

The cell biology of hearing

Martin Schwander,¹ Bechara Kachar,² and Ulrich Müller¹

¹Dorris Neuroscience Center and Department of Cell Biology, The Scripps Research Institute, La Jolla, CA 92037

²Laboratory of Cell Structure and Dynamics, National Institute of Deafness and other Communication Disorders, National Institutes of Health, Bethesda, MD 20892

Mammals have an astonishing ability to sense and discriminate sounds of different frequencies and intensities. Fundamental for this process are mechanosensory hair cells in the inner ear that convert sound-induced vibrations into electrical signals. The study of genes that are linked to deafness has provided insights into the cell biological mechanisms that control hair cell development and their function as mechanosensors.

Setting the tone: the remarkable properties of the auditory system

The inner ear responds to sound-induced vibrations of less than a nanometer, can amplify signals by more than 100-fold, and has a wide dynamic range enabling humans to perceive frequencies from 20 Hz to 20 kHz. Essential for this extraordinary capability are the mechanosensory hair cells, which together with supporting cells and accessory extracellular structures form the organ of Corti within the snail-shaped cochlea of the inner ear (Fig. 1, A and B).

The human organ of Corti harbors ~16,000 hair cells that are patterned in one row of inner hair cells (IHCs) and three rows of outer hair cells (OHCs; Fig. 1, B and C). Each hair cell contains at the apical surface its mechanically sensitive organelle, the hair bundle, which consists of dozens of stereocilia (Fig. 1, C and D; Fig. 2 A). An extracellular matrix, the tectorial membrane, covers the apical surface of the organ of Corti and is attached to the stereociliary bundles of OHCs. The cell bodies of hair cells form tight connections with support cells, which in turn adhere at their basal surface to an additional extracellular matrix, the basilar membrane (Fig. 1 B).

Hearing is initiated when oscillations in air pressure are converted into fluid pressure that travel down the cochlear duct and induce vibrations in the basilar membrane. The vibrations are transferred onto hair cells, leading to deflection of the hair bundles, the opening of mechanically gated ion channels and

hair cell depolarization. Because of gradual changes in the features of the organ of Corti, such as the height of stereocilia and the width and thickness of the basilar membrane (Lim, 1980), hair cells at different positions along the cochlear duct are tuned to different frequencies: hair cells at the base of the duct respond to highest frequencies, those at the apex to the lowest frequencies (Liberman, 1982; Müller, 1991, 1996).

Active feedback mechanisms must amplify basilar membrane motion because viscous damping in the cochlea would otherwise dissipate sound energy. The underlying process is called the cochlear amplifier and depends on OHCs (Kiang et al., 1986; Dallos, 1992). When passive basilar membrane resonance is induced by a pure tone at its corresponding frequency position along the cochlear duct, OHCs are locally activated and enhance basilar membrane vibration (Rhode, 1971). IHCs detect these vibrations and activate afferent neurons. The cochlear amplifier has a remarkable compressive nonlinearity; this ensures that soft sounds are amplified more strongly than loud sounds (Robles and Ruggero, 2001; Hudspeth, 2008).

Dramatic progress has recently been made in our understanding of the molecular mechanisms that regulate auditory sense organ development and function. Progress has largely been driven by the study of genes that are linked to hearing loss, the most common form of sensory impairment in humans (Table I). We will emphasize here advances regarding the cell biology of hair cells. Other recent reviews have summarized the mechanisms that regulate auditory sense organ development and synaptic function and will not be considered (Glowatzki et al., 2008; Kelly and Chen, 2009; Rida and Chen, 2009).

The hair cell cytoskeleton: an intricate scaffold that underlies hearing

The morphology of hair cells is optimized for their function as mechanosensors. The stereocilia within a hair bundle are organized in rows of decreasing height, where the longest stereocilia are juxtaposed next to the kinocilium (Fig. 2, A and B). The vertices of all hair bundles point away from the center of the cochlea. This polarity is critical for hair cell function as bundle deflection only in the direction of the longest stereocilia leads to

Correspondence to Ulrich Müller: umueller@scripps.edu

Abbreviations used in this paper: IHC, inner hair cell; OHC, outer hair cell; PCP, planar cell polarity.

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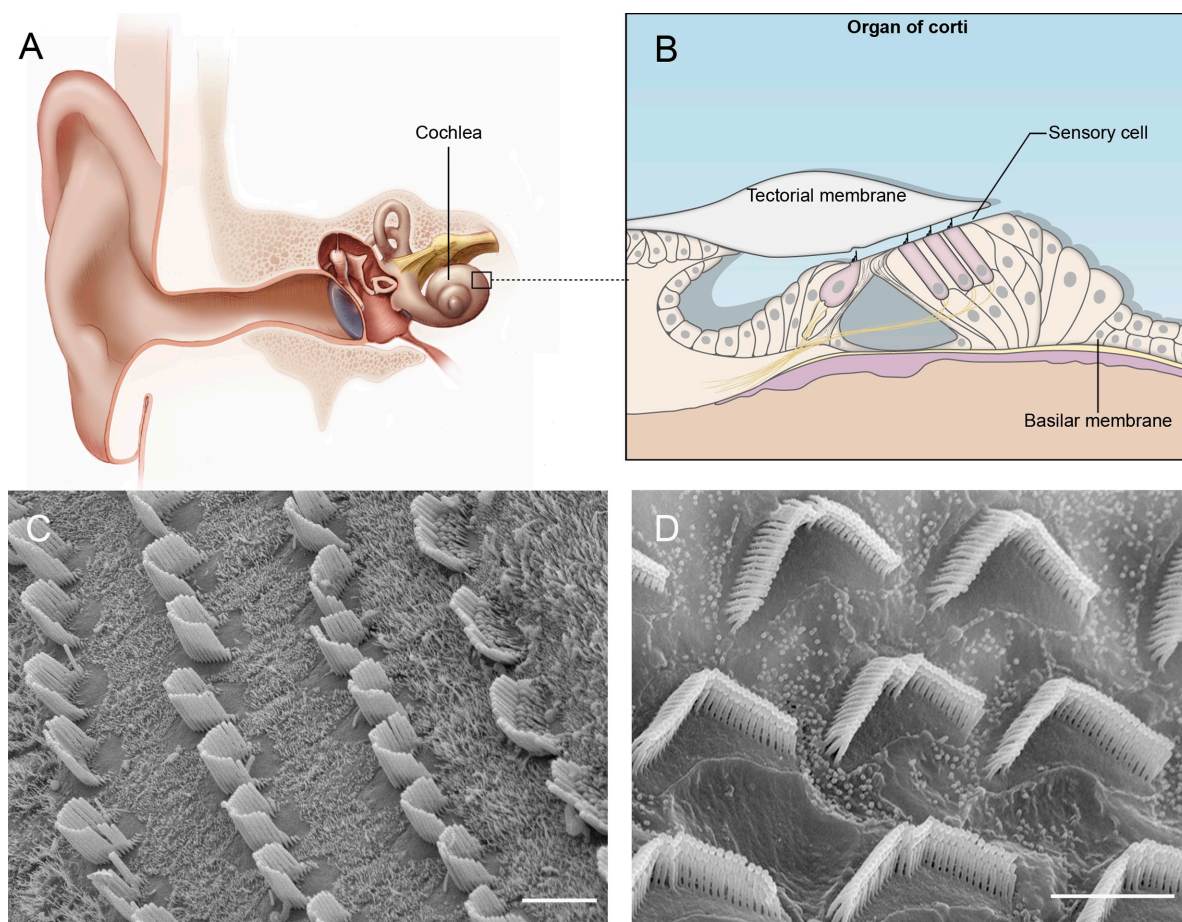


