pathologies of the inner ear. This increasing knowledge and understanding will then feed back to create new interventions and novel technologies to further enable the interventions.

2 Prevention of Hearing Loss

2.1 Timing of Interventions for Preventing Hearing Loss

There are many cases wherein a subject knowingly enters into a situation that provides a risk of generating hearing loss. Such an "initiating event" (Fig. 14.1) could be from noise in the working or recreational environment, or from drugs that are taken to treat diseases such as cancer or bacterial infection. It could then be possible to have interventions before the initiating event, shown as the prophylactic interventions in Fig. 14.1, during or immediately after the event (listed as Immediate Intervention in Fig. 14.1) before the immediate changes induced by the noise, drug, or other "event" occurs. In this case, our knowledge of the mechanisms leading to early pathology is critical to identify early interventions. However, many of the pathways that are induced continue to progress over hours, days, and even weeks, and prevention/intervention can still be possible well after the initiating event.

Outer hair cells are specialized sensory cells that actively expand and contract during acoustic transduction and thus contribute to the exquisitive sensitivity of the auditory system. Necrotic- or apoptotic-induced hair cell death represents the primary cause of hearing impairment for most, if not all, environmental stress-induced cell death (e.g., Hu, Chap. 5). In addition, many instances of genetic stress-induced cell death appear to reflect metabolically driven mitochondrial derived oxidative stress (e.g., Gong and Lomax, Chap. 9). Noise stress can be considered a representative model of environmental stress-induced inner ear cell death. During noise stress, energy demands induce mitochondrial free radical formation, causing lipid peroxidation and the upregulation of cell death pathways, producing hair cell death by necrosis or apoptosis. Free radical formation occurs in the organ of Corti and lateral wall soft tissues, and this free radical formation is enhanced by reduced blood flow during noise and a "stroke-like" rebound reperfusion after the noise. Free radical formation continues after exposure, and increased accumulations have been linked to progressive cell death over a 10-day post-noise period (Yamashita et al. 2004). Genetic- or diet-induced upregulation of endogenous antioxidant pathways, or exogenous treatment with antioxidants and vasodilators, modulates the free radical formation, subsequent cell death, and hearing loss. Similar findings show the same mechanism, mitochondrial-derived oxidative stress, underlies aminoglycoside-induced hair cell death, may underlie age-related cell death, and has been speculated as a factor in Ménière's disease, sudden sensorineural hearing loss, and trauma of cochlear implantation. From other fields, it is clearly established that free radical formation is key to hyperoxia-, hypoxia-, reoxygenation-, radiation-, cigarette smoke-, and stroke-induced cell death (Circu and Aw 2010; Roberts et al. 2010;

for recent reviews). To the extent that mitochondrial-derived oxidative stress represents a common element to the final pathway to cell death, it represents an "upstream" target of opportunity for intervention and prevention.

Most mechanism-based therapeutic strategies take one of two approaches. One approach is to mimic or enhance endogenous "good" mechanisms, those that provide protection. Three such protective pathways are discussed herein: antioxidants, neurotrophic factors, and heat shock proteins. The other approach is to block the progression of "bad" pathways, those that lead to cell death. This could involve blocking apoptotic and excitotoxic pathways, using agents such as calcium channel blockers, calpain and calcineurin inhibitors, Bcl-2 anti-apoptotic proteins, caspase inhibitors, and JNK-inhibitors. These agents were reviewed in Le Prell et al. (2007b), and a more recent discussion is provided by Abi-Hachem et al. (2010). Recent data on calcium channel blockers and JNK inhibitors are reviewed in Le Prell and Bao (Chap. 13).

2.1.1 Antioxidants

Endogenous antioxidant systems are a major protective mechanism in the cochlea that can respond to a variety of trauma, stresses, and "initiating events" such as intense noise that generates free radicals in the cochlea for hours and days after exposure, which then induce cell death signals (for detailed reviews, see Le Prell et al. 2007b; Le Prell and Bao, Chap. 13). Administration of exogenous antioxidants has great potential for therapeutic intervention. In fact, a variety of antioxidant agents have been shown to attenuate NIHL effectively in animal studies. Such agents include glutathione monoethyl ester (GSHE; Ohinata et al. 2000; Kopke et al. 2002; Miller et al. 2003b), resveratrol (Seidman et al. 2003), allopurinol (Seidman et al. 1993; Cassandro et al. 2003), superoxide dismutase-polyethylene glycol (Seidman et al. 1993), lazaroid (a drug that inhibits lipid peroxidation and scavenges free radicals) (Quirk et al. 1994), vitamin A (Ahn et al. 2005), vitamin C or ascorbate (Derekoy et al. 2004; McFadden et al. 2005), alpha-tocopherol (Hou et al. 2003), salicylate and trolox (Yamashita et al. 2005), and (R)-phenylisopropyl-adenosine (R-PIA; Hu et al. 1997). 2-Oxothiazolidine-4-carboxylate (OTC) (Yamasoba et al. 1998), N-acetylcysteine (NAC) (Ohinata et al. 2003; Duan et al. 2004), NAC and salicylate (Kopke et al. 2000), D-methionine (Kopke et al. 2002), and ebselen (Pourbakht and Yamasoba 2003; Lynch and Kil 2005; Yamasoba et al. 2005). Other potential agents such as coenzyme Q10 (Hirose et al. 2008) and ferulic acid (Fetoni et al. 2010) continue to be added.

Dietary supplements that reduce NIHL are of particular interest given their easy over-the-counter accessibility, but therapy with any single micronutrient may need to be initiated days to weeks in advance of noise exposure to obtain clinically meaningful results. Whereas a 35-day pretreatment with vitamin C significantly reduced NIHL and cochlear hair cell death (McFadden et al. 2005), vitamin C treatment initiated 48 h before noise exposure failed to prevent noise-induced cell death (Branis and Burda 1988). Pretreatment requirements may vary across micronutrients, as vitamin E reduced NIHL with treatment initiated 3 days pre-noise (Hou et al. 2003) and vitamin A reduced NIHL with treatment initiated 2 days pre-noise (Ahn et al. 2005).

Although dietary treatments may need to be provided for some longer period of time pre-noise to be maximally effective, high-dose vitamin C did not completely prevent NIHL even with 35 days pretreatment (McFadden et al. 2005), and stable plasma and tissue levels of vitamin C are obtained in humans approximately 3 weeks after beginning dietary treatment (Levine et al. 1996). Taken together, these data suggest that dietary antioxidants may be more useful in combination than as single-agent therapeutics. The work of Le Prell et al. (2007a) demonstrating robust attenuation of NIHL with 1-h pre-exposure administration of the antioxidants beta-carotene, vitamins C and E, plus magnesium, supports this view.

