"All the Zebrafish News That's Fit to Print"

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ECTODERMAL PATTERNING AND NEUROGENESIS IN THE ZEBRAFISH

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We are exploring the mechanisms directing ectodermal patterning and neurogenesis in the zebrafish. To gain access to these events we have devised an assay that permits the culture of presumptive ectoderm from blastula stage embryos *in vitro*. The explants consist of 30-50 cells and, when cultured in aggregates of ten to fifteen explants, they survive for at least 48 hrs in a balanced salt solution. The explants undergo extensive cell division during the culture period, as evidenced by a ten-fold increase in total cell number. This system is now being used to study cell interactions and signals required for induction of the ectoderm. Initially, wholemount *in situ* hybridization and quantitative PCR with a battery of molecular markers was used to assay the final fate of ectoderm explanted from blastula stage embryos. Neural marker expression was undetectable by *in situ* hybridization, but the PCR assay detects low levels of two markers (pax6 and NCAM), while four others (Zash A, Zash B, eng3 and wnt1) are essentially undetectable by PCR. Taken together, this suggests that the ectoderm has not yet received inductive signals to differentiate as neural tissue. The explants do differentiate however, as they go on to express high levels of a non-neural cytokeratin, cyt1, that we recently cloned. The explants also express only low levels of five different mesodermal markers, arguing that they are essentially free of contaminating mesoderm. Current studies are aimed at studying neural induction in these explants by culturing them in contact with putative inducing tissues (e.g. gastrula stage mesoderm) as well as by co-culture with defined factors.

Acknowledgement: We are grateful to our colleagues Greg Conway, Anders Fjose, David Grunwald, Christiane Nüsslein-Volhardt, Robert Riggleman and Eric Weinberg, for generously providing cDNA clones and sequences.

INTESTINAL *CAPILLARIASIS* IN ZEBRAFISH

By M. Pack, J. Belak, C. Boggs, M. Fishman and W. Driever; Cardiovascular Research Center, Massachusetts General Hospital and Harvard Medical School, 149 Thirteenth Street, Charlestown, MA 02129, USA.

In 1994, a progressive wasting illness associated with the presence of intestinal Nematodes was noted in our facility. Fish which appeared healthy were also infected and often infertile. In this report we provide details of our experience with this condition.

Fish raised in our facility during the first months of 1994 developed a progressive wasting illness soon after reaching sexual maturity. Pathological evaluation performed outside of our facility was normal. Because of the sporadic occurrence of elevated copper levels in Boston water a reverse osmosis water purification system was installed. Snails were introduced into all aquaria to remove excess food and prevent fungal growth. Despite these measures the percentage of affected fish increased over several months. In the summer of 1994 we repeated the pathologic evaluation and found live Nematodes in the intestinal bulb of all fish examined. Microscopic examination of eggs identified the worm as a member of the *Capillaria* species.

Capillarids are thin and transparent worms that can reach one centimeter length. The eggs have a characteristic oval shaped appearance with a plug-like structure at either end and are visible in the adult worm, and the gut or feces of infected fish. Photographs and a good description of Capillarids at different developmental stages can be found in the *Handbook of Fish Diseases*, Dieter Untergasser, Editor (TFH publications, 1989, p104-5). Worms in the intestinal bulb of adult zebrafish are motile and can be easily seen when the dissected gut, in egg water (0.03% Instant Ocean) is viewed with transmitted light using a high power (50X) dissecting microscope. Outside the fish gut Capillarids are no longer motile.

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Editorial

HOLES IN THE FISH NET?

Hey! Once again we need your help.

The zebrafish WWW server, "The Fish Net", is entering its 8th month of operation. Use has steadily increased and presently we receive more than 1000 logins per week.

We're gratified to see the Net used so extensively. But at the same time, we need more feedback from you. We know that these files must contain at least a few mistakes. You know, those annoying typos like "zebrafinch" instead of "zebrafish".

Ensuring the quality of information in the Fish Net is your responsibility, too. If you see mistakes, let us know. If there is important stuff missing (like a reference to your most recent article), send us an e-mail. If you have information to contribute, send us a copy.

We hope to upgrade the hardware in the near future. This will provide faster access and, hopefully, better service in general.

You can jump into the Fish Net at: "http://zfish.uoregon.edu".

Zebrafish Books Temporarily Out of Print; Update Packets Available

By P. Edwards, Institute of Neuroscience, 1254 University of Oregon, Eugene, OR 97403-1254 USA

We have temporarily run out of copies of *The Zebrafish Book*. Another printing will be done in late spring 1995, when the zebrafish staging series (Kimmel, et al., *Dev. Dyn.*, 1995) is available to include in the new edition. In the meantime, the information in *The Zebrafish Book*, as well as other zebrafish information, can be accessed on the World Wide Web (http://zfish.uoregon.edu)

If your 1993 version of *The Zebrafish Book* has not been updated to the 1994 Ed. 2.1 version, please contact me at the above address.I will send you an update packet is sent to you for each 1993 book you have.

