Springer Handbook of Auditory Research

Mark E. Warchol · Jennifer S. Stone
Allison B. Coffin · Richard R. Fay
Arthur N. Popper *Editors*

Hair Cell Regeneration

With 33 Illustrations

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The Acoustical Society of America

On 27 December 1928, a group of scientists and engineers met at Bell Telephone Laboratories in New York City to discuss organizing a society dedicated to the field of acoustics. Plans developed rapidly, and the Acoustical Society of America (ASA) held its first meeting on 10–11 May 1929 with a charter membership of about 450. Today, ASA has a worldwide membership of about 7000.

The scope of this new society incorporated a broad range of technical areas that continues to be reflected in ASA's present-day endeavors. Today, ASA serves the interests of its members and the acoustics community in all branches of acoustics, both theoretical and applied. To achieve this goal, ASA has established Technical Committees charged with keeping abreast of the developments and needs of membership in specialized fields, as well as identifying new ones as they develop.

The Technical Committees include acoustical oceanography, animal bioacoustics, architectural acoustics, biomedical acoustics, engineering acoustics, musical acoustics, noise, physical acoustics, psychological and physiological acoustics, signal processing in acoustics, speech communication, structural acoustics and vibration, and underwater acoustics. This diversity is one of the society's unique and strongest assets since it so strongly fosters and encourages cross-disciplinary learning, collaboration, and interactions.

ASA publications and meetings incorporate the diversity of these Technical Committees. In particular, publications play a major role in the society. *The Journal of the Acoustical Society of America* (JASA) includes contributed papers and patent reviews. *JASA Express Letters* (JASA-EL) and *Proceedings of Meetings on Acoustics* (POMA) are online, open-access publications, offering rapid publication. *Acoustics Today*, published quarterly, is a popular open-access magazine. Other key features of ASA's publishing program include books, reprints of classic acoustics texts, and videos. ASA's biannual meetings offer opportunities for attendees to share information, with strong support throughout the career continuum, from students to retirees. Meetings incorporate many opportunities for professional and social interactions, and attendees find the personal contacts a rewarding experience. These experiences result in building a robust network of fellow scientists and engineers, many of whom become lifelong friends and colleagues.

From the society's inception, members recognized the importance of developing acoustical standards with a focus on terminology, measurement procedures, and criteria for determining the effects of noise and vibration. The ASA Standards Program serves as the Secretariat for four American National Standards Institute Committees and provides administrative support for several international standards committees.

Throughout its history to present day, ASA's strength resides in attracting the interest and commitment of scholars devoted to promoting the knowledge and practical applications of acoustics. The unselfish activity of these individuals in the development of the society is largely responsible for ASA's growth and present stature.

Series Preface

Springer Handbook of Auditory Research

The following preface is the one that we published in volume 1 of the Springer Handbook of Auditory Research back in 1992. As anyone reading the original preface, or the many users of the series, will note, we have far exceeded our original expectation of eight volumes. Indeed, with books published to date and those in the pipeline, we are now set for 7 volumes in SHAR. Once volume 77 is completed, we are turning the series over to new Series Editors who will carry on with additional volumes.

We are very proud that there seems to be consensus, at least among our friends and colleagues, that SHAR has become an important and influential part of the auditory literature. While we have worked hard to develop and maintain the quality and value of SHAR, the real value of the books is very much because of the numerous authors who have given their time to write outstanding chapters and to our many co-editors who have provided the intellectual leadership to the individual volumes. We have worked with a remarkable and wonderful group of people, many of whom have become great personal friends of both of us. We also continue to work with a spectacular group of editors at Springer. Indeed, several of our past editors have moved on in the publishing world to become senior executives.

But the truth is that the series would and could not be possible without the support of our families, and we want to take this opportunity to dedicate all of our SHAR books to them. Our wives, Catherine Fay and Helen Popper, and our children, Michelle Popper Levit, Melissa Popper Levinsohn, Christian Fay, and Amanda Fay Sierra, have been immensely patient as we developed and worked on this series. We thank them and state, without doubt, that this series could not have happened without them. We also dedicate the future of SHAR to our next generation of (potential) auditory researchers – our grandchildren – Ethan and Sophie Levinsohn, Emma Levit, Nathaniel, Evan, and Stella Fay, and Sebastian Sierra.

