Hair cell regeneration

David W. Roberson, MD, and Edwin W Rubel, PhD

University of Washington, Seattle, Washington, USA

In 1988, two landmark papers definitively showed that hair cells in the mature avian inner ear can regenerate after otologic insults have destroyed the existing receptors. This discovery, not new to the vertebrate phylum but new for terrestrial vertebrates, has led to a rethinking of the assumption that hair cell loss in mammals, including humans, must necessarily be permanent. In this article, we present a brief progress report on the status of research on hair cell regeneration in birds and mammals. In the brief period since these initial publications, a great deal has been accomplished; we now know much about the process of hair regeneration in birds. We know that it does lead to a recovery of function(s), and we are beginning to get clues about the cellular and molecular events that trigger hair cell regeneration in birds. In mammals, intriguing data are emerging that suggest induction of early stages of the regeneration process, as well as the possibility that more than one cellular route for hair cell replacement may exist. These data do not provide a cure for hearing loss or dizziness, but they do provide a new beacon shining light down a long tunnel, the entrance to which has only recently been opened.

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Traditionally, it was thought that the hair cells in the inner ear of warm-blooded vertebrates were only produced prenatally [1,2]. Any hair cells lost in adult life were believed to be irreplaceable, and associated hearing or balance deficits uncorrectable. Studies published in 1986 and 1987 led to a paradigm shift and a revolution in our thinking. The avian cochlea and vestibular organs can produce new hair cells throughout life [3–9]. The avian vestibular organs produce new hair cells continuously and upregulate the production of hair cells following hair cell loss [7–9]. The avian cochlea, in contrast, does not produce new hair cells in its undamaged, quiescent state [10], but does produce new hair cells to replace those lost following acoustic or ototoxic insult [3–6].

Hair cell regeneration, in combination with reinnervation, regrowth of the tectorial membrane, and other anatomic and physiologic responses to damage, leads to significant functional recovery of hearing and balance following inner ear damage in birds. Recent reports have suggested that the initial stages of hair cell regeneration may also occur to a limited extent in the mammalian vestibular epithelium, although the details of the processes are not yet entirely clear [11,12,13••]. There is evidence that regeneration normally does not occur after damage to the mature mammalian auditory epithelium [14]. One group has reported that regeneration can be stimulated by exogenous factors in the immature mammalian auditory epithelium in vitro. However, these findings have not been replicated by other researchers, and further investigation is clearly needed [15,16•].

The rapid progress in the study of hair cell regeneration has recently been reviewed in detail by several authors [17•–19•]. This paper presents a brief overview of the current state of knowledge, including structural and functional recovery, the evidence for and against regeneration in mammalian epithelia, the identity of progenitor cells, the possibility of regeneration via a nonmitotic pathway (direct transdifferentiation), and possible inducing signals, with particular emphasis on the clinical implications of this research.

Structural recovery in the inner ear

Numerous studies have demonstrated that the avian inner ear epithelia can replace hair cells lost because of acoustic overstimulation or ototoxic chemical damage. In all avian vestibular organs, there is a low rate of ongoing production of new hair cells throughout life [7,8]. Following hair cell loss, the rate of cell division and new hair cell production in the vestibular epithe-

Abbreviations
EGF—epidermal growth factor; **TGF**—transforming growth factor.

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lia is upregulated. Ultimately, the vestibular epithelium returns to an anatomic state almost indistinguishable from the pre-injury state [9,20••].