High School Curriculum Resource List

By P. Edwards, Institute of Neuroscience, 1254 University of Oregon, Eugene, OR 97403-1254 USA

There has been an increasing amount of interest in using the zebrafish as a model in high school and elementary school classes and labs. I have begun to assemble a list of those who are interested in planning such a curriculum and those who are willing to act as resources. The list can also be used by the participants as a means to network with each other. If you would like to be included on the list and have not already done so, please contact Pat Edwards, ZF Publications Coordinator, Institute of Neuroscience, 1254 University of Oregon, Eugene, OR 97403-1254 USA; phone (503) 346-4556; FAX (503) 346-4548; e-mail EDWARDS@UONEURO.UOREGON.EDU.

Capillariasis... *(cont'd from page 1)*

To rid fish and aquaria of the Nematodes, snails, which may act as storage for Capillarid eggs, were removed, and treatment with anti-helminthic drugs was begun. We tried Levamisol (9mg/ml) first, but two treatments were ineffective. Next we tried a combination of two drugs, Trichlorfon and Mebendazole ("Fluke-tabs"; Aquarium Products, 180-L Penrod Court, Glen Burnie, MD 21061) . This combination of drugs has been reported to be extremely efficacious for removing monogenetic trematodes from fresh water fish. Trichlorfon is an insecticide with anti-cholinergic activity that is considered toxic to humans. Mebendazole, a common anti-helminthic used to treat human intestinal infections, inhibits glucose uptake and is cidal for adult helminths and embryos.

We used the dosage recommended by the supplier; one tablet per 38 liters once trials to assess toxicity were completed. The drugs were added to our recirculating system (volume of tanks, reservoirs, filters and pipes included) as a slurry (100 tablets per one liter water-let stir 10 minutes) since they are poorly soluble in water. The treatment was repeated after 24 and 48 hours with a 10% water change every day thereby increasing the effective concentration. Carbon filters were removed during treatment. UV filters were not. Fish facility personnel were required to wear plastic gloves to avoid contact with water. Carbon filters were re-installed at 72 hours which removed the drugs from the system. The treatment protocol was repeated at 10 days since the cidal effect of these drugs on freshly fertilized eggs or dauerlarvae is not reported.

A beneficial effect from the treatment was evident within one

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week as fish gained weight and regained fertility. Fish sacrificed for pathological examination were free of worms. No widespread recurrence has been noted three months after treatment and fish remain healthy. However, after four months, isolated appearance of Capillaria was observed in few tanks. The treatment was repeated. No significant adverse effects of treatment were observed.

The youngest fish in the system which received medication had been feeding on brine shrimp for at least five days. Larvae still feeding on paramecia received one treatment (one tablet per 38 liters) 24 hours before their first dose of brine shrimp and were introduced into our recirculating system 30 hours later. No toxicity was observed.

Parasitic infections are common in fresh water fish and heavy infestations can be lethal. We feel the wasting illness and reduced fertility seen in our fish was the result of nutritional deprivation since the intestinal bulb of all fish examined was fully occupied by live worms. Eradication of the parasite was coincident with weight gain and a dramatic improvement in fertility. Within a large facility, recurrence even after several months is possible, and fish with wasting syndrome should be routinely checked for parasites.

We thank Drs. B. Dixon (Haywood, CA), D. Grunwald (Salt Lake City), C. Nuesslein-Volhard (Tuebingen), J. McBain (San Diego) and R. Bullis (Woods Hole) for their assistance.

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Differential Induction of Four Msx Homeobox Genes During Fin Development and Regeneration in Zebrafish

*(*in press, *Development)*

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To study the genetic regulation of growth control and pattern formation during fin development and regeneration, we have analysed the expression of four homeobox genes, msxA, msxB, msxC, and msxD in zebrafish fins. The median fin fold, which gives rise to the unpaired fins, expresses these four msx genes during development. Transcripts of the genes are also present in cells of the presumptive pectoral fin buds. The most distal cells, the apical ectodermal ridge of the paired fins and the cleft and flanking cells of the median fin fold express all these msx genes with the exception of msxC. Mesenchymal cells underlying the most distal cells express all four genes. Expression of the msx genes in the fin fold and fin buds is transient, and by 3 days after fertilization, msx expression in the median fin fold falls below levels detectable by in situ hybridization. Although the fins of adult zebrafish normally have levels of msx transcripts undetectable by in situ hybridization, expression of all four genes is strongly reinduced during regeneration of both paired and unpaired fins. Induction of msx gene expression in regenerating caudal fins occurs as early as 30 hours post-amputation. As the blastema forms, the levels of expression increase and reach a maximum between the third and fifth days. Then, msx expression progressively declines and disappears by day 12 when the caudal fin has grown back to its normal size. In the regenerating fin, the blastema cells that develop at the tip of each fin ray express msxB and msxC. Cells of the overlying epithelium express msxA and msxD, but do not express msxB or msxC. Amputations at various levels along the proximo-distal axis of the fin suggest that msxB expression depends upon the position of the blastema, with cells of the rapidly proliferating proximal blastema expressing higher levels than the cells of the less rapidly proliferating distal blastema. Expression of msxC and msxD is independent of the position of the blastema cell along this axis. Our results suggest distinct roles for each of the four msx genes during fin development and regeneration and differential regulation of their expression.

