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

THE ZEBRAFISH SCIENCE MONITOR

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A SOLUTION FOR DELAYED *IN VITRO* FERTILIZATION OF ZEBRAFISH EGGS

By G. Corley-Smith, Institute of Molecular Biology & Biochemistry, Simon Fraser University, Burnaby, B.C., CANADA

Coho salmon ovarian fluid can be used for delayed *in vitro* fertilization of zebrafish eggs. Thus, it can be useful for sequential fertilization of small batches of eggs or for procedures where manipulations of eggs prior to fertilization are desired.

Eggs can be squeezed from a female zebrafish, placed in a small amount of coho ovarian fluid, and held at room temperature, with fertilization rates of up to 85% still obtainable after one hour. Various batches of ovarian fluid have varying abilities to preserve zebrafish eggs in a fertilizable state.

To make coho ovarian fluid available to other zebrafish researchers, we have supplied it to a commercial distributor in the USA. We collected coho ovarian fluid in Vancouver, B.C., Canada. We tested individual batches and shipped it to SeaTech Bioproducts, who are now selling it for $20 /ml. This alleviates the difficulties we were experiencing with sending the ovarian fluid through customs to zebrafish labs in the USA.

Supplier:
SeaTech Bioproducts.
141 California Street.
Newton, MA 02158-1023
USA
Telephone: (617) 965-5092
Voice: (617) 630-5145
e-mail: none yet, www: not yet
Cat # SOF-870-1 (1 ml for $20)
Cat # SOF-870-5 (5 ml for $90)

References below indicate how ovarian fluid is used.

Ovarian fluid is presently shipped by SeaTech on dry ice. As freeze dried samples are much cheaper to mail, we have freeze dried some coho ovarian fluid and will test it shortly for its efficiency

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EXPRESSION DOMAINS OF A ZEBRAFISH HOMOLOGUE OF THE *DROSOPHILA* PAIR-RULE GENE *HAIRY* CORRESPOND TO PRIMORDIA OF ALTERNATING SOMITES

Development **122**:2071-2078

By M. Müller, E.V. Weizsäcker, and J.A. Campos-Ortega, Institut für Entwicklungsbiologie, Universität zu Köln, 50923 Köln, GERMANY

her1 is a zebrafish cDNA encoding a bHLH protein with all features characteristic of members of the *Drosophila* HAIRY-E(SPL) family. During late gastrulation stages, *her1* is expressed in the epibolic margin and in two distinct transverse bands of hypoblastic cells behind the epibolic front. After completion of epiboly, this pattern persists essentially unchanged through postgastrulation stages; the marginal domain is incorporated in the tail bud and, depending on the time point, either two or three paired bands of expressing cells are present within the paraxial presomitic mesoderm separated by regions devoid of transcripts. Labeling of cells within the *her1* expression domains with fluorescein-dextran shows that the cells in the epibolic margin and the tail bud are not allocated to particular somites. However, allocation of cells to somites occurs between the marginal expression domain and the first expression band, anterior to it. Moreover, the *her1* bands and the intervening non-expressing zones, each represents the primordium of a somite. This expression pattern is highly reminiscent of that of *Drosophila* pair-rule genes. A possible participation of *her1* in functions related to somite formation is discussed.

THE ZEBRAFISH SCIENCE MONITOR

MONTE WESTERFIELD, Editor
PAT EDWARDS, Publications Coordinator

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Editorial

A PLEA FOR CONVENTIONS

Several hallway conversations at the recent Cold Spring Harbor meeting brought gripes about misuse of nomenclature to the fore.

Can you believe that some of us are still naming genes "zf-blah"? naughty, naughty. That's not allowed.

We suppose some of you figure conventions are just for old fuddy duddies; who cares about those old rules anyway? But, hey, aren't we all supposed to be scholars?

Next time you are faced with the fun task of naming a cloned gene or a new mutant, remember that there are a few simple rules to help you keep from making a fool of yourself. We summarize them in this issue of the *Monitor* and encourage you to read and remember them.

