Published in final edited form as:
Cell. 2009 October 2; 139(1): 33–44. doi:10.1016/j.cell.2009.09.010.

Mechanotransduction by Hair Cells: Models, Molecules, and Mechanisms

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Abstract

Mechanotransduction, the transformation of mechanical force into an electrical signal, allows living organisms to hear, register movement and gravity, detect touch, and sense changes in cell volume and shape. Hair cells in the inner ear are specialized mechanoreceptor cells that detect sound and head movement. The mechanotransduction machinery of hair cells is extraordinarily sensitive and responds to minute physical displacements on a submillisecond timescale. The recent discovery of several molecular constituents of the mechanotransduction machinery of hair cells provides a new framework for the interpretation of biophysical data and necessitates revision of prevailing models of mechanotransduction.

Hair cells reside in the auditory and vestibular systems of the vertebrate inner ear and in the lateral line organ of fish. These cells carry out mechanotransduction, the conversion of a mechanical stimulus into an electrical response, which is then processed by the central nervous system (for recent reviews, see Vollrath et al., 2007; Fettiplace and Hackney, 2006). Hair-cell transduction is astonishingly sensitive, yet the ear can respond to sounds over an extremely wide intensity range; this dual capability allows the organism to detect and internally represent both faint and intense environmental mechanical disturbances such as sound, head movements, and fluid motion, adding to the richness of sensory information and allowing for efficient communication.

Mechanotransduction and Adaptation

The mechanically sensitive organelle of the hair cell is the hair bundle, a cluster of ~100 actin-filled stereocilia and, in immature and vestibular hair cells, an axonemal kinocilium (Figure 1). Hair cells respond to deflections of their hair bundles by opening and closing transduction channels. Bundles are extraordinarily sensitive to deflection, responding maximally to an ~1° angular deflection (Corey and Hudspeth, 1983). At the threshold of hearing, bundles are deflected by less than 1 nm (Rhode and Geisler, 1967). Because transduction channels are cation selective (with a substantial preference for Ca^{2+}) and because hair cells sit at a resting potential of about −60 mV, channel opening induces an inward current. When all transduction channels open, their total conductance dominates other ion channels and the cell depolarizes toward ~0 mV; depolarization activates neurotransmitter release at the base of the hair cell and conveys the hair cell's excitation to the central nervous system.

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Transduction Channel Features

Hair cells respond best to stimuli directed toward the gradient of stereocilia height. In the absence of a stimulus, channels flicker between the open and closed states, with a probability of being open, P_o, of ~0.1. Deflections that tilt the bundle toward the tallest stereocilia (positive deflections) induce transduction channels to spend more time open, whereas deflections toward the shortest stereocilia (negative deflections) close channels. By contrast, perpendicular stimuli have little effect on channel gating (Shotwell et al., 1981).

Transduction channels open exceptionally rapidly. In hair cells of the bullfrog sacculus (part of the vestibular system), channels can open within ~10 μs (Corey and Hudspeth, 1979), with larger deflections gating channels much more quickly (Corey and Hudspeth, 1983). The speed of channel opening puts considerable constraints on the mechanism of transduction. Neither enzymatic conversion nor diffusion are fast enough to account for transduction (Corey and Hudspeth, 1983).

The extent of transduction channel opening depends on stimulus size and is fit well with a three-state Boltzmann distribution (Corey and Hudspeth, 1983). In the most common interpretation, the channel's free energy increases as the deflection stretches the gating spring, an elastic element in series with the channel. The data are consistent with the assumption that the gating spring is a linearly elastic spring of stiffness ~1 mN/m, and that there are two closed states and one open state of the channel (Corey and Hudspeth, 1983). As gating-spring stretch raises the energy of the closed states, the transduction channel's open state is favored, allowing a flush of ions to enter the cell. Notably, a direct transduction mechanism like this allows for a reciprocal interaction of gating and stimulus. Gating of the channel independent of a stimulus allows for reverse transduction, where a mechanical stimulus is sent out from the inner ear.

Although each stereocilium has few active transduction channels, their pores are large. The most rigorous determination of channel number found ~1.5 channels per stereocilium (Ricci et al., 2003), perhaps a modest underestimation if some channels were damaged during tissue isolation; the data are certainly consistent with two channels per stereocilium. Several estimates place the single-channel conductance at ~100 pS (Crawford et al., 1991; Géléoc et al., 1997). Interestingly, in the turtle auditory system, there is a systematic increase in conductance, increasing from ~100 to ~300 pS, between the low-frequency end of the cochlea and the high-frequency end (Ricci et al., 2003). This result suggests that the channel might consist of several different subunits, with a varying composition along the length of the cochlea.

Thus the hair cell's transduction channels are defined by their scarce numbers, which has made them difficult to find; their mechanical gating, which permits remarkable sensitivity and allows reverse transduction; their directional gating, which allows them to encode additional features of a stimulus; and their passage of prodigious numbers of ions, which allows the channels considerable control over the cell's membrane potential and subsequent auditory or vestibular nerve excitation.

