

CHAPTER

53

Molecular Biology of Hearing and Balance

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GENERAL FEATURES OF MECHANOTRANSDUCTION

Mechanotransduction is of great utility for all organisms

Most multicellular organisms exploit mechanoreceptors—specialized cells that detect external mechanical forces—to help construct their internal view of the external world. The senses of hearing, balance, and touch all rely on mechanoreceptors, as do the proprioceptive sensations that tell an organism how it is situated within the environment. Mechanoreception

is probably one of the most ancient of the senses, with mechanosensitivity existing in virtually every type of organism in all three domains—Bacteria, Archaea and Eukarya (Kloda & Martinac, 2002).

Models for mechanotransduction allow comparison of mechanoreceptors from many organisms and cell types

Mechanoreceptors use ion channels for transducing sensory information. In multicellular organisms, mechanoreceptors are

either neurons or neuroepithelial cells with synapses, which allow the cells to stimulate other neurons. Because the currency of the nervous system is the membrane potential, by opening an ion channel a cell can quickly and extensively modulate its membrane potential and hence impact neurotransmitter release, the final step in mechanoreception at the cellular level.

Ion channels open or close upon relative movement of internal domains, which can be elicited by voltage, ligand binding or force. A key question in the study of mechanotransduction is how force can influence domain movements within ion channels. Multiple models of how this could occur have been generated from the study of mechanosensitive ion channels in bacteria and other cell types. These mechanotransduction models have allowed comparison of the multiple types of mechanoreceptors that exist within different species. In one model, ion channel domains are moved by tension within the plasma membrane. Bacterial osmosensors are thought to work this way; as membrane tension increases, the channels gate to reduce tension (Sukharev & Cori, 2004). Other mechanoreceptors apparently do not sense membrane tension, however. Ion channels in these mechanoreceptors are tethered on both sides of the membrane; external forces cause a net displacement of the two anchors, transmitting force to the channel and triggering a net domain movement that opens the channel (Gillespie & Walker, 2001). These two models differ in the direction force is applied to the ion channel; in the membrane-tension model, force is felt in the plane of the membrane, while in the tether model, the force vector is perpendicular to the membrane (Fig. 53-1).

Interestingly, a third model, the “elevator model,” incorporates elements of the previous two models (Kung, 2005). In this model, a single tether—most likely extracellular—pulls on a mechanotransduction channel. Net displacement of the channel with respect to the membrane leads to altered exposure of side chains to the membrane lipids, which could lead to a conformational change that gates the channel. While bacterial mechanotransduction channels clearly conform to the membrane-tension model, they also could be seen to fit features of the elevator model. Unfortunately, we have insufficient molecular and structural information about all eukaryotic transduction channels to determine which of these models fit best.

NON-VERTEBRATE MODEL SYSTEMS

Our understanding of the general features of mechanoreceptors has been greatly assisted by the use of model systems. Bacterial mechanotransduction channels do not require any other additional molecules for gating and can be assayed in patch-clamp experiments when reconstituted into liposomes. Structures of several channels have been solved and gating mechanisms are understood at a very sophisticated level. However, because it is not clear whether the lessons learned from these channels are generally applicable to the channels used by sensory cells in multicellular organisms, many investigators have focused on mechanotransduction in both invertebrate and vertebrate model systems. Genetically tractable organisms like *Caenorhabditis elegans* and *Drosophila melanogaster* allow for analysis of interactions between genes, as well as for extensive cataloging of essential molecules.

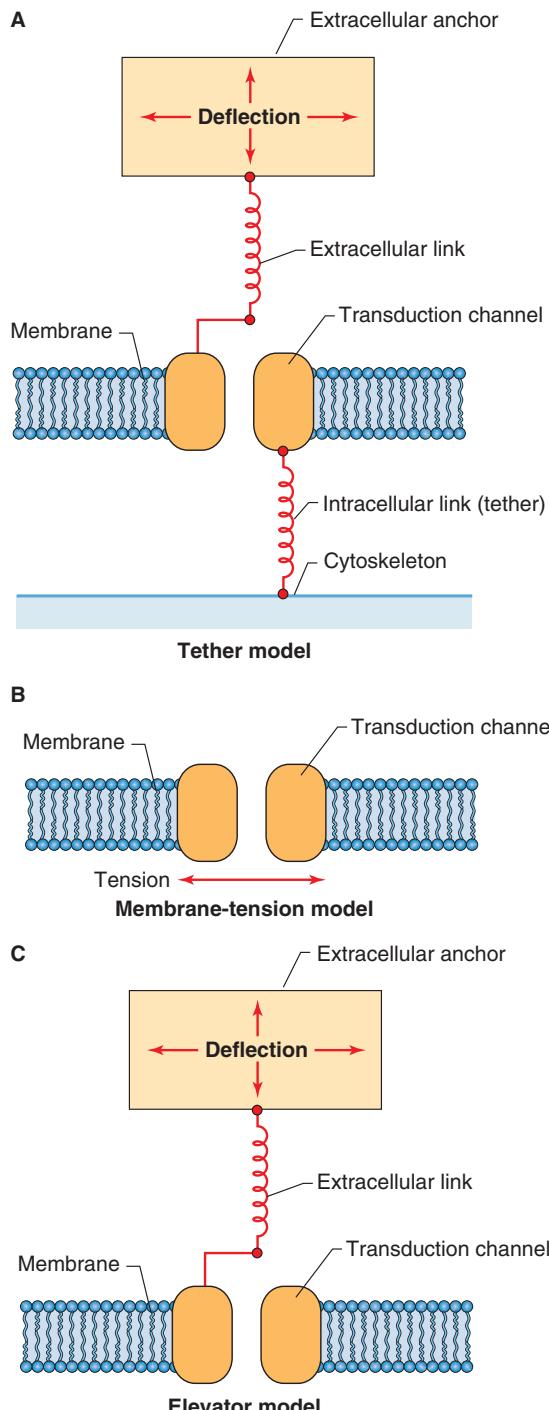


FIGURE 53-1 General models for mechanotransduction. **A**, Tether model. A transduction channel, embedded in the plasma membrane, is anchored mechanically to the cytoskeleton and to an extracellular structure that can move relative to the cell. Movements of the extracellular structure relative to the cytoskeleton cause tension to develop throughout the system, which is delivered to the gate of the channel. Increased gating tension causes channels to open. **B**, Membrane-tension model. Tension parallel to the membrane is sensed by the channel, which dilates to open. **C**, Elevator model. Extracellular anchors and links are present as in the tether model, but force perpendicular to the membrane causes a movement of the channel protein relative to the membrane, changing the interaction of the channel with the membrane and subsequently opening it.

