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

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New Method for Mutagenesis of Zebrafish: TMP Mutagenesis Procedures

By H. Ando and M. Mishina; Department of Molecular Neurobiology and Pharmacology, School of Medicine, University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-0033, Japan

(Efficient mutagenesis of zebrafish by a DNA cross-linking agent. *Neurosci. Lett*. **244**, 81-84. 1998)

We report a novel procedure for efficient mutagenesis of zebrafish. This method uses a DNA cross-linking agent, 4,5',8-trimethyl-psoralen (TMP), which is known to induce small deletions frequently in *Escherichia coli* and *Caenorhab-ditis elegans*. Our pilot screen indicated that the TMP mutagenesis procedure was efficient. At present, we are using 3 ng/ml TMP to obtain mutants. The high efficiency of the TMP mutagenesis will allow the isolation of a significant number of zebrafish mutants in a single laboratory. TMP induces small deletions by nucleotide excision and recombinational repair of the interstrand DNA cross-links provide a useful marker for cloning the mutated gene. We have successfully cloned a zebrafish DNA fragment which is absent in a mutant.

1) Prepare 3 mg/ml 4,5',8-trimethylpsoralen (TMP) in dimethyl sul-

(cont'd on page 10)

Fgf8 Is Mutated in Zebrafish *Acerebellar (Ace)* Mutants and Is Required for Maintenance of Midbrain-Hindbrain Boundary Development and Somitogenesis

By F. Reifers[1], H. Böhli[1], E.C. Walsh[2], P.H. Crossley[2], D.Y. R. Stainier[2] and M. Brand[1*]; [1]Department of Neurobiology, University of Heidelberg, Im Neuenheimer Feld 364, D-69120 Heidelberg, Germany; [2]Department of Biochemistry and Biophysics, University of California San Francisco, San Francisco, CA 94143-0554

(Development 1998, in press)

We describe the isolation of zebrafish Fgf8 and its expression during gastrulation, somitogenesis, fin bud and early brain development. By demonstrating genetic linkage and by analyzing the structure of the Fgf8 gene, we show that *acerebellar* is a zebrafish Fgf8 mutation that may inactivate Fgf8 function. Homozygous *acerebellar* embryos lack a cerebellum and the midbrain-hindbrain boundary organizer. Fgf8 function is required to maintain, but not initiate, expression of Pax2.1 and other marker genes in this area. We show that Fgf8 and Pax2.1 are activated in adjacent domains that only later become overlapping, and activation of Fgf8 occurs normally in *no isthmus* embryos that are mutant for Pax2.1.These findings suggest that multiple signaling pathways are independently activated in the midbrain hindbrain boundary primordium during gastrulation, and that Fgf8 functions later during somitogenesis to polarize the midbrain. Fgf8 is also expressed in a dorsoventral gradient during gastrulation and ectopically expressed Fgf8 can dorsalize embryos. Nevertheless, *acerebellar* mutants show only mild dorsoventral patterning defects. Also, in spite of the prominent role suggested for Fgf8 in limb development, the pectoral fins are largely unaffected in the mutants. Fgf8 is therefore required in development of several important signaling centers in the zebrafish embryo, but may be redundant or dispensable for others.

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...an informal vehicle dedicated to communicating zebrafish news. References to information appearing in the Monitor should be made as personal communications and only if explicit permission of the authors is obtained.

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Zebrafish Research: Activities at the NIH

By D.B. Henken (**dh50g@nih.gov**), for the Trans-NIH Zebrafish Coordinating Committee, National Institutes of Health, National Institute of Child Health and Human Development, Center for Research for Mothers and Children, Developmental Biology, Genetics and Teratology Branch Executive Building, Room 4B01, 9000 Rockville Pike, MSC 7510, Bethesda, Maryland, 20892-7510

Over the past few years, it has become apparent that the zebrafish as a model of vertebrate development and disease has received increased attention by the scientific community, primarily because of its value in both experimental and genetic analyses. While small groups of researchers have been working with *Danio rerio* for many years, the increase in the number of investigators using this model in recent years prompted the NIH to become more involved in assisting its development. Early in 1997, a workshop, sponsored by several NIH Institutes was held by members of the zebrafish community to assess the state of the science relating to the zebrafish as a model genome system. This group presented a report to the Director of the NIH, in spring, 1997, with the recommendation to develop the zebrafish system for genetic studies of vertebrate embryogenesis and disease. In response to these recommendations, the Director of the NIH formed the Trans-NIH Zebrafish Coordinating Committee which first met in the fall of 1997. This working group is composed of representatives from most of NIH's Institutes and Centers having an interest in promoting zebrafish as a research model. The Committee is co-chaired by Dr. Josephine Briggs of the National Institute of Diabetes and Digestive and Kidney Diseases, and Dr. Tyl Hewitt of the National Institute of Child Health and Human Development.

Activities by this group have been substantial. The initial effort of this group resulted in a Request for Applications (RFA) as part of an effort to create resources that will facilitate the mapping and positional cloning of genes in the zebrafish. The RFA (DK-98-006) was published in December 1997, and applications will be reviewed in July 1998. Successful applications will be funded by fall, 1998. This effort is being co-sponsored by 12 Institutes and Centers.

Most recently, the Coordinating Committee published a Program Announcement (PA), soliciting investigator-initiated applications using the zebrafish as a model for development and disease research. The objectives of this PA are to encourage and promote new and innovative research and approaches to identify the genes and elucidate the molecular and genetic mechanisms responsible for normal and defective development using zebrafish. This PA (HD-98-074) was published in the May 21, 1998 issue of the NIH Guide. A total of 18 Institutes and Centers are participating in this endeavor. Each of the participating Institutes and Centers has interests in using the zebrafish as a system to understand better particular processes, organs, or diseases. In addition, some may be interested in supporting development of methods, either general techniques or techniques that may particularly apply to their areas of interest. Please contact the appropriate program official listed on the PA with questions. The receipt dates for this PA are the same as for any R01 application. The URL of this PA is:

http://www.nih.gov/grants/guide/pa-files/PA-98-074.html

In addition, Institutes participating in the Coordinating Committee are assisting in the support of a Zebrafish Resource Center, overseen by National Center for Research Resources, that will act as a stock center for the maintenance and distribution of zebrafish mutants to the scientific community as well as to provide state-of-the-art informational resources via the world wide web (see article, "Zebrafish Stock Center Funded" on page 3 in this issue).

These initiatives represent one of the most coordinated and unified efforts on the part of the NIH to support research using a single animal model. This indicates an appreciation of the importance of zebrafish as a model for development by the funding components of the NIH. It is important to understand, however, that the peer review system is another important link in the funding process. As the number of applications using zebrafish increases, it is important that members of the zebrafish scientific community understand the

Cont'd on Page 3

need for them to participate as reviewers in this system. Active participation in the review process not only will enlighten their colleagues using other model systems, but also will ensure that the expertise is available on study sections for the fair and appropriate review of zebrafish research grant applications.

The Trans-NIH Zebrafish Coordinating Committee will continue to meet on a regular basis. We welcome your suggestions, comments and concerns. Please contact us and let us know how the NIH can be of help in the future.

Biochemical Identification and Tissue-Specific Expression Patterns of Keratins in the Zebrafish *Danio rerio*

By M. Conrad, K. Lemb, T. Schubert and J. Markl, Institute of Zoology, Johannes Gutenberg University of Mainz, D-55099 Mainz, Germany

Cell and Tissue Research, in press (1998)

In the zebrafish, *Danio rerio*,we have identified by two-dimensional polyacrylamide gel electrophoresis, complementary keratin blot-binding assay,and immunoblotting, a number of different type I and type II Keratins. They range from 56 kDa to 46 kDa in molecular mass and from pH 6.6 to pH 5.2 in isoelectric point. The type II zebrafish Keratins exhibit significantly higher molecular masses (56 - 52 kDa) compared to the type IKeratins (50 - 48 kDa), but the isoelectric points show no significant difference between the two keratin subclasses (type II: pH 6.0 - 5.5; type I: pH 6.1 5.2). According to their occurrence in various zebrafish tissues, the identified Keratins could be classified into "E" (epidermal) and "S" (simple epithelial) proteins. A panel of monoclonal anti-keratin antibodies was used for immunoblotting of zebrafish cyto-skeletal preparations and immunofluorescence microscopy of frozen tissue sections. These antibodies showed differential cytoplasmic expression of Keratins, which not only included epithelia, but also a variety of mesenchymally derived cells and tissues. It is concluded that previously detected fundamental differences in keratin expression patterns between higher vertebrates and a salmonid, the rainbow trout *Oncorhynchus mykiss*, are also true for the zebrafish, a cyprinid. However, in spite of principle similarities, trout and zebrafish Keratins differ from each other in many details. The present data provide a firm basis from which the application of Keratins as cell differentiation markers in the well established genetic model organism, the zebrafish, can be developed. (Supported by the Stiftung Innovation von Rheinland-Pfalz)

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Zebrafish Stock Center Funded

By M. Westerfield, Institute of Neuroscience, 1254 University of Oregon, Eugene, OR 97403-1254

Recently, a small group of representatives of the zebrafish research community obtained funds to establish an International Stock Center for Zebrafish on the University of Oregon campus. These include money from the State of Oregon for construction of a new building and a grant from the NIH (P40 RR12546) for equipment, supplies and operating expenses. An application to the NIH for additional construction money is pending review.