Figure 1. **The auditory sense organ.** (A) Diagram of the auditory sense organ highlighting the snail-shaped cochlea. (B) Diagram of the organ of Corti. (C) Scanning electron micrographs of hair bundles in the cochlea after removal of the tectorial membrane. Three rows of OHCs are shown at the left, one row of IHCs at the right. (D) Higher magnification view of OHCs. Bars, 5 μ m.

an increase in the open probability of mechanotransduction channels (Hudspeth and Corey, 1977). A single axonemal cilium, the kinocilium, is also present in the hair bundle but degenerates in cochlear hair cells after birth.

Extracellular filaments connect the stereocilia and kinocilium into a bundle (Fig. 2 B) and contribute to bundle passive mechanics (Bashtanov et al., 2004). Tip links project in the axis of mechanical sensitivity of the hair bundle and are thought to gate transduction channels at stereociliary tips (Pickles et al., 1984). In support of this model, the hair bundle loses its mechanical sensitivity when tip links are broken (Assad et al., 1991; Zhao et al., 1996).

Similar to filopodia and microvilli, stereocilia are supported by bundles of uniformly polarized actin filaments with the barbed (plus) ends pointing toward the stereociliary tips (Tilney et al., 1992). The filaments contain β - and γ -actin and are cross-linked by espin, plastin1, and T-plastin (Tilney et al., 1989; Zine et al., 1995; L. Zheng et al., 2000; Daudet and Lebart, 2002; Li et al., 2004). Unlike filopodia and microvilli, stereocilia are maintained at a constant length throughout life. β -actin, γ -actin, and espin are expressed in many tissues, but mutations in their genes affect predominantly hair bundles, attesting to the importance of different actin isoforms and their cross-linkers in stereocilia (L. Zheng et al., 2000; Zhu et al., 2003; Procaccio et al., 2006; Belyantseva et al., 2009).

The actin core of stereocilia is dynamic, at least in developing hair bundles. Actin monomers are incorporated into actin filaments at stereociliary tips and translocate toward the cell body (Schneider et al., 2002; Rzadzinska et al., 2004). To maintain stereociliary lengths, rates of actin polymerization and depolymerization must be tightly coordinated. The actin treadmilling rate in stereocilia is ~ 10 -fold slower than in filopodia (Rzadzinska et al., 2004), suggesting that specialized mechanisms control this process in hair cells (Lin et al., 2005).

Some of the actin filaments in stereocilia form rootlets, which anchor the stereocilia into a specialized actin network, the cuticular plate (Fig. 2 B). Tropomyosin and spectrin are concentrated around rootlets and might stabilize them (Corwin and Warchol, 1991; Tilney et al., 1992; Furness et al., 2008). Microtubules connect the cuticular plate to the axial cytoskeleton (Jaeger et al., 1994). An actin belt, which is attached to tight-adherens junction, surrounds the cuticular plate; the tight-adherens junctions (Nunes et al., 2006) couple hair and support cells (Corwin and Warchol, 1991; Tilney et al., 1992). An actin filament network that is cross-linked by spectrin underlies the lateral plasma membrane of OHCs and helps to maintain their cylindrical shape (Holley et al., 1992; Kalinec et al., 1992; Raphael et al., 1994).

Table 1. Genes that are linked to hearing loss

Gene	Protein	Mouse mutant	Usher syndrome subtype	Other forms of deafness in humans
<i>MYO7A</i>	Myosin VIIa	<i>shaker 1; headbanger</i>	USH1B	DFNB2, DFNA11
<i>USH1C</i>	Harmonin	<i>deaf circler</i> ; targeted mutation	USH1C	DFNB18
<i>CDH23</i>	Cadherin 23	<i>waltzer; salsa</i>	USH1D	DFNB12
<i>PCDH15</i>	Protocadherin 15	<i>Ames waltzer</i>	USH1F	DFNB23
<i>USH1G</i>	SANS	<i>Jackson shaker</i>	USH1G	–
<i>USH2A</i>	Usherin	targeted mutation	USH2A	–
<i>GPR98</i>	VLGR1	<i>Gpr98del7TM</i> ; targeted mutation	USH2C	–
<i>DFNB31</i>	Whirlin	<i>whirler</i>	USH2D	DFNB31
<i>ACTB</i>	β_{cyto} -actin	Not available	–	Syndromic hearing loss
<i>ACTG1</i>	γ_{cyto} -actin	Targeted mutation	–	DFNA20/26
<i>ESPN</i>	Espin	<i>Jerker</i>	–	DFNB36
<i>PTPRQ</i>	PTPRQ	<i>Ptpq^{-/-}</i>	–	–
<i>MYO6</i>	Myosin VI	<i>Snell's waltzer; tailchaser</i>	–	DFNA22 DFNB37
<i>RDX</i>	Radixin	Targeted mutation	–	DFNB24
<i>MYO3A</i>	Myosin IIIa	Not available	–	DFNB30
<i>MYO15A</i>	Myosin XV	<i>Shaker 2</i>	–	DFNB3
<i>SLC26A5</i>	Prestin	Targeted mutation	–	Nonsyndromic hearing loss