With respect to the propagation of oxidative stress reactions, it is clear that iron (Halliwell and Gutteridge 1986) and other transition metals (for review, see Halliwell and Gutteridge 2007) contribute to the generation and propagation cycles of free radicals. Ferrous iron (II) is known to be oxidized by hydrogen peroxide to ferric iron (III), a hydroxyl radical and a hydroxyl anion. Iron (III) is then reduced back to iron (II), a peroxide radical and a proton by the same hydrogen peroxide. This process is known as the Fenton reaction. Because iron is involved in ROS generation, iron chelators are also potential candidates to reduce NIHL. An iron chelator, deferoxamine mesylate (DFO), alone or in combination with mannitol, a hydroxyl scavenger and weak iron chelator, attenuated NIHL in guinea pigs with little evidence for additive effects (Yamasoba et al. 1999). Because an oral iron chelator is available and used safely for humans (Oliva et al. 2010), such agents may be applied prophylactically for humans, especially for those who are scheduled to be exposed to intense noise, including those in the military such as bomber crews.

2.1.2 Neurotrophic Factors

Neurotrophic factors (NTFs) provide another endogenous protective mechanism that can be mimicked or enhanced to provide therapeutic intervention in the progression toward hearing loss. NTFs have multiple functions and, therefore provide different options. For example, NTFs will scavenge free radicals, interrupt cell death pathways, and modulate calcium homeostasis; any of which may attenuate the progression toward hearing loss. Withdrawal of NTFs leads to ROS formation and initiates a cascade of events that lead to cell death (for review, see Kirkland and Franklin 2003).

Most of the exogenous NTFs delivered into the cochlea have been reported to prevent noise-induced hair cell death, which include acidic fibroblast growth factor (FGF1) (Sugahara et al. 2001), basic FGF or FGF2 (Zhai et al. 2004), glial cell line–derived neurotrophic factor (GDNF) (Ylikoski et al. 1998; Yamasoba et al. 1999), and neurotrophic factor 3 (NT3) (Shoji et al. 2000a). Brain-derived neurotrophic factor (BDNF) (Shoji et al. 2000a) and, in some studies, FGF1 and FGF2 (Yamasoba et al. 2001) did not reduce noise-induced injury, suggesting that (1) the effect is growth factor specific, which could be a consequence of different NTF receptors on the hair cells (Ylikoski et al. 1993; Pirvola et al. 1997), and (2) the protective effects are dependent on multiple factors such as optimal drug dosage and nature or severity of injury.

In addition to preserving hair cell survival after noise, NTFs have been shown to be extremely effective at preserving neural survival in the absence of surviving hair cells. In the presence of intact hair cells, damaged auditory nerve peripheral processes may be able regrow and restore auditory sensation (Puel et al. 1991, 1995; Le Prell et al. 2004), whereas with loss of hair cell targets, auditory nerve regrowth is limited (Bohne and Harding 1992; Lawner et al. 1997; McFadden et al. 2004). It has, however, recently been shown that an acoustic overexposure that causes moderate, reversible, temporary shift of hearing threshold (TTS) may leave cochlear sensory cells intact but cause loss of afferent nerve terminal connections and delayed degeneration of the auditory nerve and cell bodies (Kujawa and Liberman 2009), suggesting that regrowth can be absent or inefficient. Although delayed auditory nerve degeneration is frequently observed as a consequence of NTF deprivation that occurs when sensory cells in the organ of Corti are damaged, the finding of loss of inner hair cell–auditory nerve connections and nerve degeneration post-noise, in the presence of intact hair cells, is a component of NIHL that should not be ignored. Indeed, much of the basic research defining protection via NTFs in the auditory system has only been in the context of neural preservation after noise- or aminoglycoside-induced cell death and deafness and not considered connections to auditory nerve.

Use of growth factor combinations, or combinations of growth factors with other non-growth factor substances, enhances efficacy over single agents both in vivo and in vitro (for review see Le Prell et al. 2007b). Importantly, a single NTF or combinations of NTFs can be highly efficacious in promoting auditory nerve survival even with temporal delay in onset of treatment relative to deafening. Nerve growth factor (NGF) delivered alone (Shah et al. 1995), or BDNF, NT3, and neurotrophin-4/5 alone (Gillespie et al. 2004), each enhanced neural survival even when administration was delayed by 2 weeks. The combination of BDNF and ciliary neurotrophic factor (CNTF) enhanced auditory nerve survival even at delays of up to 6 weeks post-deafening (Yamagata et al. 2004). Consistent with an important role for FGF1 in neurite outgrowth in the immature auditory system (Dazert et al. 1998; Hossain and Morest 2000), it has recently been demonstrated that BDNF plus FGF1 was effective in promoting systematic regrowth of the peripheral process of the auditory nerve even after a 6-week period of deafening (Miller et al. 2007; Glueckert et al. 2008). Together, these results suggest post-noise treatment with NTFs may prevent neural degeneration that occurs consequent to noise-induced sensory cell death.

2.1.3 Heat Shock Proteins

The classical stress response, involving heat shock proteins, provides another endogenous pathway that could be induced to provide protection from noise or other initiating events. Heat shock proteins provide protection by stabilizing proteins and preventing stress-induced misfolding and may further interface with the endoplasmic reticulum (ER)-related pathways and pathologies. Yoshida et al. (1999) found that providing a heat stress in mice that induced the heat shock response in the cochlea provided protection from a noise exposure that might otherwise be damaging to the

cochlea and hearing. Fairfield et al. (2005) did the opposite, removing the protection by using mice with the heat shock response compromised by knockout (KO) of HSF1, the transcription factor that induces activation of the pathway. Results showed more damage and hearing loss after noise in the HSF1 KO mice compared to wild-type littermates. While heating one's ear before noise might not be practical, recently small molecules have been developed that can act at the cellular level to activate HSF1 and induce the heat shock protective response (Neef et al. 2010) providing the potential for a more applicable therapeutic intervention.