In the avian cochlea, there is very little or no cell division in the undamaged, quiescent epithelium [10]. Using ^{3}H thymidine, researchers have seen labeled hair cells within 4 days after acoustic trauma [21,22] and 5 days after ototoxic insult [23–25]. New hair cells initially have a characteristic immature morphology; only after several days do they achieve mature morphology [23,26]. After acoustic insult, afferent terminals degenerate; over a period of up to 3 months after damage, there is progression to a more normal innervation pattern, although even at 6 months, hair cell innervation is ultrastructurally different from control animals. Innervation of regenerated hair cells has been conclusively demonstrated [23,27,28]. Following ototoxic insult, the tectorial membrane is undamaged; in contrast, following acoustic insult the tectorial membrane is disrupted [26,29]. After acoustic damage, recovery of the tectorial membrane occurs during the initial 2 weeks following damage. The tectorial membrane recovers to a near-normal state, except for the lateral fibrils on its upper surface, which never reappear [30,31]. In addition, there are ultrastructural indications of damage to the tegmentum vasculosum, which recovers during the first 48 hours after noise exposure [32]. Ultimately, the damaged cochlea recovers to a near-normal anatomy; the only persistent anatomic changes that have been documented to date are the loss of tectorial membrane fibrils on the lateral surface, some disorientation of stereocilia bundles, and some ultrastructural differences in hair cell innervation patterns. Despite these apparently permanent changes, the anatomic substrates for functional recovery are clearly present.

Functional recovery

Functional recovery of hearing and balance following hair cell regeneration in birds has been documented by various measurement techniques. Early studies demonstrated recovery of evoked potentials in the avian brain stem following noise damage and aminoglycoside ototoxicity [33,34]. More recently, behavioral experiments have also shown a return of perceptual auditory thresholds [35,36•]. Recovery of vestibular nerve compound action potentials and the vestibulo-ocular reflex in chicks following vestibular hair cell loss has also been documented to be approximately parallel to that of vestibular hair cell regeneration [37,38].

Although the correlation of functional recovery with hair cell regeneration has been elegantly documented, it is not exact. Carey *et al.* [39] demonstrated that there was no simple correlation between vestibular hair cell number and vestibulo-ocular reflex recovery. Hashino *et al.* [40] found a low frequency hearing loss, which was inconsistent with the location of the hair cell damage seen. It also appears that distortion product otoacoustic emissions, a measure of hair cell function, recovered more rapidly than evoked potentials thresholds [41,42]. The studies of otoacoustic emissions suggest that hair cell regeneration and functional recovery precede the recovery of functional hearing at the central level. Niemiec *et al.* [36•] found that hearing sensitivity, measured with behavioral testing, preceded complete anatomic recovery from acoustic insult.

Mammalian regeneration

Ruben [1] demonstrated that the normal postnatal mammalian cochlea does not have mitoses in the sensory epithelia. Using ^{3}H thymidine labeling in young adult gerbils, Roberson and Rubel [14] confirmed that there was no division within the sensory epithelium after acoustic trauma. Two reports in 1993 suggested that a limited amount of hair cell regeneration might occur in the mammalian vestibular epithelia after aminoglycoside ototoxicity. Forge *et al.* [11] found ultrastructural evidence for regeneration of stereocilia bundles in guinea pig vestibular organs after damage, and Warchol *et al.* [12] reported ^{3}H thymidine labeling in supporting cells of mammalian vestibular organs after damage and several weeks in vitro. Taken together, these two reports suggest that a small amount of hair cell regeneration might be occurring in the guinea pig vestibular epithelia. Because Warchol *et al.* used a culture system, it was impossible to preserve the vestibular organs for prolonged time periods and to document reliable hair cell phenotypes among the labeled cells. Rubel *et al.* [13••], in an attempt to extend the in vitro results of Warchol *et al.* to an in vivo system, infused ^{3}H thymidine into the labyrinth of guinea pigs after ototoxic insult. Scanning electron microscopy demonstrated the immature-appearing hair cells, as seen by Forge *et al.*, and ^{3}H thymidine autoradiography demonstrated the labeling in the supporting cell layer, as seen by Warchol *et al.* However, in animals kept alive for up to 6 weeks, no labeled hair cells were seen, suggesting that the cell division seen in the supporting cell layer may not have been related to the immature-appearing stereocilia bundles seen by scanning electron microscopy. The bundles may represent recovery of damaged hair cells, or alternatively, a limited amount of hair cell regeneration may occur via direct transdifferentiation. Clearly, there is cell division in the damaged mammalian vestibular epithelium, and as discussed elsewhere in this paper, it can be upregulated via the addition of exogenous growth factors. Whether true hair cell regeneration occurs and by which route, however, remain uncertain.