NOTICE:

The "Research Positions Available" section that was originally printed in this issue of the Monitor is not included as it is no longer relevant.

Tetrahymena: an Easy Alternative for Paramecia

By J.E. Speksnijder and E. Bijmolt, Department of Genetics, Center for Biological Sciences, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

We now routinely use Tetrahymena cultures as an alternative for Paramecia to feed the smallest baby fish. Tetrahymena have two advantages, namely that the culture medium contains few components and is easy to prepare, and that sterile cultures are commercially available from the Culture Collection of Algae and Protozoa, Freshwater Biological Association, The Ferry House, Ambleside, Cumbria LA22 0LP, UK; phone 44-5394-42468. The strain we use is *Tetrahymena pyriformis* (catalogue no. CCAP 1630/1W), and the PPY culture medium is prepared as follows: Dissolve 20 g proteose peptone and 2.5 g yeast extract in 1000 ml of distilled water, and sterilize in batches of 200 ml in 500 ml bottles. To start the culture, inocculate about 6 ml of dense culture in 200 ml fresh PPY medium, and grow for 3-4 days at room temperature with bottle caps opened one turn. These cultures can then be harvested, or kept for up to several weeks at 15 C to start new cultures. To harvest, centrifuge the cultures for about 3 min at 500g, and rinse with PJ salt solution (see below). Repeat twice to remove all PPY medium. Add a small volume of PPY after the last step; the culture can now be used to feed the baby fish. Using *Tetrahymena*, we have been able to grow babyfish with survival rates comparable to those obtained with Paramecia.

PJ (Prescotts & James's) solution:

Make up the following three stock solutions, each in 100 ml distilled water: 1) 0.433 g $CaCl_2.2H_2O$ and 0.162 g KCl; 2) 0.512 g K_2HPO_4; 3) 0.280 g $MgSO_4.7H_2O$. Add 1 ml of each stock solution to 1 liter distilled water.

Stages of Embryonic Development of The Zebrafish

By C.B. Kimmel, W.W. Ballard, S.R. Kimmel, B. Ullmann, and T.F. Schilling,* Institute of Neuroscience, University of Oregon, Eugene, OR 97403; **Department of Biology, Dartmouth College, Hanover, NH 03755*

We plan to publish soon an extensively expanded revision of the staging series in the journal, *Developmental Dynamics.* The new version has more illustrations, including Nomarski views of key features we use in staging. The text is expanded, including a glossary, and the text has profited from the comments of reviewers for the journal, as well as from Monte and other colleagues. We have changed a few of the stage names for the sake of clarity (you can find the new names in the accompanying figure), and we rename the *period* of development during the second day (i.e. the time between 24 and 48 h) the *"pharyngula period"*, a name coined by Bill Ballard a number of years ago for von Baer's vertebrate phylotypic stage. I think this is a much better name, meatier and more accurate than the old name "straightening", which no one used anyway, and hope that you will use it and honor Bill in doing so.

We are making arrangements with the publisher to assure that we will be able to continue to include the series as a chapter in *The Zebrafish Book* and also keep it available on the zebrafish WWW Internet server (http://zfish.uoregon.edu). I sincerely hope that the new publication will not stifle the growth and development of the series as an evolving tool, and that the new version will soon be out of date. Recently we have been studying cartilage morphogenesis occurring late in embryogenesis and in the larva, and new staging information during these periods would certainly be useful!

Among the changes is a new set of line drawings, made from camera lucida sketches of the embryo at selected stages by Seth Kimmel (moonlighting from his job as a carpenter here in Eugene). We reproduce this set here, and we will be pleased to send you an unlettered set of very nice quality larger reproductions made onto velum upon request to Bonnie Ullmann (ULLMANN@UONEURO.UOREGON.EDU). They can also be downloaded from the zebrafish WWW server (http://zf_info/zfbook/stages/stages.html).

The animal pole is to the top for the early stages, and anterior is to the top later, except for the two animal polar (AP) views shown below with their side view counterparts for germ-ring and shield gastrulas. Face views are shown during cleavage and blastula stages. After shield stage, the views are of the embryo's left side, but before the shield arises one cannot always reliably ascertain which side is which. Pigmentation is omitted.

Arrowheads indicate the early appearance of some key diagnostic features at the following stages: **1k-cell**: YSL nuclei. **Dome**: the doming yolk syncytium. **Germ ring**: the germ ring. **Shield**: the embryonic shield. **75%-epiboly**: Brachet's cleft. **90%-epiboly**: blastoderm margin closing over the yolk plug. **Bud**: polster. **3-somite**: third somite. **6-somite**: eye primordium (upper arrow), Kupffer's vesicle (lower). **10-somite**: otic placode. **21-somite**: lens primordium. **Prim-6**: primordium of the posterior lateral line (on the dorsal side), hatching gland (on the yolk ball). **Prim-16**: heart. **High pec**: pectoral fin bud. Scale bar: 250 µm.