We anticipate that the stock center will be able to receive samples of frozen sperm and various reagents in the immediate future. Please contact Pat Edwards (**edwards@uoneuro.uoregon.edu**) for information about submitting such materials. Special arrangements for taking live fish stocks

Cont'd on Page 4

from labs that are desperate for space may also be possible. Construction of the new facility will begin immediately.

Overview

Zebrafish has emerged recently as a premiere organism to study vertebrate development and genetics. Powerful techniques allow efficient generation and recovery of zebrafish mutations affecting genes that regulate developmental patterning, organogenesis, physiology and behavior. The functions of many of these genes are conserved among vertebrate groups. Thus, analysis of zebrafish mutations provides insights into gene functions in other vertebrates, including humans.

Ongoing genetic screens have identified over 7,000 mutations; fish that carry them are distributed among more than 100 laboratories in 28 countries. The zebrafish research community desperately needs a centralized facility to preserve and track these stocks and to facilitate their distribution to researchers.

The Stock Center will have three main functions: 1) It will maintain and make available to the research community wild-type and mutant zebrafish stocks, frozen sperm, and reagents. It will organize genetic markers and maintain the genetic map. 2) The stock center will distribute information. It will maintain the ZFIN computer database, accessible via the Internet, publish a manual for the laboratory use of zebrafish, facilitate communication among zebrafish researchers, and host visits from researchers to work with stocks or learn techniques to identify and maintain mutants. 3) The stock center will develop methods to improve zebrafish health. It will establish standards and procedures for generating and maintaining healthier more vigorous strains, characterize endemic diseases, develop methods for disease control and treatment, and publish a manual of procedures for preventing, diagnosing, and treating zebrafish diseases.

Specific Plans

The last several years have witnessed an explosion in our understanding of vertebrate development, largely based on work from a few model genetic organisms. The zebrafish is the newest of these model organisms. Because the basic principles of body patterning appear similar during embryogenesis of all vertebrates, insights gained from work on embryonic zebrafish will have implications for human health and disease. Moreover, research on this organism meets the intent of the Animal Welfare Act because use of many higher vertebrates can now be replaced by use of this lower vertebrate.

Systematic genetic research on zebrafish began at the University of Oregon and for many years Eugene was the only place it was performed. Recently, however, international interest in this organism has grown tremendously (Balter, 1995; Eisen, 1996; Travis, 1996); studies of the embryology and genetics of zebrafish and the recent identification of many genetic mutations have led to a dramatic increase in the number of laboratories using this organism to study the basic mechanisms of vertebrate development. Currently these genetic stocks are distributed among laboratories around the world. To make room for new mutants, laboratories must discontinue some of their current stocks many of which are permanently lost. The zebrafish research community desperately needs a centralized site to preserve and keep track of these stocks and to facilitate their distribution to researchers, thus supporting and promoting research opportunities while preventing duplication of effort.

We will construct a facility to maintain wild-type and mutant stocks of zebrafish and to make these stocks widely available to the international research community. A stock center is needed because it can eliminate the requirement of individual laboratories to maintain stocks they are unable to study, it can provide animals at lower cost than individual laboratories, and most importantly, it can ensure the highest possible levels of quality and uniformity. Specifically we will:

♦ 1. *Establish a stock center to serve as a central repository for materials and information.* We will maintain healthy stocks of fish and frozen sperm of identified genotypes and make them widely available to the research community. We will obtain carriers of mutations from the research community and breed them to produce new generations. We will freeze and store sperm from these carriers. We will receive and store antibodies, gene probes, and markers used to identify and analyze wild-type and mutant stocks. We will receive and organize genetic markers and maintain the genetic map. Upon request, we will ship these materials to research laboratories throughout the world.

♦ 2. *Make information widely available to the research community.* We will maintain ZFIN, a computer database accessible via the WWW of the Internet, with information about the stocks. Addition-

ally, ZFIN will provide information about the genetic map, markers, molecular probes, laboratory methods, developmental staging, embryonic and adult anatomy, and gene expression patterns. Electronic links to researchers, laboratories, sources, and publications will be provided through WWW services. We will foster an electronic network of communication among laboratories using zebrafish. We will publish both hardcopy and electronic versions of a manual for the laboratory use of zebrafish and a periodical with news about zebrafish research and techniques. The stock center will host visits from researchers who wish to work with stocks, learn techniques, or learn to identify and maintain mutant stocks.

♦ 3. *Develop methods for improving health*. We will establish standards and procedures for generating and maintaining healthier and more vigorous strains. We will characterize diseases endemic to laboratory stocks. We will study these diseases to identify their sources and causes and we will develop methods for their control and treatment. We will publish a manual for the prevention, diagnosis, and treatment of diseases affecting zebrafish.

For more information about the Zebrafish Stock Center see:

http://zfish.uoregon.edu/zf_info/stckctr/stckctr.html

References

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The Zebrafish Stock Center in Tübingen

By H.G. Frohnhoefer and C. Nüsslein-Volhard, Max-Planck-Institut für Entwicklungsbiologie, Spemannstrabe 35/III, D-72076 Tübingen, GERMANY

The stock center in Tübingen keeps about 400 mutant lines of *Danio rerio*, which are publicly available for scientific research. Most of these mutants in the stock center were isolated in a screen for embryonic visible phenotypes in Tübingen between 1992 and 1994. In addition to mutants with embryonic visible phenotypes, several dominant and recessive adult visible mutants are kept (Haffter et al., 1996a & accompanying articles; Haffter et al., 1996b).

From the 1200 mutations that were analysed, the stock center keeps a representative collection as outbred lines. The mutant stocks include one or more alleles of most of the 371 genes described, as well as some wild-type and reference strains with visible markers. Most mutants that are insufficiently characterized and not assigned to complementation groups (termed “unresolved”) are kept only as frozen sperm samples. As a rule these alleles will not be made available. The collection of mutants kept as live fish in the stock center is in some flux. Current mutations may be retired as new mutations are discovered. Thus the composition of the collection is changing slightly with time.

Standardly, the stock center will ship spawns from mutant outcrosses for the recipient lab to grow. It is also possible to provide fixed mutant embryos. Because the capacity of the stock center is limited, it may not be possible to fill all requests. Priority will be given to people who can grow their own fish. If you have problems raising and keeping extra lines, you might also spend some time in our lab to carry out the experiments in-house.

Generally the transfer of living stocks will require you to sign a Material Transfer Agreement (MTA), which serves to protect the economic interests of the Max-Planck-Institute from commercial exploitation of the mutant strains. Otherwise there are no restrictions on the research done with the fish. If you can obtain the requested stocks from another lab closer to you, you should nevertheless first ask for an MTA form and return it to us.

