide array sequence analysis, reverse transcriptase PCR.

Voslarova, E., V. Pistekova, and Z. Svobodova (2006). **Nitrite toxicity to *Danio rerio*: effects of fish age and chloride concentrations**. *Acta Veterinaria, Brno* 75(1): 107-113. ISSN: 0001 7213.
NAL Call Number: SF604.B7
Abstract: The aim of this study was to compare the acute nitrite toxicity in *Danio rerio* aged 20-25 days (larval stage) and 2-3 months (juvenile stage) at various chloride concentrations. The semistatic method according to OECD 203 was used in the tests. A total of 6 experiments with different NO2- and Cl- concentrations in the diluting water were undertaken. In each test series, 5 tests of acute toxicity were made, with 10 fish used for each concentration and for the control group. The 96hLC50 NO2- values were 386.00+or-29.75 mg/litre (*D. rerio* aged 20-25 days) and 242.41+or-13.67 mg/litre (D. rerio aged 2-3 months), respectively, at 19 mg Cl-/litre in diluting water. An increase in 96hLC50 NO2- was observed in *D. rerio* of both developmental stages following an increase in Cl- concentration in diluting water. A comparison between the 96hLC50 NO2- determined for *D. rerio* aged 20-25 days and those aged 2-3 months at different chloride concentrations in diluting water (19, 50 and 100 mg/litre) showed a highly significant increase ($p<0.01$) in the 96hLC50 NO2- for the younger fish at all chloride concentrations. An exponential relationship between 96hLC50 NO2- values and chloride concentrations in diluting water (I=0.849) was demonstrated in *D. rerio* aged 20-25 days, whereas a logarithmical relationship (I=0.856) was observed in *D. rerio* aged 2-3 months. Copyright © 2008 CABI
Descriptors: zebrafish larva and juvenile, age differences, chloride, nitrite, toxicity, toxicology, *Danio rerio.*
Language of Text: Czech.

Voth, D.E., E.E. Hamm, L.G. Nguyen, A.E. Tucker, I.I. Salles, W. Ortiz-Leduc, and J.D. Ballard (2005). ***Bacillus anthracis* edema toxin as a cause of tissue necrosis and cell type-specific cytotoxicity**. *Cellular Microbiology* 7(8): 1139-1149. ISSN: 1462 5814.
NAL Call Number: QR1 .C45
Abstract: Oedema factor (OF) and protective antigen (PA) are secreted by *Bacillus anthracis*, and their binary combination yields oedema toxin (OT). Following PA-mediated delivery to the cytosol, OF functions as an adenylate cyclase generating high levels of cAMP. To assess OT as a possible cause of tissue damage and cell death, a novel approach was developed, which utilized a developing zebrafish embryo model to study toxin activity. Zebrafish embryos incubated with OT exhibited marked necrosis of the liver, cranium and gastrointestinal tract, as well as reduced swim bladder inflation. The OT-treated embryos survived after all stages of development but succumbed to the toxin within 7 days. Additional analysis of specific cell lines, including macrophage and non-macrophage, showed OT-induced

cell death is cell type-specific. There was no discernible correlation between levels of OF-generated cAMP and cell death. Depending on the type of cell analysed, cell death could be detected in low levels of cAMP, and, conversely, cell survival was observed in one cell line in which high levels of cAMP were found following treatment with OT. Collectively, these data suggest OT is cytotoxic in a cell-dependent manner and may contribute to disease through direct cell killing leading to tissue necrosis. Copyright©Thomson Reuters 2009
Descriptors: zebrafish edema toxin model, toxicology, infection, *Bacillus anthracis* infection, bacterial disease, pathology, etiology.

Wang Buhler, J.L., S.J. Lee, W.G. Chung, J.F. Stevens, H.P. Tseng, T.H. Hseu, C.H. Hu, M. Westerfield, Y.H. Yang, C.L. Miranda, and D.R. Buhler (2005). **CYP2K6 from zebrafish (*Danio rerio*): cloning, mapping, developmental/tissue expression, and aflatoxin B1 activation by baculovirus expressed enzyme.** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 140(2): 207-19. ISSN: 1532 0456.
NAL Call Number: QP901.C6
Abstract: A full-length zebrafish (*Danio rerio*) cytochrome P450 (CYP) 2K6 cDNA, was obtained (GenBank accession No. AF283813) through polymerase chain reaction cloning using degenerated primers based on a consensus CYP2 sequence and the heme-binding domain. This first CYP2K family member cloned from zebrafish had 1861 bp which contained 27 bp of 5'-untranslated region (5'-UTR), an open reading frame (ORF) of 1518 bp, and a 300 bp 3'-UTR with a poly A tail. The deduced 506 amino acid sequence of CYP2K6 had 63%, 62% and 59% identity with rainbow trout CYP2K1, CYP2K4 and CYP2K3, respectively; and 45%, 42%, and 42% identity with rabbit CYP2C1, human CYP2C19 and mouse CYP2C39, respectively. CYP2K6 mapped to 107.49cR on LG3 using the LN54 radiation hybrid panel. Its mRNA was detected at 5 days post-fertilization and in the adult liver and ovary among nine tissues examined. The ORF, including the 27 bp of the 5'-UTR, was cloned into pFastBac donor vector and then transferred into the baculovirus genome (bacmid DNA) in DH10Bac competent cells. The recombinant bacmid DNA was used to infect Spodoptera frugiperda insect cells to express the CYP2K6 protein (Bv-2K6). As its ortholog, rainbow trout Bv-2K1 [Yang, Y.H., Miranda, C.L., Henderson, M.C., Wang-Buhler, J.-L., Buhler, D.R., 2000. Heterologous expression of CYP2K1 and identification of the expressed protein (Bv-2K1) as lauric acid (omega-1)-hydroxylase and aflatoxin B1 exo-epoxidase. Drug Metab. Disp. 28,1279-83.], Bv-2K6 also catalyzed the conversion of aflatoxin B1 (AFB1) to its exo-8,9-epoxide as assessed by the trapping of a glutathione (GSH) adduct in the presence of a specific mouse alpha class glutathione S-transferase. The identity of the AFB1-GSH adduct was verified by liquid chromatography-mass spectrometry (LC-MS) and mass spectrometry-mass spectrometry (MS-MS) analysis. Although rainbow trout Bv-2K1 was capable of oxidizing lauric acid, zebrafish Bv-2K6 protein showed no activity against this substrate.
Descriptors: zebrafish aflatoxin B1 metabolism, aryl hydrocarbon hydroxylases genetics, alkane 1 monooxygenase metabolism, amino acid sequence, aryl hydrocarbon hydroxylases biosynthesis, Baculoviridae, base sequence, biotransformation, high pressure liquid chromatography, liquid chromatography, molecular cloning fish gene library, mass spectrometry, molecular sequence data, PCR, recombinant proteins metabolism, sequence alignment, spodoptera, steroid hydroxylases genetics, tissue distribution, zebrafish growth and development.