Purpose of Adaptation

Both auditory and vestibular hair cells adapt to mechanical stimuli on fast and slow timescales (LeMasurier and Gillespie, 2005). Because "fast" adaptation in vestibular hair cells can be slower than "slow" adaptation in cochlear hair cells, the relationship between the various forms of adaptation is not clear. Moreover, requirements for adaptation are likely to vary between auditory hair cells, which respond to periodic stimuli, and vestibular hair cells, which largely respond to static hair-bundle deflections. Adaptation's traditional meaning, restoration of sensitivity by decreasing the response to a maintained stimulus, is clearly relevant for vestibular hair cells. However, two other universal roles for adaptation in hair cells have been proposed.

First, adaptation sets the resting tension of the transduction channel. Given the Boltzmann relationship between tension and channel opening, a modest resting tension is essential to position channels near their most sensitive point. As a consequence, both positive and negative deflections change the current and the hair cell's transducer has no threshold (Hudspeth, 1992). In addition, the reciprocity of channel gating allows the bundle's adaptation mechanisms to control mechanical amplification by hair cells (LeMasurier and Gillespie, 2005).

Fast Adaptation

Fast adaptation occurs when Ca^{2+} enters transduction channels, then closes them within a few milliseconds or less. In auditory hair cells of the turtle, mouse, and rat, fast adaptation dominates the decline in current that follows a deflection. Fast adaptation is also present in vestibular hair cells, although its presence was not appreciated initially because early studies used relatively slow mechanical stimulators. In turtle auditory and frog vestibular hair cells, fast adaptation is associated with a hair-bundle movement opposite the direction of the stimulus, producing force that pushes back against the stimulus probe, consistent with tensioning caused by channel reclosure (Howard and Hudspeth, 1987; Ricci et al., 2000). Surprisingly, in rat cochlear hair cells, fast adaptation is associated with a bundle movement in the same direction as the stimulus, consistent with relaxation of the gating spring (Kennedy et al., 2005). These findings suggest that mechanisms of fast adaptation might differ between species and/or vestibular and cochlear hair cells.

Slow Adaptation

Slow adaptation also closes transduction channels, but on a different timescale and by a different mechanism than fast adaptation. In response to a positive deflection of the hair bundle, the hair bundle relaxes in the direction of the stimulus, corresponding to a reduction in gating-spring tension (Howard and Hudspeth, 1987). Ca^{2+} also influences slow adaptation, with its rate accelerated about 5-fold by the ion (Assad and Corey, 1992). As with fast adaptation, the rate of slow adaptation varies considerably between vestibular (time constants, or τ, of 20–50 ms) and cochlear (τ of ~10 ms) hair cells (Assad and Corey, 1992; Holt et al., 1997; Stauffer and Holt, 2007). The difference in slow adaptation rate may relate to their function as sensors for tonic and cyclic stimuli, respectively. In addition, it is possible that the underlying mechanisms for slow adaptation in vestibular and cochlear hair cells may differ.

The slow-adaptation mechanism, commonly referred to as the adaptation motor, is also thought to control the resting tension of the gating spring. Decreasing the concentration of Ca^{2+} increases the resting tension exerted by the motor, which results in hair-bundle movement in the negative direction (Assad and Corey, 1992). At rest, the tension exerted by the motor is ~10 pN, which can increase to ~25 pN under low-Ca^{2+} conditions (Jaramillo and Hudspeth, 1993).

Extent of Adaptation

Adaptation is not always complete; depending on the size of the stimulus and the preparation, transduction currents usually decline to 50%–90% of the peak. In the frog sacculus, the limits of adaptation have been examined carefully. There, the degree of adaptation is only ~80% (Shepherd and Corey, 1994). This behavior can be explained by invoking an extent spring, an elastic element that is in line with the gating spring and with ~20% of its stiffness, which limits the reduction in tension in the gating spring. Shepherd and Corey (1994) also showed a limit to adaptation following negative displacements and modeled the limit as a simple stop element that prevented the shift of the I(X) curve past a certain point. Alternatively, the extent spring and the negative limit may be the same molecular element (Yamoah and Gillespie, 1996).

Anatomy of the Hair Bundle and Tip-Link Complex

Hair cells differ fundamentally from other well-characterized mechanosensory cells, such as touch-sensitive neurons in the cuticle of nematodes, the skin of mammals, and the bristles and chordotonal organs of insects. Whereas transduction channels in mechanosensory neurons are localized to microtubule-based specializations, the mechanically sensitive stereocilia of a hair cell contain at their core stiff, hexagonally packed, unipolar, and highly crosslinked actin filaments (Tilney et al., 1992). Some of the actin filaments traverse the tapered base of the stereocilia and form rootlets, which anchor the stereocilia in the cuticular plate, a specialized actin-rich structure at the top of the cell body. Extracellular filaments such as tip, lateral, and ankle links connect the stereocilia into a bundle (Figure 1B) and contribute to bundle stiffness (Bashtanov et al., 2004).

Initial observations of hair-bundle motion in response to relatively large, low-frequency stimuli suggested that stereocilia within a bundle tilt around the pivots at the base and move as a unit (Hudspeth and Corey, 1977; Crawford et al., 1989). More recently, a high degree of coherence and negligible phase lag have been demonstrated in the movement of bullfrog saccular hair cells at subnanometer spatial and submillisecond resolution (Kozlov et al., 2007). Although hair-bundle morphology differs between species and between the cochlea and vestibule, it seems likely that coherence in stereociliary motion is a universal feature of hair bundles, ensuring high sensitivity to mechanical stimulation by synchronous gating of transduction channels across the bundle.

