

## ***THE HEARING REGENERATION INITIATIVE***

### **1. Introduction**

Sensory hair cells are located in the cochlea of the inner ear. They convert the mechanical information in sound into electrical impulses that are processed by the brain. Hair cells are fragile: they are destroyed during aging, by loud noises, by toxins, and by disease. Loss of hair cells is the leading cause of deafness. In humans, all cochlear hair cells are produced during gestation and cannot be regenerated when lost. Thus, at present, the only treatment for hearing impairment due to hair cell loss is the use of hearing aids and cochlear implants.

More than a decade ago, it was discovered that, unlike humans and other mammals, hair cell regeneration does occur in birds and other lower vertebrates. Lost hair cells are replaced (like skin), and this regeneration results in total recovery of hearing. Mechanisms used by lower vertebrates to regenerate hair cells involve non-sensory supporting cells that reside next to hair cells. When hair cells are damaged in birds, supporting cells begin dividing and replacing the lost hair cells. Supporting cells with similar potential appear to be present in the mature mammalian cochlea, but they are unable to initiate hair cell regeneration, because appropriate signals are absent, or cells lack the ability to respond.

The *Hearing Regeneration Initiative* is a collaborative program that includes basic and clinical scientists whose aim is to hasten progress toward promoting hair cell regeneration in humans. These scientists are working together to find out why the mammalian inner ear fails to trigger robust regeneration of hair cells after they are lost. There are 10 principal investigators

who are currently participating in the *Hearing Regeneration Initiative*, with appointments in several departments at the University of Washington, as well as at the House Ear Institute in Los Angeles, CA: Drs. Edwin Rubel, Olivia Bermingham-McDonogh, Clifford Hume, Elizabeth Oesterle, David Raible, Jennifer Stone (shown at right), George Gates, Thomas Reh, Neil Segil, and Andrew Groves. These investigators, with their laboratory groups, are conducting the experiments



to experimentally decipher ways to trigger hair cell regeneration in mammals, as well as to provide research tools to the international academic research community to hasten the world's progress toward developing a cure for hearing disorders. They are also committed to publicly distribute new ideas and specializations, to share early-stage results prior to publication, and to publicize research findings in a timely and widely disseminated manner, thereby promoting rapid progress toward a cure for hearing loss.

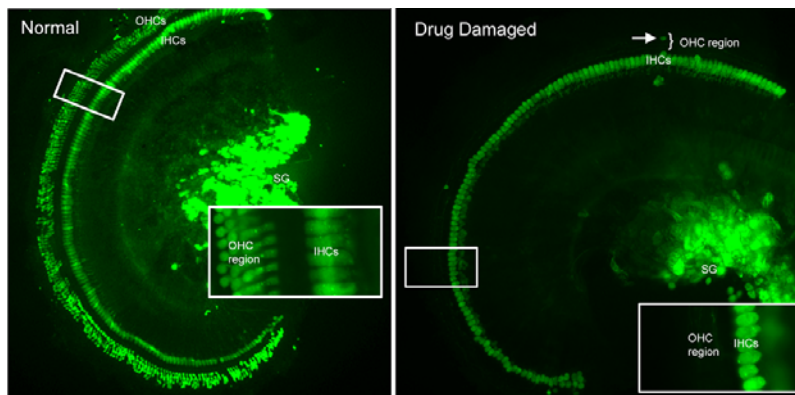
### **2. Tools for Studies of Hair Cell Regeneration in Mice**

A major challenge is to identify genes that are necessary for hair cell regeneration in mammals. This requires analysis of damaged tissue, typically in large quantities, to find potential candidate genes. Subsequently, we must determine how these genes are expressed after hair cell damage, and we must verify their role in the regenerative process. The *Hearing*

*Regeneration Initiative* is developing tools required to execute this challenge, including methods for reliably damaging hair cells and methods for altering gene function in live animals to test their applicability for inducing hair cell regeneration.

## 2A. A Model for Human Hearing Loss

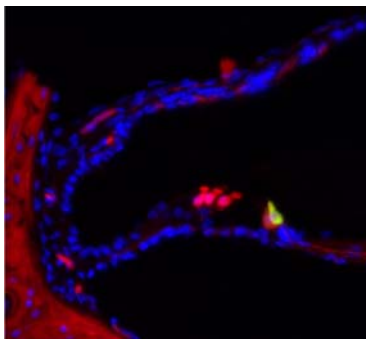
The inner ear of mice is remarkably similar to that of humans. Rapid advances in molecular biology have established mice as the model of choice for genetic studies of diseases, including hearing loss. Unfortunately, it has been surprisingly difficult to establish a simple and reliable model of acquired deafness in mice. Investigators in the *Hearing Regeneration Initiative* are addressing this problem by developing methods of deafening adult mice using similar antibiotics and chemotherapeutic agents as applied in humans (see figure below). Once established, this deafening protocol will be a starting point to test novel therapies to repair the inner ear and will be invaluable to scientists studying hair cell regeneration world-wide.