Wang, P.J., M.S. Chien, H.N. Chou, and S.J. Lee (2005). **Microcystin-LR nhibits zebrafish mbryonic development**. *Journal of the Fisheries Society of Taiwan* 32(1): 101. ISSN: 0379-4180.
NAL Call Number: SH135.T3

Descriptors: zebrafish, abnormalities, bioaccumulation, biological poisons, embryonic development, freshwater fish, inhibitors, phytoplankton, toxicity tests, *Danio rerio*, reproduction and development, effects on organisms.

Wang Pei, J., S. Chien Ming, J. Wu Fong, N. Chou Hong, and J. Lee Shyh (2005). **Inhibition of embryonic development by microcystin-LR in zebrafish, *Danio rerio*.** *Toxicon* 45(3): 303-308. ISSN: 0041 0101.

NAL Call Number: 391.8T66

Abstract: Microcystin-LR (MC-LR), a cyanobacterial toxin, is a potent inhibitor of protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A). PP1 and PP2A are critical regulators in embryonic development. However, the effects of MC-LR in embryonic development have been controversial. MC-LR has been demonstrated to be highly toxic in medaka, but not in zebrafish or rabbit embryos. The causes of difference may be due to membrane impermeability that impaired the delivery of MC-LR into cytoplasm of zebrafish and rabbit embryos. Therefore, we microinjected MC-LR directly into developing zebrafish embryos and investigated the effects of MC-LR on embryonic development. We demonstrated that MC-LR induced the lethality of zebrafish embryos in a dose- and time-dependent manner. MC-LR also induced the loss of blastomere coherence via the interference of beta -catenin and cadherins distributions. Furthermore, the MC-LR treated fry revealed various developmental defects. These results suggested that MC-LR might affect the phosphorylation equilibrium of signaling molecules, including beta -catenin and cadherins, required early in zebrafish embryonic development. Copyright © 2008 CABI

Descriptors: zebrafish embryo, blastomere, cytotoxicity, microcystin-LR, embryonic development, lethal dose, *Danio rerio.*

Wang Wei, D.J. Cai, Z.J. Shan, W.L. Chen, N. Poletika, and X.W. Gao (2007). **Comparison of the acute toxicity for gamma-cyhalothrin and lambda-cyhalothrin to zebra fish and shrimp** . *Regulatory Toxicology and Pharmacology* 47(2): 184-188. ISSN: 0273 2300.

NAL Call Number: RA1190.R42

Abstract: Gamma-cyhalothrin 15CS (GCH) contains only the active stereoisomer of the two isomers found in lambda-cyhalothrin 25EW (LCH). GCH (0.5 x rate) provides equivalent overall insect control as LCH (1 x rate). Both formulations showed high acute toxicity to zebra fish (*Brachydanio rerio* H.B.) and shrimp (*Macrobrachium nippoensis* de Haan). The 96-h LC50(zebra fish,GCH) is 1.93 micro g a.i/L and LC50(zebra fish,LCH) is 1.94 micro g a.i/L. LC50(shrimp,GCH) is 0.28 micro g a.i./L and LC50(shrimp,LCH) 0.04 micro g a.i./L. This indicates that the toxicity to shrimp is likely stereochemistry-dependent. The fates of GCH and LCH are similar in laboratory simulated rice paddy water and their concentrations decrease rapidly, with no GCH or LCH detected after 3 or 4 days. Both are toxic to shrimp in a simulated paddy irrigation reservoir even though treated return water is diluted 5 times. No shrimp fatality is shown in the GCH-treated paddy water after a 4-day holding period, and longer than 5 days is necessary to reach a zero fatality rate for LCH. This is compatible with the 7-day water holding period considered reasonable in agricultural practice. Copyright © 2008 CABI

Descriptors: zebrafish, insecticide residues, insecticides, lambda cyhalothrin, nontarget effects, nontarget organisms, toxicity, *Danio rerio*, *Macrobrachium*.

Wang, Y.H., Y.H. Chen, T.N. Wu, Y.J. Lin, and H.J. Tsai (2006). **A keratin 18 transgenic zebrafish Tg(k18(2.9):RFP) treated with inorganic arsenite reveals visible overproliferation of epithelial cells.** *Toxicology Letters* 163(3): 191-7. ISSN: 0378-4274.

NAL Call Number: RA1190.T62

Abstract: Inorganic arsenic has strong human carcinogenic potential, but the availability of an animal model to study toxicity is extremely limited. Here, we used the transgenic zebrafish line Tg(k18(2.9):RFP) as an animal model to study arsenite toxicity. This line was chosen because the red fluorescent protein (RFP) is expressed in stratified epithelia (including skin), due to the RFP reporter driven by the promoter of the zebrafish keratin 18 gene. We titrated doses of inorganic arsenite for zebrafish embryos and found that arsenite exposure at 50 microM for 120 h was suitable for mimicking a long-term, chronic effect. When embryos derived from Tg(k18(2.9):RFP) adults were treated with this arsenite dose and time of exposure, abnormal phenotypes were not noticeable under the light microscope. However, arsenic keratosis was visible in the epithelial cells under the fluorescent microscope. Morphological defects became more severe with increased dose and exposure duration, suggesting that the severity of skin lesions was dose- and time-dependent. Histochemical examination of keratosis after 4',6'-diamidino-2-phenylindole hydrochloride (DAPI) staining showed that the epithelial cells overproliferated after treatment with arsenite. Therefore, this Tg(k18(2.9):RFP) zebrafish line is an excellent model for studying toxicity induced by inorganic arsenite and may have potential for studying other environmental pollutants.