Ultrastructure of Tip Links

Tip links, the fine extracellular filaments that connect the tips of stereocilia to their next taller neighbors, are aligned with the bundle's axis of mechanical sensitivity and thus are appropriately positioned to participate in transduction-channel gating (Pickles et al., 1984). Indeed, agents that disrupt tip links abolish mechanotransduction; regeneration of tip links and recovery of mechanical sensitivity occur with a similar time course (Assad et al., 1991; Zhao et al., 1996). Tip links are composed of two strands that form a helix 150–200 nm in length with an axial periodicity of ~25 nm; each strand consists of globular structures ~4 nm in diameter. The two strands of the helix separate at the upper end of the tip link, with variation in the distance of the branch point from the membrane, indicating limited structural flexibility in the tip link. In some preparations, more than two filaments have been observed close to the membrane at the base of the tip link, suggesting that additional filaments may anchor the tip link (Kachar et al., 2000; Tsuprun et al., 2004).

Tip-Link Densities

Intracellular electron-dense areas, the lower tip-link density (LTLD) and upper tip-link density (UTLD), are juxtaposed to the lower and upper insertion site of tip links (Furness and Hackney, 1985) (Figures 1D–1F). Their positions relative to tip links and transduction channels suggest that these densities have important functions in mechanotransduction. A parallel might be the postsynaptic densities in neurons, which contain clusters of molecules essential for efficient synaptic transmission. The plasma membrane overlying the UTLD shows a characteristic cup-shaped indentation, indicative of tight coupling of the membrane to the electron-dense plaque (Figure 1D). The shape of the plasma membrane at the base of the tip link is equally remarkable; it pulls away from the UTLD in the direction of the longer stereocilium (Figure 1D). This "tenting" is not observed at the tips of the longest stereocilia and is lost when tip links are broken, indicating that it is a consequence of tip-link-mediated tension (Assad et al., 1991; Kachar et al., 2000; Schwander et al., 2009).

Identity and Localization of Molecules

Although hair-cell mechanotransduction has been studied in considerable detail by biophysical methods, description of the underlying molecular machinery has been slow in coming. Even today, the molecules that form the transduction channel are not known, although the channel's surprising location has recently been defined with high spatial resolution using Ca^{2+} imaging. A discrete number of molecules, including several cadherins, myosins, and scaffolding proteins, have also been tied to transduction and adaptation (Figure 2). Examination of these proteins reinforces the tip-link model of mechanotransduction and has revealed an unexpected molecular asymmetry in the transduction machinery, which suggests that the upper and lower ends of tip links are functionally distinct. With new understanding of the channel's location and its associated molecules, the transduction field is in considerable flux. We expect rapid progress toward identification of the channel and definitive resolution of the gating mechanism.

Localization of the Transduction Channel

Transduction channels are located at stereociliary tips. Hudspeth showed that a current sink present at stereocilia tips develops as transduction channels are opened (Hudspeth, 1982). These results indicated that the channels are within a micrometer or so of the beveled edge of the hair bundle. Subsequent mapping with channel blockers (Jaramillo and Hudspeth, 1991) and Ca^{2+} imaging (Lumpkin and Hudspeth, 1995; Denk et al., 1995) confirmed this observation. Because transduction-dependent Ca^{2+} entry could be observed in both the tallest and the shortest stereocilia, Denk and colleagues argued that channels are located at both ends of a tip link.

More recently, higher-resolution imaging of cochlear hair cells demonstrated compellingly that Ca^{2+} only enters the shorter stereocilium of a tip-link-connected pair, suggesting that transduction channels are only located at the base of the tip link (Beurg et al., 2008). This result was quite surprising, as most investigators believed that the channels would also be located at the upper end of the tip link, where they could rapidly influence the slow-adaptation motor thought to reside there. Beurg et al. suggested that Denk and collaborators saw fluorescent signals in the tallest stereocilia due to Ca^{2+} that entered from the base. Alternatively, deflection of the kinocilial links (made of cadherin 23 and protocadherin 15) might have gated Ca^{2+}-selective channels in the longest stereocilia. Because of the unexpected location of the channel and its consequences for adaptation mechanisms (see below), repetition of the Beurg et al. results in cochlear and vestibular hair bundles is essential for the proposed channel location to be fully accepted.

The molecules that form the transduction channel have remained a mystery. Members of the TRP channel family are important for the perception of several sensory stimuli in the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogastor*, including responses to mechanical signals. However, the extent to which TRP channels are directly gated by mechanical force and whether the transduction channel in hair cells belongs to this class of molecules is unclear (Vollrath et al., 2007). As the conductance of the transduction channel increases along the longitudinal axis of the cochlea, corresponding to sensitivity to increasingly higher frequencies, the channel likely consists of multiple subunits that endow it with a systematically increasing pore size. Unfortunately, the pharmacological and conductance properties of the channel are insufficiently distinct to suggest a strong candidate family (Farris et al., 2004). It is entirely possible that the transduction channel belongs to a new channel family, as was recently recognized with the store-operated Ca^{2+} channel (Lewis, 2007).