The figure shows cochleas from a normal mouse (left) and from a mouse that was treated with ototoxic drugs to induce hair cell loss. The hair cells are green in color and arranged in a C shape. In both panels, the region in the small white box is shown at a higher magnification at the bottom right corner. Nearly all outer hair cells (outer row of green cells, OHCs) were killed by the drug treatment (right).

## 2B. Tools for Gene Therapy of Hearing Loss

Current studies aim to identify genes that stimulate inner ear repair in mammals (see below). Once such candidate genes are identified, each gene will need to be tested by delivering it to the inner ear of normal hearing and deafened animals. This will require targeting each gene to the correct location in the ear and controlling when and where it is turned on.



The yellow cell is a cochlear hair cell that has been specifically infected with virus.

The *Hearing Regeneration Initiative* is developing modified forms of viruses specifically for gene therapy in the inner ear. Most of our attention is focused on adenovirus and adeno-associated viruses (AAV) because they have been shown to be safe and efficient for gene therapy in humans. AAVs have excellent safety profiles and cause minimal inflammation, but are highly efficient in transferring genetic material. By changing the surface of the virus and the genetic control elements, we can target specific cell types in the inner ear.

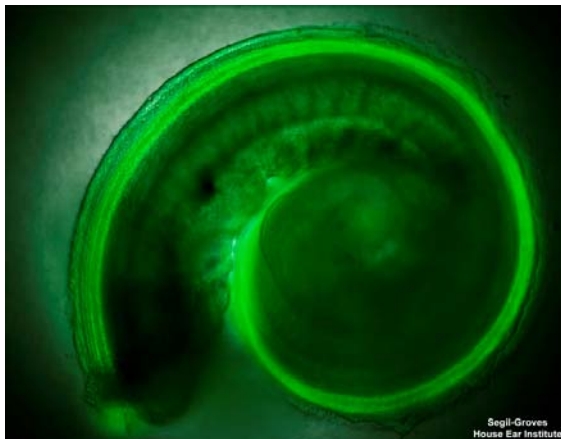
Through the use of these improved viruses and optimized surgical techniques for viral delivery, we have been able to deliver genes to the inner ear of mice without damage to the surrounding tissue or to the hearing. This is an exciting first step toward bringing advances in our understanding of the biology of the inner ear into clinical treatments for human hearing loss and dizziness.

### 3. Identification of Candidate Genes for Controlling Hair Cell Regeneration

Research has shown that hair cell regeneration is possible in the mammalian inner ear, but it is extremely rare. This means that hair cell regeneration can be achieved in the human inner ear if critical biological barriers are removed. The first step toward overcoming this barrier is to identify which cells have the capacity to divide and determine what prevents them from doing so in the normal inner ear. The *Hearing Regeneration Initiative* is using several innovative approaches for isolating enough of each type of supporting cell to understand what makes them different, outlined below. This is highly challenging, because the delicate tissues of the inner are embedded in dense bone and the supporting cells are relatively few in number.

#### 3A. Purification of Supporting Cell Subtypes from the inner ear: A Genetic Approach

Previous studies have taken advantage of mouse genetics to selectively label supporting cells in the inner ear of a special mouse called a *p27/GFP-BAC* transgenic mouse. In this mouse,



A dissected cochlea from a mouse harboring a gene that makes some supporting cells glow green. Green cells can be segregated from the cochlea to study them independently (Lee et al., *Development* 133:2817 (2006)).

all supporting cells are fluorescent green (see below), but all other cell types in the cochlea, including hair cells, are non-fluorescent. We are using fluorescent cell sorting methods to isolate the green supporting cells from the cochlea. Our studies have shown that some supporting cells retain the ability to divide and transform into hair cells in culture, at least for a limited time after birth. Current studies of the *Hearing Research Initiative* are focused on discovering new ways to purify different sub-populations of supporting cells so that their differences can be studied,

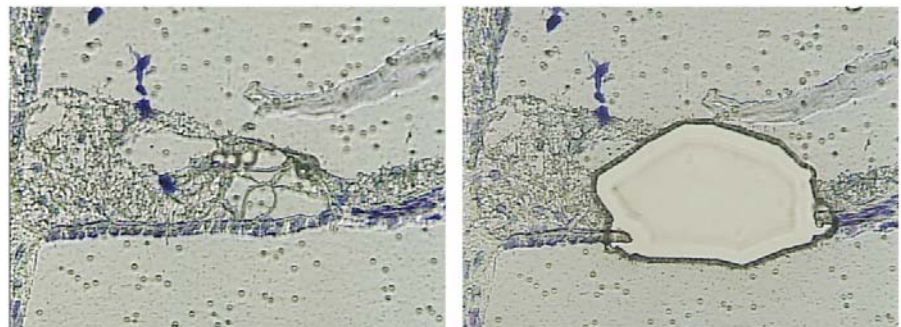
using additional genetic techniques to mark distinct sub-populations of supporting cells prior to their purification.