Descriptors: zebrafish model for arsenites toxicity, epithelial cells drug effects, keratins genetics, keratosis chemically induced, teratogens toxicity, genetically modified, cell proliferation drug effects, drug dose response relationship, drug effects, epithelial cells metabolism and pathology, keratin 18, keratosis pathology, linear models, luminescent proteins biosynthesis and genetics, fluorescence microscopy, promoter regions genetics, random allocation, skin drug effects, metabolism and pathology, toxicity tests methods.

Watral, V. and M.L. Kent (2007). **Pathogenesis of *Mycobacterium* spp. in zebrafish (*Danio rerio*) from research facilities.** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 145(1): 55-60. ISSN: 1532 0456.

NAL Call Number: QP901.C6

Abstract: One of the most common diseases that we have diagnosed in zebrafish is mycobacteriosis, caused by several *Mycobacterium* spp. The severity of the disease ranged from severe outbreaks to incidental infections. We conducted an in vivo study to evaluate the pathogenesis of six isolates of *Mycobacterium* from zebrafish with mycobacteriosis from four research facilities and one wholesale supplier of zebrafish in the United States: Mycobacterium abscessus, Mycobacterium peregrinum, *Mycobacterium chelonae* (2 isolates), and *Mycobacterium marinum*. We also included two isolates of *M. marinum* from other fishes. Fish were exposed by intraperitoneal injection at a target does of 5 x 10(4) bacteria/fish, and were held in static aquaria at 28 degrees C for 8 weeks. Fish were examined by histology and culture, and mortalities were recorded. The *M. marinum* isolates caused 100% infection and mortality between 30% and 100%. None of the other *Mycobacterium* species caused significant mortalities, but several of these fish had granulomatous lesions in visceral organs. *Mycobacteria* were consistently recovered in culture from fish exposed to *M. marinum*, and from only 9% of fish exposed to the other species. This study suggests that, of the isolates tested, only *M. marinum* is highly pathogenic and virulent to healthy zebrafish.

Descriptors: zebrafish *Mycobacterium* infections, fish diseases microbiology, fish diseases pathology, *Mycobacterium* infections microbiology, pathology and mortality, zebrafish microbiology, fish diseases mortality, granuloma microbiology and pathology, *Mycobacterium chelonae*, *Mycobacterium marinum*, laboratory facility, pathogenicity of various species.

Weber, D.N. (2006). **Dose-dependent effects of developmental mercury exposure on c-start escape responses of larval zebrafish *Danio rerio***. *Journal of Fish Biology* 69(1): 75-94. ISSN: 0022 1112.

NAL Call Number: QL614.J68

Abstract: Zebrafish *Danio rerio* embryos were exposed to 0, 25, 50 or 75 ppb Hg2+ from 0 to 24 h post-fertilization (hpf) then placed into Hg2+-free water. Inductively coupled plasma-mass spectrophotometer analysis of whole embryo Hg2+ content at 24 hpf showed a positive correlation with exposure regime (Pearson's one-tailed, r(2) = 0.698, P < 0(.)01); at 5 days post-hatch (dph), whole larval Hg2+ content was not detectable. Hg2+-induced behavioural deficits in larvae were, therefore, due to changes during embryogenesis and not to residual Hg2+ in the larvae. At 5 dph, larvae were tested for responses to different frequencies but equal intensities of vibrational stimuli generated by a remotely controlled plastic hammer. Data were recorded by high-speed videography and computer-analysed for latency of response (ms), amplitude of the response as measured by maximum initial velocity [normalized as body (standard) lengths s(-1); V-max] and duration of behaviour from initial head movement to cessation of caudal tail movement (ms). A single mechanical stimulus resulted in behavioural outcomes that were related to embryonic Hg2+ uptake. Response latency increased with exposure level and displayed an increase of x 1.5-2.5 over control values (ANOVA, P < 0.01). The V-max decreased with exposure level to a low of 71% of control at the highest Hg2+ concentration (ANOVA, P < 0.01). Duration of behaviour displayed a biphasic response pattern in which exposure to 0, 50 or 75 ppb Hg2+ did not result in a significantly different response yet exposure to 25 ppb Hg2+ caused a significantly longer time of active response (ANOVA, P < 0.01). Repeated stimulation (1, 2 or 4 hits s(-1)) resulted in a concentration-dependent increase in response failures. Regardless of stimulation frequency, larvae exposed to 0 or 25 ppb Hg2+ as embryos maintained higher V-max levels for longer intervals during the testing period than those exposed as embryos to either 50 or 75 ppb Hg2+. (c) 2006 The Author. Copyright©Thomson Reuters 2009

Descriptors: zebrafish embryos, behavior, toxicology, development, biosynchronization, mercury, embryogenesis, mechanical stimulus, vibrational stimulus, concentration sensitive behavioral response.

Weber, L.P., R.L.J. Hill, and D.M. Janz (2003). **Developmental estrogenic exposure in zebrafish (*Danio rerio*): II. Histological evaluation of gametogenesis and organ toxicity.** *Aquatic Toxicology* 63(4): 431-46. ISSN: 0166 445X.

NAL Call Number: QH541.5.W3A6

Abstract: Aquatic species can be exposed to endocrine disrupting chemicals (EDCs) in wastewater that often includes the weak estrogen, 4-nonylphenol (NP) and the potent estrogen, 17alpha-ethinylestradiol (EE). The goal of the present study was to determine concentration-dependent effects of developmental exposure to NP and EE on gametogenesis, as well as gonad, kidney and liver pathology using quantitative histological evaluation of hematoxylin/eosin-stained saggital sections of zebrafish (*Danio rerio*). The major finding of the present study was that exposure to NP (>/=100 microg/l nominal) and EE (>/=1 ng/l nominal) from 2 to 60 days post-hatch (dph) caused concentration-dependent suppression of gametogenesis in both male and female zebrafish. Severe kidney pathology was observed in 60 dph zebrafish, specifically glomerular dilation or degeneration, fibrosis, tubule enlargement and tubule necrosis, at a threshold of 10 ng/l EE. However, minor kidney histopathology indicated by increased pyknotic nuclei in kidney tubule and interstitial (hematopoietic) cells was detected at lower estrogenic exposures (>/=10 microg/l NP nominal) than delayed gametogenesis. Considering all histological parameters in the current

study, the rank order of potency for pathological effects in 60 dph zebrafish was 10 ng/l EE>1 ng/l EE=100 microg/l NP>30 microg/l NP>10 microg/l NP10 (nominal concentrations). Zebrafish from the same cohort examined in the current study that had been placed in clean water from 60 to 300 dph had histologically normal testes and no kidney or liver histopathology. However, increased ovarian follicle atresia was detected at 300 dph in zebrafish exposed developmentally to 100 microg/l NP. Therefore, we conclude that functional rather than morphological changes may be more important for future evaluations of developmental exposure to estrogens in fish, and that negative effects in female rather than male gonads may contribute to prolonged breeding impairment.