Tip-Link Cadherins

Although the tip-link filament appears in the electron microscope as a helix with no particular structural heterogeneity, it has an inherent molecular asymmetry. Two distant relatives of classical cadherins, cadherin 23 (CDH23) and protocadherin 15 (PCDH15), form tip links in vertebrates (Siemens et al., 2004; Ahmed et al., 2006). Although classical cadherins were originally identified as Ca^{2+}-dependent homophilic cell adhesion molecules, tip links are heterophilic adhesion complexes in which PCDH15 homodimers form the lower part and CDH23 homodimers the upper part of a tip link (Figure 1F) (Kazmierczak et al., 2007).

Tip-link cadherins differ in several fundamental ways from classical cadherins. Instead of the five extracellular cadherin (EC) repeats in classical cadherins, the extracellular domains of CDH23 and PCDH15 contain 27 and 11 EC repeats, respectively (Figure 2). The N-terminal EC1 domain of classical cadherins, and possibly EC2, contribute to lateral *cis*-interactions (Pokutta and Weis, 2007). In contrast, *cis*-homodimerization of CDH23 and PCDH15 involves extensive contacts between most if not all EC domains.

As in classical cadherins, adhesive *trans*-interactions between CDH23 and PCDH15 are mediated by EC1. However, tryptophan residues that are critical for *trans*-interactions between classical cadherins are not conserved, indicating that tip-link cadherins have evolved a specialized adhesive interface, which might be optimized to withstand mechanical force (Kazmierczak et al., 2007). Robust CDH23-PCDH15 interaction requires Ca^{2+} (Kazmierczak et al., 2007), which accounts for Ca^{2+}-chelator sensitivity of tip links. Presumably, Ca^{2+} in some way stabilizes the CDH23-PCDH15 interaction even in the presence of high force.

The Upper Tip-Link Density

Molecular asymmetry extends to the tip-link densities. Harmonin is a multi-PDZ domain protein that is expressed in several alternative spliced isoforms (Verpy et al., 2000). The longest isoform, harmonin b (Figure 2), is present in hair bundles and binds to myosin-7a (MYO7A), F-actin, and harmonin itself (Müller, 2008). Harmonin also interacts in vitro with CDH23 and PCDH15 but is concentrated in functionally mature hair cells at the UTLD (Figure 1F), suggesting that interactions with CDH23 are particularly important. Harmonin targeting is affected by mutations that disrupt interactions of harmonin with CDH23 and F-actin, indicating that harmonin provides at the UTLD a connection between the tip link and the cytoskeleton (Grillet et al., 2009). Harmonin localization is perturbed in MYO7A-deficient *shaker-1* mice, which also exhibit deafness, suggesting that MYO7A is involved in harmonin transport (Boeda et al., 2002).

Although the actin-based molecular motor myosin-1c (MYO1C) appears to be relatively broadly distributed in hair cells, immunogold localization studies show that it is concentrated at and above the UTLD (Figure 1F) (Garcia et al., 1998; Steyger et al., 1998). MYO1C binds to phosphatidlyinositol 4,5-bisphosphate (PIP_2), which is abundant in the membrane of stereocilia and important for mechanotransduction (Hirono et al., 2004). MYO1C also interacts in vitro with CDH23 (Siemens et al., 2004). Moreover, not only does recombinant MYO1C bind in an overlay assay to the tips of stereocilia (Cyr et al., 2002), but binding depends on calmodulin and CDH23 (Phillips et al., 2006), suggesting the possibility that calmodulin modulates at the UTLD interactions of MYO1C with CDH23.

The Lower Tip-Link Density

Several molecules have been localized to the very tips of stereocilia (Figure 1), including myosin-15a (MYO15A) and whirlin, which has a similar domain structure to harmonin (Probst et al., 1998;Mburu et al., 2003;Belyantseva et al., 2003,2005;Delprat et al., 2005). Myosin-3a (MYO3A) is localized at and below the LTLD (Schneider et al., 2006) and is the mammalian

ortholog of the *Drosophila* NINAC protein, which interacts with actin and PDZ proteins to organize the phototransduction machinery in ommatidia (Walsh et al., 2002). In hair cells, MYO3A interacts with the actin crosslinker espin (Salles et al., 2009). The extent to which these tip-localized proteins interact physically or functionally with PCDH15 at tip links is currently unknown.