Preface 1992

The Springer Handbook of Auditory Research presents a series of comprehensive and synthetic reviews of the fundamental topics in modern auditory research. The volumes are aimed at all individuals with interests in hearing research including advanced graduate students, post-doctoral researchers, and clinical investigators. The volumes are intended to introduce new investigators to important aspects of hearing science and to help established investigators to better understand the fundamental theories and data in fields of hearing that they may not normally follow closely.

Each volume presents a particular topic comprehensively, and each serves as a synthetic overview and guide to the literature. As such, the chapters present neither exhaustive data reviews nor original research that has not yet appeared in peer-reviewed journals. The volumes focus on topics that have developed a solid data and conceptual foundation rather than on those for which a literature is only beginning to develop. New research areas will be covered on a timely basis in the series as they begin to mature.

Each volume in the series consists of a few substantial chapters on a particular topic. In some cases, the topics will be ones of traditional interest for which there is a substantial body of data and theory, such as auditory neuroanatomy (Vol. 1) and neurophysiology (Vol. 2). Other volumes in the series deal with topics that have begun to mature more recently, such as development, plasticity, and computational models of neural processing. In many cases, the series editors are joined by a co-editor having special expertise in the topic of the volume.

SHAR logo by Mark B. Weinberg, Potomac, Maryland, used with permission

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*Deceased

Volume Preface

The chapters in this volume review current knowledge of the mechanisms that underly the generation of hair cells in the mature ear and discuss how such new hair cells might reestablish functional connections with the brain. Although older findings are discussed, our emphasis is on work conducted since about 2005 because much exciting work has taken place since chapters in earlier volumes of the *Springer Handbook of Auditory Research*.

Chapter 2, by Madeleine Hewitt, David W. Raible, and Jennifer S. Stone, gives an overview of hair cell regeneration in non-mammalian vertebrates (primarily fishes and birds). They review the evidence that first led scientists to conclude that hair cells can be replaced after damage, and they detail the cellular processes by which supporting cells can give rise to new hair cells. This is followed by Chap. 3, where Mark A. Rudolf and Jeffrey T. Corwin focus on the basic biophysical and mechanical properties of supporting cells during regeneration. Together, Chaps. 2 and 3 build a foundation of understanding for the remarkable capacity for hair cell regeneration shared by nonmammalian vertebrates and establish the context for the next three chapters, which focus on the mammalian ear.

In Chap. 4, Andrew Forge and Ruth Taylor give an overview of hair cell regeneration in the mammalian inner ear. The authors provide a brief review of the development and structure of the mammalian vestibular and auditory sensory epithelia as well as review the phenomenology of natural regeneration in the mature mammalian vestibular organs, how it occurs through phenotypic conversion of supporting cells, and its limitations.

One frequently proposed strategy for inducing regeneration in the mature cochlea is to reactivate the signaling pathways that produce hair cells during embryonic development. In Chap. 5, Melissa M. McGovern and Andrew K. Groves describe the molecular mechanisms that govern organ of Corti development, resulting in the establishment of correct cell phenotypes, numbers, and patterning. The authors then explain how insights gleaned from development have shaped studies of auditory hair cell regeneration.

Another approach to inducing repair in the mammalian inner ear involves the reactivation or surgical introduction of stem cells. Such efforts are reviewed by

Amanda Janesick, Stefan Heller, and Eri Hashino in Chap. 6. The chapter describes current strategies for generating inner ear cell types from pluripotent stem cells in vitro as well as somatic stem and progenitor cells that are present in the ears of non-mammalian vertebrates, in the adult mammalian vestibular organs, and in the cochleae of neonatal rodents.

In Chap. 7, Steven H. Green, Sepand Bafti, Benjamin M. Gansemer, A. Eliot Shearer, Muhammad Taifur Rahman, Mark E. Warchol, and Marlan R. Hansen summarize the causes of spiral ganglion neuron death and review several approaches to their possible regeneration.

Finally, in Chap. 8, Brandon C. Cox, John V. Brigande, and Bradley J. Walters focus on some newer approaches to the biological restoration of hearing. They provide a detailed overview of such methodology and potential applications to otic regeneration.

