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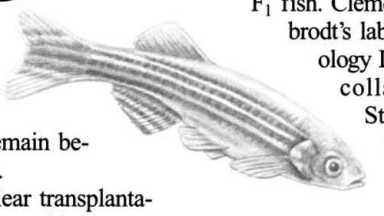
PERSPECTIVES: GENETICS AND DEVELOPMENT

Zebrafish in the Spotlight

Suresh Jesuthasan



The policewoman at the entrance to the University of Wisconsin, Madison, could hardly believe the logo on the meeting T-shirt: "You mean all these people came here to talk about a little fish?" Pioneers of the zebrafish as a model organism would probably share her surprise. At the first zebrafish meeting 10 years ago, there were fewer than 190 abstracts and the big discussion points were two developmental mutants, *spadetail* and *cyclops*. In contrast, this year's zebrafish conference featured more than 500 abstracts that discussed a wide variety of mutants and topics as diverse as learning and memory, infectious diseases, wound healing, growth control, circardian clocks, and lipid biochemistry (*1*).

With this diversity of material, it is no surprise that the talks grabbing the most attention were those reporting technical advances, particularly improved methods for manipulating gene expression and inducing targeted mutations in zebrafish. Karen Urtishak (Steve Farber's lab, Thomas Jefferson University) described a new reverse genetics tool using modified peptide nucleic acids (MPNAs) to selectively shut down the production of individual proteins. The effectiveness of MPNAs for targeted gene disruption compares well with that of morpholino antisense oligonucleotides, which were introduced at the previous zebrafish meeting two years ago. By preventing translation (knockdown) of targeted proteins, morpholinos have revolutionized our ability to test the function of genes. MPNAs (18 base pairs in length) that are complementary to specific genes such as *chordin* or *uroD* effectively prevent translation of the mRNAs encoded by these genes, resulting in abnormal development of embryos that resemble mutants. MPNAs are highly specific—mismatches of just two bases produced no phenotype (visible alteration). Although potent, these reagents are costly, and large-scale knockdown screens, in which the function of all known and predicted genes is tested by MPNA or morpholino injection, remain beyond the reach of most labs.

Cloning of fish by nuclear transplantation using donor nuclei from blastomeres has been carried out successfully in China for 50 years (*2*). Ki-Young Lee (Shuo Lin's lab, University of California, Los Angeles) reported the cloning of viable, fertile zebrafish using a zebrafish fibroblast cell line as the source of donor nuclei. The advantage of using this cultured cell line is that the zebrafish genome can be manipulated prior to cloning. In fact, Shuo Lin's group has obtained transgenic fish by transplanting nuclei from a retrovirally transformed zebrafish cell line into wild-type fish eggs. The obvious next step—transplantation of nuclei containing homologously recombined DNA—is probably one of the most eagerly awaited technical developments in the zebrafish field because it will allow the production of fish in which the expression of one or a few genes can be switched on and off at will.

Despite the unavailability of zebrafish embryonic stem cells, it is already possible to induce mutations in any given gene, as revealed in talks by representatives from the labs of Ron Plasterk (Hubrecht Laboratory) and Cecilia Moens (Fred Hutchinson Cancer Center). Plasterk's group, in collaboration with Artemis Pharmaceuticals/Exelixis Deutschland, has sequenced the *Rag-1* gene of more than 4000 F_1 progeny of fish treated with the mutagen ethylnitrosourea (ENU). Of the 15 different point mutations found in the *Rag-1* gene, one caused a premature stop codon rendering the Rag-1 protein inactive. As Erno Weinholds reported, mutant fish homozygous for this mutation were deficient in V(D)J recombination of immunoglobulin genes and presumably, like mammals carrying *Rag-1* mutations, were unable to produce antibodies. Bruce Draper from Moens's group reported on their efforts to develop a high-throughput method for identifying point mutations, borrowing the TILLING method (targeted induced local lesions in genomes) from the *Arabadopsis* world (*3*). In this approach, the CEL I endonuclease is used to identify ENU-induced point mutations in DNA from frozen F_1 fish. Clemens Grabher (Jochen Wittbrodt's lab, European Molecular Biology Laboratory) described their collaboration with Jean-Stephan Joly's lab (CNRS) to develop another method for manipulating the zebrafish genome using a meganuclease to increase transgenesis rates and improve reporter gene expression. By coinjecting the meganuclease with DNA plasmids containing a reporter gene flanked by restriction sites, germline transmission rates of up to 50% (and even nonmosaic expression) were obtained.