Models for Transduction

All features of transduction can be accounted for by assuming that transduction channels are coupled directly or indirectly to tip links (Pickles et al., 1984; Hudspeth, 1992). Although the tip link was initially proposed to be the channel's gating spring, recent structural and molecular studies indicate otherwise. Hair-bundle deflection involves a mechanical gain of ~0.14 in bullfrogs; deflection of the tip of the bundle of 100 nm would stretch the gating spring by 14 nm. In experimental settings, hair bundles are frequently deflected much further (Shepherd and Corey, 1994), indicating that the spring can stretch to a greater extent (>100 nm). This degree of stretching seems at odds with the tip link's compact coiled structure (Kachar et al., 2000). Indeed, modeling studies reveal only limited elasticity in the extracellular domains of classical cadherins (Sotomayor and Schulten, 2008), and tip links appear in the electron microscope as stiff filaments that buckle under strain (Kachar et al., 2000). In classical cadherins, binding of three Ca^{2+} molecules to each linker domain that connects adjacent EC repeats rigidifies the extracellular domain (Pokutta and Weis, 2007). Ca^{2+} also induces an extended conformation in the CDH23 and PCDH15 extracellular domains and promotes homodimerization and *trans*-interactions between them (Kazmierczak et al., 2007). A CDH23/PCDH15 hetero-tetramer contains 76 EC domains and 108 putative Ca^{2+}-binding sites. Depending on the affinity of different binding sites for Ca^{2+}, tip-link rigidity might be affected by the local Ca^{2+} concentration. Nevertheless, cadherin elasticity is likely very limited, which suggests that tip links are not the gating spring but in series with it.

How tip links open transduction channels is not clear, but two broad classes of models for channel coupling and gating have been proposed (Figure 3), which can explain the data and provide clues to the whereabouts of the gating spring.

The Tethered-Channel Model

In this model, the channel is connected to the cytoskeleton and to the tip link, so that forces in the tip link are propagated via protein-protein interactions to the channel (Figures 3A and 3B). Most models of transduction have employed the tether model, although the definitive localization of the channel to the base of the tip link has moved the position of the tether. As PCDH15 forms the lower part of the tip link, the model predicts that the transduction channel is tightly coupled to PCDH15. One of the structures in series with the channel, most likely the hypothetical tether of the transduction channel, is the gating spring. The tether could be an inherent feature of the channel or a molecule that binds to the channel. Although the presence of an intracellular filament between the tip link and underlying cytoskeleton has been proposed (Pickles et al., 1991), this observation has not been confirmed. The tethered-channel model easily explains why there are only 1–2 channels per stereocilia tip; each channel might interact with a single PCDH15 molecule. Limiting the number of tip links to one per stereocilium automatically would limit the number of channels. Using large-conductance channels, the cell could still respond with a large receptor potential.

The Lateral-Tension Model

In this model, the channel responds to tension in the stereocilia tip membrane. Increased tension in the tip link would then increase membrane tension and open channels (Figures 3C and 3D). For example, the mechanically sensitive channels of *C. elegans* are now thought to be gated

in this manner (Cueva et al., 2007). This model is plausible for hair cells given the location of the channels at stereocilia tips, where the force in the tip link pulls the membrane away from the cytoskeletal core (Assad et al., 1991).

Critical to this model is the magnitude of the resting tension of the membrane. A large membrane tension might leave transduction channels open most of the time, unable to respond to increases in tension mediated by the tip link. Prost and colleagues (Prost et al., 2007) suggest that the polymerization force of actin filaments makes this tension very high. However, they did not consider that the bulk of the filaments may be capped, with the polymerization force reduced proportionally. In any case, the force exerted on the membrane by the tip link (~10 pN at rest) is sufficiently high to distort the membrane; much larger forces may even pull the membrane into a tube. Consistent with this effect, stereocilia actin filaments are longer in the region of the tip link and undergo remodeling after tip links break (Rzadzinska et al., 2004). The data are thus consistent with the tip membrane of stereocilia having a moderate lateral tension, which can be increased significantly by external forces by way of the tip link. This is an optimal situation for a channel responsive to lateral tension. In this model, however, it is more difficult to envision how the number of channels can be limited to 1–2.

Ultimately, to distinguish between the tethered channel and lateral-tension models, it will be essential to identify the hair cell's transduction channel, define the proteins that bind to it, and test the mechanisms of channel gating in a reconstituted system. With several molecular components of the mechanotransduction machinery in hand, a directed search for the channel has become a feasible task. Viable approaches for identifying the channel include testing of plausible known channels, identification of putative channels in deafness-gene screens, and biochemical purification of channels from purified hair bundles using affinity reagents. All of these approaches are underway at present.

Models for Adaptation

For the past 20 years, slow adaptation has been modeled as resulting from a cluster of myosin molecules, together called the adaptation motor, which are attached to the upper end of the tip link and control tension in the gating spring (Figure 4) (Howard and Hudspeth, 1987;Gillespie and Cyr, 2004). At rest, the motor generates sufficient tension to partially open the transduction channel, which positions it near the point of optimal sensitivity. During a positive stimulus, the motor slides down stereocilia actin under tension conveyed by the gating spring; the kinetics of this process are controlled by Ca^{2+} entering through open transduction channels. When tension is reduced by a negative stimulus, myosin molecules climb to restore tension. As a result of slow adaptation, the transduction channel's conductance resets toward the resting value, restoring sensitivity during a sustained stimulus. Recent experiments, however, have called into question some of the features of this model.

Localization of the Adaptation Motor

During a 1 μm stimulus, the gating spring stretches 140 nm. Adaptation then reduces tension by 80%, which corresponds to a gating-spring slackening of ~110 nm (Shepherd and Corey, 1994). A movement this large most likely occurs at the upper attachment point of the tip link. Indeed, a preliminary report suggested that the UTLD, located there, could slide down the stereocilium in response to a large stimulus (Shepherd et al., 1992). Moreover, the presumptive adaptation motor, MYO1C, is concentrated in this region (Garcia et al., 1998; Steyger et al., 1998).