Descriptors: zebrafish larva toxicity, estradiol congeners toxicity, ethinyl estradiol analogs, derivatives and toxicity, gametogenesis drug effects, phenols toxicity, sex differentiation drug effects, abnormalities, drug dose response relationship, estradiol congeners pharmacology, chemically induced hermaphroditism, kidney drug effects and pathology, larva drug effects, growth and development, liver drug effects and pathology, ovary drug effects and pathology, testis drug effects and pathology, anatomy and histology.

Williams, F.E. and W.S.J. Messer (2004). **Muscarinic acetylcholine receptors in the brain of the zebrafish (*Danio rerio*) measured by radioligand binding techniques.** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 137(4): 349-53. ISSN: 1532 0456.

NAL Call Number: QP901.C6

Abstract: Muscarinic acetylcholine receptors (mAChRs) play a role in learning, memory and behavior in vertebrate animals. We measured the muscarinic cholinergic receptor levels in extracts from zebrafish (*Danio rerio*) brain by radioligand binding techniques. Saturation binding experiments with the radioligand [3H]-quinuclidinyl benzilate (QNB) were used to determine receptor number and relative affinity for several agonists and antagonists. Affinity at zebrafish brain receptors was relatively high with a K(d) of 40 +/- 5 pM. The number of receptors, represented by Bmax, was 63 +/- 16 fmol/mg protein. Oxotremorine and carbachol, agonists at muscarinic acetylcholine receptors, bound with displacement curves indicating multiple binding sites. In addition, oxotremorine bound with a higher affinity than did carbachol. The antagonist potency profile at zebrafish receptors in brain was determined to be atropine>>pirenzipine>p-fluoro-hexahydro-sila-difenidol>>otenzepad. The results obtained with zebrafish brain compare favorably to those found in insect, fish and mammalian species. Taken together, the binding results and favorable comparisons to mammalian systems indicate that zebrafish may provide a useful model organism for evaluating the role of cholinergic systems in learning, memory and behavior.

Descriptors: zebrafish cholinergic system model, brain metabolism, receptors, muscarinic metabolism, kinetics, muscarinic agonists metabolism and pharmacology, muscarinic antagonists metabolism and pharmacology, radioligand assay.

Williams, F.E., T.J. Sickelbaugh, and E. Hassoun (2006). **Modulation by ellagic acid of DCA-induced developmental toxicity in the zebrafish (*Danio rerio*).** *Journal of Biochemical and Molecular Toxicology* 20(4): 183-90. ISSN: 1095-6670.

Abstract: The ability of ellagic acid (EA) to modulate dichloroacetic acid (DCA)-induced developmental toxicity and oxidative damage was examined in zebrafish embryos. Embryos were exposed to 20 mM EA administered concomitantly with 32 mM DCA at 4 hours post-fertilization (hpf) and 20 h later. Embryos were observed through 144 hpf for developmental malformations, and production of superoxide anion (SA) and nitric oxide (NO) was determined in embryonic homogenates. DCA was shown to produce developmental abnormalities

and significant levels of SA and NO in zebrafish embryos. EA exposure alleviated the developmental malformations observed in treated embryos and decreased the levels of SA and NO in those same embryos. Less than 10% of DCA + EA exposed embryos showed developmental malformations compared to 100% of embryos treated with DCA alone. Animals in this group that developed malformations were shown to have fewer defects than those treated with DCA only. Taken together, the results confirm the involvement of oxidative stress in the developmental toxicity of DCA in zebrafish embryos, and suggest possible protection against those effects with the use of antioxidants. 2006 Wiley Periodicals, Inc.

Descriptors: zebrafish embryos, dichloroacetate toxicity, ellagic acid pharmacology, embryo drug effects, metabolism, heart rate drug effects, nitric oxide biosynthesis, superoxides metabolism.

Wright, A.D., O. Papendorf, G.M. Konig, and A. Oberemm (2006). **Effects of cyanobacterium *Fischerella ambigua* isolates and cell free culture media on zebrafish (*Danio rerio*) embryo development.** *Chemosphere* 65(4): 604-8. ISSN: 0045 6535.

NAL Call Number: TD172.C54

Abstract: The toxic effects of several species of fresh water cyanobacteria, notably Microcystis species and associated toxins, the microcystins, Anabaena species (anatoxin), *Nodularia sp. (nodularin*), and *Cylindrospermopsis raciborskii (cylindrospermopsin*), are well known. Little, however, is known about the effects of secondary metabolites other than alkaloids. Early life stage tests with zebrafish (*Danio rerio*) were used to detect bioactive properties of compounds released by healthy cyanobacteria (*Fischerella ambigua*), particularly on the early developmental stages of fish. This approach, using *F. ambigua* is probably most valuable as it shows the toxicity of healthy growing cyanobacteria. The effects of cyanobacterial secondary metabolites on the embryonic stages of fish are of considerable interest as many aquatic creatures, particularly fish, are unable to avoid the potential toxins that may be released by undesirable algal blooms or as a result of allelopathic effects. In the current study, the zebrafish (*D. rerio)* was used as a model experimental system to investigate the effects of ambigols A and C, tjipanazole D and C, 2,4-dichlorobenzoic acid, cell free culture media, and media extracts of a terrestrial/fresh water strain of the cyanobacterium F. ambigua on embryo development. Fish embryo tests performed with the cell free culture medium showed that after 3h of exposure to undiluted culture medium all fish embryos died. At a tenfold dilution the process of epiboly (formation of the gastrula) was retarded in all embryos, lesions were observed, and their general development was significantly arrested, finally followed by death. The same tests performed with extracts (dichloromethane, n-butanol, and residual cell free culture medium) of the cell free culture medium, ambigol A, ambigol C, 2,4-dichlorobenzoic acid and tjipanazole D showed only ambigol A to have an influence on zebrafish development at concentrations>or=1 mg/l (2.06 microM). After 55 h all embryos showed pectoral oedema, irregularly shaped fin folds, bent tails, and unusual circular neoplasms in the dorsal tail fin fold. Due to the high concentration of ambigol A used in this assay these effects were considered to be of minor importance when compared to those of the culture medium.