ZEBRAFISH NOMENCLATURE

Adapted from: Mullins, M. (1995) TIG Genetic Nomenclature Guide: Zebrafish. *Cambridge, UK: Elsevier Trends Journals, p. 31-32.*

By M. Mullins, University of Pennsylvania, Dept/Cell & Developmental Biology, 605 Stellar-Chance, 422 Curie Blvd., Philadelphia, PA 19104-6058 USA

Conventions

Zebrafish gene names are lower case and italicized. Abbreviations should be three Roman (no Greek) letters, or three letters with a number (no hyphens) italicized. Names are not preceded by "z" or "zf". Examples: *cyclops*, *cyc*, *engrailed2*, *eng2*.

Genes and Other Loci

For genes identified by mutation, the name chosen for the gene reflects the mutant phenotype, e.g. floating head, no tail. When mutations in different genes confer similar phenotypes, the genes should be given distinct names. Names identical to those used in other species should be avoided unless the genes are known to be homologous.

Genes identified by cloning are named according to the same principles except that gene families identified in this way may be distinguished by letters following the name, e.g. *eng1, eng2, eng3*.

Genes cloned by homology with genes in other organisms should be given the same name as their counterpart in the other organism, but designated according to the zebrafish conventions, e.g. the zebrafish homologue of the mouse *Wnt1* gene would be *wnt1*.

Alleles

Wild-type alleles are designated by a superscript plus symbol, e.g. brs^{+}. Mutant alleles may be designated generically by a superscript minus, e.g. brs^{-}. Specific mutant alleles are denoted by superscripts following the gene name. Dominant mutant alleles are designated by a "*d*" in the first position of the superscript. There follows a letter designating the laboratory in which the allele was identified, and unique characters for the particular allele. Thus, each mutant allele has a unique designation. For example, cyc^{b16}, cyc^{b13}, and cyc^{b229} are recessive alleles of cyclops identified in Eugene, Oregon (laboratory designation "*b*"). Some other laboratory designations are "*m*" for MGH, Boston; "*t*" for Tübingen; "*n*" for Newcastle.

Chromosomes and Linkage Groups

Cytological definitions of chromosomes are not yet available. The numbering system of linkage groups (I-XXV) should be used for now (Johnson et al., *Genetics* **142**:1277-1288, 1996).

Nomenclature Committee

Mary Mullins (chair, see address above), Chuck Kimmel (University of Oregon, USA), José Campos-Ortega (University of Cologne, Germany), John Postlethwait (University of Oregon, USA), Nigel Holder (The Randall Institute, King's College, University of London, UK).

The full nomenclature guidelines are available in Mullins, M. (1995) Genetic Methods: conventions for naming zebrafish genes, The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish (*Danio rerio*), Edition 3, M. Westerfield, ed., Eugene, Oregon: University of Oregon Press, p.p. 7.1-7.4. They are also available in the on-line version of The Zebrafish Book, which is part of the documentation provided by the WWW zebrafish server at the University of Oregon <http://zfish.uoregon.edu>.

Solution... ***continued from page 1***

in delaying fertilization of zebrafish eggs. We are also working towards developing a defined medium in collaboration with Derek Stemple in Wolfgang Driever's Lab. We have performed an extensive chemical analysis of coho ovarian fluid and hope by the summer of 96 to have a defined medium that has the ability to delay fertilization of zebrafish eggs . At that time we will make the formulation available to the zebrafish community.

We presume most researchers would rather buy a relatively cheap mixture than make the mixture from scratch. Thus, if and when we develop such a mixture, we hope a company such as SeaTech will distribute it at a reasonable cost. Until then, coho ovarian fluid is available, at least in a limited supply.

I do not have any affiliation with SeaTech. Collection and testing was performed at the Institute of Molecular Biology and Biochemistry, Simon Fraser University, free of charge so that the coho ovarian fluid could be made available to other zebrafish researchers at as low a fee as possible. Derek Stemple has received and tested coho ovarian fluid. It worked for him.