If you are interested in receiving mutant stocks, please check our Web Site:

http://wwweb.mpib-tuebingen.mpg.de/Abt.3/Stockcenter/

for more detailed and updated information. Please contact the stock keeper by email to make arrangements. Upon return of a signed MTA, we will usually provide outcrosses of identified carrier fish. We try to obtain two independent outcrosses with about 2 x 70 eggs for each mutation. In case we manage to get only two batches from the same spawn, one of the batches is marked as “clone”. Outcrosses are usually made against our “wild-type” lines Tübingen (Tu) and Tup- Longfin (TL). The latter carries mutations with adult phenotypes in two genes: a weak allele of leopard (former Tup, leo^{t1}) makes the striped pigmentation pattern dissolve into a spotlike pattern (recessive phenotype), and longfin (lof^{t2}) causes the growth of conspicuous long fins, both in heterozygous and homozygous mu-

tant fish. These two mutations are present in the background of the majority of our lines. More rarely, some lines contain the golden (gol^{b1}) allele, which reduces the melanin in embryos and adults. Some stocks may contain genetic backgrounds from the wild-type lines AB or WIK. The spawn will be bleached, some Pronase will be added to facilitate hatching and the embryos will be sent by UPS (Europe) or Fedex (all other areas). Please provide us with your complete address (including phone number) and a second contact email address of someone from your lab, who can receive the embryos in case you are not around upon delivery. If possible, also provide us with a customer number of the transport company servicing your area.

The eggs we send, as far as we can tell, are free from disease. Despite these precautions, we cannot take responsibility for infectious diseases that might be carried with the spawn. Egg batches of second day layers can not be bleached before the eggs undertake their journey. Those eggs however will always be explicitly marked as "not bleached". You may state beforehand whether you are prepared to receive unbleached eggs.

The time required to obtain the mutant lines will mainly depend on whether suitable carrier fish are available or whether it is necessary to wait for another generation of fish to be identified. Please indicate if some of the mutants have higher priority for you and are needed more urgently than others. We will confirm your order, tell you which of the required mutants are immediately available, and provide an estimate of when carriers of the remaining lines can be expected. If you order a large number of strains, the entire transfer may take several months. In this case, it will be useful if you restate by email which lines you still require. Please inform us if you no longer need the fish you requested or whether you obtained them from another source in the meantime. If some of the strains you received are not growing well, we recommend that you request a new batch as soon as possible, because the parents may still be available. When planning to order strains, you should also take into account that winter is a bad season for shipping eggs.

Up to now we have sent the MTA document by email and keep a general list of mutations without information about currently available lines on our website. In the future we plan to make both the MTA and an updated list of available stocks accessible via the internet.

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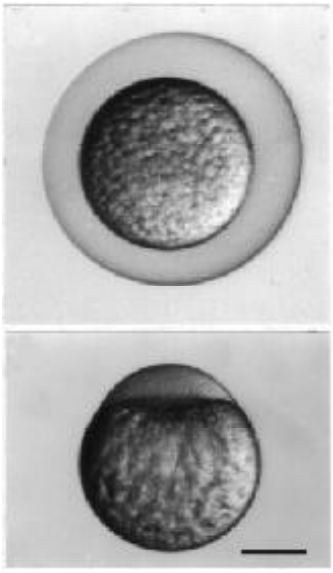

What Stage is This???

Chamber Volume Requirements for Reproduction of the Zebrafish, *Danio rerio*

E.M. Goolish, R. Evans, K. Okutake, and R. Max, Lockheed Martin Engineering & Sciences Company, Space Station Biological Research Project, NASA Ames Research Center, T20G, Moffett Field, California, 94035

(Details on the water quality and other aspects of the methods can be found in the original paper (*Prog. Fish Cult*. **60**:127-132).

The zebrafish, *Danio rerio*, has recently become a major vertebrate model for the study of developmental biology, neurobiology, and molecular genetics. As a result, most research universities have now invested considerable resources in the construction of large zebrafish facilities, where a key element in the design is maximizing the efficiency of available space. Here we report on the effects of aquarium chamber volume on the reproduction of zebrafish, with the objective of identifying the minimal volume required for normal egg production. The test chambers were supplied with flow-thru water from a large recirculating, aerated system with biofilter, etc. Flow-rate to all chamber sizes was kept uniform at 70 ml/min, so that all chambers received the same degree of dilution of metabolic wastes. Water volume turnover was very high, ranging from 8 to 42 per hour. Water quality parameters were monitored and found not to differ significantly among the various chamber sizes. Six adults

Cont'd on Page 7

(2 males and 4 females) were tested in chamber volumes of 500, 400, 300, 200, and 100 ml, and compared to a control volume of 3.5 l. Eggs were removed from the test chambers after spawning and incubated in petri dishes at 28°C. Total egg production, percent of eggs hatching, and larval length at 96h were used to evaluate breeding success. Egg production was not significantly affected by reduced aquaria volumes of 500, 400, and 300 ml compared to the control. However, mean egg production from a test volume of 200 ml was only 48% of the control egg production ($P<0.05$), and at a test volume of 100 ml, egg production was reduced to 26% of the control value ($P<0.005$). Percent egg hatch and 96h larval length were unaffected at any test volume.

"I am busy just now again on electro-magnetism, and think I have got hold of a good thing, but can't say. It may be a weed instead of a fish that, after all my labor, I may at last pull up."

Michael Faraday (1791-1867)

Coleps, Scourge of the Baby Zebrafish

By A.l Mazanec and B. Trevarrow, Institute of Neuroscience, 1254 University of Oregon, Eugene, OR 97403-1254

In the past we have noticed an intermittent high fish mortality among young larval zebrafish. Our daily monitoring of conditions has implicated vicious predators, known as "*Twirlies*" at the University of Oregon, to be the cause of much of this larval death. Over the years they have been rumored to kill entire stocks of 4-7 day old zebrafish babies.

To better characterize the problem, we have recently identified the 'twirlies' as *Coleps*, free-living freshwater protozoa (Patterson, 1996). They have barrel-shaped bodies approximately 50-80 µms in length that are reinforced by a layer of calcareous plates (Figure 1). *Coleps* are regarded as scavengers with a preference for dead or dying tissue as well as rotifer eggs (Patterson, 1996; Jones & Hollowday, 1992). Occasionally we find these predators in fish water and especially in our paramecia cultures. In small quantities they seem to be relatively harmless, but in concentrated numbers they can be deadly to small larval fish. As soon as the larvae hatch, the *Coleps* are immediately attracted to them. Within a few minutes baby zebrafish can be swarmed by hundreds of *Coleps*. It then takes this swarm only between 30 minutes and an hour to kill and completely consume the baby zebrafish leaving virtually no evidence of their heinous act (see figure). Rather than a disease or parasite we consider this an infestation of a microscopic predator.

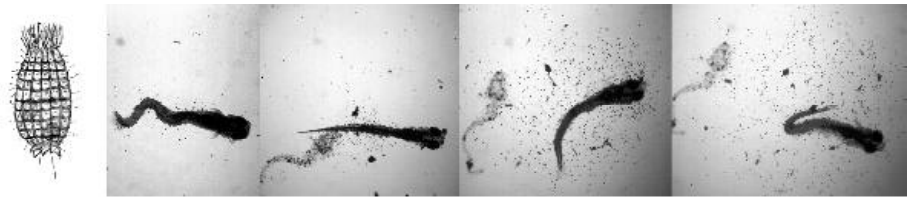

Figure 1 The first picture is a drawing of an individual Coleps. The series of 4 photos (right to left) shows the fate of two zebrafish larvae confined in a small volume of water from a Coleps culture. The first picture shows a recently killed zebrafish surrounded by a halo of Coleps (small dots). After 45 minutes (second picture), the fish in the first frame has been processed to a mere shadow of its former self (dead fish on left) and a live larva, 5 days old, has been added. The next 2 pictures are after 15 and 20 minutes. During this period, the larvae has been killed and is in the process of being eaten by a large swarm of Coleps while the first fish has become increasingly ghost-like.

We found that *Coleps* were being inadvertently concentrated when we used a 23 µm mesh to harvest the paramecia for feeding 4-9 day old babies. The *Coleps* were eliminated from the paramecia cultures when the fish water used to make up the cultures was sterilized by passing it through a 142 mm glass filter and a 0.2 µm membrane filter. This alone has almost entirely eliminated our *Coleps* problem.

References:

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Jones, K.R., Hollowday, E. D. (1992). A Note on the Consumption of a Rotifer egg by Ciliated Protozoa (Ciliophora). *Microscopy*, **36**: 718-720.

High Resolution whole-mount in situ hybridization

By C. Thisse and B. Thisse, IGBMC, 1 rue Laurent Fries, 67404 Illkirch Cedex, France

Preparation of Probe:

Note: Work has to be done using gloves and sterile tubes and buffers.