Progenitor cells

A number of laboratories have attempted to identify the cells that give rise to new hair cells. In the lat-

eral line organ, supporting cells have been shown unequivocally to give rise to new hair cells [43]. In the avian cochlea and vestibular organs, several authors have also documented that supporting cells give rise to new hair cells [20••,21,22,44,45••,46••]. Girod *et al.* [21] also have suggested that after extreme damage by noise, hyaline cells may spread to cover the denuded basilar membrane, then divide, with some progeny becoming new hair cells. Presson and Popper [47] have suggested that a population of less differentiated cells, embryonic-like neuroepithelial cells, give rise to new hair cells in the vestibular epithelium of the teleost (*Astronotus ocellatus*), the oscar. However, Presson [48] also found that the immunoreactivity of the hair cell progenitors in the oscar most closely corresponded to the immunoreactivity of supporting cells, suggesting that the progenitor cells are supporting cells or closely related to them.

It is not known if all supporting cells are potential hair cell progenitors, or if only a subpopulation of supporting cells can reenter the cell cycle and give rise to new hair cells. Although a distinct subclass of progenitor cells may exist in the oscar, no such subclass has been identified in avian species. In one attempt to determine if all supporting cells are potential progenitors, Roberson and Rubel [49] infused ^{3}H thymidine directly into the labyrinth, providing a continuous supply of the cell division marker, for 12 days after an ototoxic insult, which caused the death of 98% of the hair cells in the basal 500 μm of the basilar papilla. Despite a presumably maximal stimulus for regeneration and the continuous presence of ^{3}H thymidine, only 15% of supporting cells were labeled. In another relevant experiment, Stone and Cotanche [46••] suggested that a relatively small number of supporting cells cycled through several rounds of cell division following damage. Both experiments imply that only a minority of supporting cells participate in hair cell regeneration; whether this is because only a minority of supporting cells are capable of dividing, or because only a minority are induced to divide, remains unknown.

Direct transdifferentiation

The initial studies describing hair cell regeneration all relied on cell cycle S-phase labeling (*eg*, ^{3}H thymidine or bromodeoxyuridine) to document the production of new hair cells. More recently, a number of authors have called attention to the possibility that some new hair cells may arise without mitosis, a process we will refer to as *direct transdifferentiation*. A series of experiments in the bullfrog vestibular organs suggests that supporting cells may differentiate into new hair cells without first dividing [50]. In the continuous labeling experiment, Roberson and Rubel [49] found that approximately one third of new hair cells that repopulated the basal part of the avian cochlea were unlabeled, despite the continuous presence of ^{3}H thymidine during the post-damage period. This result also strongly suggests that cell division is not a prerequisite for the acquisition of new hair cells. These observations are consistent with accumulating data from a number of other biologic systems in which differentiated cells have also been shown to have the capability to transdifferentiate into other cell types without dividing [51]. They are also consistent with recent observations that, following laser ablation of hair cells in embryonic and neonatal mice, new hair cells can be produced to replace those lost, without mitosis [52••].

The existence of an alternate pathway for hair cell regeneration may be important both experimentally and clinically. Experiments are currently being designed to manipulate hair cell regeneration or to induce it in mammals, where it does not normally occur. In most cases, these experiments involve the addition of exogenous growth- or differentiation-inducing factors. The interpretation of such experiments may be difficult if there are, in fact, two distinct pathways that lead to the production of new hair cells, possibly regulated by entirely different factors.