Genes that are discussed in the text and linked to hearing loss. All genes are expressed in hair bundles and required for bundle development/function. DFNA, autosomal dominant mode of inheritance; DFNB, autosomal recessive. A more complete list of genes linked to hearing loss can be found at <http://hereditaryhearingloss.org> and <http://hearingimpairment.jax.org/index.html>.

The uniquely shaped hair bundle: morphogenetic events that control bundle development and polarity

Studies that were performed most thoroughly with avian hair cells (Tilney et al., 1992) provide the basis of our understanding of hair bundle development (Fig. 2 A). At the onset of hair bundle morphogenesis, the apical surface of each hair cell is covered with microvilli. These microvilli elongate and form stereocilia of comparable length; at this stage a single kinocilium is localized in the center of the apical cell surface. The kinocilium subsequently moves to the cell periphery and the stereocilia next to the kinocilium start to elongate, followed by elongation of the adjacent rows of stereocilia. Next, stereocilia cease to grow, but increase in width by adding actin filaments. The filaments in the central core extend basally to form rootlets; the basal ends of stereocilia adopt a tapered shape. Finally, stereocilia reinitiate elongation and grow to their final lengths. Upon maturation of the hair bundle, the kinocilium is lost in some hair cells.

Based on the morphological studies, the kinocilium has been proposed to be critical for the development of hair bundle polarity. The recent study of genes linked to Bardet-Biedle syndrome, a ciliopathy that affects many organs, lends support to this model. Accordingly, mice with mutations in orthologues of Bardet-Biedle syndrome proteins have misoriented hair bundles (Ross et al., 2005). Conditional inactivation of the intraflagellar and intraciliary transport protein Ift88 in hair cells also leads to underdevelopment of the kinocilium and misoriented hair bundles (Jones et al., 2008).

The movement of the kinocilium in the apical hair cell surface is nonrandom, but its final position, and therefore bundle polarity, is fine-tuned after the original polarization of the hair bundle (Dabdoub and Kelley, 2005). The molecules that

control the movement of the kinocilium and bundle polarity are not known. Candidates are proteins that regulate planar cell polarity (PCP). In support of this model, PCP pathway components are localized asymmetrically at the junctions between hair and support cells, and mice with mutations affecting the PCP pathway show defects in hair bundle polarity (Curtin et al., 2003; Montcouquiol et al., 2003, 2006; Lu et al., 2004; Wang et al., 2005; Wang et al., 2006; Qian et al., 2007; Etheridge et al., 2008; Yamamoto et al., 2008). Although evidence is lacking, it is tempting to speculate that PCP components affect polarity by regulating cytoskeleton-associated motor proteins.

Motoring to the tip: myosin motor proteins regulate stereociliary length

After the establishment of planar polarity, hair cell stereocilia elongate to form rows of graded height. Recent studies indicate that myosin motor proteins transport components of the actin assembly machinery to the tips of stereocilia to regulate their length. As stereocilia can reach up to 100 μm in length (Silver et al., 1998), regulated protein transport is an elegant solution to supply actin assembly regulators to the barbed ends of actin filaments.

Myosin 15a (MYO15A; Fig. 3 A) is one of the first proteins to be implicated in the regulation of stereociliary growth and it cooperates with the adaptor protein whirlin (Fig. 3 A) in this process. A link between MYO15A and whirlin was suspected by genetic studies, which demonstrated hearing loss in humans carrying mutation in *MYO15A* and *whirlin*; mutations in the orthologous mouse genes lead to shortened stereocilia (Probst et al., 1998; Wang et al., 1998; Mburu et al., 2003). MYO15A binds whirlin and both proteins localize to the tips of stereocilia (Fig. 3 B; Rzadzinska et al., 2004; Belyantseva