Worm mechanoreceptors use a transduction cascade that depends on epithelial sodium channels (ENaC)

Worms like *C. elegans* respond to a gentle touch on the nose by turning away. Mechanical deformation of the worm's cuticle stimulates specific cells (six body touch sensory neurons), which transmit sensory information to neural circuits that control locomotion. Genetic experiments have revealed a dozen genes involved in this response, and the identities of these genes led to the suggestion that touch reception is best exemplified by the tether model (Ernstrom & Chalfie, 2002). The touch receptor transduction channel is thought to consist of two pore-forming subunits (MEC-4 and MEC-10, members of the DEG/ENaC channel family), as well as the accessory membrane proteins MEC-2 and MEC-6 (O'Hagan et al., 2005). Because touch reception requires several extracellular matrix molecules (MEC-5, MEC-9) and an underlying microtubule network, consisting of unique 15-protofilament microtubules (tubulin subunits encoded by the *mec-7* and *mec-12* genes), it has been proposed that movement of the cuticle relative to the microtubule network mechanically gates the transduction channel directly, admitting cations that depolarize the cell. However, localization of the touch channels showed that they are not close to either extracellular matrix molecules required for transduction or the ends of the specialized microtubules (Cueva et al., 2007). These results have suggested that instead of opening channels by tether stretching, *C. elegans* touch receptor transduction channels instead respond to membrane tension, applied to the membrane distant from the channels.

Touch neurons are not the only mechanoreceptors in *C. elegans*, however. For example, other mechanoreceptors rely on a different type of channel, the *transient receptor potential* (TRP) channels (Ch. 4). Neurons involved in the basal slowing response rely on the channel TRPN1, also known as TRP-4, for mechanotransduction (Kang et al., 2010). Polymodal sensory neurons in *C. elegans* also seem to fall into the TRP class of mechanoreceptors as they apparently rely on several TRP channels for mechanosensation (Tobin et al., 2002). These sensory neurons respond to nose touch, hyperosmolarity, and volatile repellents by triggering a backward response in the worm; the OSM-9 and OCR-2 channels apparently mediate these responses, but the pathways for each sensory modality differ. Little is known, however, about the transduction mechanism in these cells and there is insufficient information to decide which of the general models for gating applies.

Fly mechanoreceptors use molecules similar to those of hair cells

Surface mechanoreceptors on insects have been studied for many years. Kernan and Zuker developed a screen for mechanoreceptor mutants in *Drosophila* (Kernan et al., 1994) that identified several dozen mutants deficient in mechanoreception; however, only some of the mutant genes have been identified. The most important molecules so far identified from this screen include the transduction channel, an extracellular molecule that could gate channels, and several molecules known to be important for axonemal structure and function.

Although the set of molecules is less complete than that identified for *C. elegans* touch receptors, the diversity of mechanotransduction in *Drosophila* and the apparent similarity of these receptors to those in vertebrates, including hair cells, demonstrates the significance of this model system.

Drosophila TRPN1 appears to be the major transduction channel in fly bristles and in the Johnston organ, an organ that is used for sound detection (Walker et al., 2000). Bristle mechanotransduction remarkably resembles hair-cell transduction in its speed, polarity and adaptation (Walker et al., 2000), suggesting the possibility of a close evolutionary relationship between these mechanoreceptors. While two other TRP channels, Nanchung and Inactive, are essential for hearing, all available evidence suggests that TRPN1 is the mechanically gated channel and Nanchung and Inactive play a role in signal amplification (Göpfert et al., 2006).

The gene *nompA* encodes a multi-domain extracellular protein that is expressed by support cells that cradle the mechanically sensitive neuron within type 1 sensory organs in the fly peripheral nervous system (Chung et al., 2001). *NompA* is localized to the dendritic cap, which covers the mechanically sensitive outer segment of the neuron. The failure of mechanotransduction in *nompA* mutants and its presence within the dendritic cap make *nompA* a good candidate for a protein that couples mechanically sensitive channels to external stimuli.

Strikingly, insect mechanoreceptors and vertebrate hair cells share evolutionary relationships at the molecular level. In flies, the proneural gene *ataonal* controls development of mechanoreceptors, whereas *Atonal homolog 1* (*Atoh1*) plays a similar role in mice. Indeed, *ataonal* can fully substitute for *Atoh1* in mice and *Atoh1* can fully substitute for *ataonal* in flies (Wang et al., 2002), suggesting that these two disparate systems might share a common genetic program used for development. As another example, *Drosophila* mechanoreceptors and vertebrate hair cells each rely on the molecular motor myosin-VIIA (Todi et al., 2005).

HAIR CELLS

Hair cells are the sensory cells of the auditory and vestibular systems

Hair cells are the sensory cells of the internal ear, essential for the senses of sound and balance. The hair cell's transduction apparatus, the molecular machinery that converts forces and displacements into electrical responses, can respond to mechanical stimuli of less than 1 nm in amplitude and tens of kilohertz in frequency. Indeed, our hearing is ultimately limited by Brownian motion of water molecules impinging on the transduction apparatus.

Even though well characterized at a biophysical level, the mechanical transduction mechanism of hair cells is still not fully understood in molecular terms. This discrepancy is in part due to the extreme scarcity of hair cells; instead of the millions or even hundreds of millions of receptor cells that the olfactory and visual systems possess, only a few tens of thousands of hair cells are found in the internal ears of most vertebrate species. Moreover, the key molecules of transduction are thought to be exceptionally scarce within hair cells; for

example, there may only be 100 active transduction channels, the ion channels at the heart of hair-cell transduction. Finally, transduction ensues without the biochemical cascades seen in the auditory and olfactory systems, increasing the challenge in detecting transduction molecules. The small number of hair cells, rarity of key molecules and distinct transduction mechanism have ensured that molecular biological and biochemical characterization of hair-cell transduction has lagged well behind description of vision and olfaction.