2.1.4 Blockers of Excitotoxicity

Although prevention of cell death is a major target of interventions to prevent hearing loss, there can also be excitotoxicity leading to loss of connections between inner hair cells (IHCs) and auditory nerve, contributing to hearing disorders. Although regrowth and reconnection of lost processes to surviving IHCs has been shown (Puel et al. 1998; Pujol and Puel 1999), recent studies show this reconnection is not always efficient (Kujawa and Liberman 2009), and loss of these connections could contribute to reduced speech comprehension, particulary in a noisey environment. Prevention of excitotoxicity must, therefore, also be a goal for therapeutic interventions. Excitotoxic trauma and the development of novel calcium channel blockers as potential therapeutics for prevention of NIHL are reviewed in detail in Le Prell and Bao (Chap. 13) and are not discussed further in this chapter.

2.1.5 Blood Flow Promoting Drugs

Trauma-mediated changes in cochlear blood flow influence the progression of hearing loss and interventions influencing blood flow can also be a therapeutic target. While in most other tissues increased metabolism is associated with increased blood flow to provide additional oxygen to stressed cells; in the cochlea, intense noise decreases blood flow and is followed by a subsequent rebound and overshoot in blood flow (for review see Le Prell et al. 2007b). The decreased blood flow in the cochlea is associated with noise-induced reductions in blood vessel diameter and red blood cell velocity (Quirk et al. 1992; Quirk and Seidman 1995). This appears to be caused by a byproduct of noise-induced free radical formation, particularly in tissues associated with the cochlear vasculature (lateral wall) (Miller et al. 2003b) and reducing the vasoconstriction that occurs with ROS production could contribute to the reduction of NIHL achieved by antioxidants. Agents that reduce vasoconstriction or have vasodilating effects such as hydroxyethyl starch (HES, e.g., Lamm and Arnold 2000) or magnesium (e.g., Scheibe et al. 2000), have been shown to reduce NIHL (Le Prell et al. 2007b for review). The protective effects of enhancing blood flow during noise exposure may be based on reducing the noise-induced blood flow reduction directly or by blocking the subsequent blood flow rebound and overshoot that follows the noise-induced reduction. In addition to the well-characterized effects

on vasodilatation, biochemical effects of magnesium include modulation of calcium channel permeability, influx of calcium into cochlear hair cells, and glutamate release (Gunther et al. 1989; Cevette et al. 2003). Regardless of the specific mechanism, magnesium clearly attenuates NIHL and is safe for use in humans within the recommended dose range.

2.1.6 Post-trauma Interventions

The question of timing for therapeutic interventions along the progression of noise-induced damages (Fig. 14.1) is a critical one. How late can interventions be applied in the process and pathway to cell death to prevent the cell from dying? Will the preserved cell be completely healthy and functioning if it is saved late in the process? This may depend on the mechanism applied for the intervention and how far along a cell is in the apoptotic pathway; however, this question remains to be carefully studied.

One exciting development is that because cell death pathways progress over a period of time, it is possible to intervene well after the initiating event and still prevent cells from progressing toward the end state of cell death. Noise-induced oxidative stress begins early and becomes substantial over time (first suggested by Ohlemiller et al. 1999), which would explain observations of hair cell death that accelerates with time after exposure for a period of up to 14 days (Bohne et al. 1999; Yamashita et al. 2004). Yamashita et al. (2004) found peak ROS and RNS production in cells of the organ of Corti was at 7–10 days after noise insult, and the final extent of damage to cochlear tissues could reflect cell death pathways initiated by late-forming free radicals in the inner ear. Therapeutic interventions after noise exposure have proven to be effective. Treatment with salicylate and vitamin E initiated 24 h after noise exposure was almost as effective as pretreatment in preventing loss of sensory elements and treatment initiated 3 days postexposure also reduced NIHL and sensory cell death relative to untreated controls (Yamashita et al. 2005). Treatment delayed 5 days relative to noise insult was not effective. D-Methionine reduced NIHL and cochlear damage when provided 1 h after noise overstimulation (Campbell et al. 2007), and all-trans retinoic acid could reduce NIHL and cochlear damage when provided up to 2 days after a noise overstimulation (Shim et al. 2009), though efficacy decreased over time. These studies suggest there is a window of opportunity of several days after noise overstimulation where therapeutic intervention can provide benefit, even if pretreatment or treatment shortly after the noise is most effective.

2.1.7 Combination Effects

Given that none of the interventions tested to date completely prevents NIHL and noise-induced sensory cell death, it would seem reasonable to seek an additive effect with a combination of factors that intervene at multiple sites in the biochemical cell death cascade. When the effect of a combination of an antioxidant (mannitol,

a hydroxyl scavenger), a neurotrophic factor (GDNF), and an iron chelator (deferoxamine mesylate [DFO]), each of which individually attenuate NIHL, was evaluated, there was little evidence for additive effects; that is, treatment with a combination of agents yielded no greater protection than the most effective agent delivered alone (Yamasoba et al. 1999).

Other studies similarly failed to find evidence for additive or synergistic effects. Another study that evaluated the potential for additive effects of various combinations of antioxidants and vasodilators, including betahistine, vitamin E, and a combination of these agents, and salicylate, vitamin E, and a combination of these agents also demonstrated no evidence for additive effects (Miller et al. 2006). When the individual and combined effects of creatine, a cellular energy enhancer, and tempol were compared in guinea pigs exposed to noise, the effects of the combination treatment were similar to those treated with creatine alone (Minami et al. 2007).

Only recently, with combinations of antioxidant vitamins and magnesium, have additive effects on prevention of NIHL or otoxicity been demonstrated. Yeum et al. (2009) have shown additive effects with β-carotene and α-tocopherol, ascorbic acid and α-tocopherol, and β-carotene and ascorbic acid on antioxidant activity in reconstituted human serum. A robust additive effect on protection from NIHL was demonstrated with the combination of β-carotene, vitamins C and E, and magnesium (Le Prell et al. 2007a). The identification of specific combinations of agents that act in additive or synergistic (i.e., multiplicative) ways is a compelling goal for future research activities. Because activation of calcineurin depends on ROS production and ROS-induced deficits in calcium homeostasis (Huang et al. 2001; Gooch et al. 2004; Rivera and Maxwell 2005), one might predict that blocking early ROS production would reduce activation of the calcineurin-initiated apoptotic pathway. If so, pretreatment with antioxidant agents that are highly efficient hydroxyl radical scavengers, in combination with FK506 to directly intervene in the calcineurin pathway, might more effectively reduce NIHL and noise-induced cell death. This hypothesis has not been directly tested, and identification of the most effective combinations remains a challenge for future research efforts.