This location for the adaptation motor made sense when the channel was thought to be located nearby; Ca^{2+} entering through the transduction channel could readily bind the Ca^{2+}-sensitive adaptation motor (Assad and Corey, 1992). Localization of the channel only at the lower end

of the tip link demands a new model for Ca^{2+} regulation of the adaptation motor. We suggest that Ca^{2+} entering through a transduction channel will affect the adaptation motor hooked up to the next tip link lower down in the same stereocilium (Figure 5). An important conclusion is that because the tallest row of stereocilia does not admit Ca^{2+} through transduction channels, adaptation motors in those stereocilia must see minimal changes in Ca^{2+} during stimulation and are not likely to show Ca^{2+}-dependent adaptation.

In turtle hair cells, Ca^{2+} diffuses substantially farther to affect both resting channel conductance and slow adaptation than it does to mediate fast adaptation (Ricci et al., 1998; Wu et al., 1999). Indeed, Ca^{2+} likely diffuses several hundred nanometers to affect the slow-adaptation motor, which is consistent with the distance from the base of a tip link at the top of a stereocilium to the upper end of the next tip link lower down the side of the same stereocilium (Figure 5). Moreover, MYO1C appears to be distributed several hundred nanometers above the upper tip-link insertion in bullfrog saccular hair cells (Garcia et al., 1998), reducing the distance Ca^{2+} needs to diffuse (Figure 5). Interestingly, the distance from the tip of a stereocilium to the upper end of the tip link down the side of that same stereocilium varies substantially. For example, in turtle hair cells, which show faster overall adaptation, this distance is short (~0.4 μm) (Hackney et al., 1993), whereas in slower-adapting frog saccular hair cells, this distance is longer (~1 μm) (Jacobs and Hudspeth, 1990). Thus a hair cell may control both its resting transduction channel conductance and its rate of slow adaptation by adjusting the tip-to-tip distance between adjacent stereocilia.

MYO1C Participates in Slow Adaptation

As the adaptation motor can exert force to move a hair bundle (Assad and Corey, 1992), at least one component of the motor is likely to be a myosin, the only motor proteins that work on actin filaments. Indeed, resting channel conductance and adaptation rate can be dramatically influenced by dialysis of hair cells with agents that affect myosin motors (Gillespie and Hudspeth, 1993; Yamoah and Gillespie, 1996). Although the location of MYO1C near the upper end of the tip link implicates it in slow adaptation (Garcia et al., 1998; Steyger et al., 1998), the most compelling evidence for a role for MYO1C in adaptation comes from introduction into hair cells of mutant MYO1C motors that are sensitized to ADP analogs (Holt et al., 2002; Stauffer et al., 2005). As the appropriate ADP analogs slow adaptation in hair cells with mutant MYO1C but not control hair cells, MYO1C is associated clearly with slow adaptation.

The suggestion that adaptation (and thus setting of resting tension) will vary between stereocilia pairs is admittedly unsettling. Because a hair cell will be maximally sensitive when all transduction channels are in the same mechanical state, differing motor force between bundle transduction complexes will broaden the displacement-response curve and make the cell less sensitive. Although it is conceivable that slow adaptation occurs at the lower end of the tip link, it is difficult to incorporate MYO1C into such a model. Reconciling the Ca^{2+} entry results of Ricci and coworkers with the role and location of MYO1C is thus a critical issue to be addressed in the field.

Harmonin and Slow Adaptation

Previous models suggested that clustered MYO1C molecules compose the UTLD, which controls the position of the transduction complex along the stereocilium (Gillespie and Cyr, 2004). Recent findings have challenged this interpretation, however, and have implicated the multi-PDZ-domain protein harmonin in slipping adaptation. Harmonin is a component of the UTLD (Grillet et al., 2009) and binds in vitro to both CDH23 and F-actin, suggesting that it links tip-link CDH23 to the cytoskeleton. Deaf circler (*dfcr*) mice express a mutant harmonin protein that lacks the actin-binding domain. Bundle morphology and tip links are preserved in

dfcr mice, but the UTLD is absent. The resting conductance of the transduction channel is reduced and the current displacement function shifted rightward and broadened (Grillet et al., 2009).

No simple model can explain the function of harmonin in mechanotransduction, but the results suggest a central role for harmonin in slow adaptation. Given its actin-binding capacity (Boeda et al., 2002), harmonin itself could be a damping element responsible for slipping adaptation. If so, MYO1C would act in parallel as a force producer, controlling channel conductance at rest. However, this model cannot explain why slow adaptation in cochlear hair cells of *dfcr* mice is slower than that in wild-type mice. Perhaps harmonin also regulates, directly or indirectly, force production by MYO1C. Alternatively, it could increase the sensitivity of MYO1C to Ca^{2+}, facilitating detachment of the motor complex from the cytoskeleton under tension. Harmonin's presence appears to be required for a cup-like curvature of the membrane at the UTLD (Grillet et al., 2009), which could be required for appropriate engagement of MYO1C motors.