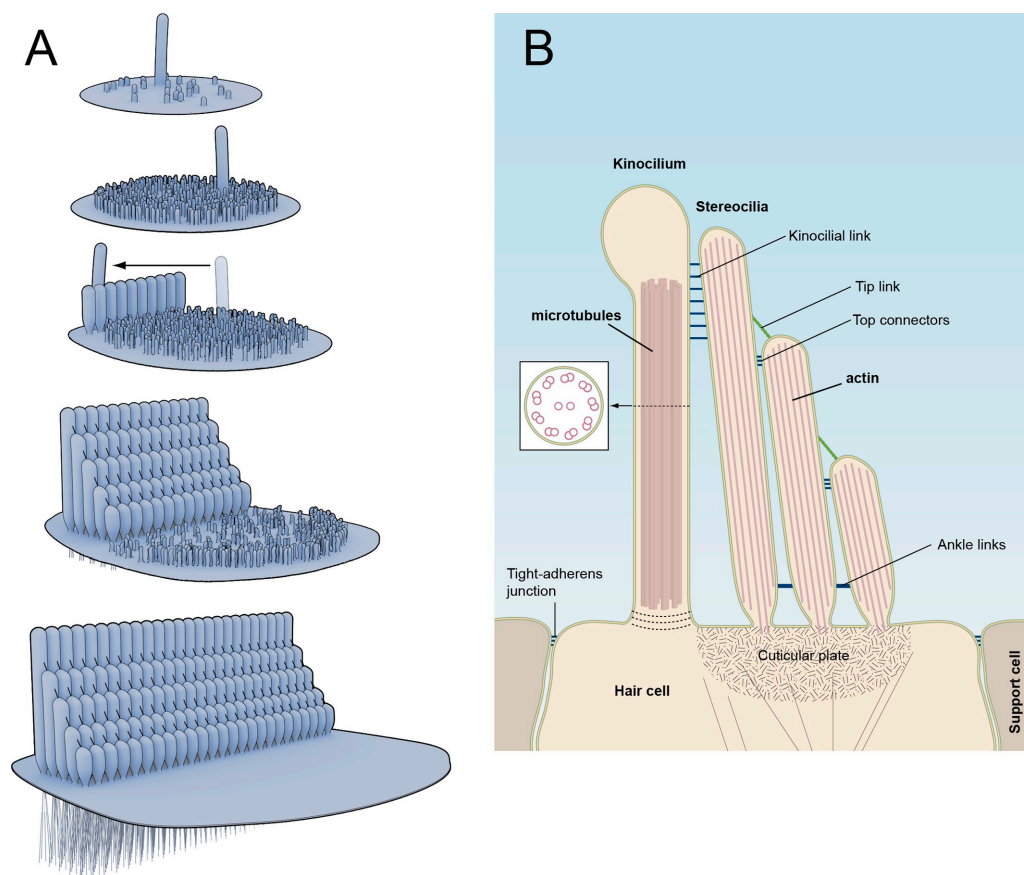


Figure 2. **Hair bundle development and structure.** (A) Diagram of sequential stages of hair bundle development. At the onset, the apical hair cell surface contains microvilli and one kinocilium. The microvilli grow in length. The kinocilium moves to the lateral edge of the hair cell. Some microvilli elongate to form stereocilia of graded heights. (B) Cross section through a hair bundle and apical hair cell surface indicating the kinocilium, stereocilia, and cuticular plate. Some of the linkages in hair bundles are highlighted.

et al., 2005; Delprat et al., 2005; Kikkawa et al., 2005; Schneider et al., 2006). Whirlin is no longer present at tips of stereocilia in *Myo15a*-deficient mice, suggesting that MYO15A transports whirlin (Belyantseva et al., 2005). Whirlin binds MAGUK protein p55, and protein 4.1R, which are expressed in OHCs; IHCs seem to express 4.1B, a 4.1R homologue (Mburu et al., 2006). Some of these proteins regulate actin assembly in erythrocytes and neurons and might have a similar function in hair cells (Marfatia et al., 1995; Biederer and Sudhof, 2001).

Mutations in genes for myosin 3a (*MYO3A*) and espin (*ESPN*) (Fig. 3 A) cause hearing loss in humans (Walsh et al., 2002; Naz et al., 2004; Donaudy et al., 2006) and the two proteins are implicated in stereociliary growth (L. Zheng et al., 2000; Rzadzinska et al., 2004, 2005a; Sekerková et al., 2004; Schneider et al., 2006; Salles et al., 2009). *Myo3a* mutant mice have not been described, but *Espn* mutant mice have abnormal hair bundles (L. Zheng et al., 2000; Rzadzinska et al., 2005a). Espin is expressed throughout stereocilia where it likely bundles actin filaments (Bartles et al., 1998; Chen et al., 1999; L. Zheng et al., 2000; Li et al., 2004; Sekerková et al., 2004). Espin contains ankyrin repeats, binding sites for monomeric actin, SH3 domains, ATP, and PIP₂ (Fig. 3 A), suggesting that the protein also has an active role in actin assembly (L. Zheng et al., 2000; Sekerková et al., 2004). Consistent with the model, espin

overexpression leads to lengthening of stereocilia (Salles et al., 2009). Intriguingly, an alternatively spliced espin isoform, espin-1 (Fig. 3 A), forms a thimble-like crown at stereociliary tips. MYO3A has a similar distribution in stereocilia and binds espin-1. Co-expression of MYO3A and espin-1 leads to greater elongation of stereocilia compared with overexpression of either protein alone (Salles et al., 2009). These findings suggest that MYO3A transports espin-1 to stereociliary tips to regulate stereociliary length.

MYO7A is a third motor protein implicated in the regulation of stereociliary growth (Fig. 3, A and B). MYO7A mutations lead to hearing loss, defects in hair bundle morphology, and excessive elongation of stereocilia (Gibson et al., 1995; Liu et al., 1997a,b; Weil et al., 1997). Actin treadmilling is increased in the absence of MYO7A, suggesting that MYO7A regulates F-actin rearward flow (Prosser et al., 2008). MYO7A might also regulate the transport of proteins that restrict actin assembly. One candidate cargo protein is twinfilin-2 (Fig. 3 A), which binds to MYO7A and caps and severs actin filaments (Palmgren et al., 2001; Paavilainen et al., 2007; Rzadzinska et al., 2009). Twinfilin is no longer targeted to the tips of stereocilia of *Myo7a*-deficient mice and overexpression of twinfilin-2 reduces stereocilia length, establishing a potential functional link to MYO7A (Peng et al., 2009; Rzadzinska et al., 2009).