1. Prepare DNA:

- Linearize 5 µg of DNA by digesting with the appropriate restriction enzyme for 2h.
- Stop the reaction using first a mix of phenol/chloroform and then chloroform.
- Precipitate the DNA with Ethanol, centrifuge, and wash with RNAse free 70% Ethanol.
- Resuspend the DNA in 10 mM Tris and 1 mM EDTA.
- Test an aliquot on agarose gel.

2. Synthesis of the antisense RNA probe. Incubate 2h at 37°C in transcription mix:

Transcription mix:

- 1µg linearized DNA
- Transcription buffer (T3 or T7 RNA polymerase) - 4 µl
- NTP-DIG-RNA (Boehringer) - 2 µl
- RNAse inhibitor (35 units/µl) - 1 µl
- T3/T7 RNA polymerase (20 units/µl, Stratagene) - 1 µl
- Sterile water to 20 µl total

3. Digest the template DNA by adding 10 µl RNAse free DNAse for 15min at 37°C.

4. Stop the synthesis reaction and precipitate the RNA for 30 min with:

- 1 µl EDTA 0.5M pH 8
- 2.5 µl LiCl 4M
- 75 µl Ethanol 100% at -70°C

5. Centrifuge at 4°C for 30min at 12,000 rpm

6. Wash with 70% ethanol, dry and resuspend in 20 µl sterile DEPC water.

7. Test 1 µl on agarose gel (generally 1 µl will be used for the hybridization).

Fixation and storage of embryos:

1. Remove chorions by pronase treatment (for embryos older than 18 somites) or manually (for earlier stages).

2. Fix embryos in 4% paraformaldehyde (PFA) in PBS overnight at 4°C.

3. Transfer embryos into 100% Methanol (MeOH), store them at -20°C (2h-several months).

In situ Day 1:

1. Rehydration: Transfer embryos into small baskets and rehydrate by successive incubtions in:

- 75% MeOH - 25% PBS for 5 min
- 50% MeOH - 50% PBS for 5 min
- 25% MeOH - 75% PBS for 5 min
- 100% PBT (PBS/Tween20 0.1%) 4 x 5 min

2. Digest with Proteinase K (10 µg/ml).

- blastula and gastrula: 30 seconds
- early somitogenesis: 1 min
- late somitogenesis (14 to 22 somites): 5 min
- 24h embryos: 15 min
- 36h/48h embryos: 30 min

3. Refix in 4% PFA-PBS, 20 min.

4. Wash in PBT, 5 x 5 min.

5. Preadsorb the anti-DIG antibody (Boehringer) in a 1:1000 dilution in PBT-sheep serum 2%-BSA (2mg/ml) for several hours at RT with a batch of previously fixed embryos. Use about 500 embryos for 10 ml of antibody.

6. Prepare the Prehybridization and Hybridization mix:

Prehyband Hybridization mix (HM):

- Formamide 50-65%
- 5 x SSC
- Tween20 0.1%
- Citric acid to pH 6.0 (460 µl of 1M for 50 ml)
- Heparin 50 µg/ml
- tRNA 500 µg/ml

Note: Add tRNA and Heparin to the prehybridation and hybridization only (not the wash solutions). Vary the % of formamide according to the desired stringency.

7. Prehybridize embryos in 800 µl of hybridization mix, 2 to 5 hrs at 70°C.

8. Remove prehybridization mix, discard, and replace with 200 µl of hybridization mix containing 100 - 200 ng of antisense RNA probe.

Hybridize overnight in a waterbath at 70°C.

In situ Day 2:

Washes:

1. 100% HM at 70°C, very brief wash

2. 75% HM/25% 2 x SSC at 70°C, 15 min

3. 50% HM/50% 2 x SSC at 70°C, 15 min

4. 25% HM/75% 2 x SSC at 70°C, 15 min

5. 2 x SSC at 70°C, 15 min

6. 0.2 SSC (when 50% formamide is used during the first day) or wash in 0.05 SSC (when 65% formamide is used), 2 x 30 min

7. 75% 0.2 (or 0.05) x SSC/25% PBT at RT, 10 min

8. 50% 0.2 (or 0.05) x SSC/50% PBT at RT, 10 min

9. 25% 0.2 (or 0.05) x SSC/75% PBT at RT, 10 min

10. PBT at RT, 10 min

11. PBT/2% sheep serum/2mg:ml BSA at RT, several hrs

Incubation with anti-DIG antiserum:

Incubate in antibody solution overnight with agitation at +4°C.

Anti-DIG antibody solution:

- Preadsorbed anti-DIG, 1:5000 dilution (final concentration) in PBT
- 2% sheep serum
- 2mg/ml BSA

In situ Day 3:

Washes:

Remove antiserum, discard, and then wash extensively:

1. PBT at RT, very brief wash

2. PBT at RT, 6 x 15 min

3. Staining buffer (100 mM tris HCl pH9.5, 50 mM MgCl2, 100 mM NaCl, 0.1% tween 20), 3 x 5 min

Staining:

1. Inclubate embryos in staining solution at RT and monitor with a dissecting microscope.

Staining solution:

- NBT 50 mg/ml - 225 µl
- BCIP 50 mg/ml - 175 µl
- Staining buffer - 50 ml

(NBT stock: 50 mg Nitro Blue Tetrazolium in 0.7 ml of Dimethylformamide anhydre + 0.3 ml H2O. BCIP stock: 50 mg of 5-Bromo 4-Chloro3Indolyl Phosphate in 1mlanhydrous Dimethylformamide).

2. Stop the reaction by removing the staining solution and washing the embryos in:

Stop solution:

- PBS pH5.5
- EDTA 1mM

3. Store the embryos in stop solution at +4°C in the dark.

Mounting:

1. For observation using a dissecting microscope, mount embryos directly in stop solution and methylcellulose.

2. For observation using a compound microscope, mount embryos in 100% glycerol.

3. For embryos at early development stage (up to 18h), dehydrate in 100% methanol, clear for a few minutes in methylsaly-cilate, and mount in Permount.

Materials and supplies:

- PFA: paraformaldehyde (Sigma)
- 10 x PBS
- MeOH: methanol
- Tween20 (Sigma P1379)
- Proteinase K (Boehringer 1000144)
- Anti DIG antibody - alkaline phosphatase Fab fragment (Boehringer 1 093 274)
- BSA fraction V protease free (Sigma A-3294)
- Formamide (deionized, high purity grade)
- 20 x SSC
- Heparin at 5 mg/ml (Sigma H3393)
- RNAse free tRNA (Sigma R7876, 50 mg/ml resuspended in H2O and extensively extracted several times in Phenol/CHCl3 to remove protein)
- Citric acid 1M
- Normal Sheep serum (Jackson Immunresearch 013-000-121)
- Tris HCl pH9.5 1M
- MgCl2 1M
- NaCl 5M
- NBT 50 mg/ml (made from powder, Sigma N6876)
- BCIP 50 mg/ml (made from powder, Sigma B8503)
- PBS pH5.5
- EDTA 0.5M
- Glycerol 100%
- Methylsalycilate (Sigma M6752)
- Permount (Fisher SP15-100)

This protocol is adapted from:

Thisse, C., Thisse, B., Schilling, T. F., and Postlethwait, J. H. (1993). Structure of the zebrafish snail1 gene and its expression in wild-type, spadetail and no tail mutant embryos. *Development* **119**, 1203-1215.

A Putative Pp2c Homologue That Shows Female-Specific Expression in the Adult Zebrafish

By P. Stothard, A. Manning and D. Pilgrim, University of Alberta, Edmonton, Alberta, Canada, T6G 2E9

Members of the serine/threonine phosphatase 2C (PP2C) family have been shown to be components of a diverse range of signal transduction pathways, including pathways that regulate sex determination in *C. elegans,* hormone responses in *Arabidopsis*, and osmotic balance in yeast. We have cloned three putative PP2C homologues from zebrafish using a PCR-based approach. One homologue is 80% identical to mammalian PP2C-alpha and another homologue is 74% identical to mammalian PP2C-beta. Recent experiments suggest that mammalian alpha and beta PP2C's may be involved in phototransduction. The third homologue is 52% identical to the bovine pyruvate dehydrogenase phosphatase (PDP). We have examined the expression of the zebrafish PP2C homologues using Northern analysis. The homologue with greatest similarity to the mammalian PP2C-beta shows expression of a 2.0 kb transcript in both male and female adult zebrafish. Interestingly, the homologue with the greatest similarity to PDP shows expression of a 1.9 kb transcript in female adult zebrafish, but no transcript is detected in male adult zebrafish. The same expression pattern is supported by RT-PCR experiments. The expression of the PDP homologue in females only suggests that it is involved in a sex-specific pathway. Based on previous findings we hypothesize that it may also have a role in sex determination or differentiation.