Harmonin appears at the upper end of the tip link relatively late during development of cochlear hair cells (Lefevre et al., 2008), and its presence is correlated with an increased rate of fast adaptation, increased channel conductance at rest, and increased sensitivity of transduction (Waguespack et al., 2007; Lelli et al., 2009). This correlation suggests that harmonin is required for maximal sensitivity of cochlear hair cells.

Indeed, harmonin's late appearance at the UTLD, which requires MYO7A (Boeda et al., 2002), may explain the observation that mice mutant for MYO7A show shifted displacement-response curves and altered adaptation (Kros et al., 2002). This physiological phenotype closely resembles that of the *dfcr* mice, suggesting that MYO7A is not a direct player in adaptation but instead is necessary for transport of molecules essential for normal transduction, such as harmonin (Gillespie, 2002).

Location and Mechanism of Fast Adaptation

The fast-adaptation mechanism clearly is associated intimately with the transduction channels. Because fast adaptation depends absolutely on Ca^{2+} entry through transduction channels, occurs within a few hundred microseconds, and is not slowed by Ca^{2+} buffers with intermediate association rates, the adaptation mechanism must be within ~20 nm of the pore (Ricci et al., 1998; Wu et al., 1999). The fast mechanism must be at the lower end of the tip link if the channels are located there. Two models have been proposed for fast adaptation, the channel-reclosure model and the tension-release model.

Channel-Reclosure Model

In the reclosure model, Ca^{2+} entering through the transducer channel binds to the channel and changes the relationship between gating-spring tension and channel conductance at rest. In support of this model, fast adaptation in turtle and frog vestibular hair cells is associated with a hair-bundle movement opposite the direction of the stimulus, producing force that pushes back against the stimulus probe. Models arising from this observation suggest that Ca^{2+} binds to the channel and makes it more difficult to open (Howard and Hudspeth, 1987; Ricci et al., 2000). However, the reclosure model is inconsistent with some studies in frog and rat hair bundles (see below) and a recent analysis of transduction in MYO15A-deficient *shaker-2* mice. Deflection of *shaker-2* hair bundles leads to the activation of transduction current with normal amplitude. Slow adaptation appears unaffected, but inner hair cells no longer exhibit fast adaptation. These findings are most easily explained with structural changes that are caused by loss of MYO15A and uncoupling of the transduction channel from a Ca^{2+}-sensitive release element; alternatively, perhaps there very short bundles were not deflected along their

sensitivity axis as supposed. Curiously, adaptation in outer hair cells (the electromotile cells involved in cochlear amplification of sound vibration in mammals) is not affected, indicating a differential requirement for MYO15A in the two hair cell types (Stepanyan and Frolenkov, 2009).

Tension-Release Model

In this model, Ca^{2+} binds to a site near the channel and causes the release of a mechanical element that is in series with the transduction apparatus. As a result of a decrease in tension, the channel closes rapidly. This model is supported by the biphasic mechanical response of frog hair bundles to transepithelial stimulation; Ca^{2+} enters stereocilia and binds to and decreases the stiffness of an elastic reclosure element allowing the hair bundle to move in the positive direction. As a result, gating-spring tension is reduced, leading the channel to close. As the channels close, gating-spring tension increases modestly, pulling the bundle into the negative direction (Bozovic and Hudspeth, 2003; Martin et al., 2003). The release model is also consistent with the observation that fast adaptation in rat cochlear hair cells is associated with a bundle movement in the same direction as the stimulus (Kennedy et al., 2005). The role of the release mechanism in frog hair cells is controversial, however, as one thorough examination of frog hair-cell mechanics failed to reveal a significant contribution by this mechanism (Cheung and Corey, 2006).

MYO1C has been proposed to be the release element. Using hair cells expressing a mutant MYO1C motor that is sensitized to ADP analogs, Stauffer et al. (2005) demonstrated that an allele-specific MYO1C inhibitor significantly reduces the rate of fast adaptation. Based on these results, fast adaptation was interpreted to arise from a conformational change in the motor that relieves tension. These data need reinterpretation in light of the observation that Ca^{2+} enters transduction channels at the LTLD, far from the proposed side of action for MYO1C at the UTLD. Instead, fast adaptation may be perturbed in mice mutant for MYO1C because of alterations in resting tension, which is subtly elevated.

Amplification

In the auditory system, although hair cells respond to deflections of only ± 100 nm, they can encode stimulus amplitudes that range over six orders of magnitude, corresponding to a 10^{12} range in stimulus power. Similarly, vestibular hair cells can detect mechanical oscillations of peak acceleration 10^{-6}-fold that of *g*, the constant acceleration of gravity felt by these cells (Narins and Lewis, 1984). This remarkable sensitivity requires nonlinear amplification, where low-intensity signals are amplified prior to hair-cell detection whereas high-intensity signals are not (Hudspeth, 2008). Amplification is also remarkably frequency specific, giving rise to extraordinary frequency discrimination.