Many groups are using the zebrafish to answer interesting biological questions. Joseph Yost (University of Utah) described how forerunner cells—a mysterious cell population that appears in the dorsal side of the embryo during gastrulation—are essential for establishing left-right asymmetry in the developing zebrafish. These cells express mRNA encoding left-right dynein and eventually form a spherical structure called the Kupfer's vesicle. Cilia in this vesicle all beat in the same direction in wild-type zebrafish embryos, possibly establishing a gradient of growth factors within the embryo that may be responsible for establishing left-right asymmetry. Loss of forerunner cells or the Kupfer vesicle cilia abolishes left-right asymmetry in the developing zebrafish.

Christoph Seiler and Samuel Sidi (Teresa Nicolson's lab, Max Planck Institute for Developmental Biology) discussed zebrafish circler mutants that are unable to keep their balance. These investigators have applied positional cloning to the circler mutants and have identified mutations in an adhesion protein and ion channel. The Nicolson lab has strikingly combined physiological analysis (specifically microphonics, which measures the extracellular potential of sensory hair cells), genetics, and microscopy, to unravel the function of these two proteins. They show that the adhesion protein and ion channel are both required for the mechanotransduction activity of sensory hair cells in the zebrafish inner ear, where they help maintain balance, and in the skin, where they detect motion in the water. Indeed, the ability to combine in

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vivo analysis of neuronal activity with genetics is what makes the zebrafish a powerful model system for studying vertebrate neurobiology. This is exemplified by the work of Patrick Page-McCaw (Herwig Baier's lab, University of California, San Francisco), who has devised a way to screen zebrafish for learning abnormalities. When exposed to a series of taps, zebrafish larvae eventually learn not to be startled. They are not simply tired and therefore unable to respond, as a mild electric shock startles them even when they are oblivious to taps. Physiological analysis, combined with positional cloning of mutants isolated in this learning screen, should provide new insights into the neural processes controlling learning in vertebrates.

The zebrafish field is clearly maturing. A sentiment from the previous meeting—"How exciting! So many new genes!"—was not heard at this meeting. Perhaps the deluge of data from the expressed sequence tag and genome-sequencing projects has quenched our thirst for assigning new names to specific bits of genetic code. With the positional cloning of so many mutants already complete, labs are now turning their efforts to understanding the molecular pathways that are disrupted in these mutants. In vitro assays for ubiquitination, growth cone turning, and microscopy of green fluorescent fusion proteins are just some of the methods being avidly adopted to study zebrafish physiology and cell biology.

But when will the zebrafish contribute to a major breakthrough in understanding vertebrate biology, or even to the discovery of a fundamentally new phenomenon? This question came up in a discussion of the new edition of *Molecular Biology of the Cell*, which barely featured the zebrafish. For all the enthusiasm in the field, so much knowledge of cell and developmental biology has already been learned from other organisms that it is not clear what this fish can tell us. Now is the time for newcomers to the field to think hard about major questions that the zebrafish is uniquely suited to answer. For example, questions about development and function of the vertebrate brain or disease susceptibility that can best be answered through a combination of genetics and in vivo analysis for which the zebrafish is ideal. Hopefully, the enthusiasm and cooperative spirit of the fish community, combined with the tools and resources already available, will soon translate into exciting discoveries. Organizers of the next meeting might do well to remove the "development" tag from the title, because the zebrafish has become a model for much more. Perhaps we shall all be surprised by what the zebrafish will reveal about vertebrate biology to those with receptive minds asking the right questions.