Hair cells are neuroepithelial cells; their large basolateral surface includes synaptic contacts with afferent and efferent nerve fibers, while the mechanically sensitive hair bundle is located on their apical surface. The hair bundle is an ensemble of ~100 actin-filled stereocilia and a single axonemal kinocilium (Fig. 53-2). The kinocilium, present during development in all hair cells, eventually degenerates in those cells sensitive to high auditory frequencies. Stereocilia range in height from 1 μm, for auditory detection of very high frequencies, to 50 μm or more in some vestibular systems. A consistent and critical feature of the hair bundle is that stereocilia are arranged in ranks of increasing heights, with the tallest stereocilia adjacent to the kinocilium. This arrangement establishes an axis of mirror symmetry, which is also the axis of maximal mechanical sensitivity.

Stereocilia contain hundreds of cross-linked actin filaments throughout their length; as the stereocilium approaches the apical surface of the hair cell, that number systematically declines to a dozen or two. Stereocilia are thus mechanically stiff throughout most of their length, but are flexible at the insertion point. Stereocilia do not flex independently, however, as they are

cross-linked together by a variety of linkages, including ankle links, lateral links, and tip links. The consequence of the mechanical properties of the stereocilia and their interconnection by flexible linkages is that when the bundle is deflected by a mechanical stimulus, the bundle moves as a whole. Moreover, individual stereocilia slide with respect to each other, a movement that underlies mechanical transduction (Fig. 53-3).

Hair cells are exposed to unusual extracellular fluids and potentials

The apical surfaces of hair cells are exposed to an unusual extracellular fluid called endolymph. Endolymph is relatively similar in ionic composition to cytoplasm: it is high in K⁺ (~150 mM), low in Na⁺ (~2 mM), and relatively low in Ca²⁺ (~100 μM). Endolymph may have evolved to remove from hair cells the energy expenditure required as a consequence of ion entry into hair cells. Normally, an excitatory cell like a neuron must pump out all ions that entered during an excitatory response. The ear separates this pumping task away from the hair cell. K⁺, the major current-carrying ion, enters hair cells down an electrical gradient (hair cells are typically -60 mV) through transduction channels; when channels are closed, however, K⁺ readily leaves through basolateral K⁺ channels down the normal K⁺ electrochemical gradient. The energy expenditure required for transduction is thus passed on to the stria vascularis cells, which establish the high K⁺ concentration in the endolymph.

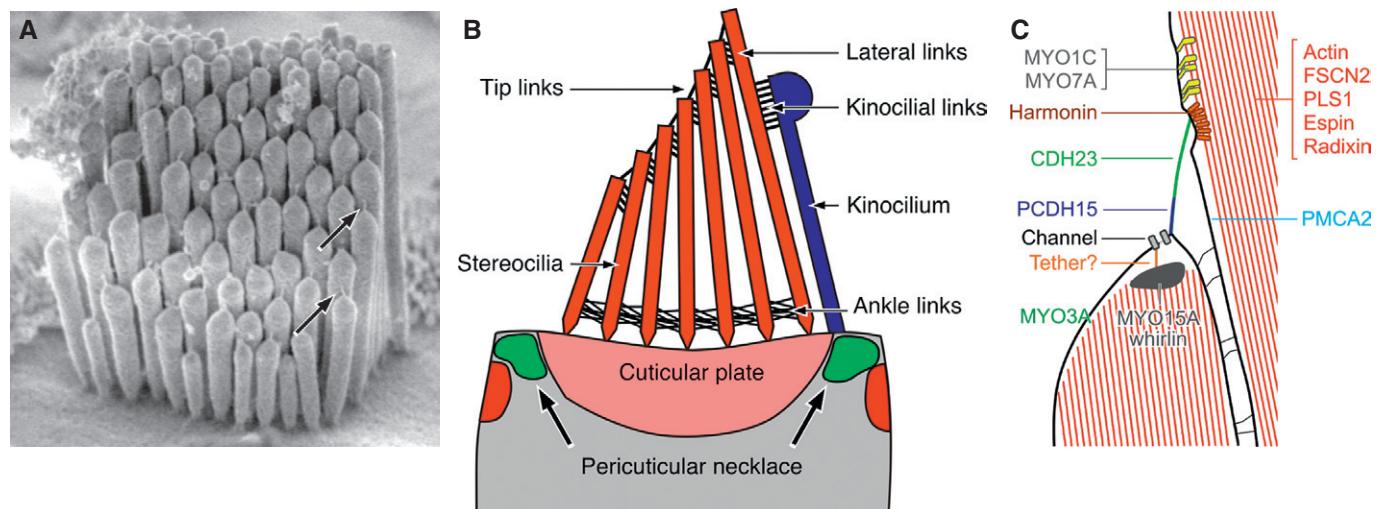


FIGURE 53-2 Hair-bundle structure. **A.** The hair bundle, consisting of ~100 stereocilia, protrudes from the apical surface of this chicken-cochlea hair cell. Deflection of the bundle towards the taller stereocilia imparts tension on to tip links (indicated by arrows), which gate transduction channels. **B.** Schematic depiction of a hair bundle. Actin-rich structures (e.g., stereocilia and cuticular plate) are indicated in red. The axonemal kinocilium, a microtubule-based cilium, is indicated in blue. The pericuticular necklace, a location of membrane vesicles bearing proteins targeted to the bundle, is indicated in green. Links between the stereocilia and between the tallest stereocilia and the kinocilium are also indicated. **C.** Known molecules participating in transduction in hair cells are shown. Actin filaments, cross-linked by fascin-2 (FSCN2), plasmin-1 (PLS1), and espin, are the substrate for slow adaptation. Radixin cross-links stereocilia actin to the membrane. The transduction channel is gated, perhaps directly, by the tip-link molecules CDH23 and PCDH15. CDH23 is anchored at the upper end of the tip link by harmonin, while PCDH15 may be coupled through the transduction channel and a hypothetical tether to the stereocilia actin. A cluster of myosin molecules (MYO1C and/or MYO7A) makes up the adaptation motor. Ca²⁺ entering through open transduction channels modulates adaptation; it is rapidly buffered by mobile Ca²⁺ buffers (not shown) and is eventually pumped out of the stereocilia by the Ca²⁺ pump PMCA2. The MYO3A motor positions espin at stereocilia tips for control of actin elongation; the MYO15A-whirin complex also regulates actin polymerization.

The endolymphatic compartment of the auditory system is at an elevated potential (about +80 mV); this endocochlear potential increases the driving force on K^+ yet more, producing additional transduction current. Some mutations that cause deafness affect either K^+ levels in the endolymph or the endolymphatic potential itself.