Mutagenesis...

(cont'd from page 1)

foxide (DMSO) and store in aliquots at -70° C in the dark.

2) Anesthetize 3-5 males by immersion in tricaine for 1-3 min according to the standard procedure from *The Zebrafish Book*.

3) When anesthetized, rinse the fish with system water, wipe and hold the fish upside-down in a slit in a sponge block.

4) Collect sperm from 3-5 fishes with 20 µl capillary and suspend in 100 µl Hank's saline containing 3-30 ng/ml TMP and 1% DMSO. Incubate the suspension which appears slightly white on ice for 5 min.

5) Drop 10 µl each of the sperm suspension onto a plastic petri dish.

6) Irradiate the suspension through the bottom of the dish (make the thickness 2 mm by placing the petri cover plate under the dish) at 312 nm and 0.02 J/cm2 with UV transilluminater (TFL-20M, Vilber Lourmat, France).

7) Transfer the suspension into a new tube on ice.

8) Expel 100 µl of the mutagenized sperm suspension onto eggs, and fertilize *in vitro* according to the standard procedure (*The Zebrafish Book*).

9) Incubate the fertilized eggs at 28.5° C in the dark for 12 h.

Ventral and Lateral Regions of the Zebrafish Gastrula, Including the Neural Crest Progenitors, are Established by a *bmp2b/swirl* Pathway of Genes

By V.H. Nguyen[1], B. Schmid[1], J. Trout, S.A. Connors, M. Ekker[2], and M.C. Mullins[3], University of Pennsylvania School of Medicine, Department of Cell and Developmental Biology, 605 Stellar-Chance, 422 Curie Blvd., Philadelphia, PA 19104-6058, and [2]University of Ottawa, Loeb Institute for Medical Research, 725 Parkdale Ave., Ottawa, Ontario, Canada

A bone morphogenetic protein (BMP) signaling pathway is implicated in dorsoventral patterning in *Xenopus*. Here we show that three genes in the zebrafish, *swirl*, *snailhouse*, and *somitabun* function as critical components within a BMP pathway to pattern ventral regions of the embryo. The dorsalized mutant phenotypes of these genes can be rescued by overexpression of *bmp4*, *bmp2b*, an activated BMP type I receptor, and the downstream functioning *Smad1* gene. Consistent with a function as a BMP ligand, *swirl* functions cell non-autonomously to specify ventral cell fates. Chromosomal mapping of *swirl* and cDNA sequence analysis demonstrate that *swirl* is a mutation in the zebrafish *bmp2b* gene. Interestingly, our analysis suggests that the previously described non-neural/neural ectodermal interaction specifying the neural crest occurs through a patterning function of *swirl*/*bmp2b* during gastrulation. We observe a loss in neural crest progenitors in *swirl/bmp2b* mutant

Cont'd on Page 11

embryos, while *somitabun* mutants display an opposite, dramatic expansion of the prospective neural crest. Examination of dorsally- and ventrally-restricted markers during gastrulation reveals a successive reduction and reciprocal expansion in non-neural and neural ectoderm, respectively, in *snailhouse*, *somitabun*, and *swirl* mutant embryos, with *swirl/bmp2b* mutants exhibiting almost no non-neural ectoderm. Based on the alterations in tissue specific gene expression, we propose a model whereby *swirl/bmp2b* acts as a morphogen to specify different cell types along the dorsoventral axis.

"Research is what I'm doing when I don't know what I'm doing."

Wernher Von Braun

"Only two things are infinite, the universe and human stupidity, and I'm not sure about the former."

Albert Einstein

"Never doubt that a small group of thoughtful, committed citizens can change the world. Indeed, it's the only thing that ever has."

Margaret Mead

The ZFIN Database: A Forecast for Good Fishing

By E. Doerry, Department of Computer and Information Science, 1202 University of Oregon, Eugene, OR 97403-1202

Stop and think about how you do research today. How much time do you spend tracking down information on mutations, genes, researchers, publications, supplies, materials and so on? How much time do you invest in keeping abreast of new developments, e.g., scanning publications and networking with colleagues? How often have you been frustrated by the lack of timely, accurate information?

You'll probably discover that you spend a surprising amount of time on these kinds of *information retrieval and integration* tasks. One reason for this is that information relevant to zebrafish is scattered across a variety of media, ranging from huge general-purpose databases like MEDLINE and GENBANK to dusty lab notebooks in the archives of individual labs.

The goal of the ZFIN database is to bring together these many disparate kinds of information in an easily-accessible, highly-integrated format. The ZFIN database has been publicly accessible for nearly a year now; many of you have probably used it at least once or twice to find information on zebrafish publications, researchers and labs. This archive of 'community information', however, represents only a small fraction of the information that will soon be accessible through ZFIN. During the past year, we have been working very hard to design efficient interfaces and upload and organize a variety of experimental data, primarily centered around zebrafish mutations and genetic maps. Thanks to the energy and commitment of information producers at zebrafish labs around the world, this effort is approaching fruition. A ZFIN component cataloguing information on mutations is currently in beta-testing and should be available by the end of June and a component devoted to zebrafish genomics will become publicly accessible by the end of the summer. For the longer term, we are developing tools for searching and viewing a graphical anatomical atlas and staging series, and for performing more advanced genomic analyses.

Although ZFIN is divided conceptually into the distinct components mentioned above, our goal is to integrate them tightly and seamlessly within an interface that is so easy to use that it has no user manual and requires no special training. Thus, a researcher with a new mutant will be able to search for mutations with similar phenotypes, examine the genetic map to find candidate genes (including potential conserved syntenies from other species), read the abstracts of relevant publications, and contact the labs and researchers that produced the related mutants or mapping information all within a single ZFIN session.

One of the most novel aspects of ZFIN is that it is designed to be a true *community database*, directly supported and maintained by the research community. Unlike databases for other species, which primarily rely on an army of data editors to extract and enter information from publications, ZFIN will allow researchers to submit their experimental results directly. This paradigm has a number of advantages: information can be updated

and disseminated almost instantaneously; researchers can make available all of their experimental results (e.g., images of a mutation) rather than the small fraction allowed by journal articles. The idea of user-submitted data, particularly prior to publication, is clearly quite controversial. Our goal in designing ZFIN is to find ways to accommodate the social realities of the science explicitly within the design, while at the same time challenging the community to find ways to accommodate this new information-sharing paradigm.

Another novel feature of ZFIN is that, as a community database, its goal is to support not only the information archiving and retrieval capabilities traditionally associated with scientific databases, but also the informal critique, discussion, and information exchange that lies at the heart of modern scientific research. For instance, we are experimenting with "data commentaries" that allow researchers to attach critique or commentary to data records; other researchers may respond, attaching their own comments and critique. These mini-newsgroups provide a focused, searchable record of the critical discussion related to a specific data item, allowing researchers to survey current issues quickly and join the discussion. It will also be possible to "tag" data records or abstract queries so that automatic notifications are sent when the data record or query result changes in the future. Finally, we are developing a shareable laboratory notebook for documenting interactions with ZFIN and discussing them with colleagues.

In sum, we see ZFIN as a resource that will change the nature of zebrafish research, reducing the amount of effort spent chasing data and bringing together a geographically distributed community. But we can't achieve this goal without your help. Your comments, critique, and suggestions are crucial in our effort to make this a truly useful and 'user-friendly' resource. Please visit ZFIN at **http://zfish.uoregon.edu/ZFIN** and let us know what you think!!!

ZFIN Timeline Summary

Fall 1995 Start of Project

1996 Research and design of database system and interface

Spring 1997 Beta-testing of first component

Fall 1998 Information on researchers, labs, and publications publicly available

June 1998 Comprehensive information on zebrafish mutations publicly available

July/August 1998 Beta-testing of genomics component

Fall 1998 Comprehensive, integrated information on genetic map available.