Descriptors: zebrafish embryos, biphenyl compounds toxicity, chlorobenzenes toxicity, chlorobenzoates toxicity, cyanobacteria chemistry, embryonic development drug effects, phenols toxicity, biphenyl compounds isolation and purification, carbazoles isolation and purification and toxicity, cell free system, chlorobenzenes isolation and purification, chlorobenzoates isolation and purification, culture media , drug effects, molecular structure, phenols isolation and purification.

Xi Wei, P. and X.e.a. Huang Yi (2004). **Acute toxicity to *Brachydanio rerio* of qiangluocide powder**. *Chinese Journal of Schistosomiasis Control* 16(1): 63-64. ISSN: 1005 6661.
Descriptors: zebrafish, pesticides, qiangluocide powders, toxicity, *Danio rerio.*
Language of Text: Chinese.

Xu, C., J. Wang, W. Liu, G. Sheng, Y. Tu, and Y.u.n. Ma (2008). **Separation and aquatic toxicity of enantiomers of the pyrethroid insecticide lambda-cyhalothrin**. *Environmental Toxicology and Chemistry* 27(1): 174-181. ISSN: 0730-7268.
NAL Call Number: QH545A1E58
Descriptors: zebrafish, benzoic acid, c.d., cellulose, cyhalothrin, edema, embryos, enantiomers, ethanol, high performance liquid chromatography, insecticides, mortality, pesticides (carbamates), pollutants, pyrethroids, temperature effects, toxicity, yolk sac, stationary phase, *Danio rerio*, agrochemicals.

Yang, F.X., Y. Xu, and Y. Hui (2006). **Reproductive effects of prenatal exposure to nonylphenol on zebrafish (*Danio rerio*).** *Comparative Biochemistry and Physiology. Part C: Toxicology and Pharmacology* 142(1-2): 77-84. ISSN: 1532 0456.
NAL Call Number: QP901.C6
Abstract: Mature female and male zebrafish were separated and exposed to nonylphenol (NP) at 0.1, 1, 10, 50, 100 and 500 microg/L, respectively, for 3 weeks. Gonadosomatic index (GSI) in both sexes and vitellogenin (VTG) induction in males was measured as the bioindicators for the impairment to the parents. The results indicated that 50 microg/L of NP was the non-observed effect concentration (NOEC) for GSI and VTG induction. Afterwards, the 50 microg/L NP exposed females and males, and the control females and males were cross-wise pair-bred in the control water for one week to examine the reproductive effects. The embryonic cathepsin D (CAT D) activity, eggshell thickness, fecundity, hatching rate and malformation (vertebral column flexure) rate of offspring were determined in the four pair-bred groups. While endpoints remained unchanged in the groups with exposed males, prenatal exposure of females to 50 microg/L of NP resulted in the impairment of reproduction in groups with exposed females including inhibition of CAT D activity ($P < 0.05$), decrease of eggshell thickness (by 23.6%) and elevation of malformation rate ($P < 0.001$). These results suggested NP could induce reproductive damage to zebrafish at NOEC for parents. The results also imply that alterations of CAT D activity and eggshell thickness may be more sensitive biomarkers to indicate the reproductive effects caused by endocrine disrupting chemicals.
Descriptors: zebrafish, endocrine disruptors pharmacology, phenols pharmacology, reproduction drug effects , drug induced abnormalities etiology, body weight drug effects, cathepsin Dmetabolism, gonads anatomy, histology and drug effects, larva drug effects, organ size drug effects, ovum drug effects, vitellogenins metabolism.

Yang, H.P., C. Carmichael, Z.M. Varga, and T.R. Tiersch (2007). **Development of a simplified and standardized protocol with potential for high-throughput for sperm cryopreservation in zebrafish *Danio rerio*.** *Theriogenology* 68(2): 128-136. ISSN: 0093 691X.
NAL Call Number: QP251.A1T5
Abstract: Sperm cryopreservation offers potential for long-term storage of genetic resources. However, the current protocols for zebrafish *Danio rerio* are cumbersome and poorly reproducible. Our objective was to facilitate adoption of cryopreservation by streamlining methods from sperm collection through thawing and use. First, sperm activation was evaluated, and motility was completely inhibited when osmolality of the extender was >=295-300

mOsmol/kg. To evaluate cryoprotectant toxicity, sperm were incubated with dimethyl sulfoxide (DMSO), N,N-dimethyl acetamide (DMA), methanol, or glycerol at 5, 10, and 15% concentrations. Based on motility, DMSO, DMA, and methanol (<=10%) were less toxic; therefore, sperm were cryopreserved using these cryoprotectants at cooling rates of 10 and 20 degrees C/min. The highest motility (mean+or-S.D.) (35+or-23%; P<=0.0001) and fertility (13+or-8%; P<=0.001) in thawed sperm were obtained with the combination of 8% methanol and a cooling rate of 10 degrees C/min. Further evaluations of 8% methanol and 10 degrees C/min were performed with males from populations with high (2.05+or-0.24) and low (1.18+or-0.12) body condition (P=0.0001). Motility of thawed sperm from the two populations was 38+or-16% (range, 10 to 60%) and 78+or-10% (50 to 90%) (P=0.0001), and fertilization was 6+or-6% (0 to 18%) and 33+or-20% (5 to 81%) (P=0.0001). These values were positively related with body condition factor. Overall, this study simplified and standardized sperm cryopreservation, and established a protocol using French straws as a freezing container and an extender without powdered milk. This protocol can be readily adapted for high-throughput application using automated equipment, and motility and fertility comparable to previous reports were obtained. Male variability and sperm quality remain important considerations for future work, especially in mutant and inbred lines. Copyright © 2008 CABI

Descriptors: zebrafish, body condition, collection, cooling, cryopreservation, cryoprotectants, dimethyl sulfoxide, fertility, fertilization, glycerol, methanol, methodology, semen diluent additives and preservation, spermatozoa, techniques, thawing, *Danio rerio.*

Yang, N., Z. Liu, H. Wang, and J.i. Li (2006). **Joint toxicity effects of PCP,2-CP and 2,4-DCP to zebrafish**. *Research on Environmental Science/Huanjing Kexue Yanjiu* 19(6): 145-148. ISSN: 1001-6929.