Mechanical transduction depends on activation of ion channels linked to extracellular and intracellular structures

A comprehensive model for hair-cell transduction has emerged, derived primarily from biophysical and morphological investigations. Residing in the mechanoreceptive organelle of a hair cell, the hair bundle, the transduction apparatus consists of at least three components: the *transduction channel*, a mechanically gated ion channel; the *tip link*, an extracellular filament that transmits force to the channel's gate; and the *adaptation motor*, a mechanism that maintains an optimal tension in the tip link so that the channel can respond to displacements of atomic dimensions (Fig. 53-3). The tip link is probably connected in series with the *gating spring*, an elastic element through which stimulus energy can affect the transduction channel. Although highly specialized for the internal ear, the channel, gating spring, and adaptation mechanism are likely to be general requirements for any mechanical transduction apparatus; detailed characterization

of the transduction apparatus of the hair cell may therefore eventually illuminate other transduction systems.

In this model, deflection of the hair bundle in the excitatory direction—towards the tallest stereocilia—causes a shorter stereocilium to slide down its neighbor's side, stretching the gating spring. Tension in the gating spring is transmitted to the transduction channel, which responds by increasing the frequency with which it opens and the duration of the openings.

Transduction channels are located, at least in cochlear hair cells, at the base of the tip link (at the tip of a shorter stereocilium). Ca^{2+} imaging experiments demonstrated that when transduction channels of a hair bundle open, rapid elevation of Ca^{2+} is seen only in the shorter ranks of stereocilia (Beurg et al., 2009).

Transduction channels do not remain open, even if a deflection is maintained; using two independent processes, the hair cell adapts to a sustained mechanical stimulus (Fettiplace & Ricci, 2003). Rapid channel reclosure (sometimes called fast adaptation) occurs on a time scale of a few milliseconds or less; Ca^{2+} entering open transduction channels binds to a site on or nearby the channel, causing the channel to close (Fig. 53-3A). Because closing channels exert a negative-directed force on the hair bundle, the fast adaptation mechanism can mechanically amplify an input; if the negative force occurs during the negative phase of a sinusoidal stimulus, the forces add and bundle movement is enhanced.

A slower mechanism, operating on the time scale of tens of milliseconds, adjusts the transduction apparatus so that

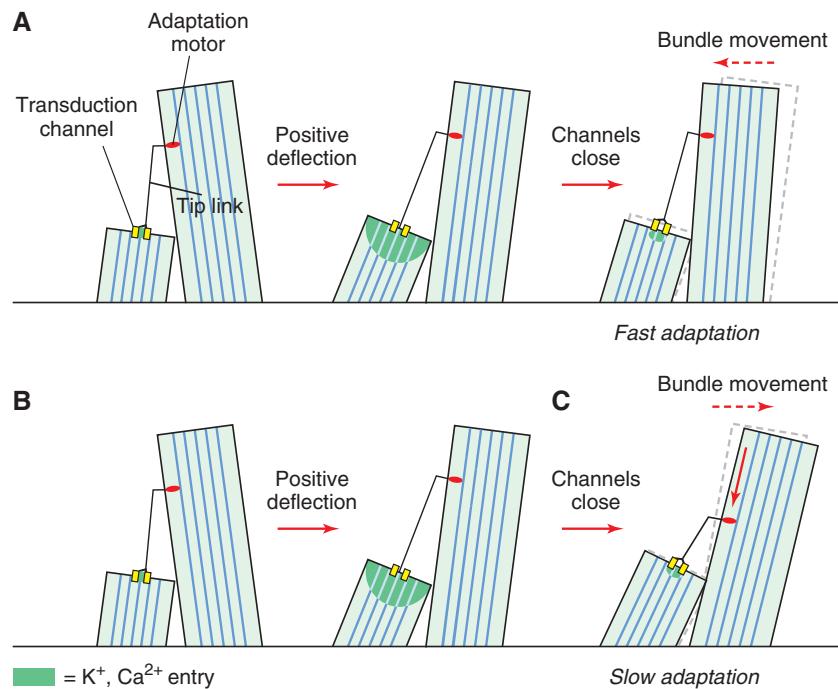


FIGURE 53-3 Hair-cell transduction and adaptation. Only two stereocilia out of a bundle are shown. **A**, The transduction channel (yellow) is gated by the tip link. At rest, channels spend ~10% of the time open. An excitatory deflection moves the bundle towards the taller stereocilia; increased tension in the tip link leads to channel opening and entry of K^+ and Ca^{2+} . **B**, fast adaptation. After a positive (excitatory) deflection, Ca^{2+} enters transduction channels, binds to a site at the channel, yanking the channel shut. The movement of the channel's gate increases tension in the tip link, and the bundle is moved in the negative direction. **C**, slow adaptation. The adaptation motor responds to Ca^{2+} and high force by slipping towards the base of the stereocilium (red vertical arrow).

the open probability at rest is optimal and fast adaptation is enhanced. Slow adaptation is mediated by a cluster of myosin molecules (Gillespie & Cyr, 2004); the myosins exert a resting tension, but the oppositely directed force in a stimulus overcomes the resting tension, dragging the myosin complex down the stereocilium (Fig. 53-3C). As the motors descend the stereocilium, tension in the gating spring is reduced, and the transduction channels close; the bundle then relaxes further in the direction of the stimulus (Fig. 53-3B). See also myosin and actin in Ch. 6.

Some of the molecules responsible for transduction have been identified

Several key molecules have been identified as part of the transduction complex (Fig. 53-2C). Strikingly, most of these molecules were identified by cloning “deafness genes,” genes that when mutated cause deafness (see Box 53). Together, identification and characterization of these transduction proteins has provided an increasingly detailed picture of the structure of the transduction complex. Several key pieces are still missing, most notably the transduction channel itself, but most of the fundamental elements have been described.

The tip link, a complex of two braided glycoprotein filaments (Kachar et al., 2000), is made from a protocadherin 15 (PCDH15) dimer on the lower end (Ahmed et al., 2006; Kazmierczak et al., 2007) and a cadherin 23 (CDH23) dimer on the upper end (Siemens et al., 2004; Kazmierczak et al., 2007). These cadherins are Ca^{2+} -dependent cell adhesion molecules, which likely explains the loss of tip links when Ca^{2+} concentrations are reduced to below a few micromolar. PCDH15 is located near detectable transduction channels, suggesting that this cadherin could be directly connected to the channel.