References:

Corley-Smith, G.E., C.J. Lim and B.P. Brandhorst (1995) Delayed *in vitro* fertilization using coho salmon ovarian fluid. In *The Zebrafish Book - A Guide For The Laboratory Use Of Zebrafish (Danio rerio)*, edition 3, edited M. Westerfield, Institute of Neuroscience, University of Oregon, pp. 7.22-7.26.

Corley-Smith, G.E., C.J. Lim, and B.P. Brandhorst (1995) Delayed *in vitro* fertilization of zebrafish eggs using coho salmon (*Oncorhynchus kisutch*) ovarian fluid. *The Zebrafish Science Monitor* **3**(5): 9-10.

We have a copy of the protocol for use of ovarian fluid on our web site. http://darwin.mbb.sfu.ca/imbb/brandhorst/ofzfbook.htm

PREPARATION OF DNA-NLS COMPLEXES FOR MICROINJECTION INTO FERTILIZED ZEBRAFISH EGGS

By P. Collas and P. Aleström, Department of Biochemistry, Norwegian College of Veterinary Medicine, PO Box 8146 Dep., N-0033 Oslo, Norway

Introduction

We have previously reported the binding of the nuclear localization signal (NLS) of SV40 T antigen to plasmid DNA, and the induction of transgene expression following injection of DNA-NLS complexes into the yolk of fertilized zebrafish eggs (Collas et al., 1996). NLS peptides were bound to DNA by simple ionic interaction at a 100:1 molar ratio of NLS:DNA. NLS peptides bound to plasmid DNA efficiently target DNA-NLS complexes to nuclei. Transient reporter gene expression with 10^4 plasmid copies injected per egg using this system is similar to that with 10^6-10^7 copies injected without the NLS peptide (see, e.g., Stuart et al., 1988; Culp et al. 1991; Collas et al. 1996). Binding NLS peptides to 10^6 plasmid copies or more is detrimental to transgene expression and zebrafish embryo survival (Collas et al., 1996).

We present here the procedure used in our laboratory to prepare plasmid DNA-NLS complexes for cytoplasmic injection into zebrafish eggs. Complexes are prepared at a ratio of 100 moles of NLS per mole of DNA. It is assumed that 10,000 plasmid copies are injected per egg, in a volume of 250 pl. Note that plasmid DNA can be in supercoiled or linear form. The recipe is for the following NLS peptide: CGGPKKKRKVG-NH2 (Collas et al., 1996a).

Procedure

1. Calculate the intended concentration of plasmid DNA in the injection solution, taking into account DNA copy number and volume to be injected per egg. Prepare a DNA solution at 100x this concentration in H_2O.

2. Calculate the number of moles of NLS peptide required to obtain a final NLS:DNA molar ratio of 100:1. Calculate the corresponding number of grams of NLS (M = 974), and prepare a 5x NLS solution in H_2O (from an initial frozen stock dissolved in ddH_2O at 1 mg/ml).

3. Prepare 1 ml of 1 M KCl in H_2O, and 1 ml of 1% phenol red in H_2O.

4. Prepare the injection solution by mixing together:

Sterile H_2O	68 µl
1 M KCl	50 µl
1% Phenol red	40 µl
DNA solution	2 µl
NLS solution	40 µl
TOTAL	200µl

and incubate for 30 min at room temp.

5. Filter the solution and proceed with injections (Collas et al., 1996b). This solution can be stored for at least two weeks at 4°C.

References

Collas, P., H. Husebye, and P. Aleström (1996a) The nuclear localization sequence of the SV40 T antigen promotes transgene uptake and expression in zebrafish embryo nuclei. *Transgen. Res*. **5**:in press.