Including Your Record in ZFIN

To include your record in ZFIN, supply the following information to Pat Edwards, ZF Admin. Coord. (**edwards@uoneuro.uoregon.edu**)

To Include Your Personal Record in ZFIN

- Name
- Full mailing address
- Phone number
- FAX number
- E-mail address
- Website address (url)
- Lab Affiliation (if applicable)
- Statement of research interests
- Photo or scanned image (optional)

Becoming a Registered ZFIN Submitter

Anyone may visit the ZFIN site and browse the data archived there. However, only *registered ZFIN submitters* are able to add or update records at the site. This includes the ability to update the information (e.g. address, phone #, etc.) in your personal ZFIN record; in the future, registered submitters will be able to add and update experimental data records as well. To cecome a registered ZFIN submitter, supply Pat Edwards with the username and password that you wish to use. Once it has been entered, you may make changes to your own record only.

Keeping your person/lab data current is up to you. Pat is happy to help if necessary, but each person should learn how to update their own records.

Other Zebrafish On-line Resources

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

The Fish Net

(**http://zfish.uoregon.edu/**)

The Fish Net is an official zebrafish research website. The server is located at the University of Oregon, and mirror sites have been set up for easier access for researchers in other parts of the world, as well.

The urls for these sites are:

http://www.grs.nig.ac.jp:6060/index.html (Asia & Pacific Rim)

http://www-igbmc.ustrasbg.fr/index.html (Europe)

Contents

The Fish Net contains information about and links to:

- Embryonic and larval anatomy - an anotated anatomical atlas
- Genomics - genetic map and sequence projects
- Informatics - ZFIN, the on-line database of zebrafish information; links to zebrafish WWW sites
- Genetic Strains - zebrafish wild-type and mutant strains
- Molecular Probes - DNA libraries, cloned genes, and antibodies
- Publications and news - books, lab manuals, jobs, funding, general information
- References & Community - zebrafish research publications, addresses
- Staging Series - embryonic and early larval developmental stages
- SEARCH the Fish Net website - Keyword search of all documents available here

The Zebrafish Information Server

(**http://zebra.biol.sc.edu/**)

The Zebrafish Information Server has existed since 1994 for the Zebrafish Research Community; think of it as the friendly bulletin board at your local grocery store. It is maintained by Richard Vogt (vogt@biol.sc.edu) from the Department of Biological Sciences at the University of South Carolina.

Contents

- Special Announcements - meetings, courses, special journal issues, etc.
- Job Announcements - jobs being offered and those being sought
- K-12 Program Resources & Information of Interest
- Zebrafish Bionet Methods Archive
- Zebrafish News Group Archive and Link
- Links to Zebrafish Resources and Websites
- U.S. Federal Government Resource Links

Zebrafish News Group Zebrafish Discussion Group

(**bionet.organisms.zebrafish**)

The zebrafish news group is an on-line discussion group for those interested in zebrafish research. You can access it through most programs that you use to access the World Wide Web (WWW) such as Netscape or Internet Explorer. There is also a link to it from the Zebrafish Information Server.

Posting messages

To post a message or question to the news group, select: **bionet.organisms.zebrafish** from the list of news groups. Click on the "New Message" or "Send Message" button and a screen will appear that looks like an e-mail send screen with the news group address already inserted onto the "To:" line. Type in your message and remember to put a subject on the reference line. Send it as you would an e-mail message.

Because the news group is moderated to filter out "spams" or unrelated and unwanted messages, it will go first to the news group's moderator (currently Pat Edwards) who will then forward it to the full subscription list. It also will be posted at the **bionet.organisms.zebrafish** site for those who are browsing the list.

Subscribing

To subscribe to the news group so that the incoming messages automatically come to your e-mail address, you will need to send an e-mail message to the following address:

biosci-server@net.bio.net

Include the words "subscribe zbrafish" in your message. (Note that the "e" in "zebrafish" is not used.) No other message needs to be included... just "subscribe zbrafish" without the quotation marks.

Responding to Posted Messages

If you wish to reply to a message, you may opt either to reply to the sender directly or allow your reply to go to the whole group so that others interested in the same subject can benefit from your response. You are strongly encouraged to respond to messages that are of interest to the whole group via the group option.

Unsubscribing

If you no longer wish to receive the news group postings automatically, you may "unsubscribe" in the same way that you subscribed to the same address **(biosci-server@net.bio.net**).To unsubscribe, however, you would type in the words "unsubscribe zbrafish" within the body of your message.

Announcements

Midwest Regional Zebrafish Meeting

The First Semi-Annual Midwest Regional Zebrafish Meeting is being planned. We are tentatively defining the Midwest as any region in the U.S. or Canada not currently served by a regional fish meeting or other appropriate venue. The meeting will be held in Chicago during the summer of 1999. Chicago was chosen for the inaugural meeting because it can be reached quickly and inexpensively from virtually anywhere. The exact dates and location in Chicago are to be decided. We are currently seeking to gauge the level of interest in terms of the number of likely participants. To this end, we have sent out an announcement and are asking any interested labs who have not yet responded to contact the organizers by e-mail and to supply the number of people who are likely to attend, and the number of these people who are likely to present their work (**beattie.24@osu.edu**; please indicate zebrafish meeting in the subject heading). This information will be used to determine the length of the meeting, where it is held and to compile a mailing list. Based on our initial survey, the interest in a regional meeting alternating with the Cold Spring Harbor meeting has been high. We encourage all suggestions as to how to make the first meeting a success (**henion.1@osu.edu**; indicate suggestions in the subject heading). We look forward to hearing from you!

Meeting Organizers:

Vicky Prince, University of Chicago
Christine Beattie, Ohio State University
Paul Henion, Ohio State University

Meeting Announcement: Reminder! The Development of Sense Organs

British Society for Developmental Biology Autumn Meeting
University of Sussex
16 - 18 September 1998

Registration deadline JULY 1st (after which a late registration fee is payable)

The programme will include talks on the development of sense organs both of vertebrates and of invertebrates, including eyes, ears, noses, bristles and skin. Recent advances in our understanding of the molecular mechanisms of sense-organ development have been spectacular, and several of these structures have become important paradigms for wider issues in developmental biology.

At the meeting, we hope both to take stock of the rich variety of types of sense organs, and to see to what extent one can now identify unifying themes and conserved mechanisms in their development, with emphasis on the peripheral organs rather than the central connections.

All participants are encouraged to present a poster, and/or volunteer a contributed talk at the meeting. Speakers for a small number of contributed talks will be selected on the basis of abstracts received.

Invited speakers include:

M. Freeman (Cambridge)
V. van Heyningen (Edinburgh)
S. Wilson (London)
A. Jarman (Edinburgh)
D. Fekete (Purdue)
C. Haddon (London)
K. Steel (Nottingham)
M. Fitzgerald (London)
L. Barlow (Denver)
P. Mombaerts (New York)
D. Strutt (Sheffield)
W. Harris (Cambridge)
Y-N. Jan (San Francisco)
A. Ghysen (Montpellier)
T. Whitfield(Sheffield)
G. Richardson (Sussex)
G. Lewin (Berlin)
E. Macagno (New York)
F. Guillemot (Strasbourg)
J. Lewis (London)

For a full programme and registration forms, contact:

Ms Amrit Khalsa (BSDB Conference),
ICRF, P.O. Box 123,
Lincoln's Inn Fields,
London WC2A 3PX, UK.
Tel: (0171)-269-3356 / Fax: (0171)-269-3417
e-mail: khalsa@icrf.icnet.uk

Details will also soon appear in the BSDB summer newsletter, and on **http://www.ana.ed.ac.uk/BSDB/meetings.html**

The organisers gratefully acknowledge sponsorship from: Defeating Deafness, The Wellcome Trust, Elsevier Trends Journals, Zeneca, SmithKline Beecham, Pfizers, Boehringer Mannheim, British Neuroscience Association, Hybaid, Calbiochem-Novabiochem and Wisepress.

More Announcements

The Company of Biologists Limited is pleased to announce the availability of the special edition Zebrafish CDROM.

(Information located at http://www.biologists.com/cdrom/zebrafish/)

This CDROM contains:

- The entire contents of *Development* volume **123** (the Zebrafish Issue), and 74 other papers from *Development*
- The 37 papers of *Development* **123** are no longer available anywhere else - the printed journal has sold out, and the papers are not available on the WWW.
- In addition, other papers from *Development* volumes 117 to 124, and part of volume 125 were chosen as significant and relevant to the body of Zebrafish literature - 74 papers in all.
- All the papers are indexed and can be searched using the Acrobat Reader software (included).
- The Flipbook Movie of Zebrafish Embryogenesis Created by Rolf Karlstrom and Donald Kane, this movie originally featured as a 'flipbook' animation in the printed journal.
- The Mutants Database
- HTML and Filemaker-Pro versions of the mutants database, first published as the appendix to Haffter et al. *Development* **123** 1-36.
- The CDROM can be used on PC (Windows), Macintosh or Unix computers. You will need a web browser (Navigator 3, 4 or MSIE 4 recommended), but an Internet connection is not required.