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PERSPECTIVES: ASTRONOMY

X-ray Astronomy—40 Years on

Herbert Gursky

On 18 June 1962, the rocket that first detected cosmic x-rays was launched from the White Sands Missile Range in New Mexico (*1*). Today, cosmic x-ray emission, a signature of hot plasmas and relativistic electrons, is known to be ubiquitous in nature, especially from neutron stars and black holes.

The team responsible for the 1962 launch—Riccardo Giacconi, Frank Paolini, and I—then worked for American Science & Engineering (AS&E), a company now best known for airport x-ray inspection machines. The suggestion to study cosmic x-ray emission had come from Bruno Rossi, a professor at MIT and the chairman of AS&E's board of directors. As a member of NASA's Space Science Board, he was familiar with discussions relating to the potential of x-ray observations.

AS&E's principal business at that time was to measure the effects of nuclear weapons, mostly relating to x-rays. In June 1962, almost the whole technical staff, including Giacconi and Paolini, were in the Pacific, preparing for the Starfish high-altitude nuclear test that took place a month later.

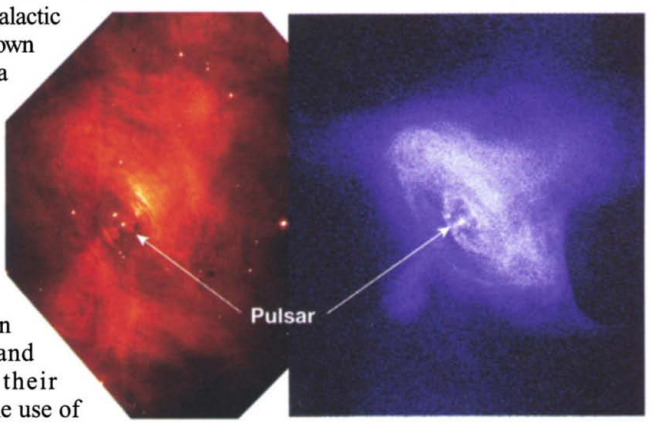
The Moon was the principal target of the June 1962 rocket flight. After calculating its emission based on fluorescence of its surface by incident solar x-rays, we decided that it could be detectable, if barely, during a rocket flight. The data revealed a very strong peak of radiation in the south. But it could not be the Moon: It was much too strong and did not line up with the Moon's direction as seen in an optical photometer.

I initially thought the radiation might be the result of local, energetic electrons. But after a few weeks of analysis, we concluded that the most likely source of the observed radiation was from outside the solar system and that the strong peak was from the general direction of the galactic center. The peak is now known to be caused by Sco X-1, a faint object in the constellation of Scorpius.

The results were certainly a surprise, but not an accident. In 1962, AS&E already had an active research program devoted to the study of x-ray radiation from space. Giacconi and Rossi had published their landmark paper (*2*) on the use of grazing incidence reflectors to study cosmic x-rays and had cited the Crab Nebula as a potential source. Two other groups were developing rocket instruments for studying cosmic x-ray emission: Herbert Friedman's group at the Naval Research Laboratory and a group headed by Phillip Fisher at Lockheed Missiles and Space Company. Albert Baez had even organized a conference on the subject of observing cosmic x-rays.

At the time, no one had a good reason to expect strong x-ray sources. But considering the advances taking place in radio astronomy, everyone expected this new window into the universe to yield its share of discoveries.

Within a few years, Sco X-1 and Cygnus X-2 were identified with faint blue stellar objects. This observation led to the idea that the x-ray emission originated from accretion from a normal star onto an unseen compact companion, such as a



The interior of the Crab Nebula. (**Left panel**) Visible light image of the Crab Nebula obtained with the Hubble Space Telescope taken in March 1994 at about 550 nm (green). (**Right panel**) Chandra x-ray image of the same region taken in August 1999 at about 3 KeV. The images are about 2 light years across.

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