2.1.8 Novel Therapeutic Tools: Hydrogen Gas and Water

Molecular hydrogen (hydrogen gas and hydrogen-rich water) was recently established as a unique antioxidant that selectively reduces the hydroxyl radical, the most cytotoxic ROS, but that does not react with other ROS that possess beneficial physiological roles. Inhalation of hydrogen gas markedly suppresses brain injury induced by focal ischemia and reperfusion by buffering the effects of oxidative stress in rats (Ohsawa et al. 2007). Further, the inhalation of hydrogen gas suppressed hepatic injury caused by ischemia-reperfusion in mice (Fukuda et al. 2007) and limited the extent of myocardial infarction in rats (Hayashida et al. 2008). In the nervous system, hydrogen-rich water was shown to prevent superoxide formation in brain slices of vitamin C-depleted senescence marker protein 30/gluconolactonase-knockout mice (Sato et al. 2008) and to prevent stress-induced impairments in learning tasks

during chronic physical restraint in mice (Nagata et al. 2009). Moreover, a clinical study showed that consuming hydrogen-rich pure water improves lipid and glucose metabolism in type 2 diabetes patients (Kajiyama et al. 2008).

Hydrogen gas is permeable to cell membranes and can target organelles, including mitochondria and nuclei. This is especially favorable for inner-ear medicine, because many therapeutic compounds are blocked by the blood–labyrinthine barrier and can not get access to the inner ear. In a recent ex vivo study, hydrogen gas markedly decreased oxidative stress by scavenging ROS and protected cochlear cells and tissues against oxidative stress (Kikkawa et al. 2009). When antimycin A was applied to organotypic explant cultures of mouse auditory epithelia, incubation with a hydrogen-saturated medium significantly reduced ROS generation and subsequent lipid peroxidation. Reduced free radical insult increased survival of the hair cells. Considering the safety and easy accessibility of hydrogen to cells in the inner ear, hydrogen gas or hydrogen-rich water seems to be a promising agent to investigate for potential prevention of NIHL in human subjects exposed to noise.

3 Treatment of Hearing Disorders

Although prevention of hearing disorders would clearly be optimal, protective treatments have not yet been shown to work in human trials, are not yet approved by the FDA for hearing protection, and even once they are more developed they may be too late or insufficient for many subjects. Therefore, treatment of hearing loss and hearing disorders remains an important and critical goal, the last intervention target in Fig. 14.1. Treatments fall into two general categories of "maintenance" and "restoration." Maintenance can involve prevention of further pathology, where it overlaps with preventions. Restoration rests upon the three Rs of "repair," "regeneration," and "replacement." Repair involves treating remaining cells in the damaged ear to return the auditory pathways to their condition before the hearing loss. Regeneration requires treatments to induce repopulation from endogenous progenitors or redifferentiation of cells remaining in the damaged ear, although replacement could involve a variety of approaches ranging from the use of exogenous cell implants to replace lost cells to cochlear prostheses to bypass lost cells. The combination of repair, regeneration, and replacement is frequently termed "tissue engineering."

3.1 Maintenance

3.1.1 Survival Factors: Neurotrophic Factors

Just as NTFs can have multiple roles in protection, they also have roles in maintenance, repair, and restoration. NTFs have an important function as survival factors, and deafferentation can result in NTF deprivation for the auditory nerve that can

lead to free radical formation and the upregulation of cell death pathways (NTF hypothesis; Mattson 1998 for review). Thus, hair cell loss results in a secondary and progressive loss of auditory nerve and its spiral ganglion neurons (SGN). If exogenous NTFs such as BDNF, NT-3, and GDNF are supplied to the auditory nerve to replace lost endogenous NTFs, they will promote maintenance and survival (e.g., Ernfors et al. 1996; Staecker et al. 1996; Miller et al. 1997; Green et al. 2008). Supplying NTFs will enhance not only the survival of SGN (Green et al. 2008 for review) but also the electrical responsiveness of the neurons (Maruyama et al. 2008). Today, the cochlear prosthesis offers an important treatment option for patients with severe hair cell loss. Because efficacy of the cochlear prosthesis is dependent on the number and functionality of the remaining SGN (e.g., Nadol et al. 1989; Incesulu and Nadol 1998), it is of therapeutic interest to prevent degeneration of auditory sensory neurons, and neurotrophic treatment has been suggested for use with cochlear prostheses to protect and support the SGN.

3.1.2 Survival Factors: Electrical Stimulation

Electrical activity within the auditory nerve provides another important survival factor (Green et al. 2008), and providing electrical stimulation to the auditory nerve has been shown to increase SGN survival after the deafferentation associated with IHC loss (Green et al. 2008 for review). The combination of chronic cochlear electrical stimulation and application of NTFs has been shown to be more effective than either alone (for examples, see Kanzaki et al. 2002; Scheper et al. 2009).

3.1.3 Regrowth of Auditory Nerve Peripheral Processes

An early event on the long-term path to SGN death after loss of IHCs is the relatively rapid degeneration of the deafferented peripheral processes of the auditory nerve, first to the level of the habenula perforata and later to the soma (Webster and Webster 1981; Spoendlin 1984; Spoendlin and Schrott 1990). If hair cell replacement becomes possible, then regrowth of the peripheral process will need to be successfully induced. Moreover, it will ultimately be necessary to connect the new hair cells to the cochlear nucleus via regrown peripheral processes when hair cell restoration or replacement becomes possible (see next section). In the present, SGN peripheral process regrowth might provide benefit to cochlear prostheses. Regrowth of the SGN peripheral process to the vicinity of the electrode would provide a closer target for cochlear electrical stimulation that would allow lower thresholds for excitation, a larger dynamic range of responsiveness, and provide less current spread and better channel separation. Lower thresholds would require less energy, allowing more complex signal processing strategies and increased battery life.

Several NTFs including BDNF, NT-3, GDNF, fibroblast growth factor (FGF), and CNTF play a role in inducing, directing, and modulating connections in the cochlea during normal development (Fritzsch et al. 1997 for review) and have also

been shown to induce a robust growth of neurites in cultured SGN (Staecker et al. 1995; Green et al. 2008 for reviews). Several NTFs have been shown to induce regrowth of afferent and efferent peripheral processes into the cochlea after hair cell loss in vivo when provided either intrascalar by mini-osmotic pumps (Altschuler et al. 1999; Miller et al. 2007; Glueckert et al. 2008) or more recently after gene transfer (Shibata et al. 2010).

