Regeneration and replacement in the vertebrate inner ear

Jonathan I. Matsui, Mark A. Parker, Brenda M. Ryals and Douglas A. Cotanche

Deafness affects more than 40 million people in the UK and the USA, and many more world-wide. The primary cause of hearing loss is damage to or death of the sensory receptor cells in the inner ear, the hair cells. Birds can readily regenerate their cochlear hair cells but the mammalian cochlea has shown no ability to regenerate after damage. Current research efforts are focusing on gene manipulation, gene therapy and stem cell transplantation for repairing or replacing damaged mammalian cochlear hair cells, which could lead to therapies for treating deafness in humans.

Jonathan I. Matsui
Mark A. Parker
Douglas A. Cotanche*
Laboratory for Cellular and Molecular Hearing Research, Department of Otolaryngology, Children's Hospital, Boston, MA 02115, USA
*e-mail: douglas.cotanche@childrens.harvard.edu
Mark A. Parker
Douglas A. Cotanche
Department of Otology and Laryngology, Harvard Medical School, Boston, MA 02114, USA
Jonathan I. Matsui
Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA
Douglas A. Cotanche
Harvard–MIT Division of Health Sciences and Technology
77 Massachusetts Avenue, E25-519, Cambridge, MA 02139, USA
Brenda M. Ryals
Department of Communication Sciences and Disorders, James Madison University, Harrisonburg, VA 22807, USA

Hair cells are the mechanoreceptors found within the inner ear that detect sound and head movements. Serious hearing and balance impairments can occur through the loss of hair cells by aging, environmental stresses, such as loud noises, or exposure to chemotherapeutic drugs, such as cisplatin or aminoglycoside antibiotics. At least 28 million Americans have a hearing impairment but only one out of five people who could benefit from a hearing aid actually wears one (www.nidcd.nih.gov). Because a large proportion of hearing loss involves the loss of hair cells, regeneration or replacement of these cells is a possible alternative to prosthetic devices.

Scientists once believed that warm-blooded animals had a full complement of hair cells at birth and, if lost, the damage was permanent. Over 15 years ago, several studies demonstrated that avians can regenerate their sensory hair cells [1–4]. Other studies demonstrated that the regenerated sensory hair cells were functional (reviewed in [5,6]). Nowadays, the regeneration phenomenon is better understood but the signaling mechanisms regulating hair cell regeneration remain unknown.

The sensory epithelium of the inner ear comprises two different general cell types: sensory hair cells and nonsensory supporting cells. There are several specialized types of hair cells in the mammalian inner ear: in the auditory system (inner and outer hair cells) and in the vestibular system (type 1 and 2 hair cells). These cells can be distinguished by their location in the organ, their morphologies and by the type of neurons that innervate the hair cell. There are also different types of supporting cells found within the mammalian auditory system (e.g. Deiters' cells and pillar cells) that each express unique structural and molecular signatures. However, in the vestibular system, the supporting cells appear to be relatively homogeneous, and scientists have yet to find morphological, molecular or physiological differences between the supporting cells.

Many events occur when the hair cells in the inner ear are damaged or killed. For example, the sensory epithelium is capable of repairing itself when hair cells in the sensory epithelium are damaged with a sub-lethal stimulus [7–9]. However, if the damage is more severe, it normally leads to the death of some or all of the hair cells in the sensory epithelium. Dying hair cells undergo programmed cell death (apoptosis) [10,11] and are either ejected from the sensory epithelia [12] or engulfed by

1359-6446/05/$ – see front matter PII: S1359-6446(05)03577-4

neighboring cells. Following the death of the hair cells, the neurons from the VIIIth cranial nerve retract their synaptic terminals. In birds and lower vertebrates, a signal from the dying hair cell induces regeneration by triggering the neighboring supporting cells to either proliferate or transdifferentiate into an immature hair cell. Proliferating cells then respond to environmental, molecular or genetic cues to differentiate into hair cells or supporting cells. Finally, nerve fibers from the VIIIth cranial nerve reconnect the hair cell to the central nervous system so that the animal can process the sensory information.