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TEMPERATURE-SENSITIVE MUTATIONS THAT CAUSE STAGE-SPECIFIC DEFECTS IN ZEBRAFISH FIN REGENERATION

By S.L. Johnson and J.A. Weston, Institute of Neuroscience, 1254 University of Oregon, Eugene, Oregon 97403-1254 USA

When amputated, the fins of adult zebrafish rapidly regenerate the missing tissue. Fin regeneration proceeds through several stages, including wound healing, establishment of the wound epithelium, recruitment of the blastema from mesenchymal cells underlying the wound epithelium, and differentiation and outgrowth of the regenerate. We screened for temperature-sensitive mutations that affect the regeneration of the fin. Seven mutations were identified, including five that fail to regenerate their fins, one that causes slow growth during regeneration, and one that causes dysmorphic bumps or tumors to develop in the regenerating fin. reg^5 mutants fail to regenerate their caudal fins, whereas reg^6 mutants develop dysmorphic bumps in their regenerates at the restrictive temperature. Temperature shift experiments indicate that reg^5 and reg^6 affect different stages of regeneration. The critical period for reg^5 occurs during the early stages of regeneration before or during establishment of the blastema, resulting in defects in subsequent growth of the blastema and failure to differentiate bone forming cells. The critical period for reg^6 occurs after the onset of bone differentiation and during early stages of regenerative outgrowth. Both reg^5 and reg^6 also show temperature-sensitive defects in embryonic development or in ontogenetic outgrowth of the juvenile fin.

ZEBRAFISH STOCK CENTER PLANNED

By. W. Driever, MGH East 4, 13th Street, Bldg. 149, Charlestown, MA 02129 USA

Following on the heels of discussion at the Cold Spring Harbor meeting, a committee has begun contacting various funding agencies who may be able to support a zebrafish stock center in North America. Currently, groups from Boston and Eugene are working with both the National Science Foundation and National Institutes of Health.

Based the results of the survey which was conducted during the meeting, this group is compiling statistics about the need for and potential use of a stock center. If you did not attend the meeting or did not have a chance to fill out this survey, please do so now. A form is included with this issue of the Monitor.

It will also be extremely important for success of the funding applications to receive letters of support from you. Please take a moment to write a short note to Wolfgang Driever. State your view on the importance of establishing a centralized stock center that can maintain a collection of mutant and wild-type zebrafish and that can distribute these fish to the scientific community.

The committee would also like to hear from anyone else who is interested in planning the stock center or who may also be thinking about establishing a stock center so that efforts can be coordinated for highest efficiency and for best meeting the needs of the community.

Please address your letters to:

Wolfgang Driever
MGH East 4
13th Street, Bldg. 149
Charlestown, MA 02129
email: driever@helix.mgh.harvard.edu
fax: 617-726-5806

POSTER VIEWING AS A CONTACT SPORT

By B. Jones, Cancer Research Labs, Queen's University, Kingston, Ontario K7L 3N6, CANADA

The recent zebrafish development meeting at Cold Spring Harbor was, from my point of view, very successful, an intellectual feast served up against the background of spring on Long Island. To northerners still experiencing the final assault of winter at home, the sight of tree blossoms and flowers, the sound of birds and frogs, and the feel of the warm spring sunshine, is seductive. It is hard to imagine that in this idyllic setting danger lurks, but it does. You may be thinking of the devastating allergic reactions brought on by those innocent blossoms, and all the itchy eyes and palates and noses you saw or heard or experienced, but the most dangerous thing at Cold Spring Harbor actually is poster-viewing.

Once in the crowded aisles of the Bush Lecture Hall between the posters, you are in immediate danger of being stepped on, tripped up, elbowed, jostled, speared, or overcome by heat or claustrophobia. It is particularly difficult in this group dominated by tall Americans and northern Europeans, if you happen to be short and are caught in a group of people single-mindedly pursuing disparate routes to target posters in different parts of the room. It is that awful feeling of being trapped in the hypoblast while the epiblast walks all over you.

Successful poster viewing, is best treated as a contact sport. The following tips will help you reach your poster destinations, get you within range of the presenter, and provide you with a view of the written material.

1. Do not wear open-toed sandals. There may be many viewers out there who are taller and heavier and wearing sturdier footwear (fortunately stiletto heels are not in vogue for poster-viewing). Remember when you are trodden that the offenders are probably not malicious, they just have a poor view of the floor through the thicket of legs.