In preclinical implant studies, treatment with these factors has been shown to enhance electrical responsiveness, increasing both threshold sensitivity and dynamic range of electrical auditory brain stem responses (ABR) (Miller et al. 2002; Yamagata et al. 2004; Maruyama et al. 2007, 2008). In these studies, it is not clear to what extent this enhanced responsiveness reflects maintenance of SGN and to what extent it reflects regrowth of peripheral afferent processes; it is likely that both factors contribute. Electrical stimulation may also induce regrowth of peripheral processes (Altschuler et al. 1999), and NTF-induced regrowth has been shown to be further enhanced by antioxidants (Maruyama et al. 2007, 2008). Immediately after implantation, it may be appropriate to infuse NTFs to initiate a burst of neurite regrowth, followed (or accompanied by) electrical stimulation with particular parameters for the first weeks, followed then by different parameters of electrical stimulation for maintenance of the connection and signal processing. Antioxidants may be used over a period before and after implantation to enhance regrowth, as well as protect from the trauma of implantation (Abi-Hachem et al. 2010), with little risk.

3.2 Restoration

3.2.1 Regeneration

The exciting discovery of hair cell regeneration after sensory cell death in the chick (e.g., Corwin and Cotanche 1988; Ryals and Rubel 1988) provided the great promise that key factors driving regeneration in birds could be introduced in mammals, including humans. Although this task has not yet been fully accomplished, great progress has been made. These efforts have spawned a set of strategies to identify and analyze the inducing factors, and the first steps toward creating new hair cells in the damaged mammalian ear have been taken. In species that naturally regenerate sensory cells when damaged, the source appears to be the supporting cells, and the mechanism often involves a dedifferentiation, reentry to cell cycling, and division, with one daughter cell becoming a hair cell and the second maturing to a replacement supporting cell, thus maintaining the mosaic of the sensory epithelium critical to mechanoelectric transduction (Kwan et al. 2009; Cotanche and Kaiser 2010 for recent reviews). If the factors that induce, modulate, and guide regeneration in the chick can be induced in mammals, perhaps a comparable regeneration can occur.

Important guidance has also come from an increased understanding of the transcription factors, their downstream pathways, and the molecular mechanisms that control the normal development of the mammalian cochlea and guide an eventual

hair cell versus supporting cell fate decision. Atoh1 is a key transcription factor in the hair cell fate choice (Maricich et al. 2009), and forced upregulation of Atoh1 by gene transfer can induce supporting cells into a hair cell phenotype in the mature cochlea in the profoundly deafened guinea pig with nerve fiber innervation and, remarkably, the return of hearing (Izumikawa et al. 2005). These findings provide a key validation of our understanding of many of the mechanisms involved in hair cell development and repair. However, translation to human application will be technically difficult when involving gene therapy (see Sect. 3.4.1 for further discussion), and a gene product (protein) approach affecting other key events in the differentiation process is also discussed later (Sect. 3.4.2).

3.2.2 Replacement: Cellular

An alternative to gene therapy for replacement of lost sensory cells or auditory nerve is use of exogenous cell implants. This approach has been applied to the neurodegenerative disorder Parkinson's disease, with initial promising results (see Winkler et al. 2005 for a review). Although technical hurdles need to be resolved before cell therapy becomes a realistic clinical tool for the treatment of Parkinson's disease, the promise of this strategy is clear. Importantly, the same approach could be applied to the dysfunctional inner ear. One could implant exogenous hair cells or auditory neurons or implant progenitor cells that are induced to become sensory cells or neurons. However, because the cochlea has an extremely complex three-dimensional structure, every cellular element needs to be precisely placed and oriented to achieve proper function. It is therefore difficult to imagine externally applied cells reaching the appropriate location and assuming the necessary functional connections to adequately replace missing hair cells and provide a functional replacement. The more common approach to restoring sensory cells in the inner ear has, therefore, focused on repair (as previously described) rather than replacement. Because the structural organization of the spiral ganglion is much less restrictive, it is conceivable to imagine a cell therapy approach focusing on the SGN being successful (see Li et al. 2004; Ulfendahl et al. 2007; Altschuler et al. 2008; for reviews).

Several cell types have been tested for the purpose of implantation into the inner ear for nerve or hair cell replacement. These range from the most immature embryonic stem cells to well-differentiated neural tissue (Ulfendahl et al. 2007; Altschuler et al. 2008; Edge and Chen 2008; for reviews). Stem cells are characterized by their capacity for self-renewal and give rise to many different cell types. Embryonic stem cells have been a major focus of research as transplantation candidates because they are both proliferative and capable of generating all tissues of the mammalian body. The cells replicate indefinitely in vitro, which makes it possible to culture them on a large scale and could create a nearly unlimited source of transplantable cells for auditory nerve replacement. Adult stem cells are found also in several tissues of the adult organism, where they normally produce new differentiated cells necessary for restoring degenerated cells.

The challenge in the use of undifferentiated stem cells, whether embryonic or adult, is to induce them to the appropriate phenotype. This could be done before placement in

the target site or after placement. The cochlear fluids can provide an avenue for infusion of agents to influence phenotype when stem cells are placed into scala tympani. Embryonic stem cells naturally differentiate into neurons and glia; however, the percentage reaching neuronal phenotype is small when no further treatment is applied. Gene transfer of the neuronal transcription factor Neurogenin2 (Ngn2) improved the percentage reaching a neuronal phenotype (Hu et al. 2005b). Mouse embryonic stem cells engineering for inducible expression of neuronal transcription factor Neurogenin1 (Ngn1) allowed for more natural transient expression. Twenty-four hours of induced Ngn1 expression was followed by infusion of GDNF and BDNF, which are the NTFs naturally received by SGN during development. This induced the majority of the implanted stem cells into a glutamatergic neuronal phenotype both in vitro and in vivo after placement into guinea pig scala tympani (Reyes et al. 2008).

An alternative to undifferentiated stem cells is to use progenitor cells; these are more specialized cells that will develop into mature, differentiated cells of a specific type that could reduce the risk of uncontrolled proliferation after transplantation. Such cells have been found in both auditory and vestibular components of the developing inner ear (Li et al. 2003a,b; Martinez-Monedero et al. 2008; Oshima et al. 2010). However, the number of progenitor cells rapidly declines after birth and only relatively small numbers remain in the sensory epithelium of the mature mammalian cochlea (Lopez et al. 2004). Interestingly, progenitor cells have been isolated from adult human modiolus removed during surgeries (Rask-Andersen et al. 2005); these progenitor cells formed neurospheres in vitro, and could be valuable for human application. Unfortunately, as in the animal studies, the populations of stem or progenitor cells in adult tissues are relatively small and do not proliferate as readily as embryonic stem cells, and thus may not be able to give rise to enough cells for cell replacement therapies.