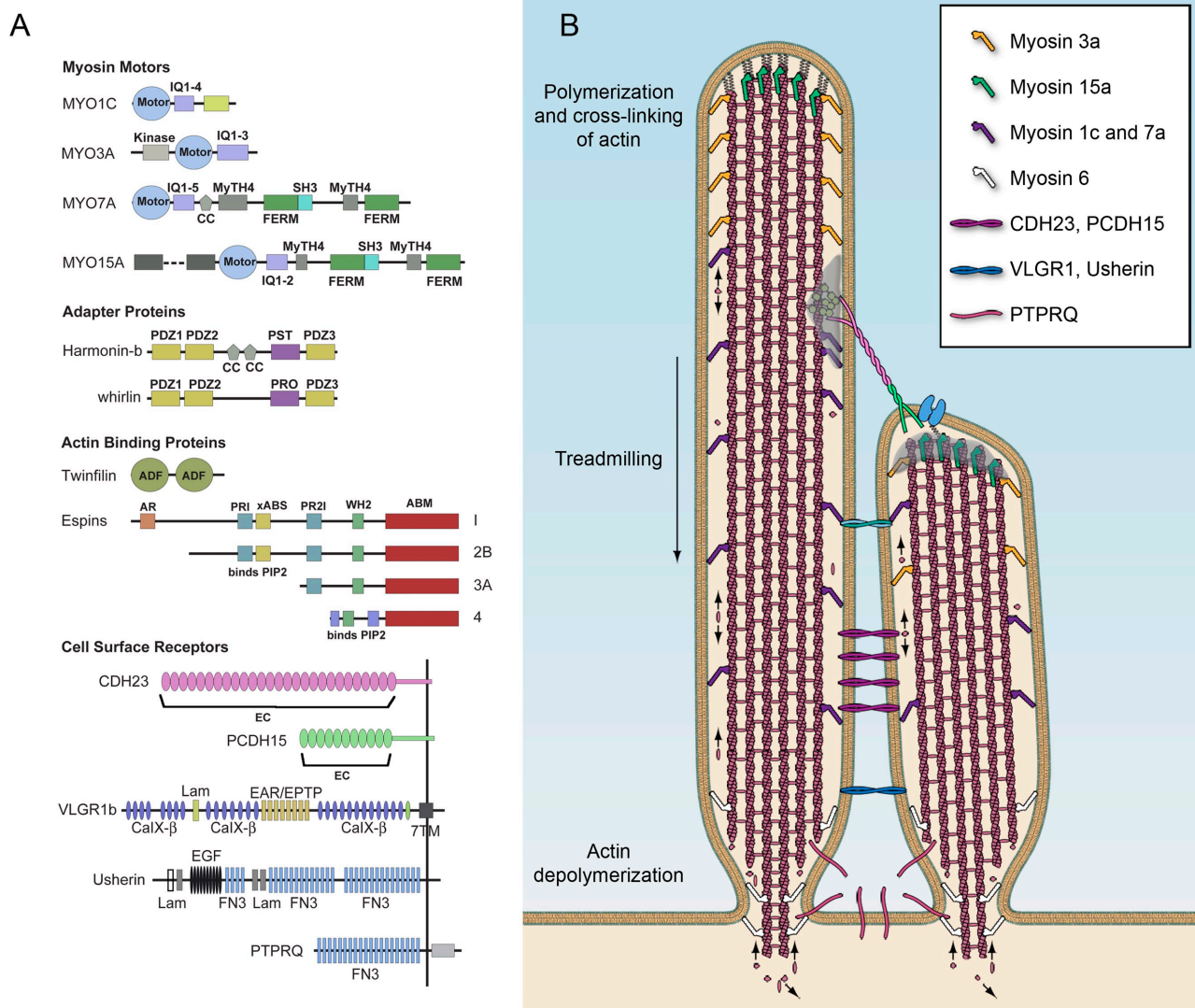


Figure 3. Hair bundle proteins. (A) Domain structure of proteins discussed in the text. Abbreviations: CC, coiled-coil domain; FERM, protein 4.1, ezrin, radixin, moesin domain; IQ, calmodulin-binding IQ domain; MyTH4, myosin tail homology 4 domain; PDZ, PSD95/SAP90, Discs large, zonula occludens-1 domain; PST, proline, serine, threonine-rich domain; PRO, proline-rich domain; SH3, src homology 3 domain; ADF, actin-depolymerization factor; AR, ankyrin-like repeat; PR, proline-rich peptide; ABS, F-actin-binding site; WH, WASP homology 2 domain; ABM, actin bundling module; EC, extracellular cadherin repeat; CalX-β, Ca²⁺-binding calcium enhancer β modules; Lam, Laminin GL or NT domain; EAR/EPTP, putative β-propeller folding domain; EGF, laminin-type epidermal growth factor-like domain; FN3, fibronectin type 3 repeat. (B) Diagram of two stereocilia indicating the distribution of some of the molecules discussed in the text.

Whirlin and espin-1 are more strongly expressed in the longest stereocilia of the hair bundle; twinfilin-2 is more abundant in the shortest (Delprat et al., 2005; Peng et al., 2009; Rzadzinska et al., 2009; Salles et al., 2009). The relative expression levels of whirlin, espin-1, twinfilin-2, and other actin-binding proteins might ultimately determine stereociliary length. One attractive hypothesis is that the PCP pathway determines the graded distribution of these proteins across the hair bundle.

Constricting the base: myosins shape the taper of stereocilia

Stereocilia are tapered at their base (Fig. 2 B, Fig. 3 B; Kimura, 1975; Tilney et al., 1980; Itoh, 1982), a feature that is critical for their function as stereocilia pivot around the taper and move as a unit during mechanical stimulation; this ensures a synchronized

response of mechanotransduction channels across the bundle (Hudspeth and Corey, 1977; Crawford et al., 1989; Kozlov et al., 2007).

Recent studies suggest that the minus end-directed molecular motor myosin 6 (MYO6; Fig. 3 A) and the protein tyrosine phosphatase receptor Q (PTPRQ; Fig. 3 A) regulate taper formation/maintenance. MYO6 and PTPRQ are localized at the base of stereocilia (Fig. 3 B) and mutations in their genes lead to deafness that is associated with a loss of the taper (Hasson et al., 1997; Goodyear et al., 2003; Sakaguchi et al., 2008). In MYO6-deficient mice, PTPRQ is broadly distributed throughout stereocilia (Sakaguchi et al., 2008), suggesting that MYO6 is required to retain PTPRQ, and possibly other proteins such as radixin (Fig. 3 A; Pataky et al., 2004), in the taper region where they might connect the membrane and cytoskeleton. PTPRQ might

also regulate actin remodeling; it contains a phosphatidylinositol phosphatase (PIPase) and tyrosine phosphatase domain (Wright et al., 1998; Oganessian et al., 2003). The PIPase activity of PTPRQ hydrolyzes phosphatidylinositol (4,5) bisphosphate (PIP₂), a key regulator of actin remodeling (Takenawa and Itoh, 2001).