Descriptors: zebrafish, chemical pollutants, marine fish, organic compounds, toxicity, *Danio rerio.*

Yeo, M.K. and M. Kang (2006). **Photodecomposition of bisphenol A on nanometer-sized TiO2 thin film and the associated biological toxicity to zebrafish (*Danio rerio*) during and after photocatalysis.** *Water Research* 40(9): 1906-14. ISSN: 0043-1354.

NAL Call Number: TD420.W3

Abstract: We investigated the relationship between the TiO2 photocatalytic decomposition of bisphenol A and biological toxicity to zebrafish (*Danio rerio*). TiO2 particles, which prepared using a solvothermal method, were applied to produce a nanometer-sized TiO2 thin film. An alcoholic solution containing the TiO2 particles and an inorganic binder was directly coated on the UV-lamp substrate. It was equipped in a photoreactor that was manufactured in our laboratory. The attachment of the thin TiO2 film to the UV-lamp substrate resulted in a stable and transparent coating. The TiO2 particles on the thin film were approximately 20-30 nm in size, and the resulting film thickness was approximately 200 nm after a single coat. The bisphenol A, which was eluted from epoxy resin in a drinking water tank, was completely degraded by the TiO2 photocatalysis. We initially detected approximately 7.8 ng/ml of bisphenol A in the epoxy-resin tank, but its concentration was undetectable after a 48-h photocatalytic reaction over TiO2. We observed a decreased survival rate in zebrafish that were reared in water exposed to the leaching process of the epoxy resin. After the photocatalysis, however, no toxic effects on the hatching rates or morphogenesis of the zebrafish were observed. In summary, toxicity during the TiO2 photocatalysis was observed; however, toxicity was no longer observed once the bisphenol A was completely decomposed by the TiO2 photocatalysis. On the basis of these experimental observations, we suggest that TiO2

photocatalysis can be adopted as a treatment method to purify an epoxy-resin tank.
Descriptors: zebrafish bisphenol A toxicity model, environmental monitoring methods, phenols chemistry, titanium chemistry, bisphenol A, water purification methods, phenols toxicity, photochemistry, ultraviolet rays.

Yu, R.M., C.C. Lin, P.K. Chan, E.S. Chow, M.B. Murphy, B.P. Chan, F. Muller, U. Strahle, and S.H. Cheng (2006). **Four-dimensional imaging and quantification of gene expression in early developing zebrafish (*Danio rerio*) embryos.** *Toxicological Sciences* 90(2): 529-38. ISSN: 1096-6080.
NAL Call Number: RA1190.F8
Abstract: Four-dimensional (4D) imaging is a powerful tool for studying three-dimensional (3D) changes in an organism through time. Different imaging systems for obtaining 3D data from in vivo specimens have been developed but usually involved large and expensive machines. We successfully used a simple inverted compound microscope and a commercially available program to study and quantify in vivo changes in sonic hedgehog (shh) expression during early development in a green fluorescence protein (GFP) transgenic zebrafish (*Danio rerio*) line. We applied the 4D system to study the effect of 100 microM cadmium exposure on shh expression. In control zebrafish embryos, shh:GFP expression was detected at about 9 h post-fertilization (hpf) and increased steadily in the next 7 h, peaking at about 17 hpf and decreasing in the following 4 h. In the same time period, different shh expression volumes were observed in cadmium-treated and control embryos. Embryos affected by cadmium-exposure demonstrated a down-regulation in shh expression. The number of GFP-expressing cells measured by flow cytometry decreased, and expression of neurogenin-1, a downstream target of the shh signaling pathway, was down-regulated, providing additional supporting data on the effects of cadmium on shh. In summary, we demonstrated the setup of a 4D imaging system and its application to the quantification of gene expression.
Descriptors: transgenic zebrafish cadmiu toxicity, basic helix loop helix transcription factors metabolism, embryonic development drug effects, nerve tissue proteins metabolism, trans activators metabolism, proteins metabolism, genetically modified, basic helix loop helix transcription factors genetics, drug effects, developmental gene expression regulation drug effects, green fluorescent proteins, Hedgehog proteins, fluorescence microscopy methods, nerve tissue trans activators genetics.

Zeruth, G. and R.S. Pollenz (2007). **Functional analysis of cis-regulatory regions within the dioxin-inducible CYP1A promoter/enhancer region from zebrafish (*Danio rerio*).** *Chemico Biological Interactions* 170(2): 100-13. ISSN: 0009 2797.
NAL Call Number: QH345.C5
Abstract: In vitro mutagenesis was utilized to render the various xenobiotic response elements (XREs) within the zebrafish CYP1A promoter/enhancer region non-functional either independently or in combination. Reporter gene assays revealed that only XRE4, XRE7, and XRE8 contributed to maximal TCDD-mediated induction of luciferase and that the contribution of each XRE to maximal induction was not equal. XRE4 and XRE7 were capable of functioning independently, while XRE8 alone could not support TCDD-mediated induction but was required for the ability of XRE4 and XRE7 to support maximal induction. These results were observed in cell lines derived from human, mouse and zebrafish. Mutagenesis of 3' nucleotides flanking the non-functional XRE5, and functional XRE4 did not alter the function of these XREs in cell culture. In silico analyses revealed the presence of putative Sp1, AP2, CREB, and two HNF-3 transcription factor binding sites that were localized to common positions within the enhancer region of both the mouse and zebrafish CYP1A

genes. In vitro mutagenesis of the binding sites showed that loss of the Sp1 or AP2 sites had minimal impact on TCDD-mediated gene induction while loss of the putative CREB site resulted in a modest decrease in basal and inducible activity and mutation of the HNF-3 reduced inducible activity by >90% of controls. Collectively, these findings suggest that the presence of XREs is not the sole determinant for regulation of aryl hydrocarbon receptor (AHR)-mediated gene and do not function in an additive manner.
Descriptors: cell lines of zebrafish, humans and mice, analysis, cis-regulatory, dioxin, CYP1A, promoter, enhancer.