An ideal situation would be to use tissue from the receiving subject itself, so-called autografting. An autologous graft essentially eliminates the host reaction. Naito et al. (2004) applied an autologous graft to the inner ear with promising results. The recent technique for reprogramming somatic cells into induced pluripotent stem (iPS) cells (Takahashi and Yamanaka 2006) is exciting. This method would allow iPS cells, derived from the recipient, to be transplanted back to the same individual after necessary modifications and without the risk of rejection. Nishimura et al. (2009) have recently applied the technique to the inner ear, although they did not transplant the cells back to the same individual.

If the challenge of generating replacement cells with appropriate sensory hair cell phenotype is met, there are still three remaining challenges: survival, integration into an appropriate location/niche, and finally, forming central nervous system (CNS) connections and achieving function. Survival of new neural connections may require the same or similar neurotrophic or maintenance factors as required by endogenous auditory nerve SGN (Ulfendahl 2007; Altschuler et al. 2008). Indeed, excellent in vivo survival of mouse embryonic stem cells implanted into guinea pig cochlea was found when exogenous NTFs were provided into scala tympani (Altschuler et al. 2008; Reyes et al. 2008). Cell survival was also greatly enhanced with a cografting approach in which, in addition to the embryonic stem cells, embryonic neural tissue was implanted (Hu et al. 2004b, 2005a). Because electrical activity has been shown to enhance SGN survival after deafness in vivo (Miller et al. 2003a) or

in vitro (Hansen et al. 2001, 2003; Green et al. 2008), it may also be that stem cells that reach a neuronal phenotype will have improved survival if they become activated by either cochlear electrical stimulation with a cochlear prosthesis or if they connect to remaining IHCs.

Although there is a challenge for integration into appropriate location and niche, the scala tympani provides access to the entire perilymphatic fluid compartment, and implanted donor cells may be able to travel to functionally relevant locations throughout the cochlea. Although the perilymphatic compartment is anatomically separated from the spiral ganglion, the barriers are literally "full of holes." Indeed, the separating bone structures contain microscopic fenestrae, canaliculae perforantes (Küçük et al. 1991), which provide a path for the implanted cells to reach the spiral ganglion region. An alternative, and possibly less damaging route, would be to access the perilymphatic compartment via the lateral semicircular canal of the vestibular part of the inner ear, as has been demonstrated by Iguchi et al. (2004).

For cells to replace or supplement SGN they must also bridge the connection between the ganglion region and the cochlear nucleus in the brain stem. Recent experiments have shown that embryonic stem cells or dorsal root ganglion cells transplanted to the transected auditory nerve migrated along the nerve fibers in the internal auditory meatus and, in some cases, even reached close to the cochlear nucleus in the brain stem (Hu et al. 2004a). Interestingly, embryonic brain tissue transplanted to the acutely transected ventral cochlear tract resulted not only in regeneration but also functional recovery (Ito et al. 2001). However, there are many chemical factors that produce a barrier between peripheral and central nervous system and could impede the ability of central processes of replacement neurons to make a connection in the cochlear nucleus. The central connection would also need to connect to cochlear nucleus neurons in a tonotopic manner.

It has been hypothesized that if the SGN population were to be supplemented with exogenous cells, the efficiency of the cochlear prosthesis would improve. Hu et al. (2009) reported on experiments in which embryonic dorsal root ganglion cells were implanted into the inner ears of deafened animals fitted with a scala tympani electrode for monitoring hearing function using electrically evoked ABR. NGF was infused to provide trophic support for the implanted cells. Indeed, extensive neurite projections were observed to extend from the implanted cells, through the thin bony modiolus, to the host spiral ganglion. However, no significant difference was seen in the electrical thresholds or input/output functions. The negative results could be due to the low survival rate of the implanted cells, or lack of functional contacts between the implanted cells and the host nervous system.

3.3 Replacement

3.3.1 Prostheses

Although cochlear prostheses represent one of the major treatment success stories, restoring hearing to thousands of the profoundly deaf, there are still major advances

remaining in the future. The patient population continues to increase as benefits are being shown from placing prostheses into patient ears with remaining hearing, and then providing a hybrid of acoustic and electrical stimulation to those patients. In patients with significant residual hearing, but low scores in speech discrimination tasks, implants can be of remarkable benefit, yielding improved abilities to understand speech (Lenarz 2009). These patients typically will demonstrate little or no hearing at 1 kHz and above; but will have significant remaining low-frequency hearing, showing losses in the 30–40 dB range below 1 kHz. To provide electrical hearing and preserve residual acoustic hearing, implants have been modified from long, scalar filling, and modiolar hugging; to short, thin, free-floating, with the recent addition of amplified acoustic stimulation of the low frequencies, in a "hybrid" device (Woodson et al. 2010 for recent review). Enhanced performance is seen in these ears with electrical stimulation, presumably because of a more physiologic auditory nerve, reflecting functioning hair cells throughout a major apical portion of the cochlea, which is further enhanced by the acoustic stimulation, the latter contributing significantly to sound localization and discrimination of speech in noisy backgrounds. There may also be a contribution from electromotile responses of surviving hair cells (e.g., Grosh et al. 2004).

One major area of challenge for current cochlear prostheses is to improve speech discrimination in noise. Many patients demonstrate remarkable speech discrimination in quiet but their scores rapidly deteriorate in noise (Munson and Nelson 2005; for general discussion of challenges resolving speech in noise, e.g., Shrivastav and Still, Chap. 7). Another long-standing challenge is to allow improved appreciation of music (Gfeller et al. 2008). There is increasing bilateral implantation of prostheses, providing a potential for improved sound localization. Increasingly, the benefits observed have offset the earlier reservations about bilateral implantation. In the past, unilateral implants were encouraged with the hope of reserving one ear for potential later technical improvements in the implant. However, the ease of replacement surgery in the vast majority of cases where required has reduced concerns related to bilateral implantation.