Other proteins present in the transduction complex with the cadherins include two scaffolding proteins, harmonin (Verpy et al., 2000) and SANS (Weil et al., 2003), both identified by the deafness gene approach (see Box 53). These two proteins each possess several protein–protein interaction domains, several of which are used to form a stable complex between them (Yan & Liu, 2010). In addition to interacting with SANS, harmonin self-associates and may form the upper tip-link insertion density (Grillet et al., 2009); appropriately, the intracellular domain of CDH23 also interacts with harmonin (Siemens et al., 2002).

Myosin molecules clearly play essential roles in hair-cell transduction, and hair cells express a variety of myosin isoforms, including myosin-1c (MYO1C), myosin-IIIa (MYO3A), myosin-VI (MYO6), myosin-VIIa (MYO7A) and myosin-XVa (MYO15A). Because it is located at the tips of the stereocilia, near the tip-link anchors, MYO1C is a strong candidate for the adaptation motor. Selective inhibition of a sensitized MYO1C mutant with an ADP analog proved that this myosin participates in adaptation in vestibular hair cells (Holt et al., 2002), although contribution by other myosins has yet to be ruled out. Moreover, CDH23 can interact directly or indirectly with MYO1C, suggesting that these two molecules form part of the transduction complex in hair cells (Siemens et al., 2004). However, mice with near-null mutations in myosin-7a have defects in auditory transduction that are consistent with alterations in the adaptation

machinery, suggesting a central role for this myosin too (Kros et al., 2002). Notably, MYO7A interacts with harmonin, suggesting that it could anchor the upper end of the tip link (Boeda et al., 2002). MYO7A also interacts with some splice forms of PCDH15 (Senften et al., 2006), suggesting that this motor could be involved in forming the complexes at both ends of the tip link.

As both fast and slow adaptation mechanisms are regulated by Ca^{2+} , the stereociliar mechanisms that control the free concentration of this ion play central roles in transduction. Entering Ca^{2+} is thought to be buffered very rapidly by the mobile buffers parvalbumin, calbindin and calretinin (Heller et al., 2002; Hackney et al., 2003). Even before bound Ca^{2+} can diffuse out of stereocilia, it is pumped back out into the endolymph by isoform 2w/a of the plasma-membrane Ca^{2+} -ATPase (PMCA2) (Lumpkin et al., 1998; Dumont et al., 2001; Hill et al., 2006). See also PMCA in Ch. 3.

Although the transduction channel has attracted much attention, it has yet to be identified. It is known that the transduction channel passes cations, including Ca^{2+} , and can be inhibited by aminoglycoside antibiotics and amiloride derivatives. Unless controlled by tension applied through the gating spring, the channel remains shut. Most in the field feel that the transduction channel belongs to the TRP channel family. TRP channels are remarkably diverse in structure and function and include many channels central for sensory function, including those involved in vision, pheromone sensing, temperature detection and mechanical responsiveness. Indeed, as mentioned earlier, TRP channels have been shown to be important in worms and insects for mechanotransduction. In particular, the TRP channel TRPN1 appears to be the major transduction channel in fly mechanoreceptors involved in mechanosensation and hearing as well as in certain types of mechanoreceptors in *C. elegans*. Moreover, two TRP channels of the TRPV family, *nanchung* and *inactive*, are also important for fly hearing.

Are any of these TRP channels found in hair cells? The answer is yes for TRPN1, although the details are unexpected. TRPN1 is found in zebrafish hair cells, where it has been shown to be essential for transduction (Sidi et al., 2003). Surprisingly enough, however, TRPN1 has yet to be identified in mammalian genomes. This result suggests the possibility that TRPN1 is just one of several channel subunits required, and that other TRP channels could contribute to the transduction channel in mammals. Although several TRPV channels have been localized to hair cells in mammals, gene knockout experiments suggest that these channels do not play a role in mechanotransduction.

Other hair cell molecules control stereocilia actin

The molecules that make up the transduction apparatus represent only a small fraction of all the proteins present within the hair cell bundle. The hair bundle is predominantly composed of stereocilia actin, and molecules that control actin filament formation and stability can also play an important role in hair cell function. Stereocilia actin is dynamic; in early postnatal animals, actin molecules incorporate at stereocilia tips and are transported to bases in 24–48 hours (Rzadzinska et al., 2004). These results suggest that the elaborate and stereotyped structure of the stereocilia actin core is not static, but

DEAFNESS GENES

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About 1 in 1,000 children are born deaf, and another 1 in 1,000 develop deafness by adolescence. Although some deafness can arise during development due to external sources, much deafness in children is due to mutations in specific genes. As many forms of deafness are nonsyndromic—there is no other associated abnormality—identification of the responsible genes can lead to characterization of proteins that are essential for the auditory system, and often for the vestibular system, too.

The genes responsible for ~90 recessive and ~60 dominant nonsyndromic deafness syndromes have been identified in humans (<http://hereditaryhearingloss.org>), and similar numbers have been identified in mice (Dror & Avraham, 2010). Moreover, a dozen or so syndromes with deafness—associated with other phenotypic abnormalities besides deafness—have also been identified. Some genes are associated with both recessive and dominant deafness, and others are associated with both syndromic and nonsyndromic deafness. Because most genes identified in humans have also been identified in mice, this tally corresponds to about 200 different genes, although the total number of genes associated with deafness may be as large as 500 to 1000.

The deafness syndrome most common in humans, DFNB1, can be caused by mutations in either of two gap-junction connexin genes that are tightly associated on chromosome 13, *GJB2* (connexin 26) and *GJB6* (connexin 30). These connexins play a critical role in establishment of a gap-junction network in supporting cells of the cochlea, which is thought to be important for recycling K⁺ ions that enter (and leave) hair cells during transduction (Nickel & Forge, 2008).

About one-third of the genes responsible for nonsyndromic deafness encode proteins that are expressed in stereocilia. Some of these encode structural proteins of the stereocilia (e.g., gamma-actin, TRIOBP and radixin); others encode proteins that regulate the actin cytoskeleton (e.g., myosin-3a, myosin-15a and espin). Additional genes encode proteins that are expressed in stereocilia but have unknown function (e.g., GRXC1).