This volume conveys many of the critical advances in our understanding of hair cell regeneration that have occurred since the 2005 SHAR volumes. We have framed these advances in the context of our shared goal of defining ways to stimulate regeneration, and recovery of hearing and balance function, in humans. In addition, this volume discusses many of the cutting-edge approaches that are being applied, or soon will be applied, to hasten progress toward this goal.

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Chapter 1
Sensory Regeneration in the Inner Ear: History, Strategies, and Prospects

Mark E. Warchol and Jennifer S. Stone

Abstract Hair cells are the sensory receptors of the vertebrate inner ear that detect sound vibrations (to initiate hearing) and head movements (to facilitate balance). In humans, injury or loss of these cells can cause permanent hearing impairment, disequilibrium, and/or improper visual reflexes. For this reason, the development of methods to promote the regeneration of lost hair cells is a topic of great translational interest. The demonstration that the ears of nonmammalian vertebrates can regenerate hair cells after injury has led to considerable research focused on understanding the biological basis of this regenerative process. Other work has attempted to induce repair in the mammalian inner ear via gene therapy or introduction of stem cells. Such research is still ongoing, and it is not clear which approaches will ultimately yield clinical strategies for restoring hearing and motion perception. This chapter provides a brief history of this discipline and summarizes the contents of this volume.

Keywords Hair cell · Cochlea · Vestibular · Basilar papilla · Acoustic trauma Ototoxicity · Regeneration

1.1 Introduction

The inner ear is a complex region of the body that houses specialized end organs for the auditory and vestibular senses. Injury or pathology can affect numerous cell types in the inner ear, ultimately leading to hearing loss or disorders of balance and equilibrium. Hair cells are one of the most critical elements of the auditory and

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vestibular sensory systems. They detect mechanical motion generated by either sound vibrations or head movements and provide synaptic input to the afferent neurons that convey information to the brain. In humans, the loss of hair cells is the leading cause of sensorineural deafness. The death of hair cells can also be an underlying cause of vestibular dysfunction.

The focus of the present volume is on biological processes that regulate the regeneration of hair cells after injury. Although the mammalian ear has a very limited ability for regeneration, the ears of nonmammalian vertebrates are capable of spontaneously regenerating hair cells and such regeneration is accompanied by functional recovery (reviewed by Warchol 2011; Burns and Corwin 2013). Hair cell-containing organs of some vertebrates also show evidence for cell turnover, in which hair cells die under apparently normal conditions (i.e., without damaging stimuli or agents), and new hair cells are then formed to replace them. Such turnover has been demonstrated to occur in lateral line neuromasts of fishes (Williams and Holder 2000; Cruz et al. 2015) and in the vestibular organs of birds and mammals (Jørgensen and Mathiesen 1988; Kirkegaard and Jørgensen 2000; Bucks et al. 2017) and may yield insights into the possibility for regeneration after injury.

This book reviews current research on sensory regeneration in the inner ear, including some of the key questions under investigation, the scientific approaches that are being applied, and the insights that have been gleaned. It has been roughly 15 years since hair cell regeneration was covered by this book series and, during that time, the field has evolved considerably. We have invited some of the leading investigators in the field to describe the progress that has been made across a range of research topics. This book should serve to update seasoned investigators and to help early-stage scientists establish a foundation of knowledge on hair cell regeneration and the factors controlling hair cell development, hair cell innervation by spiral ganglion neurons, and other topics closely connected to hair cell regeneration. The authors provide insights into how research progress has shaped the questions we are now asking, and how technological advances have enabled more sophisticated and efficient approaches to address these questions.

Research into the possibility of promoting regeneration in the inner ear was first stimulated by the observations that the ears of fishes and amphibians continue to produce new hair cells throughout mature life and that birds can spontaneously regenerate hair cells after injury (see Chap. 2). Many efforts are aimed at inducing similar forms of biological repair in the human ear. Such work is still in its early stages, and there is no consensus as to which particular approach will ultimately lead to restoration of inner ear function. For this reason, the present volume contains reviews of several differing conceptual and methodological strategies for promoting regeneration in the cochlea and vestibular organs.