2. Drink plenty of liquids before the poster session (this is particularly important if you have been training in the bar the previous night; see item 6). It will be hot and sweaty and it may be some time before you are able to navigate out to the drinking fountain. Also, a well-lubricated throat has a better chance of projecting sound above the general din.

3. Do some stretching and flexibility exercises before attempting to view the posters. You will be in much better shape to twist and squat and take advantage of gaps at any level in front of your chosen poster.

4. As with other spectator sports, it may be useful to bring a pair of small binoculars in case you are unable to work your way in to close range. Note that meeting decorum calls for confining binocular use to the posters.

5. If you're not nimble enough to avoid being knocked off your feet, bring along a couple of sure-footed friends to block for you.

6. The best form of ross-training may be a few sessions in the bar when the evening talks have ended. There you will have plenty of practice squeezing into small open spaces, moving against the body tide, and attracting the attention of the distant bartender. If you have perfected the "elbows out" technique and have survived without spilling a drink, you are ready for the poster hall.

7. And finally, if you wish to resort to trickery to clear a space for yourself, just ask loudly if it is expressed in the nose.

EARLY DEVELOPMENT OF FUNDULUS HETEROCLITUS

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Provided by J.P. Trinkaus, Department of Biology, Yale University, New Haven, CT 06511 USA

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JANE MARION OPPENHEIMER
1911-1996

By Margaret Hollyday, Bryn Mawr College

Jane Marion Oppenheimer, who died on 19 March 1996 in her 84th year, achieved distinction as an embryologist and historian of science. She was born in Philadelphia on September 19, 1911. After graduating from Bryn Mawr College in 1932, where she majored in zoology, she became a graduate student at Yale University. There she was influenced by John Spangler Nicholas, her Ph.D. advisor, whom she credits with focusing her intellectual interests on both embryology and its history, and by Ross Granville Harrison, director of the Osborn Zoological Laboratories, the leading American embryologist of the day. She received her Ph.D. in zoology from Yale University in 1935. The work she began as a graduate student formed the basis for her experimental career. Nicholas introduced her to the embryo of the common minnow or killifish, Fundulus heteroclitus. Nicholas had previously devised a method for dechorionating the embryo, thus making it possible to perform precise experimental manipulations on teleost embryos for the first time. Miss Oppenheimer independently pursued the work on Fundulus, while Nicholas pursued his own interests in rat embryology and nervous system function.

Dr. Oppenheimer joined the faculty of Bryn Mawr College in 1938 as an instructor in Biology. She retired from the College faculty in 1980 as the William R. Kenan, Jr. Professor of Biology and History of Science. She belonged to numerous professional and scholarly societies. She was president of the American Society of Zoologists (1973) and a fellow of the American Association for the Advancement of Science. Her many honors included election to the American Philosophical Society, of which she was secretary from 1987 to 1992, and the Academy of Arts and Letters. Among her national and international awards were the Otto H. Hafner Award from the American Association of the History of Medicine and Medical Library Association, the Karl Ernst von Baer Medal from the Estonian Academy of Sciences, and the Wilbur Lucius Cross Medal from the Yale Graduate Alumni Association. Bryn Mawr College recognized her teaching with the Christian R. and Mary F. Lindback Award for distinguished teaching in 1976. Professor Oppenheimer was named a "Distinguished Daughter of Pennsylvania" in 1981.

Dr. Oppenheimer made a number of important contributions to teleost embryology. A group of seven papers published from 1934 to 1937 is especially noteworthy. She showed that induction of a secondary axis including neural structures can and does occur in Fundulus when grafts of vitally stained dorsal lip material from young gastrulae are implanted into hosts the same stage as the donor. Those grafting experiments demonstrated that the dorsal lip of the fish embryo showed the same organizer activity as did the dorsal lip of amphibian embryos. Dr. Oppenheimer also performed fate mapping experiments of the fish embryo blastoderm and described cell movements of gastrulation. She published a staging series for Fundulus embryos. These early papers provide a wealth of information about the early development of the fish embryo of interest to contemporary workers. Throughout her career as an embryologist, Dr. Oppenheimer continued to exploit the methods of experimental embryology to explore questions of inductions, differentiation capabilities and regulation. One of her last Fundulus projects involved sending embryos into space to study the effects of zero gravity on embryonic development. She participated in the first joint U.S.-U.S.S.R. cooperative venture; Fundulus embryos were included on the 1975 Apollo-Soyuz space shuttle mission.