Ordering

Costs are as follows:
EC countries: UK£31.73 (inc VAT)
Non EC countries: UK£27 / US$41 Prices include postage and packing.

Payment:
In US$ or £ sterling cheque, payable to The Company of Biologists Limited By Credit Card (American Express, Access, MasterCard, Eurocard, VISA).

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Email: **sales@biologists.com**

Positions Available

Job Opening: Director of International Zebrafish Stock Center

University of Oregon

The University of Oregon seeks a Director for an International Zebrafish Stock Center which will be built on the University campus. The Stock Center will maintain and make available to the research community wild-type and mutant zebrafish stocks, frozen sperm, and reagents. It will organize genetic markers, maintain the genetic map, and distribute information. The Director will work with an advisory committee of zebrafish researchers and local planners to design and construct the new facility, purchase equipment, set up and maintain a large-scale aquarium system, hire and train facility staff, and manage the activities of the Stock Center. With help from the advisory committee, the Director will be responsible for deciding which mutations to keep in the Stock Center as live fish and which as frozen sperm samples. The Director will need to interact efficiently with the zebrafish research community to solicit submission of mutant strains, reagents, and genetic map markers. Candidates should have an advanced degree and/or training in biology, experience with embryology and genetics (preferably zebrafish), and strong organizational and management skills. Salary comensurate with experience. For priority consideration of applicaton, file must be complete by July 1, 1998.

For more information about this position and the Stock Center, see
WWW: http://zfish.uoregon.edu/zf_info/dir.html:

To apply, contact:

Ms. Ellen McCumsey
Institute of Neuroscience
1254 University of Oregon
Eugene, OR 97403-1254
Email: ellenm@uoneuro.uoregon.edu
Telephone: 541-346-3191

AA/EO/AOA institution committed to cultural diversity

RESEARCH POSITIONS at the Laboratory of Fish Biotechnology,

Institute of Molecular Agrobiology (IMA), Singapore

Four positions, two Research Fellows (postdoctoral) and two Assistant Research Officers are available immediately to undertake research in applied fish molecular biology/biotechnology.

The recently established Laboratory of Fish Biotechnology will study the molecular basis of sex determination in fish and will apply methods of molecular biology for possible improvements in current aquaculture protocols. Ornamental and farmfish species with importance for the aquaculture of South-East Asia will be the primary target of our investigations, but model species (e.g. zebrafish, medaka) will also be studied. Methods to be applied include various PCR techniques, isolation and characterization of DNA markers, analysis of differential expression, gene transfer into fish eggs, gene expression studies in fish embryos/larvae as well as generation and analysis of transgenic lines of fish. We are looking for young, dedicated, hard-working scientists who have experience with most of these techniques. Previous experience with fish is an advantage, but candidates with mol. biol. experience on other species will also be considered, provided that they demonstrate genuine interest in fish research.

Further details on the positions are available from the IMA website

http://www.ima.org.sg

Applicants should send their full CV, reprints or summary of 2-3 relevant publications in English and names of three references to:
Laszlo ORBAN, Ph.D.
Laboratory of Fish Biotechnology
Institute of Molecular Agrobiology
1, Research Link
Singapore 117604
Tel: 65-872-7413
Fax: 65-872-7007
E-mail: **orban@ima.org.sg**

Deadline for the arrival of applications to IMA is June 15, 1998. The AROs are expected to start their term at IMA as soon as possible, while RFs preferably before the end of August, 1998.

An Opinion of Interest...

"Let us suppose that an ichthyologist is exploring the life of the ocean. He casts a net into the water and brings up a fishy assortment. Surveying his catch, he proceeds in the usual manner of a scientist to systematise what it reveals. He arrives at two generalisations:

(1) No sea-creature is less than two inches long.

(2) All sea-creatures have gills.

These are both true of his catch, and he assumes tentatively that they will remain true however often he repeats it.

In applying this analogy, the catch stands for the body of knowledge which constitutes physical science, and the net for the sensory and intellectual equipment which we use in obtaining it.

The casting of the net corresponds to observation; for knowledge which has not been or could not be obtained by observation is not admitted into physical science.

An onlooker may object that the first generalisation is wrong. "There are plenty of sea-creatures under two inches long, only your net is not adapted to catch them." The icthyologist dismisses this objection contemptuously. "Anything uncatchable by my net is ipso facto outside the scope of icthyological knowledge. In short, "what my net can't catch isn't fish."

Or--to translate the analogy--"If you are not simply guessing, you are claiming a knowledge of the physical universe discovered in some other way than by the methods of physical science, and admittedly unverifiable by such methods. You are a metaphysician.

Bah!"

Sir Arthur Eddington
(1882-1944) b. England

Ph.D. Student Positions in Evolutionary Biology at the University of Konstanz

Two positions as graduate students in evolutionary biology are available immediately in a new lab in evolutionary biology in the Department of Biology at the University of Konstanz. Diverse subdisciplines are represented and include molecular evolution/phylogenetics, developmental biology, molecular ecology, conservation genetics, and the evolution of developmental mechanisms. Our current research interests include the evolution of gene families (particularly developmental control genes such as Hox genes), comparative developmental evolution involving the zebrafish model and cichlid fishes, molecular evolution and molecular phylogeniesparticularly of diverse groups of fishes, evolution of social systems, and population level questions based on microsatellite analyses.

The University of Konstanz and the Department of Biology are among the most highly rated in Germany and provide a lively, and academically outstanding research environment. The charming town of Konstanz is located on Lake Constance one hour from Zürich and is close to both Switzerland and France.

Student stipends are paid based on 1/2 BAT II salary of approximately (depending on age and marital status) 34.000 DM (19.000 $US) annually. These positions will remain open until filled, and review of applications will begin on July 1, 1998.

Additional information can be obtained from **axel.meyer@uni-konstanz.de**, tel +49 7531 88 4163, fax +49 7531 88 3018.

Applications, including a statement of research interests, a full CV, publications if available, and up to three letters of recommendation, should be sent to: Prof. Dr. Axel Meyer, Department of Biology, University of Konstanz, D-78457 Konstanz, Germany

Post-doctoral Research in the Developmental Genetics Programme at the Krebs Institute, Sheffield, U.K.

A post-doctoral position supported by a European Union TMR Network entitled "Gastrulation and the Vertebrate Body Plan" is available in the laboratory of Phil Ingham from August 1st 1998. The Network is a multi-national collaboration between Phil's lab and the labs of Tony Durston (Utrecht), Dado Boncinelli (Milan), Jean-Paul Thierry (Paris) and John Gurdon (Cambridge) and offers a unique opportunity for interactions between these five groups.

Applicants should have a strong background in developmental biology and preferably have previous experience with zebrafish: they must be EU nationals not currently resident in the U.K.

To apply for this position, send a full C.V. and names and addresses of two academic referees to: Prof. P. Ingham, Developmental Genetics Programme, The Krebs Institute, Firth Court, Western Bank, Sheffield S10 2TN, U.K.

The closing date for applications is June 30th 1998. For further information about the Developmental Genetics Programme visit our Web Site at: **http://www.shef.ac.uk/uni/academic/A-C/biomsc/research/dgp.html**

Postdoctoral Research Position

A three year Postdoctoral position funded by the Muscular Dystrophy Group is available from 1 October 1998 in the laboratory of Dr. Peter D. Currie within the Developmental Genetics Section of the MRC Human Genetics Unit in Edinburgh. The project aim is to understand the role of secreted peptides in inducing structural muscle proteins within the developing zebrafish myotome. Applicants should have a background in developmental and molecular biology. Experience with zebrafish as a model system is preferred but not essential. The laboratory is fully equipped for zebrafish development biology including a recently completed state of the art 600 tank aquarium.