One straightforward approach to the development of regenerative therapies is to identify the genetic and molecular signals responsible for such regeneration in nonmammals and to then use such information to guide methods for inducing similar forms of repair in the ears of mammals. A related approach is inspired by the observation that, during embryonic development, the mammalian ear is capable of some degree of self-repair but this ability is lost during maturation (reviewed in Chap. 4).

Identifying the changes in gene expression and cell signaling that accompany this transition may reveal which signaling pathways are permissive or inhibitory for regeneration. There is also much interest in the use of stem cell technologies to induce repair in the ear, either by activating endogenous stem cells that might remain in the mature ear or by transplantation of stem cells that have been guided toward becoming replacement sensory receptors (e.g., Chap. 6). Finally, new methods for gene therapy offer the potential to reprogram cells within the damaged ear to undergo self-repair or to change phenotype into new sensory receptors (see Chap. 8). A major objective in assembling the present volume was to provide a thorough review of the scientific issues relevant for this field of study and background information on the various approaches currently being employed.

1.2 Historical Overview of Otic Regeneration

1.2.1 Postnatal Generation of Sensory Receptors in Vertebrates

A pioneering study of the developing mouse inner ear, conducted in the 1960s by Robert Ruben, had demonstrated that the proliferation of hair cell precursors terminates at around the time of birth (Ruben 1967). This finding suggested that all mammalian hair cells were produced during embryogenesis and implied that the ear may lack the ability to generate new hair cells later in life. In contrast, studies of other sensory modalities indicated that, in some circumstances, other types of receptor cells could be produced throughout life. For example, adult mammals can regenerate neurons of the olfactory epithelium and olfactory bulb (Altman 1969; Graziadei 1979; Graziadei and Monti Graziadei 1985). Gustatory receptors also exhibit a normal turnover throughout life and can be replaced after injury or loss (Farbman 1980).

When considering these observations, it is notable that the sensory receptors for both taste and smell are directly exposed to the external environment, making them particularly vulnerable to damage, and it is possible that their capacity for renewal and regeneration is a consequence of evolutionary pressure to maintain proper sensory function. However, not all generation of sensory cells in mature animals appears to be linked to injury and repair. The retinae of fishes and amphibians, for example, continue to add photoreceptors throughout life. These animals also continue to grow throughout mature life (albeit at increasingly slower rates with age), and such cell addition is common in many organ systems as they grow larger. In the case of the fish retina, addition of new photoreceptors parallels the overall growth of the animal (Johns 1982) and results in slightly enhanced visual acuity with age (Fernald 1990). It is further notable that the growing retina also adds new ganglion cells (Johns and Easter 1977), which extend axons to the optic tectum. Age-related growth and addition of neurons also occurs in the tectum so that the increase in retinal input is matched by a corresponding increase in the numbers of target cells in the CNS (Meyer 1978; Raymond and Easter 1983).

1.2.2 Postnatal Addition of Hair Cells in Cold-Blooded Vertebrates

Several classic studies demonstrated that hair cells in the lateral line neuromasts of aquatic vertebrates could be produced or replaced throughout life. For example, certain salamanders show a remarkable ability to regenerate lost body parts, such as limbs or tails (e.g., Brockes and Kumar 2008). Studies of axolotls, conducted in the 1930s, demonstrated that amputation of distal tail tips that contained a lateral line neuromast resulted in a regenerated tail segment that also contained a newly formed neuromast (Stone 1937). Such observations might have been interpreted as evidence for the possibility of hair cell regeneration, but the generation of new neuromasts in salamanders occurs as part of a more global regenerative process, and it was not clear how – or whether – these findings could be applied to the sensory receptors of inner ear.

The potential for postnatal hair cell production in the vertebrate inner ear was first demonstrated by Jeffrey Corwin, who showed a dramatic age-related increase in hair cell numbers in elasmobranchs. Quantitative studies demonstrated that the maculae of young charcharinid sharks contained about 40,000 hair cells, while the number of hair cells in the maculae of mature animals could exceed 200,000 (Corwin 1977, 1981). Later, morphological observations by Li and Lewis (1979) suggested that the inner ears of bullfrogs (*Rana catesbeiana*) might also add new hair cells during maturity, and postembryonic addition of hair cells was subsequently demonstrated in other cold-blooded vertebrates, including rays (*Raja clavata*; Corwin 1983), teleost fish (*Astronotus ocellatus*; Popper and Hoxter 1984), and toads (*Bufo marinus*; Corwin 1985).