Usher syndrome is a deafness-blindness syndrome that is caused by mutations in a number of genes that are essential for stereocilia development and function. Usher syndrome is divided into three classes depending on the severity of the phenotype. Usher I produces profound deafness and vestibular dysfunction, as well as adolescent blindness; in Usher II, deafness is less profound. Usher III is less common; in these patients, hearing and vision loss develop progressively, and vestibular dysfunction is variable.

Of the seven mapped genes underlying Usher type I, five have been identified: *CDH23*, *PCDH15*, *MYO7A*, *USH1C* (harmonin) and *SANS*. Biochemical evidence and phenotypic

similarity suggests that these proteins may assemble into a complex (Adato et al., 2005; Lefevre et al., 2008), although conclusive evidence for such a complex is lacking. Moreover, all of these proteins are important for both hair-bundle development and mechanotransduction, suggesting an intimate relationship between the two processes. Usher type II is caused by mutations in at least four genes, three of which—*USH2A*, *GPR98* and *WHRN*—have been identified. The products of the *USH2A* and *GPR98* genes, usherin and the very large G-protein-coupled receptor 1, are large transmembrane proteins that may make up the ankle links, links that interconnect stereocilia at their bases. Whirlin, the product of the *WHRN* gene, is a scaffolding protein that has been implicated in the control of stereocilia actin dynamics. One gene responsible for Usher type III is *CLRN1*; clarin-1 is a four-transmembrane-domain protein that plays a role in stereocilia development (Yan & Liu, 2010).

Understandably, disruption of hair-cell function at many levels can lead to deafness, so it is expected that deafness genes include those involved in inner-ear development, in ion balance in the endolymph, and in structural integrity of hair cells. However, the deafness-gene approach has not been successful in identifying some constituents of the transduction apparatus, most notably the transduction channel. This is a surprising observation, as the genetic approach relies on few assumptions about the nature of the systems studied. Perhaps the lesson here is that some of the important molecules for transduction play essential roles elsewhere in the organism during development, a sobering observation for those relying exclusively on deafness-gene identification to reconstruct the transduction apparatus of hair cells.

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is continuously being remodeled. Whether actin dynamics are so rapid in mature hair cells is not known.

The crosslinking protein espin is essential for proper formation of stereocilia actin (Zheng et al., 2000); interestingly other

cross-linkers, like plastin-1 and fascin-2, apparently are much more abundant (Shin et al., 2010), suggesting that espin exerts its profound effects on stereocilia actin at low stoichiometry. Like actin, espin incorporates into the stereocilia actin core at

tips; the motor MYO3A, also found at stereocilia tips, apparently transports espin to tips (Salles et al., 2009). Humans with an apparently null mutation in the *MYO3A* gene exhibit progressive hearing loss that develops over decades (Walsh et al., 2002).

While mice with mutations in the *Myo6* and *Myo15a* genes are also deaf, these isoforms apparently mediate other essential hair-cell functions besides adaptation. MYO6 is an unusual isoform as it moves along polarized actin filaments in a direction opposite from all other well-characterized myosins (see Ch. 6). MYO6 is located near the base of the hair bundle and could play a role in anchoring the apical membrane to prevent fusion along the stereocilia shafts (Avraham et al., 1995); in addition, MYO6 appears to localize proteins to stereocilia bases (Sakaguchi et al., 2008).

MYO15A is located at stereocilia tips and apparently controls the rate of actin filament growth (Rzadzinska et al., 2004) in a complex with the protein whirlin; stereocilia with more MYO15A grow faster than those with lesser amounts of the myosin. Hair cells with no functional MYO15A protein grow stereocilia that are very short and stubby, indicating that MYO15A is important for actin filament elongation but not for the specification of the positions of the stereocilia.

Taken together, these data suggest that control of stereocilia actin structure is complex, with several motors and cross-linking proteins involved. As yet, proteins responsible for catalyzing actin monomers into stereocilia actin filaments have not been identified. The well-characterized Arp2/3 complex is unlikely to be involved, as that complex is responsible for branched actin networks, however members of the formin family could conceivably control stereocilia actin elongation. Higher level control of actin dynamics, for example through the Rho-Rac-Cdc42 family of proteins, is likely to be important but has not yet been defined in hair bundles.

HAIR CELLS IN THE INNER EAR

In vertebrates, hair cells are used in the inner ear's balance and hearing organs; in amphibians and fish, hair cells also are the mechanoreceptors of the lateral line, which detects nearby fluid movement. In all of these varied organs, hair cells and their hair bundles differ in their morphological and physiological properties. However, despite these differences, hair cells within the inner ear appear to share the same overall mechanism of mechanotransduction, and thus study of both the balance and hearing organs have contributed to our molecular understanding of bundle structure and function.

BALANCE: VESTIBULAR ORGANS

Vestibular organs detect head rotation and linear acceleration

Balance and head movement are detected by a series of organs within the inner ear that together comprise the mammalian vestibular system. The vestibular system consists of three

semicircular canals, which detect angular acceleration (that is, head rotation), as well as the saccule and utricle, which detect linear acceleration (that is, head translation) (Fig. 53-4). Hair cells within each of these organs are stimulated when a lateral force is applied to the hair bundles via the movement of an overlying auxiliary structure. Hair cells within swellings (or ampullae) of the semicircular canals are covered by a gelatinous structure called the cupula, which is displaced by fluid movement in the canal as the head is rotated. In the utricle and saccule, the tips of the hair bundles are embedded in an otolithic membrane, which contains deposits of calcium carbonate called otoconia or otoliths. The inertia of the otoconia causes displacement of bundles in response to small changes of head position.

Vestibular hair cells are polarized such that movement of the hair bundle towards the kinocilium depolarizes the hair cell, while movement away from the kinocilium hyperpolarizes them. Within the different vestibular organs, bundle orientation varies systematically, visualized readily by noting the kinocilium position relative to the stereocilia. In each of the semicircular canals, the kinocilia of all the hair cells are oriented in only one direction such that all hair cells in a given canal will respond to a specific direction of angular acceleration. In the utricle and saccule, however, hair cells are oriented in different directions. A specialized structure called the striola divides utricular and saccular hair cells into two populations with opposing polarities (Fig. 53-4). This spatial organization ensures that hair cells can respond to any direction of otolithic displacement.