Keeping it together: transmembrane receptors promote hair bundle cohesion

Fine proteinaceous filaments connect the stereocilia and kinocilium of a hair cell (Fig. 2 B; Fig. 3 B). These linkages are remodeled during development, suggesting that they actively shape the hair bundle. Prominent linkages in developing cochlear hair cells of mice are transient lateral links, ankle links, and kinociliary links; functionally mature cochlear hair cells contain tip links and horizontal top connectors (Goodyear et al., 2005).

Some of the components of the linkages have been identified by the study of genes that are linked to Usher syndrome (USH, deaf-blindness). USH1, the most severe form of the disease, is caused by mutations in genes encoding the transmembrane receptors cadherin 23 (CDH23, USH1D) and protocadherin 15 (PCDH15, USH1F), the adaptor proteins harmonin (USH1C) and sans (USH1G), and the motor MYO7A (USH1B) (Fig. 3 A; Kremer et al., 2006). CDH23 and PCDH15 are expressed in developing hair bundles where they localize to transient lateral links and kinociliary links (Siemens et al., 2004; Lagziel et al., 2005; Michel et al., 2005; Rzadzinska et al., 2005b). Developing hair bundles also express a harmonin splice variant, which binds CDH23, PCDH15, MYO7A, and F-actin, suggesting that harmonin establishes a link between cadherins and F-actin (Verpy et al., 2000; Boëda et al., 2002; Siemens et al., 2002; Adato et al., 2005b; Reiners et al., 2005; Senften et al., 2006). As hair bundles are disrupted in mice carrying severe alleles of USH1 genes, these transmembrane complexes are thought to regulate hair bundle cohesion and morphogenesis (Gibson et al., 1995; Alagramam et al., 2001; Di Palma et al., 2001; Wilson et al., 2001; Johnson et al., 2003; Kikkawa et al., 2003).

Mutations in the genes linked to USH2, a less severe form of deaf-blindness, also affect hair bundle morphology in mice (Mburu et al., 2003; McGee et al., 2006; Liu et al., 2007). These genes encode the G protein-coupled receptor 98 (GPCR98, VLGR1); usherin, a protein that is expressed in secreted and transmembrane isoforms; and whirlin, which is an adaptor protein with homology to harmonin (Fig. 3 A; Eudy et al., 1998; Weston et al., 2000, 2004; Ebermann et al., 2007). Antibodies to VLGR1 and usherin stain the base of developing stereocilia (Fig. 3 B), and ankle links are absent in VLGR1-deficient mice (Adato et al., 2005a; McGee et al., 2006; Michalski et al., 2007), suggesting that VLGR1 and usherin are likely ankle link components. Whirlin binds to VLGR1 and usherin and is transiently expressed in the ankle link region (Delprat et al., 2005; Michalski et al., 2007). In analogy to the role of harmonin at transient lateral links and kinociliary links, whirlin might provide a connection between ankle links and the cytoskeleton.

The study of USH proteins has also provided insights into the mechanisms by which transmembrane receptors are transported into stereocilia, which contain no transport vesicles.

Harmonin, whirlin, VLGR-1, PCDH15, and usherin are no longer present in the stereocilia of MYO7A mutant mice. This suggests that receptors are inserted into the stereocilia at their base and then transported by MYO7A (Boëda et al., 2002; Senften et al., 2006; Michalski et al., 2007; Lefèvre et al., 2008). Sans binds to harmonin and MYO7A (Weil et al., 2003; Adato et al., 2005b; Yan et al., 2010) and is required for the localization of harmonin to stereocilia (Lefèvre et al., 2008), suggesting that the three proteins are part of a transport complex. In *Drosophila* follicle cells, sans colocalizes with the syntaxin avalanche to endocytotic vesicles, consistent with a role in vesicle transport (Demontis and Dahmann, 2009).

Notably, CDH23 is still transported in stereocilia of MYO7A-deficient mice. CDH23 interacts with MYO1C, suggesting that this motor protein might carry out CDH23 transport (Siemens et al., 2004).

At the heart of hearing: the mechanotransduction machinery of hair cells

At the heart of hearing is the mechanotransduction process. The transduction channel is thought to be gated by an elastic element, the “gating spring,” which is stretched in response to mechanical stimuli, leading to channel opening and the influx of Ca²⁺ and K⁺ ions into stereocilia (Fig. 4 A; Corey and Hudspeth, 1983). After channel opening, hair cells adapt to maintain their sensitivity to stimulation (Fig. 4 A). Adaptation progresses on a fast and slow time scale and is regulated by Ca²⁺ that enters stereocilia upon stimulation. Fast adaptation is thought to depend on binding of Ca²⁺ to the transduction channel or an element near the channel, which leads to rapid channel reclosure. Slow adaptation is thought to depend on an adaptation motor, which has been proposed to consist of a cluster of myosin motor proteins that is attached to the upper tip-link end. According to the model, the motor generates at rest tension within the gating spring. During activation, tension in the gating spring increases and is conveyed to the motor in a Ca²⁺-dependent manner; the motor slides down the actin filaments, relaxing tension and leading to channel closure. Subsequently, the motor complex climbs up the stereocilium and restores tension (Fig. 4 A; LeMasurier and Gillespie, 2005; Ricci et al., 2006; Jia et al., 2007; Gillespie and Müller, 2009).