1.2.3 Sensory Regeneration in the Avian Inner Ear

Together, these earlier studies demonstrated that cold-blooded vertebrates continue to produce new sensory hair cells well into maturity, but they did not demonstrate regeneration per se. Injury-induced production of new hair cells was first observed in the hearing organ of birds, known as the basilar papilla. This sensory organ shares many similarities with the mammalian cochlea in that it contains hair cells that are arranged in a tonotopically organized array, resting upon a basilar membrane that vibrates in response to sound input (reviewed by Fettiplace 2020).

Like many scientific advances, the demonstration of hair cell regeneration in the avian ear occurred through an indirect route. Frequency tuning in the basilar papilla is accomplished (in part) by a traveling wave that propagates along the basilar membrane, with a locus of peak displacement that varies in a tonotopic fashion (von Békésy 1960). It had further been suggested that the frequency map of the basilar papillae underwent a systematic shift during development, such that the basal/proximal regions initially detect low-frequency sounds and then become best sensitive to higher frequencies as the ears matured (e.g., Rubel 1978). Evidence for such remapping was first provided by Rubel and Ryals (1983), who showed that the position of maximal damage caused by exposure to a high-intensity pure tone shifted along the basilar membrane, from proximal to distal, during maturation. In addition, the neural frequency map within the auditory brainstem (which is determined by the pattern of afferent input from the cochlea) also undergoes a similar shift during this same developmental period (Lippe and Rubel 1983).

Age-related changes in susceptibility to noise injury were further studied by Cotanche and colleagues, who used scanning electron microscopy to image the patterns of hair cell loss across the surface of the basilar papilla after exposure to intense pure tones (Cotanche et al. 1988). In a subsequent study, Cotanche quantified the loci of such lesions both immediately following the noise exposure, and also after several days of recovery. As expected, papillae that were examined shortly after the sound exposure contained large regions that were devoid of hair cells. But after several days of recovery, these lesioned regions were found to contain numerous hair cells that possessed morphological features characteristic of immature hair cells (Cotanche 1987). This finding constituted strong and dramatic evidence that the injured hair cells had been replaced. At nearly the same time, Cruz, Lambert, and Rubel were studying patterns of aminoglycoside ototoxicity in the chick inner ear. In mammals, systemic treatment with gentamicin permanently destroys auditory hair cells. However, at several weeks after such treatments, in chicks, Cruz et al. (1987) found that hair cells had apparently recovered in the basilar papilla.

The demonstration that the avian inner ear could regenerate hair cells triggered great interest among auditory researchers but also raised many questions. Were new hair cells being produced or did damaged hair cells undergo some form of repair? Was cell division occurring in the injured papillae or were other cells converting into a hair cell phenotype? These issues were partly resolved by parallel studies that showed evidence for the injury-evoked proliferation of supporting cells and the subsequent differentiation of some of the progeny as replacement hair cells (Corwin and Cotanche 1988; Ryals and Rubel 1988). A similar form of hair cell regeneration was also demonstrated to occur in the vestibular organs of birds, both after spontaneous hair cell death (Jørgensen and Mathiesen 1988) and after injury by ototoxic antibiotics (Weisleder and Rubel 1993). In addition, subsequent studies found that renewed proliferation was not the only mechanism that can generate new hair cells after injury. In some case, supporting cells can undergo a direct change of phenotype, thereby becoming hair cells without first dividing (e.g., Adler and Raphael 1996; Baird et al. 2000). Finally, evidence emerged for a limited – but demonstrable – recovery of hair cells in the vestibular organs of mammals (Forge et al. 1993), which was accompanied by a low level of supporting cell proliferation (Warchol et al. 1993). These findings motivated numerous efforts to determine whether the mammalian cochlea also possessed some degree of regenerative ability. The outcomes of such studies were uniformly negative (e.g., Roberson and Rubel 1994), and it is currently believed that the mature mammalian cochlea lacks any ability to replace sensory cells after injury.

1.3 Overview of Contents

The chapters in this volume review current knowledge of the mechanisms that underlie the generation of hair cells in the mature ear and discuss how these new hair cells might reestablish functional connections with the brain. Although older findings are discussed, our emphasis is on work conducted since 2008.

