Aquarium to Bedside:
The Zebrafish Role in Fatal Disease Research

John Postlethwait, researcher and professor of biology at the University of Oregon Institute of Neuroscience, has demonstrated the power of translational science. With NCRR support and resources, he and his team established a zebrafish model system for studying Fanconi anemia (FA), a fatal, inherited disorder that prevents the body from making enough new blood cells to work normally. FA leads to bone marrow failure and increases the risk of certain cancers and other serious health problems. In the U.S. and Europe, about one in every 360,000 children is born with FA. The average lifespan for people with the disease is about 25 years, and children who inherit FA are at a higher risk for birth defects.

NCRR's zebrafish resources helped Postlethwait and his team achieve critical successes at the basic research level. "We were fortunate to have NCRR support as we began our initial attempts to map the zebrafish genome starting some 15 years ago," he said. "Having the genetic map made it possible for everyone who studies zebrafish models of human diseases to map their mutations, facilitating cloning and molecular identification of zebrafish mutants." As a result, the zebrafish is a valuable model for human disease. Postlethwait's work has been published in a series of high-impact articles, one of which was published in *Genetics* in 1999 and has since been cited 1,500 times in other published studies.

BRIDGING THE TRANSLATIONAL RESEARCH GAP

Having shown that the zebrafish is a valuable model of human biology, Postlethwait sought ways to make his zebrafish research more translational. That is, he wanted to apply his findings to the search for new drugs, interventions and therapies that could improve human health. A personal connection led him to apply his zebrafish model to the study of FA: The three daughters of David B. Frohnmayer, then-president of the University of Oregon (where Postlethwait's lab is located), all had FA; two have since died.

Knowing that zebrafish and humans have some genetic material in common, Postlethwait started to probe the zebrafish genome. "DNA in any organism is under constant environmental assault that causes breaks and other damage to DNA

■ John Postlethwait and his team are screening thousands of drug candidates for activity against mutations that cause Fanconi anemia (FA), a rare genetic blood disease. With NCRR support, they have developed a model system in zebrafish that carries the human genes for FA and exhibits structural changes that parallel some of those found in humans with the disease.

WHY ZEBRAFISH?

One of the more widely used, well-characterized animal models is *Danio rerio* – the zebrafish, a popular denizen of home aquariums. These tiny animals are providing remarkable insights into the biology of a larger creature – *Homo sapiens.* It turns out that humans and zebrafish have large chromosome segments in common that have been conserved for 430 million years, since the divergence of the two evolutionary lines.

This similarity makes them excellent research models for studying human disease. In fact, as zebrafish develop, the way their organs form is nearly indistinguishable from the way organs develop in humans. Researchers also are able to capitalize on some of the unique features offered by zebrafish. For example, fertilized eggs develop outside of the mother, so investigators can access the embryos directly. The embryos are transparent, making it easy to view internal structures or label cells with fluorescent compounds. And the fish reproduce quickly, so it is possible to study successive generations in a short period of time.

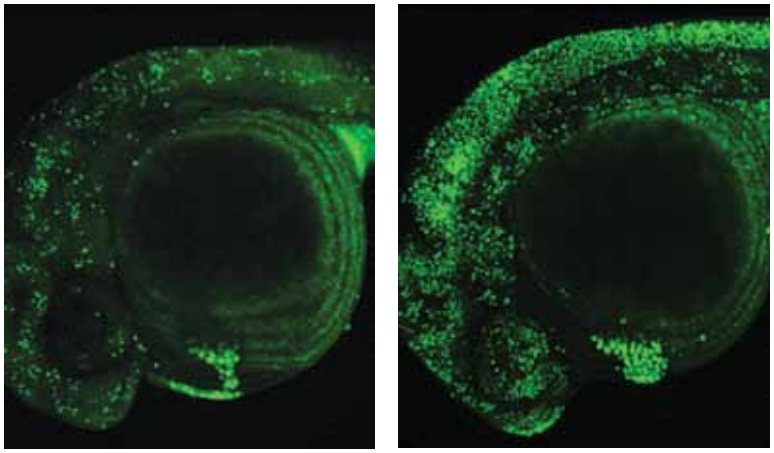
To ensure that researchers have access to these critical resources, NCRR supports the Zebrafish International Resource Center (ZIRC) located at the University of Oregon, which houses more than a million live zebrafish and preserves known zebrafish mutations as frozen sperm. Every week, ZIRC staff send hundreds of fish to researchers around the world. NCRR also funds the Zebrafish Model Organism Database called ZFIN. With this resource, investigators have easy access to a complete catalog of zebrafish literature, genes and mutations as well as a genetic map for zebrafish with corresponding human genes.