One solution to provide better speech discrimination in noise and allow appreciation of music and language nuances depending on tonal modulations would be an improved channel separation, allowing an increased number of stimulation sites on the prosthesis and dividing the signal into more channels. Directed regrowth of peripheral processes toward stimulation sites or using stem cells to provide a closer target for stimulation are also potential solutions. Another approach is to place prostheses directly in the auditory nerve (Middlebrooks and Snyder 2007), providing more intimate contact of electrode to neural element, or to place prostheses in central auditory system sites such as the cochlear nucleus (Colletti and Shannon 2005; Schwartz et al. 2008) or inferior colliculus (Lim et al. 2008, 2009). Implantation into the central auditory system further increases the implant patient candidate pool, as it allows prostheses for those with unimplantable cochleae or lost auditory nerve populations. The remarkable plasticity of the central auditory system (e.g., Kaltenbach, Chap. 8) suggests the potential for successful "remapping" of these tonotopically organized nuclei with the advent of electrical stimulation via a central auditory system implant.

With electrical stimulation benefits in part dependent on hair cell survival and acoustic hearing completely dependent on hair cell survival, primary concerns have focused on reducing the trauma of cochlear implantation (hence smaller implants, with much smaller fenestrae) and eliminating any negative long-term effects of the implant or stimulation. The same strategies used for protection and repair from NIHL and ototoxicity could also be used to reduce loss of residual hearing from cochlear implantation trauma. This could include use of NTFs, immunosuppressants, cell death pathway inhibitors (Bcl-2 genes, JNK inhibitors) (Van de Water et al. 2010), antioxidants (Abi-Hachem et al. 2010), and agents that may enhance cochlear blood flow. Acute delivery into the cochlea at the time of surgery in forms that allow delayed release over time may be possible, however, risk factors should be taken into consideration (Garnham et al. 2005). The antioxidants, with and without vasodilators, that are being evaluated in multiple human trials for prevention of NIHL could also be considered for trials to improve postimplant hearing preservation and have the advantage of oral delivery, low cost, and minimal or no systemic side effects when used at recommended intake levels.

These considerations lead to a final area in the future frontiers of cochlear prostheses: the use of drug delivery systems coupled with cochlear prostheses. The use of drug interventions coupled with implants to preserve residual hearing is based on the same strategies discussed to preserve and regrow the auditory nerve. Future implant frontiers will include the integration of drug delivery with implants with the ability to deliver locally and safely NTFs, proteins, and other agents, in some cases with biopolymer–nanoparticle encapsulation of drugs, in systems that will allow burst, delayed, and sustained release. In the future, biopolymer and nanoparticle systems will be used to deliver genetically designed cells fixed to implants that can release growth factors and serve as targets for nerve growth, or extend neurites that will grow into the auditory nerve and enhance connectivity to the CNS.

3.4 Methods

3.4.1 Gene Therapy

Gene therapy technology has improved in recent years, making it a promising technique for treating inner ear disorders; the inner ear holds several unique advantages as a model for gene therapy. First, the cochlea is anatomically well suited for in vivo gene therapy both accessible and with a fluid compartment (Salt and Plontke 2009 for review). The relative isolation of the cochlear compartments minimizes unwanted effects of the introduced gene into other tissues. The inner ear is fluid filled, allowing all functionally important cells to be accessed by a transfection reagent. The concentration and dosage of complexes introduced to the cochlea can easily be modulated with a single injection or longer infusion via an osmotic pump. Cochlear endolymph and perilymph volumes have been characterized in guinea pigs, rats, mice, and also humans (e.g., Thorne et al. 1999), so adverse effects of high volume

and pressure can be avoided. In addition, a variety of precise physiological measures, such as otoacoustic emissions, compound action potentials, evoked potentials, and ABR, have been developed to monitor the function of specific cells, which makes reliable assessment of efficacy and safety of gene therapy practical. Finally, many genes have been recently cloned in the mouse and human cochlea. More than 100 different genes have been identified that affect inner ear development or function, as well as many loci known to be involved in deafness (see also Gong and Lomax, Chap. 9). A transgenic technique has been demonstrated in shaker-2 mice to correct deafness (Probst et al. 1998).

Gene therapy with NTFs has been the most frequent application of gene therapy in inner ear animal research. For example, inoculation of an adenoviral vector encoding human GDNF gene (Ad.GDNF) into guinea pig cochleae via the round window membrane 4 days before injection of the ototoxic aminoglycoside antibiotic kanamycin (KM) and the loop diuretic ethacrynic acid (EA) provided better hearing and less hair cell damage compared with controls (Ad.lacZ vector) (Yagi et al. 1999). Coinoculation of two vectors, one encoding human TGF-beta1 gene and the other encoding human GDNF gene, into guinea pig cochleae 4 days prior to injection of the same ototoxic agent combination (KM and EA) provided better hearing and less hair cell loss compared to inoculation of only Ad.GDNF (Kawamoto et al. 2003). Endogenous antioxidant systems can be upregulated in the same way as endogenous NTF systems, with similarly protective benefits. Adenoviral vectors for overexpression of catalase and Mn superoxide dismutase (SOD2) protected hair cells and hearing thresholds from a combination of KM and EA when given 5 days before ototoxic insult. After inoculation, there was a significant increase in catalase and a moderate elevation in SOD2 levels in tissues of the cochlea inoculated with the respective vectors (Kawamoto et al. 2004). Gene therapy to prevent NIHL has been more challenging, perhaps because of the more complex mechanisms of cell death being initiated (e.g., Henderson et al. 2006; Hu, Chap. 5, for reviews). While exogenous GDNF administered intracochlearly can protect the inner ear from NIHL (Shoji et al. 2000a, b), Kawamoto et al. (2001) reported no difference in the protection afforded by Ad.GDNF versus control Ad. lacZ vectors.

As described previously, Atoh1 overexpression after gene transfer can promote hair cell regeneration from supporting cells after hair cell destruction (Izumikawa et al. 2005). Other more preliminary data suggest overexpression of Atoh1 may also promote recovery of the stereocilia of the cochlear hair cells after noise (Yang et al., Association for Research in Otolaryngology Meeting, 2010). The hair bundle is susceptible to acoustic trauma and ototoxic drugs, and mammalian cochlear hair cells lose the capability to regenerate the stereocilia spontaneously once lost. Atoh1 inoculated within the first week after noise exposure, however, induced stereociliary regeneration and the newly regenerated stereocilia were functional, as ABR and CM measured 1 and 2 months after Atoh1 inoculation showed significant hearing threshold improvement. These findings imply that Atoh1-based gene therapy has the potential to restore hearing after noise exposure (Izumikawa et al. 2005; Husseman and Raphael 2009).