The second chapter, Nonmammalian Hair Cell Regeneration: Cellular Mechanisms of Morphological and Functional Recovery, by Madeleine Hewitt, David W. Raible, and Jennifer Stone, gives an overview of hair cell regeneration in nonmammalian vertebrates (primarily fishes and birds). They review the evidence that first led scientists to conclude that hair cells can be replaced after damage, and they detail the cellular processes by which supporting cells can give rise to new hair cells. This chapter also describes progress in understanding how hair cell regeneration is controlled at the molecular level, such as which cellular signaling systems, transcription factors, and epigenetic regulators trigger supporting cells to form new hair cells after damage and promote precursor cells to acquire the hair cell-specific features required to restore function.

The third chapter, Cell Junctions and the Mechanics of Hair Cell Regeneration, by Mark A. Rudolf and Jeffrey T. Corwin, focuses on the basic biophysical and mechanical properties of supporting cells. The morphology of supporting cells is significantly altered by the loss of hair cells, and such mechanical cues may be a key factor that either triggers or limits the induction of regeneration. Supporting cells in the ears of nonmammals (i.e., fishes, amphibians, and birds) undergo shape changes in response to loss of hair cells, and the extension of these cells may enable them to leave the quiescent state and divide. In contrast, supporting cells of the mammalian ear possess large cytoskeletal elements that have been hypothesized to limit their proliferative ability. In other cell types, such mechanical factors are known to activate the Hippo and Wnt pathways, which regulate cell division. As such, it is likely that cellular mechanics is one factor that may help explain the differences in the regenerative ability of the ears of nonmammals vs. mammals. Together, Chaps. 2 and 3 build a foundation of understanding for the remarkable capacity for hair cell regeneration shared by nonmammalian vertebrates and establish the context for the next three chapters, which focus on the mammalian ear.

In the fourth chapter, Mammalian Hair Cell Regeneration, Andrew Forge and Ruth Taylor give an overview of hair cell regeneration in the mammalian inner ear. As noted above, the mammalian cochlea does not regenerate hair cells, and only a limited amount of regeneration occurs in the vestibular organs. Current research in this area is directed toward uncovering a possible latent potential for hair cell regeneration, either by negating putative signals that might prevent spontaneous regeneration or by inducing pro-regenerative responses. The authors give a brief review of the development and structure of the mammalian vestibular and auditory sensory epithelia. Studies have shown that the immature inner ears of mammals possess some ability to regenerate but that this capacity is lost with maturation. Much current research is aimed at identifying factors that are permissive for a regenerative response in immature ear but limit regeneration in mature ears. The authors also review the phenomenology of natural regeneration in the mature mammalian vestibular organs, how it occurs through phenotypic conversion of supporting cells, and its limitations. They further describe current approaches to enhance or stimulate hair cell regeneration in adult mammals and the outcomes of such attempts. Finally, the authors review the structural reorganization that occurs in the mature organ of Corti after hair cell damage and how it may impact possible regenerative strategies.

One frequently proposed strategy for inducing regeneration in the mature cochlea is to reactivate the signaling pathways that produce hair cells during embryonic development. In Chap. 5, Specification and Plasticity of Mammalian Cochlear Hair Cell Progenitors, Melissa M. McGovern and Andrew K. Groves describe the molecular mechanisms that govern organ of Corti development, resulting in the establishment of correct cell phenotypes, numbers, and patterning. The authors then explain how insights gleaned from development have shaped studies of auditory hair cell regeneration. Of particular interest is the identification of signals that might suppress pro-regenerative behaviors by supporting cells in the mature cochlea after damage. They further discuss evidence that age-dependent modifications of chromatin might block the activation of genetic programs that have the potential to drive hair cell regeneration. This chapter highlights how an understanding of the mechanisms that regulate the phenotype and proliferation of supporting cells is a critical step toward the development of regenerative therapies.