Zhao Hua, Li Kang, Wu ShengGan, Wu ChangXing, Xu Hao, Hu XiuQing, and Wu Min (2004). **Evaluation on toxicity and safety of chlorpyrifos to environmental organisms**. *Acta Agriculturae Zhejiangensis* 16(5): 292-298. ISSN: 1004 1524.
Abstract: The toxicity of chlorpyrifos to 8 species viz., bee (*Apis mellifera*), silkworm (*Bombyx mori*), fish (*Brachydanio rerio [Danio rerio*]), bird (*Coturnix coturnix*), parasites (*Trichogramma nubilale and T. ostriniae*), frog (*Rana limnocharis*), soil microorganisms and earthworm (*Eisenia foelide*) was determined based on the experimental guidelines for environmental safety evaluation of chemical pesticides. Chlorpyrifos was highly toxic to bee, silkworm, fish, bird, parasites and frog and only slightly toxic to earthworm and soil microbe. Therefore, when chlorpyrifos is used in fields, it must be ensured that the pesticide flows in rivers and ponds. Neither should it be used near mulberry fields or regions where bees, birds and parasites are abundant. Copyright © 2008 CABI
Descriptors: toxicity testing of zebrafish and other species, chlorpyrifos, insecticides, nontarget effects, nontarget organisms, poultry, silkworms, Apidae, *Apis mellifera*, *Bombyx mori*, *Danio rerio*, *Eisenia*, *quails*, *Rana limnocharis*, *Trichogramma nubilale*, *Trichogramma ostriniae.*
Language of Text: Chinese.

Zhi, J.H., B. Tang, and L.F. Li (2007). **Efficacy and toxicity of tetrakis(hydroxymethyl) phosphonium sulfate in indoor air disinfection**. *Journal of Environment and Health* 24(3): 156-158. ISSN: 1001-5914.
Descriptors: zebrafish and other species toxicity testing, benefits, bioaccumulation, *Daphnia*, disinfection, freshwater crustaceans, mice, mortality causes, pollution indicators, sulfates, temperature effects, testing procedures, toxicity, toxicity tests, water pollution effects, *Cladocera*, *Cladocera crustacea*, *Crustacea*, *Danio rerio*, *Daphnia magna*, water fleas, industrial chemicals, effects on organisms, water treatment.

Zhou, Y., H.X. Yu, X. Ding, L.S. Wang, Z.B. Wei, L. Zhu, and S.J. Shi (2003). **Effect of combined toxicity of chlorobenzenes on zebrafish embryo**. *Journal of Agro-Environment Science* 22(3): 340-344. ISSN: 1672-2043.
NAL Call Number: S589.75.N86
Descriptors: zebrafish embryos, aromatics, benzene, benzenes, bioassays, chloramphenicol O-acetyltransferase, chlorine compounds, chlorobenzene, embryonic growth stage, embryonic development, embryos, enzymatic activity, enzymes, fish, pollution effects, superoxide dismutase, toxicity tests, *Danio rerio*, chlorobenzenes, structure activity relationships, chronic exposure, effects on organisms, effects of pollution, water pollution, control & remediation monitoring.

Zhu, X., L. Zhu, Y. Li, Z. Duan, W. Chen, and P.J. Alvarez (2007). **Developmental toxicity in zebrafish (*Danio rerio*) embryos after exposure to manufactured nanomaterials: buckminsterfullerene aggregates (nC60) and fullerol.** *Environmental Toxicology and Chemistry*

26(5): 976-9. ISSN: 0730 7268.
NAL Call Number: QH545A1E58
Abstract: The present paper summarizes, to our knowledge, the first study regarding the developmental toxicity of stable buckminsterfullerene aggregates suspended in water (nC60) using zebrafish (*Danio rerio*) as a vertebrate model. Zebrafish embryo survival, hatching rate, heartbeat, and pericardial edema were noted and described within 96 h of exposure. Fullerol (a hydroxylated C60 derivative, C60(OH)16-18) at 50 mg/L did not exert toxicity to zebrafish embryos. In contrast, nC60 at 1.5 mg/L delayed zebrafish embryo and larval development, decreased survival and hatching rates, and caused pericardial edema. Toxicity was mitigated by adding an antioxidant (glutathione), which suggests that a free radical-induced mechanism or another form of oxidative stress played a role in developmental toxicity.
Descriptors: zebrafish, drug effects, fullerenes toxicity, nanostructures toxicity, oxidative stress drug effects, chemical water pollutants toxicity, antioxidants pharmacology, drug dose response relationship, edema embryology, edema pathology, physiology, free radicals metabolism, glutathione pharmacology, heart rate, oxidative stress physiology, survival rate, time factors.

Zodrow, J.M., J.J. Stegeman, and R.L. Tanguay (2004). **Histological analysis of acute toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in zebrafish.** *Aquatic Toxicology* 66(1): 25-38. ISSN: 0166 445X.
NAL Call Number: QH541.5.W3A6
Abstract: Previous studies have demonstrated that acute exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) by injection leads to inhibition of caudal fin regeneration in zebrafish. Since the TCDD exposure in these studies is systemic, it is possible that pathology in organs other than the fin could result in inhibition of fin regeneration. Therefore, histopathology of adult zebrafish (*Danio rerio*) organs was characterized following abdominal cavity injection of a TCDD dose (70ng/g). The most pronounced histopathologic changes 5 days post-injection included lipidosis and hypertrophy of liver hepatocytes and hypertrophy of gill lamellae. Effects of TCDD exposure on immunolocalization of the zebrafish aryl hydrocarbon receptor nuclear translocator (ARNT2), the heterodimer partner of the aryl hydrocarbon receptor (AHR2), and an AHR regulated gene cytochrome P450 1A (CYP1A) was also determined. ARNT2 was immunolocalized to the gastrointestinal tract, gill lamellae, kidney, ventricle of the heart, caudal fin, brain and liver of zebrafish. TCDD exposure had no measurable effect on ARNT2 abundance or localization. CYP1A was immunolocalized in TCDD exposed fish as a biomarker for cells with an activated AHR pathway. CYP1A was not detected in any tissue from vehicle exposed fish. Significant TCDD-dependent induction of CYP1A was detected in the proximal tubules of the kidney, in liver hepatocytes and in the gastrointestinal tract of TCDD exposed fish. Significant but lower TCDD-dependent CYP1A expression was evident in the gill, caudal fin and ventricle of the heart. Overall, TCDD exposure in adult zebrafish leads to histopathology similar to that reported in other fish species, and it appears unlikely that the histopathology in these organs completely explains the inhibition of fin regeneration.
Descriptors: zebrafish, cytochrome P450 CYPA1A1 metabolism, environmental pollutants toxicity, gills drug effects, hepatocytes drug effects, tetrachlorodibenzodioxin toxicity, metabolism, acute toxicity tests, aryl hydrocarbon receptor nuclear translocator, cytochrome P450 enzyme system drug effects and genetics, gills pathology, hepatocytes pathology, immunohistochemistry, organ specificity, aryl hydrocarbon receptors metabolism, transcription factors metabolism.