Hair bundles display varying morphology and physiology

Hair bundles of vestibular hair cells differ not only in orientation but also in their varying morphological and physiological properties. Two general types of vestibular hair cell, Types I and II, have been described within the system based on differences in their afferent nerve terminals. Type I and II hair cells also differ in the number and length of stereocilia in their hair bundles. Differences in hair bundle morphology have also been detected based on the spatial location of different hair cells within the utricle. Hair bundle structure is intimately connected to bundle mechanics and thus to properties of the mechanotransduction complex. Thus, these different cell types will exhibit varying physiological properties based on differences in transduction currents as well as their innervation characteristics. Vestibular hair cells also differ in their morphological and physiological characteristics compared to auditory hair cells, although the basic principles of hair cell function remain similar for both.

HEARING: COCHLEA

The cochlea detects sound and is tonotopically organized

The cochlea is the auditory sense organ within the inner ear that is responsible for hearing. The cochlea is a coiled, fluid-filled

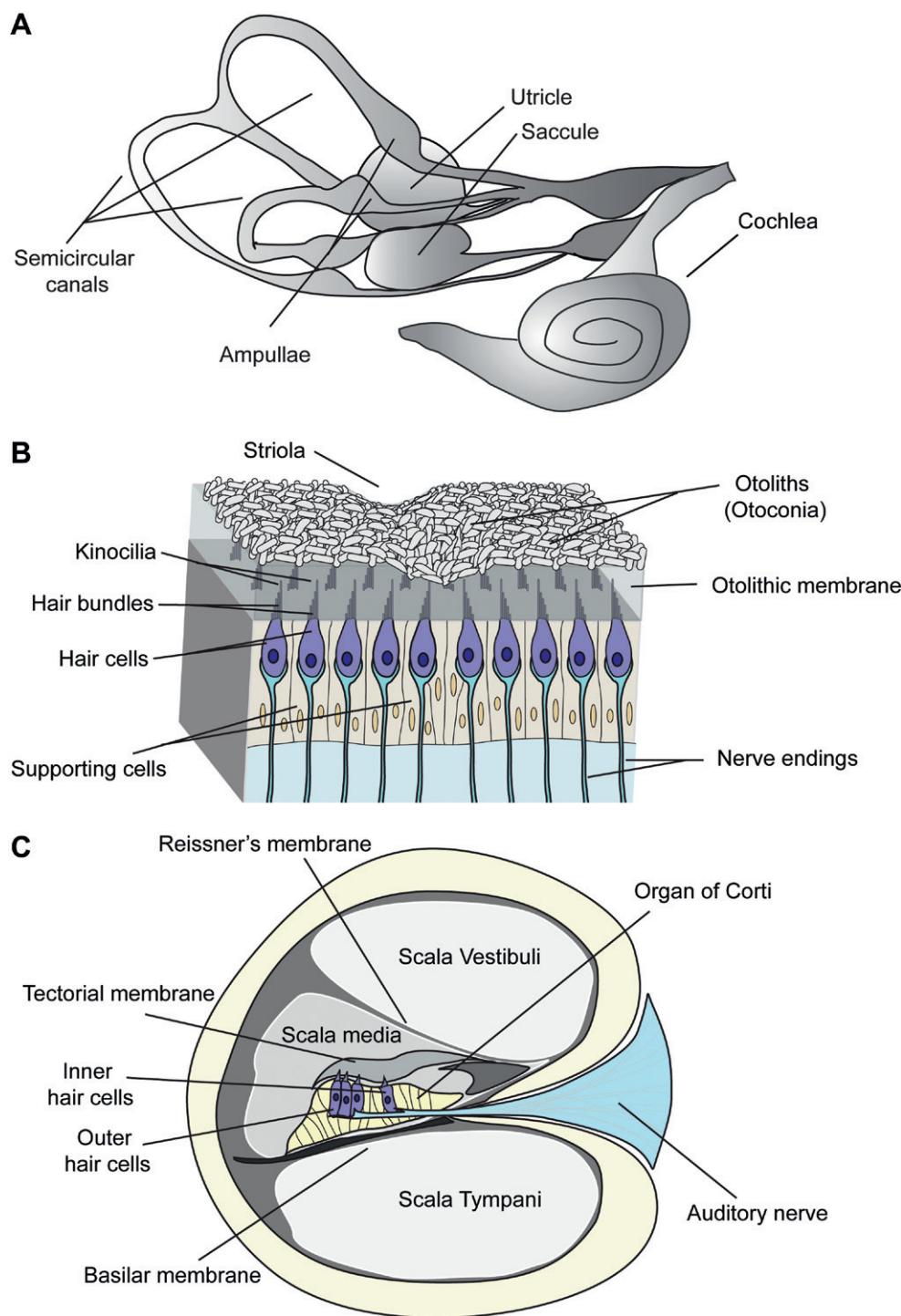


FIGURE 53-4 Anatomy of the inner ear. **A.** Diagram of the vestibular and auditory organs within the inner ear. **B.** Cross-section of the utricle, indicating the hair cells (purple) with their hair bundles embedded in the otolithic membrane (gray) and their kinocilia oriented towards the striola. **C.** Cross-section of the cochlear duct, indicating the outer and inner hair cells (purple) within the organ of Corti (yellow), the two membranes dividing the duct (Reissner's membrane and the basilar membrane), and the three scalae, with the scala media differentiated by the darker shading of its extracellular solution (the endolymph).

tube that is split into three chambers by two membranes, Reissner's membrane and the basilar membrane (Fig. 53-4). The outer two chambers, the scala vestibuli and scala tympani, are contacted by two windows on the middle ear—the oval and

round windows—and form a continuous channel that allows sound vibrations to travel through the cochlear fluids. The middle chamber, or scala media, lies between the two outer chambers and holds the organ of Corti, where the hair cells are located.

The organ of Corti sits on top of the basilar membrane and is covered by a gelatinous matrix called the tectorial membrane. Sound vibrations traveling down the cochlear fluid cause the basilar membrane to vibrate, which then stimulates the hair cells by displacing their hair bundles relative to the tectorial membrane.

Individual cochlear hair cells respond to specific sound frequencies based on their positioning along the basilar membrane. The basilar membrane is not uniform in structure, but rather is narrow and stiff at the basal end of the cochlea and wider and more flexible at its apical end. Due to this gradient in size and stiffness, the basilar membrane vibrates with maximal amplitude at different positions along its length as a function of the frequency of the sound vibration. The basilar membrane, and the hair cells that sit on top it, are thus tonotopically organized such that cells along its basal end respond best to high frequencies and cells at its apical end respond to low frequencies.

