Zebrafish
A Serendipitous Solution

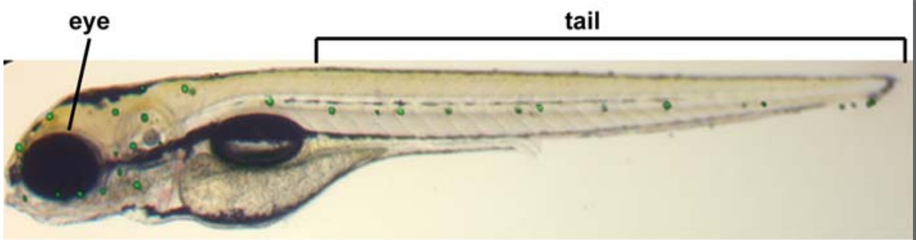
BY KELLY OWEN, PH.D., ALLISON COFFIN, PH.D., DAVID RAIBLE, PH.D., AND EDWIN RUBEL, PH.D.

Some of the most fruitful avenues of research have arisen from new combinations of ideas and people. One such collaboration started in early 2001, when Edwin Rubel and two of his postdoctoral fellows, Lisa Cunningham and Alan Cheng, ventured from their lab in the University of Washington's Virginia Merrill Bloedel Hearing Research Center across the street to meet with David Raible, a neuroscience professor in the university's Department of Biological Structure. Raible uses small zebrafish to study development of the nervous system, while Rubel studies the auditory system in birds and mammals, looking at how hearing damage occurs and how it might be prevented. Eight years later, a large group of researchers in both labs are still working together, trying to understand – and someday prevent – human hearing loss.

Most hearing loss is due, at least in part, to loss of the sensory cells in the inner ear that are responsive to sound. Each human cochlea (the hearing organ in our ears) has only a few thousand of these tiny sensory hair cells, so-called because they have a little hair-like tuft sticking out of the top of each cell. These hair cells are exquisitely sensitive to sound and enable us to hear a huge range of sounds, from the whisper of the wind to the roar of a jet engine. Unfortunately, they are also extremely sensitive to damage from noise and some types of drugs and also are lost as we age. In humans, this is a permanent condition; once hair cells die, we can't grow more of them.

But some animals and some humans seem resistant to noise and drugs and some humans hear perfectly until old age. What grants this protection? Do some people have genetically "tough" ears and others have "weak" ears? If so, what are the genes responsible for this difference and can we use them to protect hearing?

Like humans and other vertebrates, fish have ears, although you wouldn't know it just by looking at one! There are no outer ears sticking out from the sides of their heads, but their inner ears are remarkably similar to our own, including the possession of a full complement of hair cells. Fish also have a second hair cell-bearing system called the lateral line, which is a series of hair cell clusters (and surrounding supporting cells) grouped in rows along the head and body of the fish, with the little hair-like tufts sticking out



Picture of a five-day-old zebrafish with eye and tail indicated. The clusters of hair cells along the lateral line are labeled with a dye that shows up in the picture as a series of green dots on the head and body of the fish.
Photo courtesy of David Raible Lab, Univ. of Washington

into the water.

Fish use their inner ears like we use ours, for hearing and balance, while they use the lateral line to detect water movement around their bodies. These hair cells are the same type of cells that detect sound and provide balance cues in our inner ear. Zebrafish, commonly found in pet stores, are particularly useful as a model system for research. These fish are small, inexpensive to keep and will reproduce year-round, making for a constant supply of larvae for study. And if that weren't enough, the larvae are easy to genetically engineer and are transparent, allowing researchers to watch events inside the fish as they happen.

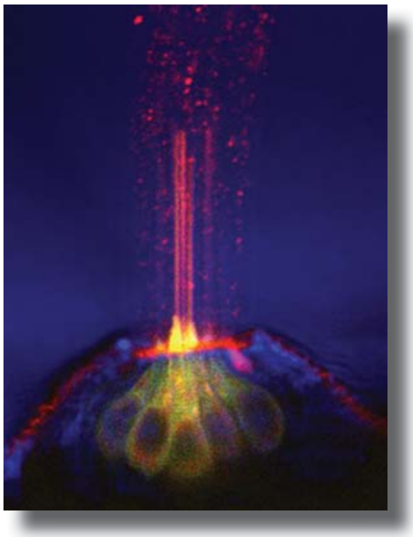
Here was an animal that conveniently displays its hair cells externally, getting around the problem of all that tough bone that encases the hair cells in people and other mammals. Rubel wondered if the zebrafish lateral line system might provide a direct look at what happened to a hair cell when it was damaged and offer a way to screen for genes that might confer protection. After one short meeting and an evening experimenting with the hair cells of these fish, Rubel and Raible were convinced that they had found the system for a new approach toward research aimed at preventing hearing loss.

Enter Julie Harris, a Ph.D. student who joined the University of Washington's neurobiology graduate training program just as the collaboration between the Rubel and Raible labs was established. In a remarkably productive series of experiments, Harris confirmed that lateral line hair cells of zebrafish were damaged by "ototoxic" drugs (drugs that damage the inner ear). This occurred in the zebrafish the same way as it does in people and other mammals. Now one could test many zebrafish in a short time (needed for genetic screening) and carefully study how the hair cells die and how these lateral line hair cells regenerate. Zebrafish really were a good model for hair cell studies, opening up all kinds of new possibilities for research.