Clinically, the existence of an alternative route for hair cell regeneration makes the possibility of ultimately being able to induce regeneration in mammals more likely. Preliminary evidence in the mammalian vestibular organs suggests that the small amount of regeneration seen may be due to direct transdifferentiation [11,13••]. If this pathway already operates in the mammal, albeit at a very low level, it may be more easily inducible than mitotically regulated regeneration. In addition, there are practical concerns about the long-term effects of administering mitogenic substances to humans; in contrast, it is possible that factors that induce direct transdifferentiation might not be mitogenic and might, therefore, be safer for human use.

Inducing factors

A number of laboratories have shown that hair cell regeneration occurs in isolated sensory organs cultured in vitro [53,54]. Thus, it is clear that the signals inducing hair cell regeneration operate locally and are not dependent on neural or systemic signals. Two possibilities exist. The trigger may be the physical absence of hair cells, or it may be a local chemical stimulus. The progenitor cells might either be stimulated to divide based on hair cell absence, or inhibited from dividing by hair cell presence. Tsue *et al.* [55••] recently presented evidence that at least part of the signal represents a positive chemical effect. The authors showed that supporting cell proliferation is increased in organs cultured adjacent to regenerating organs, compared with those cultured adjacent to undamaged organs. In addition, Oesterle *et al.* (Unpublished data) have demonstrated an increase in proliferation in cultured avian vestibular organs following the addition of exogenous IGF-I (insulin-like growth factor-I), and

by exogenous insulin. In mammalian utricle, Lambert [56••] and Yamashita and Oesterle [57••] documented increases in proliferation in mouse vestibular epithelia when exogenous transforming growth factor (TGF)-α or epidermal growth factor (EGF) plus insulin were added to culture media. Both these experiments revealed a small fraction of labeled nuclei in the upper portion of the epithelium, normally occupied by hair cell nuclei. However, neither presented clear-cut morphologic evidence of new hair cells.

It is unlikely that the above-mentioned molecules (IGF-I, TGF-α, EGF, insulin) are responsible for induction or regulation of hair cell regeneration in the avian sensory epithelium, or that they will hold the key for producing such effects in the human inner ear. Molecular approaches will be required to determine what factors are able to regulate these processes in vivo. A number of molecular studies are currently in process. However, that supporting cell mitosis can be upregulated in both avian and mammalian species does provide encouraging evidence that it may ultimately be possible to induce regeneration in order to treat human vestibulocochlear dysfunction due to hair cell loss.

Clinical relevance

The possibility of inducing robust hair cell regeneration in mammals and of treating human vestibulocochlear dysfunction is still distant. However, our attempts to examine this possibility are very new, and a number of findings in the past 2 years have made it significantly more likely that it may ultimately be possible. There is clearly a small amount of cell division in the damaged mammalian vestibular organ, and there is at least suggestive evidence that new hair cells arise after damage, possibly via direct transdifferentiation. First, the existence of a second, nonmitotic pathway for regeneration offers an alternate route to pursue should it prove impossible or unsafe to induce regeneration in humans via mitosis. Second, several researchers have demonstrated the ability to increase the rate of division in both undamaged and damaged vestibular epithelium, in both avian and mammalian species, by the addition of exogenous growth factors to cultured epithelia. Third, a number of ongoing studies are attempting to identify the regulatory factors responsible for regeneration using molecular technology, and others are investigating novel delivery systems.

The success of cochlear implants, which rely on a very limited number of transmitting channels, in treating profound sensorineural deafness has made it clear that it would not be necessary to generate a complete organ of Corti or a full complement of normal hair cells to make a major impact on the life of people with profound sensorineural hearing loss. All these facts make it increasingly conceivable that human vestibulocochlear dysfunction due to hair cell loss may one day be treatable via the induction of new hair cell growth in the inner ear.

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Edwin W Rubel, PhD, Virginia Merrill Bloedel Hearing Research Foundation, Box 356515, Department of Otolaryngology–Head and Neck Surgery, University of Washington, Seattle, WA 98195, USA.