#### 3B. Purification of Non-Sensory Supporting Cell Subtypes from the Mature cochlea: A Microdissection Approach

An alternative to the genetic approach for isolating supporting cells is a surgical approach, where each set of supporting cells is physically dissected out and prepared for further study using a powerful and advanced new

dissection method called Laser Microdissection. Using this method, we can

use newly developed genetic and protein analyses technologies to develop “fingerprints” each



Thin sections through the cochlea of an adult mouse before (left) and after (right) the cochlear epithelium containing hair cells and supporting cells was removed using laser microdissection. Arrow points to region where cells were removed.

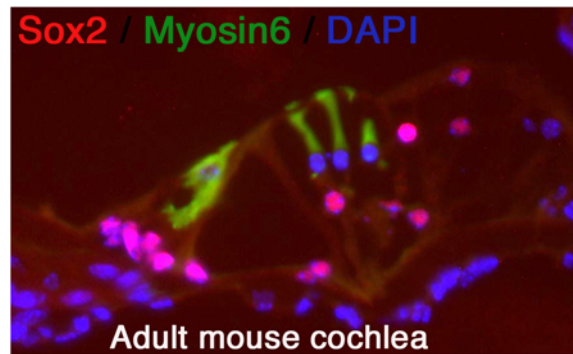
unique type of supporting cell. This information will be compared to data from animals that can recover their hearing (e.g., chickens and zebrafish, see below) in order to identify where mammalian supporting cells fall short in the regenerative response.

### 3C. Recovery of Hearing in Birds: A Model for Hair Cell Regeneration

The *Hearing Regeneration Initiative* is using modern genetic techniques including microarrays to identify the genes that enable hair cell regeneration to occur spontaneously and robustly in birds. Our hope is to piece together a map of the genes that are activated or inactivated in avian supporting cells after hair cell damage and to compare that map to one for mammals. Genes that differ between the two maps are candidates for key genes that regulate hair cell regeneration. These studies may eventually lead to the identification of new molecules that can trigger hair cell regeneration in mammals.

### 3D. The Mammalian Response to Deafening

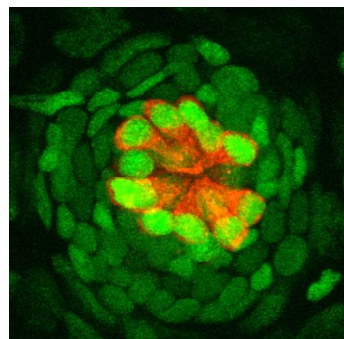
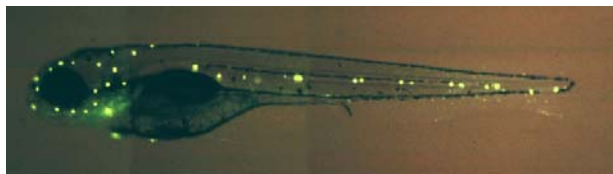
Tissues such as the liver or skin that exhibit a regenerative response to trauma share a common feature: they possess a cell type that can revert to an embryonic-like state, expressing many of the genes that are active in developing cells, a process is called de-differentiation. In cases where regeneration fails, such as the mammalian inner ear, it is possible that progenitor cells are not capable of reverting to the embryonic pattern. The *Hearing Regeneration Initiative* is exploring the role of genes that control the progenitor (supporting) cell response to hair cell damage in mice. Early results show mature supporting cells are genetically distinct from embryonic supporting cells. Modification of these genes may promote the transition of progenitor cells to a more embryonic state, improving the likelihood of repair.



This figure shows expression of one candidate regulatory gene, Sox2 (pink), in supporting cells in the adult mouse organ of Corti. This gene regulates stem cell behavior in other tissues.

### 3E. Lateral Line Hair Cells in Zebrafish: A Model for Hair Cell Regeneration

Hair cells similar to those found in the inner ear of mammals are also found on the body surfaces of fish, in organs called lateral line neuromasts. Neuromast hair cells also regenerate spontaneously after damage. Because they are easily visualized, lateral line hair cells are highly useful for studies of hair cell structure, function and regeneration that are not possible in mammals or birds. *The Hearing*



**Hair cells in zebrafish.** These panels show the hair cell-containing neuromasts located on the head and body of the zebrafish, in low magnification (above) and high magnification (left). Neuromasts are labeled green in both panels; hair cells are red in the left panel.

*Regeneration Initiative* is performing large-scale genetic screens in zebrafish to identify genes that regulate supporting cell division and hair cell differentiation. Once we identify genes that affect hair cell regeneration in zebrafish, we will study the effects of modifying the activity of these genes in the mouse inner ear.