Another approach to inducing repair in the mammalian inner ear involves either the reactivation or surgical introduction of stem cells. Such efforts are reviewed by Amanda Janesick, Stefan Heller, and Eri Hashino in Chap. 6, Inner Ear Cells from Stem Cells – a Path Toward Inner Ear Cell Regeneration. The investigation of otic regeneration has been limited by the difficulty in accessing the sensory organs of the inner ear and by the relatively small numbers of hair cells and supporting cells that are present in the mammalian ear. Large numbers of cells are necessary for employment of techniques such as high-throughput in vitro drug screens to identify pro-regenerative drugs and gene therapies with the potential to promote regeneration. This chapter describes current strategies for generating inner ear cell types from pluripotent stem cells in vitro. Also discussed are somatic stem and progenitor cells that are present in the ears of nonmammalian vertebrates, in the adult mammalian vestibular organs, and in the cochleae of neonatal rodents. Extracting knowledge from these naturally proliferative cell populations may lead us closer to the possibility of reawakening regenerative potential in the ears of adult mammals.

Hair cells are not the only type of sensory cell that can be damaged or lost from the mature ear. The neurons of the spiral ganglion convey information from cochlear hair cells to the nuclei of the auditory brainstem. These neurons can be injured or lost by many of the same insults that kill cochlear hair cells. Moreover, the loss of spiral ganglion neurons (SGNs) can also occur after exposure to noise levels that do not kill hair cells (Kujawa and Liberman 2006, 2009). SGNs are essential for both normal hearing and for the restoration of hearing provided by cochlear implants. In Chap. 7, Spiral Ganglion Neuron Regeneration in the Cochlea: Regeneration of Synapses, Axons, and Cells, Steven H. Green, Sepand

Bafti, Benjamin M. Gansemer, A. Eliot Shearer, Muhammad Taifur Rahman, Mark E. Warchol, and Marlan R. Hansen summarize the causes of SGN death and also review several approaches to their possible regeneration. The degeneration of SGNs often occurs slowly, and it is possible that restoring their synaptic connections with hair cells may prevent their loss. Current research emphasizes the use of certain neurotrophic factors to promote such regrowth. However, it is also possible that SGNs can be replaced by transplantation of new neurons derived from stem cells. The chapter discusses current efforts to generate SGNs from stem cells and the challenges facing these attempts.

The final chapter, Genetic and Epigenetic Strategies for Promoting Hair Cell Regeneration in the Mature Mammalian Inner Ear, by Brandon C. Cox, John V. Brigande, and Bradley J. Walters, focuses on some newer approaches to the biological restoration of hearing. The development of successful strategies for inducing regeneration will likely involve the genetic manipulation of experimental animals, in order to either delete or overexpression of certain genes or to introduce novel genetic constructs into cells of the inner ear. Chapter 8 provides a detailed overview of such methodology and potential applications to otic regeneration. Inducing repair in the mature inner ear will be a complex process, requiring the manipulation of multiple genes and/or alterations of epigenetic modifiers. The chapter reviews techniques such as CRISPR/Cas and CreER/lox methodology. Complementary strategies using virus-mediated gene transfer, small interfering RNA, antisense oligonucleotides, and microRNAs are also highlighted.

1.4 Conclusions

As noted earlier, the investigation of hair cell regeneration is currently proceeding along several distinct and parallel paths. We hope that readers will find this book to be a useful resource for initiating or continuing research into sensory regeneration and that it will enhance their understanding of the rationale, potential, and limitations of the current approaches and methodologies. We further hope that this information will stimulate researchers to uncover new facts and develop more effective techniques that will guide this field toward its ultimate objective: the biological repair of the human inner ear.

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Chapter 2
Nonmammalian Hair Cell Regeneration: Cellular Mechanisms of Morphological and Functional Recovery

Madeleine N. Hewitt, David W. Raible, and Jennifer S. Stone

Abstract This chapter provides an overview of hair cell regeneration in nonmammalian vertebrates. First, we review the early foundational research on hair cell replacement. Then, we discuss research in the past 15 years that underlies our current understanding of the mechanistic basis of hair cell regeneration, including the identity, properties, and epithelial locations of supporting cells, the progenitors to new hair cells. We also describe some of the new approaches used to study hair cell regeneration, and we discuss molecules that have been found to either block or drive supporting cells to generate new hair cells after damage. This chapter focuses on chickens and zebrafish, which are the most commonly used animal models for this type of research.

Keywords Hair cell · Supporting cell · Regeneration · Molecular regulation Zebrafish · Chicken

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