Zodrow, J.M. and R.L. Tanguay (2003). **2,3,7,8-tetrachlorodibenzo-p-dioxin inhibits zebrafish caudal fin regeneration**. *Toxicological Sciences* 76(1): 151-161. ISSN: 1096 6080.
NAL Call Number: RA1190.F8
Abstract: Adult zebrafish [*Danio rerio*] completely regenerate their caudal fins following partial amputation. Fin regrowth can easily be monitored in vivo and regenerating tissues can be used to study this dynamic developmental process. In this study we determined that fin regeneration is significantly affected by exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Zebrafish caudal fins were partially amputated, and the fish received intraperitoneal (ip) injection of 2.8, 14, or 70 ng/g weight TCDD or vehicle control. By 7 days postamputation, fish exposed to the highest concentration of TCDD regenerated 15% of their fin compared to 65% regrowth in control fish. To determine if this effect was stage specific, zebrafish were exposed to 70 ng/g TCDD on 1, 2, 3, or 4 days postamputation. Fin regeneration was significantly inhibited at all time points following TCDD exposure. TCDD exposure also induced hyperpigmentation in de novo tissue. Zebrafish were dosed with BrdU, following fin amputation and TCDD exposure, to study changes in cell proliferation. By 4 days postamputation, cell proliferation rates were significantly lower in TCDD-exposed fish. TCDD toxicity is mediated through the aryl hydrocarbon receptor (AHR), and RT-PCR experiments confirmed AHR2, ARNT2b, and TCDD-dependent CYP1A expression in the regenerating tissue. These results demonstrate that zebrafish caudal fin regeneration is a unique model to investigate molecular mechanism(s) of TCDD toxicity. Copyright © 2008 CABI
Descriptors: zebrafish caudal fin regeneration model, biochemical receptors, biological development, developmental stages, exposure, pesticide residues, pesticides, *Danio rerio.*

Zoller, U. (2008). **Distribution profiles of endocrine disrupting PAHs/APEOs in river sediments: is there a potential ecotoxicological problem?** *Water Science & Technology* 57(2): 237-242. ISSN: 0273-1223.
NAL Call Number: TD420.A1P7
Descriptors: zebrafish, endocrine disruption, PAHs, APEOs, river sediments, ecotoxicologicaly.

Zoller, U., I. Plaut, and M. Hushan (2004). **The case of the nonionic alkylphenol ethoxylates in the Mediterranean Sea region: is there a problem?** *Water Science and Technology* 50(5): 79-84. ISSN: 0273 1223.
NAL Call Number: TD420.A1P7
Abstract: The concentration profiles of the potential endocrine disrupting nonionic alkylphenol ethoxylate (APEO) surfactants in Israel's rivers, groundwaters and coastal water of the eastern Mediterranean Sea, were found to be within the range of 12.5-74.6, trace-20.2 and 4.2-25.0 micro g/L respectively. Determination of the APEO's homologic distribution revealed "skewing" towards the more toxic shorter-chain ethoxylates. Egg production of zebrafish, *Danio rerio*, exposed to these actually found the environmental concentrations range of the APEOs decreased, after 20 days, to 89.6+or-2.1, 84.7+or-3.9 and 76.9+or-2.2% of the baseline levels, compared with control, in concentrations of 10, 25 and 75 micro g/L respectively. These results suggest that, (a) there is a potential health problem, particularly in countries in which the "hard"/environmentally persistent APEOs are still in use; and (b) the related health-risk is seasonally-dependent, particularly in semi-arid regions where the fluctuations in the water quantities in surface- and groundwater are substantial. Copyright © 2008 CABI

Descriptors: zebrafish water pollution model, coastal areas, groundwater, rivers, surfactants, toxicity, water quality, *Danio rerio*.

Zou, J.X., X.K. He, C.J. Tao, X.Y. Piao, and H. Jiang (2006). **Acute toxicity and bio-concentration of fluoroglycofen-ethyl to *Brachydonio rerio***. *Chinese Journal of Pesticide Science* 8(4): 375-378. ISSN: 1008-7303.
Descriptors: zebrafish, bioaccumulation, freshwater fish, mortality causes, pesticides , pollution indicators, test organisms, toxicity tests, *Danio rerio*, effects on organisms.

Selected Web Sites

The Zebrafish Information Network (ZFIN)
University of Oregon
http://zfin.org/cgi-bin/webdriver?MIval=aa-ZDB_home.apg
An online resource for zebrafish researchers that provides genetic, genomic and developmental information; maintains data sets of zebrafish research informaiton; facilitates the use of zebrafish as models for human biology; and links the zebrafish information to corresponding data in other databases.

Zebrafish Husbandry Association
http://www.zhaonline.org
A non-profit organization devoted to promoting and developing zebrafish husbandry standards through education, collaboration, and publication.

The Zebrafish Model
Animal Lab News
http://www.alnmag.com/article/zebrafish-model
This paper discusses the history of using zebrafish as research models.

Zebrafish: The Challenge of Standardization in a Maturing Research Model
Animal Lab News
http://www.alnmag.com/article/zebrafish-challenge-standardization-maturing-research-model
Discussion of scientifically-based care and use standards for zebrafish.

Evolution of Standards in the Care and Use of Zebrafish
Animal Lab News.
http://www.alnmag.com/article/evolution-standards-care-and-use-zebrafish
The authors describe standards for the care of zebrafish used as animal models in research settings.

Zebrafish Developmental Staging Series
Zebrafish Information Network.
http://zfin.org/zf_info/zfbook/stages/index.html
Images and information from 4th edition of The Zebrafish Book.

Trans-NIH Zebrafish Initiative
National Institutes of Health.
http://www.nih.gov/science/models/zebrafish/index.html
Provides a central information resource, focusing on major NIH-organized zebrafish initiatives.

Zebrafish K-12
University of Oregon.
http://www.neuro.uoregon.edu/k12/zfk12.html
Facts and information about zebrafish (*Danio rerio*), the popular model used for developmental and genetics research.