3.4.2 Protein Transduction Therapy

The objective of gene therapy is gene delivery followed by expression of gene products that either possess a therapeutic biological activity or induce an altered cellular phenotype. Gene therapy approaches to a number of genetic disorders require long-term and appropriately regulated expression of the transgene. The short-term requirement for the presence of the therapeutic gene product raises the possibility of achieving the same objective by direct delivery of the gene product itself, rather than the gene. Recent developments in protein transduction (delivery of protein into cells) suggest this is now a realistic approach (see Tilstra et al. 2007).

Protein transduction domains (PTDs), or cell-penetrating peptides, are small peptides that are able to carry much larger molecules such as oligonucleotides, peptides, full-length proteins, 40 nm iron nanoparticles, bacteriophages, and even 200-nm liposomes across cellular membranes. They have proven useful in delivering biologically active cargoes in vivo and, remarkably, have the ability to transduce nearly all tissues, including the brain, following intraperitoneal administration of fusion proteins. At least three classes of PTDs have been described, including positively charged transduction domains (cationic), protein leader sequence–derived domains (hydrophobic), and peptides identified by phage display that are able to transduce cells in a cell-type-specific manner (tissue-specific). The positively charged cationic PTDs are the most efficient and the best characterized. These cell penetrating peptides (CPPs) include a TAT (transactivator of transcription) derived from human immunodefiency virus type 1 (HIV-1) that contains numerous cationic amino acids, where positive charges interact with the negatively charged cell membrane to facilitate permeability (Patsch and Edenhofer 2007 for review).

As described previously, a significant role of Bcl-2 genes has been implicated in NIHL as well as recovery from other auditory trauma. FNK, which has been constructed from Bcl-xL by site-directed mutagenesis based on the high-resolution crystal structure of the rat Bcl-xL, has three amino acid substitutions, Tyr-22 to Phe (F), Gln-26 to Asn (N), and Arg-165 to Lys (K), in which three hydrogen bonds stabilizing the central $\alpha 5$–$\alpha 6$ helices (the putative pore-forming domain) are abolished (Asoh et al. 2002). Compared with Bcl-xL, FNK protected cultured cells more potently from cell death induced by oxidative stress (hydrogen peroxide and paraquat), a calcium ionophore, growth factor withdraw (serum and IL-3), anti-Fas, cell cycle inhibitors (TN-16, camptothecin, hydroxyurea, and trichostatin A), a protein kinase inhibitor (staurosporine, STS), and heat treatment (Asoh et al. 2000). When FNK was fused with Tat-PTD of the HIV/Tat protein and added into culture media of human neuroblastoma cells and rat neocortical neurons, it rapidly transduced into cells and localized to mitochondria within 1 h and protected against staurosporine-induced apoptosis and glutamate-induced excitotoxicity (Asoh et al. 2002). When injected intraperitoneally, TAT-FNK gained access into mouse brain neurons and prevented delayed neuronal death in the gerbil hippocampus caused by transient global ischemia (Asoh et al. 2002). Similarly, TAT-FNK was diffusely distributed in the cochlea after an intraperitoneal administration to guinea pigs; the distribution was most prominent in the hair cells and supporting cells, followed by the SGN and

peaked 3 h after the injection (Kashio et al. 2007). Further, the TAT-FNK protein intraperitoneally injected for 8 h (3 h pre-insult, 5 h post-insult) significantly attenuated ABR threshold shifts and the extent of HC death induced by a combination of EA and KM, and it significantly reduced the amount of cleaved poly-(ADP-ribose) polymerase-positive HCs compared with that in the vehicle-administered controls (Kashio et al. 2007). When TAT-FNK was topically applied on the round window membrane of guinea pigs, this protein penetrated through the membrane, distributed diffusely throughout the cochlea with the greatest expression 6 h after application and continuing up to 24 h, and significantly reduced hair cell death and caspase-9 expression induced by a combination of KM and EA (Kashio et al., ARO meeting, 2010).

Recently, to increase the biological activity of transduced protein in cells, novel carriers that transduce the target protein in its active native structural form have been designed. For example, when a PEP-1 peptide carrier, which consists of three domains – a hydrophobic tryptophan-rich motif, a spacer, and a hydrophilic lysine-rich domain – was mixed with the target protein (e.g., GFP, β-gal) and then overlaid on cultured cells, the nondenatured target protein was transduced (Morris et al. 2001). PEP-1 peptide carriers fused with SOD1 have been shown to protect cells from paraquat-induced oxidative stress in vitro and dopaminergic neuronal cell death in vivo in paraquat-induced Parkinson disease mouse models (Choi et al. 2006). Considering the rapid progress in protein transduction technology, delivery of the therapeutic gene products (e.g., anti-apoptotic agents, antioxidants, and NTFs) to the inner ear for the optimal short period seems to be promising and needs to be studied more intensively with the goal of human application.

4 Summary and Conclusions

As detailed in this chapter, and other chapters in this volume, there have been many remarkable advances in our understanding of the mechanisms associated with NIHL that have illuminated paths toward its prevention and treatment. More basic research is still needed to choose the best paths and navigate their initial hurdles, to provide guidance on which of the many approaches discussed will be the most effective, and which combinations of therapies acting by different mechanisms can provide greatest benefit. Clearly the "dirty work" of translational research is now demanded. There is sufficient knowledge of mechanisms and there are interventions with sufficient safety to begin studies in humans. There is a need for the difficult-to-fund parametric dose–response measurements of efficacy and safety, in animals and then in people; and a need to move to clinical trials. The field is much further along in some paths than others. Cochlear prostheses are, of course, already a success story, with wide application and they continue to be refined and improved. Antioxidant clinical trials are already testing for protection from noise or ototoxins. Other approaches such as stem cell therapy or induced hair cell regeneration have shown great promise on the benchtop but have yet to move from it. The fact that such a large number of approaches are being considered for prevention and treatment

provides both a large opportunity and challenge for the future. They must all be tested, compared, and contrasted under the different conditions of noise and the different resulting pathologies. All the tools and knowledge are available to begin and complete that task. The promise is great; once the initial translational efforts bear fruit, there will be safe and effective measures that reduce the prevalence of deafness and tinnitus resulting from noise and other stressors. In addition, with the demonstration that NIHL can be medically treated, a paradigm change in perspective will lead to prevention and treatment of many other causes of hearing impairment.

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