High-frequency sound detection requires specialized structures and molecules

There are two classes of hair cell within the mammalian cochlea: inner hair cells and outer hair cells. These two cell types are spatially segregated into one row of inner hair cells and three rows of outer hair cells. Inner and outer hair cells each have distinct morphological and innervation characteristics as well as highly divergent functions. The inner hair cells are the actual sensory cells of the cochlea and are responsible for relaying sound information to the brain. The more abundant outer hair cells, on the other hand, mainly act as mechanical amplifiers by converting changes

in their membrane potential into a mechanical change in their cell length. Outer hair cells also receive efferent input from the brain, which allows neural control over auditory amplification.

The specialized function of outer hair cells in the mammalian cochlea is thought to have evolved out of a selective pressure to improve sound detection and localization at higher frequencies (Heffner & Heffner, 2008). High-frequency sounds are severely damped by the viscosity of cochlear fluids, posing a challenge for the detection of these sounds, particularly at low levels. By acting as "cochlear amplifiers," outer hair cells allow the mammalian cochlea to overcome these limitations by increasing both the amplitude and frequency selectivity of basilar membrane vibrations. Upon deflection of their hair bundles, outer hair cells produce a longitudinal contraction along their cell bodies, which serves to shorten the cell and pull up on the basilar membrane (Fig. 53-5). Outer hair cells achieve this somatic electromotility through the expression of a voltage-sensitive membrane protein called prestin along the lateral cell walls (Fig. 53-5). While prestin (SLC26A5) is a member of the SLC26A family of anion transporters, it does not appear to function as a transporter. Prestin does, however, contain an anion-binding site that can bind intracellular chloride ions, which induces a conformational shift in the protein that increases its surface area and elongates the cell. Thus, in the hyperpolarized state, chloride ions are thought to be bound to prestin, maintaining an elongated hair cell; in the depolarized state, chloride ions dissociate from prestin, prestin reverses its conformation, and the hair cell shortens. The mechanical response of these cells is extremely rapid, and thus outer hair cells can potentially

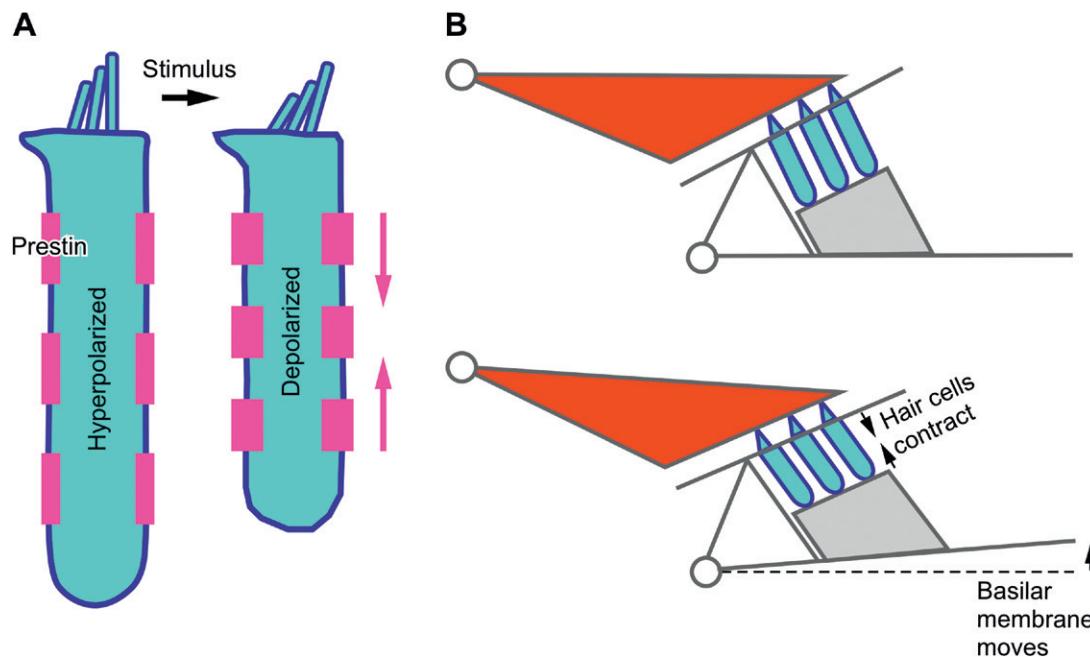


FIGURE 53-5 Prestin electromotility. **A**, When an outer hair cell is hyperpolarized, prestin (magenta boxes) has a large cross-sectional area in the membrane. Depolarization, such as happens after a mechanical stimulus, causes prestin membrane area to decrease. Sufficient levels of prestin are expressed in outer hair cells and the cytoskeleton is arranged such that this area-decrease shortens the cell. **B**, Contraction of outer hair cells can move the basilar membrane. Figure is after Fig. 5 of Fettiplace & Hackney, 2006.

alter their length in synchrony with the sound stimulus, and thereby amplify basilar membrane vibrations for all frequencies in the auditory range.

Cochlear hair cell mechanotransduction is similar to that of vestibular hair cells

While inner and outer hair cells have distinct morphological and functional features, the general mechanism of mechanotransduction within their hair bundles is thought to be very similar. Indeed, the mechanotransduction apparatus appears to be highly conserved among the cochlear and vestibular hair cells in the inner ear; for example, mutations in many of the molecules known to be essential for hair cell transduction lead to both auditory and vestibular deficits in humans and/or mouse models (see Box 53). These include mutations in MYO7a, CDH23, PCDH15 and harmonin. Many key transduction molecules are also known to have similar localization patterns within both vestibular and cochlear hair bundles. Despite these similarities, it is likely that differences in protein isoform expression will emerge between hair cells of varying types as more of the major transduction proteins continue to be identified.

CONCLUSIONS

Although not all hair-bundle proteins have been identified, many of these clearly play important roles for transduction and bundle structure. The increasing speed of identification of important proteins suggests that a thorough accounting of the major transduction proteins for hair cells should not be too far off. The next and more interesting phase of characterization of hair-cell transduction will then ensue, determining how these molecules interact and how the hair cell assembles them during development and following normal protein turnover in order to make a remarkably sensitive transduction apparatus.

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