The mechanotransduction channel of vertebrate hair cells has a conductance of ~100 pS (Crawford et al., 1991; Géléoc et al., 1997). Conductance increases in the turtle from ~100 to ~300 pS from the base to the apex of the cochlea (Ricci et al., 2003). This suggests that the channel consists of multiple subunits that vary in stoichiometry along the cochlear duct. The molecules that form the channel are not known, but its localization in hair cells has been determined by analyzing the site of Ca²⁺ entry into stereocilia after their deflection. Initial studies suggested that transduction channels are located at both tip-link ends (Denk et al., 1995), but high-resolution imaging provides compelling evidence that the channel is only located at the lower tip-link end (Fig. 4, A and B; Beurg et al., 2009).

Proteins that regulate the function of the transduction channel in hair cells have been identified. Among these are PCDH15 and CDH23, which are not only components of transient lateral links and kinociliary links but also of tip links (Siemens et al.,

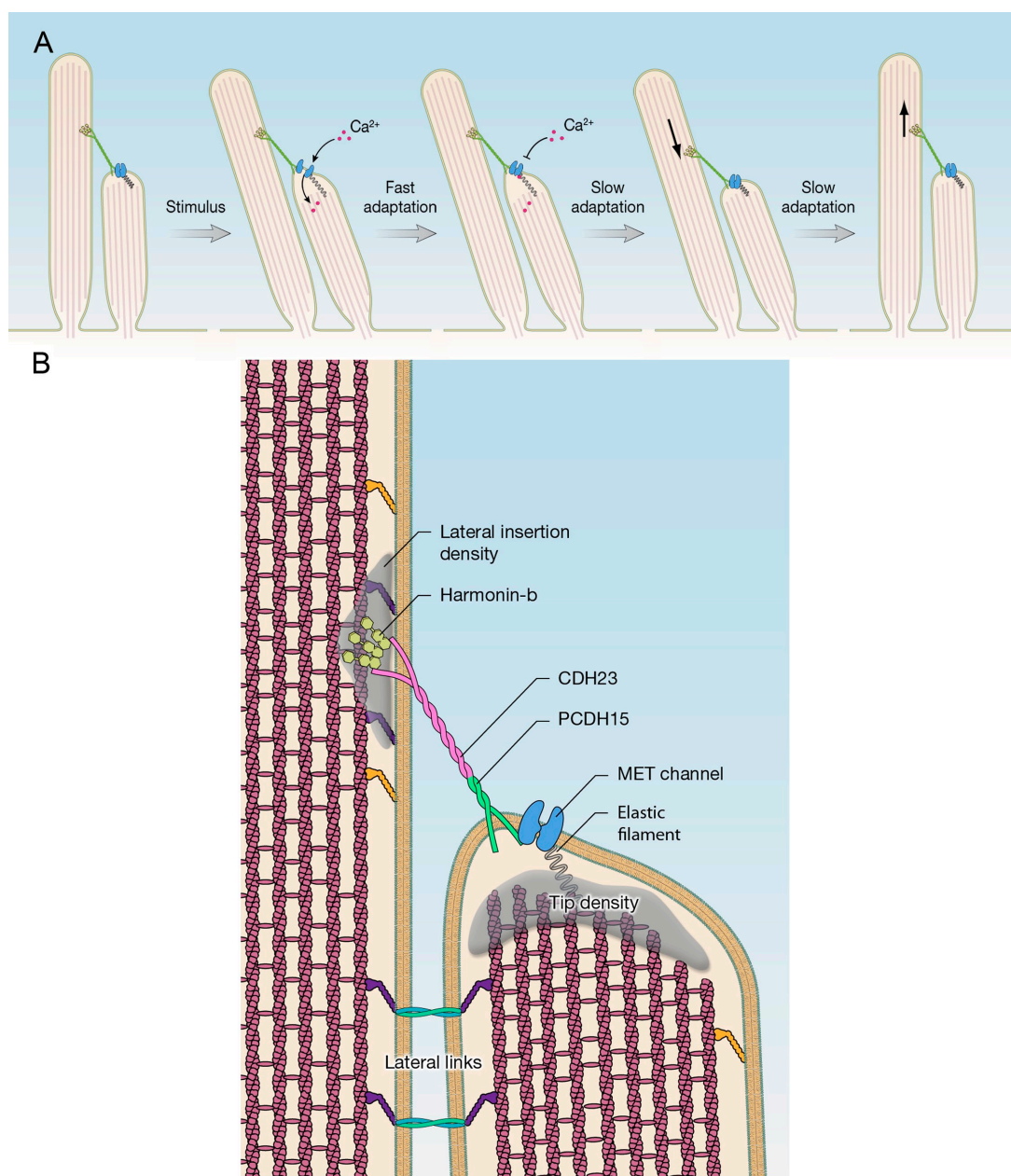


Figure 4. Hair bundles and mechanotransduction. Model of transduction and adaptation. Deflection of hair bundles in the direction of the longest stereocilia leads to the opening of transduction channels at the lower ends of tip links. Ca^{2+} enters the transduction channel and binds to the channel or a side near the channel and leads to channel closure (fast adaptation). The adaptation motor at the upper end of tip links subsequently detaches from the actin cytoskeleton and slides down the stereocilium, leading to release of tension in the transduction machinery (slipping phase of slow adaptation). Next, the motor complex climbs up the stereocilium, reestablishing tension (climbing phase of slow adaptation). (B) Molecular components of the mechanotransduction complex in stereocilia.

2004; Ahmed et al., 2006; Kazmierczak et al., 2007). The upper part of a tip link is formed by CDH23 homodimers and the lower part by PCDH15 homodimers (Fig. 4 B); the two cadherins interact at their N termini to form a tip-link filament (Kazmierczak et al., 2007). This asymmetric distribution of CDH23 and PCDH15 at tip links suggests that transient lateral links and kinociliary links might have a similar asymmetric structure.

The extracellular domain of classical cadherins such as N-cadherin is rigidified by binding Ca^{2+} molecules to each linker domain that connects adjacent EC repeats (Pokutta and Weis, 2007). These Ca^{2+} -binding motifs are conserved in CDH23 and

PCDH15 (Kazmierczak et al., 2007), suggesting that tip links are rigid and not the elastic gating spring for the transducer channel. In agreement with these findings, tip links appear in the electron microscope as stiff filaments that buckle under strain (Kachar et al., 2000).