Fish and chicks: 'lower vertebrate' model systems to study hair cell regeneration

In the past fifteen years, two different mechanisms have been proposed for sensory hair cell regeneration in avian and other non-mammalian species: mitotic proliferation and direct, nonmitotic, transdifferentiation. Many studies indicate that the supporting cells adjacent to a dying hair cell receive a signal to enter the cell cycle. The supporting cells that undergo mitotic proliferation migrate to the luminal surface of the sensory epithelium, duplicate their DNA and then divide into two daughter cells (reviewed in [6,13–15]). The daughter cells then proceed through symmetrical differentiation to produce two hair cells or two supporting cells [16–18], or through asymmetrical differentiation to produce one hair cell and one supporting cell [17–20]. Alternatively, hair cells can transdifferentiate from neighboring supporting cells by non-mitotic mechanisms [21–26]. Transdifferentiation is a switch in gene expression in the supporting cell so that it expresses markers that are characteristic of a developing hair cell. Although non-mitotic transdifferentiation of supporting cells is a simpler way of replacing lost hair cells, it results in the loss of the supporting cells; and often large numbers of hair cells and supporting cells are needed to repopulate the sensory epithelia. Mitotic cell division maintains the structural integrity of the organ by producing hair cells and supporting cells, whereas direct transdifferentiation results in a significant loss of supporting cells if they all transdifferentiate into hair cells.

Functional studies using avians

When it was discovered that birds could regenerate their sensory hair cells, the next logical question was: are the regenerated cells functional? Not only do the supporting cells need to differentiate into hair cells but the newly regenerated hair cells also need to be re-innervated by the VIIIth cranial nerve fibers. Moreover, the animal must use the new sensory information to produce behaviorally meaningful responses. Two recent reviews on functional recovery after sound and drug-induced damage have comprehensively covered these issues [5,6].

Although many studies have examined the physiology of recovered sensory hair cells, few have examined the complex properties of perceptual processing and behavioral plasticity. The recognition and production of vocal signals depend on hearing and are necessary for communication. Budgerigars, *Melopsittacus unduratus*, have been used to examine the renewal of vocal production and complex auditory perception after hair cell regeneration [27,28]. These birds mimic sounds and readily learn new vocalizations throughout life, which has been likened to language acquisition in humans. The birds were trained to match precisely their vocalizations to specific acoustic templates. Aminoglycoside treatment disrupted auditory perception and vocal production. Behavioral tests of auditory sensitivity showed that audiometric thresholds returned to near-normal levels (within 20 dB) within four weeks of deafening. More-complex perceptual tasks, such as vocal call discrimination and/or recognition, took up to five months to return to normal levels. Precision in vocal production initially declined but was restored to pre-treatment levels before the recovery of auditory function. Therefore, relatively little acoustic feedback from a few regenerated hair cells was necessary to guide full recovery of vocal precision.

Another series of studies examined complex communication behavior in male Bengalese finches, *Lonchura striata*, which learn a single sequence of 'syllables' early in life and reliably produce the same song throughout their lifespan [29–32]. After recording each bird's song and verifying its stability, they were treated with a combination of aminoglycosides and sound exposure to induce hearing loss and their songs rapidly deteriorated [33]. Once hearing was restored by hair cell regeneration [30], the song returned to its pre-exposure structure [31]. Restoration of hearing allowed each bird to access a stored 'template' of its own learned vocalization, and gradually match this new vocalizations to the stored memory.

Compensatory behaviors, such as gaze, oculomotor and postural responses, that occur during movement largely depend on a functioning vestibular system. The vestibular ocular reflex and the vestibular colic reflex disappear after the vestibular hair cells of birds are destroyed with aminoglycoside antibiotics [34–38]. However, these reflexes reappear as the hair cells regenerate [35–37,39]. Dickman and Lim [40] trained adult pigeons to run along a chamber and peck an illuminated key. Multiple behavioral measures assessing performance, posture, and head stability were quantified. Once normative values were obtained, the animals received aminoglycosides, which killed the vestibular hair cells and resulted in severe postural and head instability. As the regeneration process progressed, the tremor and head shakes diminished and spatial orientation and navigation ability improved to pretreatment levels.

Zebrafish: a new model system to study apoptosis and hair cell regeneration

Recently, there has been much interest in using zebrafish, *Danio rerio*, as an animal model for studying the inner ear. Zebrafish have sensory hair cells in the vestibular organs