Learn more at http://zebrafish.org and http://zfin.org.

PHOTO BY AMANDA RAPP

molecules," Postlethwait said. But healthy individuals have cellular mechanisms that repair such damage to prevent genetic mutations and disease. "Humans have at least 14 FA genes (*FANC* genes) that interact to help repair damage to DNA," he continued. "In FA, these genes are mutated so that the DNA repair mechanisms do not work properly, allowing damaged cells to proliferate."

It was thought that the FA gene system existed only in mammals, but Postlethwait and his team found all 14 genes in zebrafish. "Not only that, but the genes seemed to act the same way in the fish as they do in humans," he said. As is the case in humans with FA, zebrafish with FA mutations had impaired DNA repair mechanisms and exhibited some characteristics of the human disease. Postlethwait knew then that the zebrafish model could make significant contributions to improving the understanding of the disease and perhaps aid in the search for treatments.



■ FA mutations change DNA repair mechanisms so that they do not work properly. The normal zebrafish embryo on the left shows a small amount of fluorescence, indicating that the cells repaired many DNA breaks induced by chemical treatment. The zebrafish embryo on the right has an FA mutation. It shows high-intensity fluorescent staining, indicating that the cells are unable to repair such breaks.

Postlethwait received additional research funds from NCRR and from the Fanconi Anemia Research Fund, which was established by David and Lynne Frohnmayer. With the funding and access to the NCRR-supported Zebrafish International Resource Center (ZIRC) and a zebrafish database called ZFIN, his research team quickly generated two FA mutations and obtained three more that other researchers had made. Postlethwait's team now had five of the 14 mutated FA genes in its zebrafish model.

The next question was: Could the zebrafish model of FA aid in the search for therapies that could prevent or reverse the disease? "Once we discovered that our zebrafish with mutations in FA genes were unable to repair breaks in the DNA of their cells, we were able to develop a screening test for drug candidates that could restore the cells' DNA repair mechanisms," said Postlethwait.

For the screen, investigators treat fertilized eggs from both normal and FA-mutated zebrafish with a chemical to cause DNA breaks. Then, they add acridine orange dye to the developing embryos. The dye binds to areas with broken DNA, causing them to fluoresce with a green glow. The degree of fluorescence is proportionate to the extent of DNA breakage. Embryos with intact DNA repair mechanisms have few DNA breaks and do not fluoresce very much, as only a small amount of the dye is bound. In contrast, zebrafish embryos with FA mutations are not able to repair the DNA breaks, leaving many areas open for the dye to bind. FA-mutated embryos, therefore, fluoresce more brightly (see photo).

Next, Postlethwait's team adds test drugs to the embryos with FA mutations. A reduction in the amount of green fluorescence indicates that the drug is restoring the cells' ability to repair DNA damage. Drugs that can restore cells' ability to repair DNA may merit further study as potential FA treatments. Postlethwait and his team are screening about 1,000 drugs that already are approved for treating other diseases. Currently, they are making the screening test more efficient with automation and 3-D imaging. The team then will expand its efforts to test a library of 5,000 drug-like molecules. Any promising drugs will be tested further. Collaborating scientists at Harvard University and Oregon Health & Science University will test these drug candidates in mice and in human cell lines.

It's likely that the FA zebrafish model will have wider implications for human health. Defects in DNA repair underlie not only FA but also other human diseases, including cancer. "Many cancers involve problems with damaged or inhibited DNA repair mechanisms," explained Postlethwait. "An effective therapy for Fanconi anemia could hold promise for treating cancer." In particular, for cancers caused by environmental exposure, such as radiation, targeting DNA repair might be a promising approach. "If repair could be stimulated after DNA damage occurs, it could have a positive effect on health," he added.

Postlethwait credits NCRR for its critical role in his translational journey. "Progress that might have taken decades has been accomplished in only a few years thanks to the support and resources made available through NCRR's Division of Comparative Medicine," said Postlethwait. "Animal models [like the zebrafish] are accelerating the rate at which we take discoveries in the lab and translate them into a fuller understanding of human biology to find new treatments for human diseases like Fanconi anemia." ■

—KAREN EDDLEMAN

ADDITIONAL READING:

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Titus, T.A., Yana, Y-L, Wilson, C., et al. The Fanconi anemia/BRCA gene network in zebrafish: Embryonic expression and comparative genomics. *Mutat Res* 668 (1–2):117–132, 2009.

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