Other molecules besides cadherins and transduction channels are asymmetrically distributed at tip links. As described above, harmonin, which can bind to CDH23 and PCDH15, is broadly distributed in developing hair bundles (Verpy et al., 2000; Boëda et al., 2002). However, it is concentrated at the upper end of tip links in mature hair bundles (Fig. 4 B; Grillet et al., 2009).

In mice that express a harmonin protein with a mutation in its F-actin-binding domain, the kinetics of transducer current activation and adaptation in cochlear hair cells is slowed down; gating of the transducer channels in different stereocilia within a hair bundle appears less well coordinated (Grillet et al., 2009). Similar observations have been reported for cochlear hair cells of mice with a different harmonin allele; unlike in cochlear hair cells, adaptation in vestibular hair cells was accelerated (Michalski et al., 2009). These findings link harmonin to mechanotransduction and suggest that mutations in its gene can affect cochlear and vestibular hair cells in different ways.

Although the mechanism by which harmonin affects transducer channel gating and adaptation still needs to be determined, it seems likely that harmonin establishes a connection between CDH23 and the actin cytoskeleton and might affect the slow adaptation motor (Fig. 4 A). Previous studies suggest that the adaptation motor consists of a cluster of MYO1C molecules (García et al., 1998; Steyger et al., 1998). In vestibular hair cells from mice that were engineered to express a mutant MYO1C motor protein that is sensitized to ADP analogues, adaptation is slowed down by ADP analogues, demonstrating that MYO1C is required for adaptation (Stauffer et al., 2005). Harmonin might affect MYO1C motor activity and its interaction with the cytoskeleton, but it could also act by other means. For example, adaptation is affected in MYO7A mutant mice (Kros et al., 2002). As harmonin binds MYO7A (Boëda et al., 2002), it might affect MYO7A activity. However, MYO7A may indirectly affect adaptation, as it is required for the transport of several proteins, including harmonin, into stereocilia (Boëda et al., 2002; Senften et al., 2006; Michalski et al., 2007; Lefèvre et al., 2008).

The localization of the transducer channel to the lower tip-link end raises several questions. As the adaptation motor is thought to be located at the upper end of tip links, how can Ca^{2+} regulate its activity? Does Ca^{2+} entering through a transduction channel affect the adaptation motor hooked up to the next tip link lower down in the same stereocilium? In this scenario, adaptation motors in the longest stereocilia would see minimal changes in Ca^{2+} during stimulation. An additional question concerns the nature of the gating spring. As tip-link cadherins appear too stiff to form the gating spring, this elastic element is likely located elsewhere, most probable in proximity to the transducer channel (Fig. 4, A and B). Finally, which molecules form the transducer channel?

Amplification of mechanical stimuli by hair cells: can you hear me now?

Auditory hair cells respond to mechanical stimuli, but they also amplify them. Low intensity signals are amplified more strongly than high intensity signals, endowing the ear with an astonishing sensitivity: auditory hair cells can encode stimulus amplitudes that range over six orders of magnitude; vestibular hair cells can detect mechanical oscillations that are 10^{-6} -fold that of g , the constant acceleration of gravity felt by these cells (Narins and Lewis, 1984). Amplification in the cochlea depends on OHCs (Dallos et al., 2008; Hudspeth, 2008).

Two mechanisms, somatic electromotility and active hair bundle motility, have been proposed for amplification. Somatic electromotility is a term that describes the property of the OHC

soma to change its lengths in response to changes in membrane potential (Brownell et al., 1985; Kachar et al., 1986; Ashmore, 1987). Using a screen for molecules that are specifically expressed in OHCs but not IHCs, J. Zheng et al. (2000) identified prestin, a protein that is localized in the lateral wall of OHCs (Belyantseva et al., 2000; Adler et al., 2003; He et al., 2010). Prestin mutations cause hearing loss in humans (Liu et al., 2003). Prestin shows voltage-dependent conformational changes (J. Zheng et al., 2000; Oliver et al., 2001). Sound amplification and frequency discrimination are lost in mice lacking prestin (Liberman et al., 2002). A similar phenotype is observed in mice expressing a mutant prestin devoid of electromotility (Dallos et al., 2008). Finally, when OHC stereocilia are detached from the tectorial membrane, amplification near threshold is abolished; however, basilar membrane motions can still be elicited by electrical stimulation (Drexler et al., 2008). Somatic electromotility alone is therefore sufficient to enhance basilar membrane motion, indicating that prestin-mediated electromotility is important for amplification.

Hair bundles from mammalian and nonmammalian species also show active motility, which leads to significant force production (Fettiplace, 2006; Hudspeth, 2008). This motility depends on Ca^{2+} influx through transduction channels and appears to be linked to fast adaptation (Ricci et al., 2000). Provided that the motion of the bundle is timed appropriately, an oscillatory stimulus could be magnified, leading to amplification. An important distinction between active hair bundle motility and somatic electromotility is that only the former is frequency selective. The cochlear amplifier might therefore depend on interplay between hair bundle motility and somatic electromotility (Fettiplace, 2006).

Concluding remarks

The study of genes that are linked to deafness has led to remarkable progress in our understanding of the cell biology of hair cells. Several important concepts can be derived. Myosin motor proteins are central to the function of auditory hair cells where they control diverse processes such as protein transport and the activity of mechanotransduction channels. The identification of components of the mechanotransduction machinery has revealed an unanticipated molecular asymmetry in this complex molecular machine. Finally, the study of the prestin molecule has provided insights into the mechanism of how changes in membrane potential can be translated into changes in cellular architecture. Undoubtedly, we will see in the coming years rapid progress in our understanding of the mechanisms that are essential for our ability to hear. As in the past, it is anticipated that the study of genes that are linked to deafness will remain instrumental in this process.

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