Our approach, and that of many other research labs, is based on the idea that if we know the cellular details of why hair cells die with exposure to noise or ototoxic drugs, or as we age, we can prevent it or slow it down in humans. Where the zebrafish model has made a huge difference is that it lets us approach the question of why some people are so sensitive and some so resistant to hearing loss. We can search for the genes involved in cell death responses; Brock Roberts and Kelly Owens of our group have used a genetic screen (a technique for randomly identifying mutations in genes) to search for genetic alterations that protect hair cells from damage by ototoxic drugs. So far we have identified five genetic mutations that potently protect hair cells from damaging drugs. One of these genes, which we call "sentinel," encodes a novel protein whose function was previously not described. This is a good example of the ability of genetic screening to identify genes whose potential role in hair cell biology was previously unknown.

As more people joined this project at the University of Washington and as other centers became aware of it, new ideas emerged for using zebrafish lateral line hair cells to address hearing impairment issues. In parallel with our genetic screening, we have tested libraries of chemical compounds as well as libraries of approved drugs to determine whether they can prevent hair cell death, or in the case of approved drugs, whether they might actually

This photograph is a magnified view of one volcano-shaped cluster of hair cells labeled with a yellow vital dye in a living zebrafish larvae.
Photo by Glen MacDonald



cause hair cell death. Felipe Santos, an otolaryngology fellow who joined our group, rapidly screened over 10,000 chemicals from a library of small drug-like compounds and identified two promising chemicals that we call PROTO-1 and PROTO-2, which act to prevent hair cell loss in the zebrafish lateral line. But would these molecules protect mammalian hair cells? To test this idea, Santos, along with Lisa Cunningham, treated mouse inner ear hair cells with PROTO1 and 2 and the ototoxic drug neomycin. Most of the mammalian hair cells survived! While much additional research will be required before PROTO1 and 2 might be used as therapeutic drugs, these experiments demonstrate the potential power of this approach for therapeutic drug development.

To potentially shorten the process between identification of a protective treatment and its use in human patients, Henry Ou, another member of our group, tested drugs that already have FDA approval for use in humans for other medical treatments and found 10 FDA-approved drugs that prevented hair cell loss in zebrafish. These drugs are currently being tested in mammals. Allison Coffin, Kelly Owens and others in our group are using protective drugs and protective genes to profile pathways of hair cell death. That is, we want to determine distinctions between the cellular pathways and molecules that the hair cell uses in response to different ototoxic drugs. Our group and others have also used the screening methods to ask about other, unknown causes of hearing loss. For example, Miguel Allende and colleagues in Chile and John Incardona and colleagues at the National Oceanic and Atmospheric Administration have examined the effects of common environmental agents. Lynn Chiu and Henry Ou have begun testing common therapeutic drugs for previously unrecognized damage to hair cells and found over 20 with potentially dangerous levels of toxicity.

Members of "the fish group" from the Rubel and Raible labs, left to right: (front) David Raible, Edwin Rubel, (middle) Eva Ma, Heather Brignull, Allison Coffin, Frederica Mackert, Arminda Suli, (back) Yoshinobu Hirose, Parhum Namdaran, Kelly Owens, Lauren Clancey, Dale Hailey, Nick Coley
Photo courtesy of Eva Ma

But what about individuals whose hair cells no longer function properly? Clearly, prevention is only half of the picture. Although many tissues in the human body can repair themselves, including replacing damaged cells, our inner ears do not produce new hair cells. In fact, hair cells are not regenerated in any mammal – but they are regenerated in fish, birds and amphibians. One aspect of our research is to understand how hair cells are regenerated in the zebrafish so that we can someday stimulate replacement of hair cells in human ears. To this end, Eva Ma, a graduate student in the Raible lab, has determined that lateral line hair cells can regenerate within 48 hours and that these new hair cells appear to be generated from dividing cells that lie beneath them. Replacing hair cells in the ear is only useful if they are functional and can be connected to the rest of the nervous system. The labs of James Hudspeth of the Howard Hughs Medical Institute and Hernan Lopez-Schier from the Centre for Genomic Regulation in Barcelona are focused on understanding how newly made hair cells are connected to the nervous system.

A lot has happened since that fateful meeting eight years ago. Many fish hair cells have been protected from drugs, and new hair cells have grown to replace damaged ones. But our real business is to come up with ways to protect human hair cells and regenerate those lost due to genetic anomalies, ototoxic drugs, noise or aging. Today, we cannot take drugs to protect our hair cells or to grow new ones but hopefully we will be able to do this in the future. We have entered a new and exciting era of biomedical research. The zebrafish has opened new and promising approaches toward prevention and treatment of hearing loss. With other approaches already underway and those not yet imagined, the next two decades are likely to dramatically change the therapeutic options available. ■

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Kelly Owens, Ph.D., is an instructor at the University of Washington and mother of an energetic teenage son. She is an avid reader, enthusiastic painter and perpetually hopeful gardener.
David Raible, Ph.D., has been a faculty member at the University of Washington since 1995. When not in the lab, he is riding his bike or watching his girls play soccer.
Edwin Rubel, Ph.D., has studied normal and abnormal development of the ear and auditory pathways of the brain since 1971. He has enjoyed Northwest living since 1986. When not working, he plays with his wife of 45 years, his adult children and his four grandchildren.