Starting salary will be in the range £17,500-£22,000 subject to age and experience. Applications including a full CV and the names of three academic referees should be sent to the Personnel Office quoting reference no. 98/170 at the above address. Informal inquiries can be addressed to **petec@hgu.mrc.ac.uk**. Further information can be obtained from our web page: **http://www.hgu.mrc.ac.uk** or from the Personnel Office 0131 539 7679. Closing date 6 July 1998.

The MRC is an equal opportunities employer.

Postdoc: Zebrafish Genomics/Development

A position is available to investigate comparative and functional aspects of zebrafish genomics, and the role of duplicate genes in developing embryos. Focusing on genome regions that first, include many developmental regulatory genes, that second, are duplicated in the zebrafish genome, and that third, conserve syntenies with the human genome, we will investigate the evolution of essential functions in duplicated genes. The approach combines molecular genetics, genomics, mutant screening, and embryological analysis.

Please send a letter describing background and goals, along with a curriculum vita and 3 letters of reference to:

Dr. John H. Postlethwait
Institute of Neuroscience
1254 University of Oregon
Eugene OR 97403-1254

An equal opportunity, affirmative action institution committed to cultural diversity and compliance with the American in Disabilities Act

POSTDOCTORAL RESEARCH; Penn State College of Medicine

POSTDOCTORAL RESEARCH opportunities are available at the Jake Gittlen Cancer Research Institute, Penn State College of Medicine. We are using the zebrafish as a new vertebrate model system to study genomic instability, cell differentiation (not development), and cancer.

We invite one fellow to characterize, map, and clone our recently identified zebrafish mutants (unpublished). Expertise in molecular biology, cell biology, cancer biology and/or histopathology is needed preferably by June 1 to study our cellular differentiation mutants and to determine their potential role as tumor suppressor genes. Candidates must be highly-motivated team players with excellent thinking and communication skills (including fluency in spoken and written English).

We are in a growing academic medical center with a superb living environment. Send c.v., statement of short/long-term career goals, and references to:

Keith C. Cheng, M.D., Ph.D.
The Jake Gittlen Cancer Research Institute, H059
Penn State University College of Medicine
P.O. Box 850
Hershey, PA 17033.
kcheng@psu.edu

AA/EOEP

Tenure Track Positions at University of Wisconsin

Just to let folks know informally: there will an advertisement in Science in the next few months announcing three tenure-track faculty openings in zebrafish biology at U. Wisc. in Madison. Faculty tenure homes will by in the departments of Anatomy (Medical School), Comparative Biosciences (Vet.School), Genetics (Agriculture and Life Sciences), or Zoology (Letters and Sci.). We anticipate getting permission to hire both begining and mid-level positions, and we are open to a wide range of approaches and interests. This is part of a strategic hiring initiative at U.W., and isbeing coupled with similar efforts in Genomics (led by Fred Blattner) and Biophotonics and Imaging (led by John White).

There will be application information in the advertisement, but if anyone has questions just email me at the address below.
Seth S. Blair
Department of Zoology
University of Wisconsin
250 N. Mills St.
Madison, WI 53706
email: **sblair@macc.wisc.edu**

THREE POSITIONS: University of Pennsylvania:

Three positions are currently available in my laboratory at the University of Pennsylvania:

A POSTDOCTORAL position is available to study the genetic and cellular basis of developmental patterning in the zebrafish brain. Projects include specification of regional cell fates, timing of commitment to regional fates, the role of homeodomain proteins (including the Otx proteins) in formation of the anterior brain. A strong background in molecular biology is required.

A RESEARCH SPECIALIST position is available to carry out basic molecular and embryological experiments in our laboratory. The projects involve research into formation of the brain, heart, and inner ear. The work will employ techniques such as plasmid preparation, cloning, PCR, in situ hybridization, fate mapping using lineage tracers, immunohistology, and genetic mapping. The position also requires participation in the care, raising, and breeding of our zebrafish stocks, and coordination of general laboratory organization. Prior experience in a molecular biology lab is essential.

We are also looking for a part-time AQUARIUM SPECIALIST to help run our zebrafish facility. The position entails approximately 20 hours work per week.

These positions are available immediately. Please send a CV and the names of three references to:

Eric Weinberg
Department of Biology
University of Pennsylvania
Philadelphia, PA 19106-6017
Fax: 215-898-8780
eweinber@sas.penn.edu

Job Announcement: Laboratory Tech II

An NIH-funded Laboratory Technician II position is available at the

University of Georgia. This position requires hands-on experience in the culture of marine and/or freshwater fish. The individual must demonstrate an understanding of the fundmaental aspects of fish husbandry including, but not limited to: fish biology, monitoring and maintaining water quality, proper diet and feeding, and maintenance of re-circulating fresh and saltwater systems. The position requires coordination of culture activities with other members of a research team with regard to breeding and perpetuation of selected genetically modified (transgenic) lineages and non-transgenic stocks (e.g. medaka, Fundulus and zebrafish). Fish care duties will be required on an alternating weekend schedule to be coordinated with members of the research team. The technician will also assist in performing various general laboratory and molecular biological procedures associated with the production of transgenic fish as models for environmental and biomedical applications, including: DNA isolation, DNA quantitation, PCR, electrophoresis, mutagen exposure experiments, etc. Experience in or an expressed willingness to learn these procedures is required.

Education/training: A bachelor of science degree or combination of college coursework, and work experience in related fields such as biology, marine science, aquatic science, genetics, or molecular biology. Position is for 12 months with possible renewal depending upon funding.

- Position availability: Immediate
- Salary: Base $18,090 annually w/ full-time employee benefits

To apply submitt a cover letter of interest and resume to:
University of Georgia
Employment Department
c/o Laboratory Technician II
Human Resources Building
Athens, Georgia 30603-4135

Also submit a copy of letter of interest and resume to: Dr. Richard Winn, CAIS, University of Georgia, 120 Riverbend Road, Athens, Georgia 30602

Univ. of Georgia is an EOC/AA institution.

SARS INTERNATIONAL CENTRE FOR MOLECULAR MARINE BIOLOGY, BERGEN, NORWAY

2-4 Sr and Jr Group Leaders (duration 6 years)
3 Postdoctoral Fellows (duration 3 years)
3 PhD students (duration 3 years)

The Sars Centre is a new division of the Bergen University Research Foundation (UNIFOB) funded by the Norwegian Research Council, the Ministry of Education, Research and Church Affairs and the University of Bergen, to promote basic research in molecular marine biology in Norway and in the international community. When full the centre will accommodate 5-7 independent research groups. Three of these groups are already in place. Research priorities defined by the Director-General in collaboration with the Scientific Advisory Committee (SAC) include molecular studies of adaptation to marine life, comparative studies of molecular mechanisms between invertebrates and vertebrates, genomic studies of marine animals and microbial marine biology. Research projects on marine organisms but not in the above mentioned areas will also be considered.

Group Leaders, whose level of appointment and salary will depend upon experience and qualifications, have a budget guaranteed for six years, for equipment, consumables and salaries. Senior and Junior Group leaders can appoint three to four and one to two persons, respectively. The laboratory space allows them to accommodate students and additional employees with external funding. The activity of the Sars Centre and its research groups is periodically evaluated by an international group of experts.

Postdoctoral positions (Salary level 46, gross salary 293.000 NOK per annum plus benefits related to expatriation and family situation) and the PhD positions (Salary level 30, gross salary 223.000 NOK per annum plus benefits) are available in the three groups already established:

- Group Molecular Genetics
(**Daniel.Chourrout@sars.uib.no**)
- Group Molecular Immunology
(**Charlie.Cunningham@sars.uib.no**)
- Group Chromatin and Gene Expression
(**Eric.Thompson@sars.uib.no**)

UNIFOB has an insurance agreement for their employees.

Deadline for application is July 1 for Postdoctoral and PhD positions, and July 15 for Group Leaders. Applicants must indicate the position and, where appropriate, the projects they wish to apply for. In addition, all applicants must include a detailed CV, a list of publications, the names of 3 references and a description of research interests (2 pages maximum). Applications for Group Leader positions must also contain an outline of the research strategy to be carried out within the Sars Centre (5 pages of text plus literature references).

Applications should be sent to: Head of Administration, Sars International Centre, HIB, Thormoehlensgt. 55, N-5008 Bergen, Norway. Specific information about the Sars Centre and the positions is available on the homepage (**http://www.uib.ni/fa/sars**) and through Head of Administration, Dr. Kjersti Birkeland (**Kjersti.Birkeland@sars.uib.no**).