As an historian of science and medicine, Dr. Oppenheimer wrote numerous articles and reviews, many on the origins of embryology. She enjoyed intellectual history and was motivated to write about her library discoveries believing that "life in our laboratories is made more meaningful to us when we know something about our intellectual forebears." A topic of special interest to her was the relationship of embryological data to evolutionary theory. She also relished telling dramatic stories about early physiological and surgical discoveries. Her biographical work included some of the great historical figures in embryology such as Karl E. von Baer, Curt Herbst and Ross Harrison. The influence of her work can be seen in a number of disciplines including developmental biology and the history of science, anthropology, evolutionary biology and psychology. Professor Oppenheimer's writings will continue to be a source of valuable information and inspiration for others similarly interested in following threads of modern ideas to their historical precedents, especially for more recently educated American scientists whose lack of foreign language training will prevent them from reading original sources.

Jane M. Oppenheimer was an exacting scholar whose attention to detail was noteworthy; she was also a prodigious book reviewer with more than 400 published reviews. She respected others who were erudite and had little patience for those who lacked rigor in scholarship. Colleagues who were creative and imaginative as well as rigorous gained her admiration. In recent years, she was delighted by the resurgence of interest in teleost embryology, and she followed modern work on zebrafish development with considerable interest. As much as she appreciated the recognition given her once overlooked Fundulus embryological work done more than one-half century ago, she would have been dismayed by the incorrect attributions and bibliographic errors which occur not infrequently in modern journal articles. As an historian and a scientist, she took pains to get all her facts right and to place them in the context of a broader conceptual framework. Borrowing a sentence from one of her own book reviews: "The qualities of her work are inseparable from those of her person."

ZEBRAFISH STOCK CENTER QUESTIONNAIRE

1. Do you currently actively work with zebrafish?

2. Does your laboratory keep wild type zebrafish stocks only? (Y / N)

3. In case your lab keeps mutant stocks, how many stocks do you maintain at present?

4. Given what you have learned at the zebrafish meeting about the mutations recently generated, and available in this summer, how many different mutations would you like to obtain for your research?

5. Would you pursue new avenues of research if mutant stocks would be readily available? (Y / N)

6. Do you think a stock center for the zebrafish community should be established? (Y / N)

7. Given the large number of mutations currently generated (> than 2000), resources for the stock center will likely be limited. What is most important to you with regard to the stock center
(Y / N ; check one in A, one in B):

   A. Frozen Sperm sample resource:

      ☐ All mutant alleles ever generated maintained as frozen samples:

      ☐ One representative allele per mutant locus maintained as frozen sample:

   B. Stocks to be kept alive for ready distribution (check 1):

      ☐ One representative allele per locus kept as live fish for ready distribution:

      ☐ Only the 100 most frequently requested ones kept alive, others as frozen samples, which require three to four months before adult fish can be shipped:

      ☐ Only the 200 most frequently requested stocks kept alive:

8. Should a stock center also engage in breeding and distributing different wild type genetic strains?

Would you buy these fish from the stock center at cost, or prefer to breed them yourself?

9. Since we plan on submitting a stock center grant to NIH, we will likely need information on whether a stock center could save expenses on current grants (like RO1, etc.). Do you have NIH or NSF grants?

10. If mutant stocks would be readily available (say, stocks of fish within three months of submission of request), would you plan to order them as needed, or try to maintain them yourself?

11. In 1997, when all mutations from the screen will be available, how many mutants would you use but NOT keep continuously if a stock center is available?

12. For those labs currently keeping stocks: How much do you estimate you will spent (in US$) per year on keeping one mutant line alive in your lab (supplies, food, salaries, fringe benefits and overhead)?

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