Outer Hair Cells and Amplification

Hair cells in the cochlea are patterned in three rows of outer hair cells (OHCs) and one row of inner hair cells (IHCs). They sit on the basilar membrane, which oscillates in response to sound. Sound induces basilar-membrane oscillations, with high frequencies exciting the base and low frequencies the apex. As far back as 1948, Gold predicted that the cochlea would require an active feedback mechanism for sound amplification (Gold, 1948). Without an active mechanism, viscous damping would dissipate most of the sound energy. The underlying active process, the so-called cochlear amplifier, depends on the function of OHCs, which add energy back into the system to enhance the basilar-membrane motions, where OHCs along the cochlear duct are tuned to specific frequencies (Hudspeth, 2008).

Somatic Electromotility

Two mechanisms have been proposed for cochlear amplification in mammals: somatic electromotility (Dallos, 2008), whereby the soma of an OHC changes length in response to membrane potential, and active hair-bundle movement, in which bundles respond to a stimulus by producing force (Fettiplace, 2006; Hudspeth, 2008). Electromotility has been observed in OHCs of several mammalian species, but not in IHCs or in hair cells from nonmammalian species. It is caused by voltage-dependent changes in the conformation of molecules of prestin, an ion-transporter relative expressed in the lateral wall of OHCs but not IHCs (Dallos, 2008). In support for a role of prestin in cochlear amplification, sound amplification and frequency discrimination are lost in mice lacking prestin or expressing a mutant prestin protein devoid of electromotility (Liberman et al., 2002; Dallos et al., 2008). An additional line of evidence stems from the study of mice with a mutation affecting the tectorial membrane, which is connected with the hair bundles of OHCs but not IHCs. In these mice, stereocilia do not contribute to amplification near threshold (Drexl et al., 2008). However, basilar-membrane motions can be elicited by electric stimulation. These motions are nearly indistinguishable from acoustically or electrically elicited responses in wild-type mice, providing strong evidence that somatic motility alone can cause enhancement of basilar-membrane motions (Drexl et al., 2008).

Active Hair-Bundle Motility

Active hair-bundle motility has been detected in bundles of mammalian, amphibian, and reptilian hair cells, and this form of motility is thought to be universal in hair cells (Fettiplace, 2006; Hudspeth, 2008). In vitro, spontaneous and evoked bundle movements occur that lead to force production. For example, in frog and turtle hair cells, deflection of hair bundles with a flexible stimulus probe evokes a bundle twitch that produces work opposing the probe deflection. If the timing of the twitch is appropriate, the work exerted by the bundle could augment an oscillatory stimulus and lead to amplification.

Hair-bundle active motility requires the influx of Ca^{2+} through transduction channels and seems to be linked inextricably with fast adaptation (Ricci et al., 2000). Because the timing of bundle motility is influenced by the extracellular Ca^{2+} concentration as well as by intracellular factors, this form of motility is thus intrinsically frequency selective. By contrast, prestin-based electromotility is not. It is conceivable that bundle and somatic motility cooperate to compose the cochlear amplifier (Fettiplace, 2006).

The Future

The hair-cell transduction field is at a critical juncture. The most pressing question, what are the molecules that make up the transduction channel, may well be solved in the next few years. Identification of the channel will allow us to determine how the channel's conductance is controlled. Critically, is the channel tethered or does it respond to lateral membrane tension? If the former is true, what is the identity of the tether, and is it the gating spring? Fast adaptation clearly is associated with the channel, so how is it controlled?

At the other end of the tip link, the relationship between CDH23, harmonin, and MYO1C must be clarified. In addition, Ca^{2+} diffusion from stereocilia tips to the site of slow adaptation needs to be mapped in OHCs, particularly those with longer tip-to-tip spacing. Because there should be little control of Ca^{2+}-dependent MYO1C in the tallest row (of only two) of stereocilia in cochlear hair cells, is MYO1C-dependent slow adaptation absent from these cells? Does MYO1C nevertheless control resting tension and if so, how is it controlled?

In addition, what is the mechanism of amplification in hair bundles? Is it the same for auditory and vestibular hair cells, and for mammalian and nonmammalian hair cells? What is the contribution of bundle-based amplification to the active process in cochlear hair cells?

Finally, molecular detail has lagged biophysical detail, largely for technical reasons. Improved methods for localization of molecules, including subdiffraction imaging, will have a major impact on our understanding of transduction. Delivery of foreign DNA, RNA, and protein to hair cells has proven challenging (for a notable success, see Gubbels et al., 2008). However, this technique would facilitate molecular dissection of transduction. Critically, the field must achieve routine delivery of RNA interference constructs coupled with measurements of mechanotransduction. More sophisticated approaches allowing functional manipulation of molecules (Stauffer et al., 2005) will be even more important in the long run.

In its most basic sense, mechanotransduction describes a phenomenon in which mechanical force is an allosteric modulator of protein structure. A transduction channel is coupled with multiple mechanical elements, which together exert force on the channel. This force must lead to an atomic rearrangement within the channel, which opens its pore. Adaptation leads to channel reclosure and terminates ion flux. An ultimate understanding of mechanotransduction therefore requires an understanding of the mechanisms by which mechanical force affects protein structure. Currently, these changes are inferred from indirect measures, such as the measurements of transducer currents or the forces exerted by movement of hair bundles. Ultimately, however, more direct measures are desirable.

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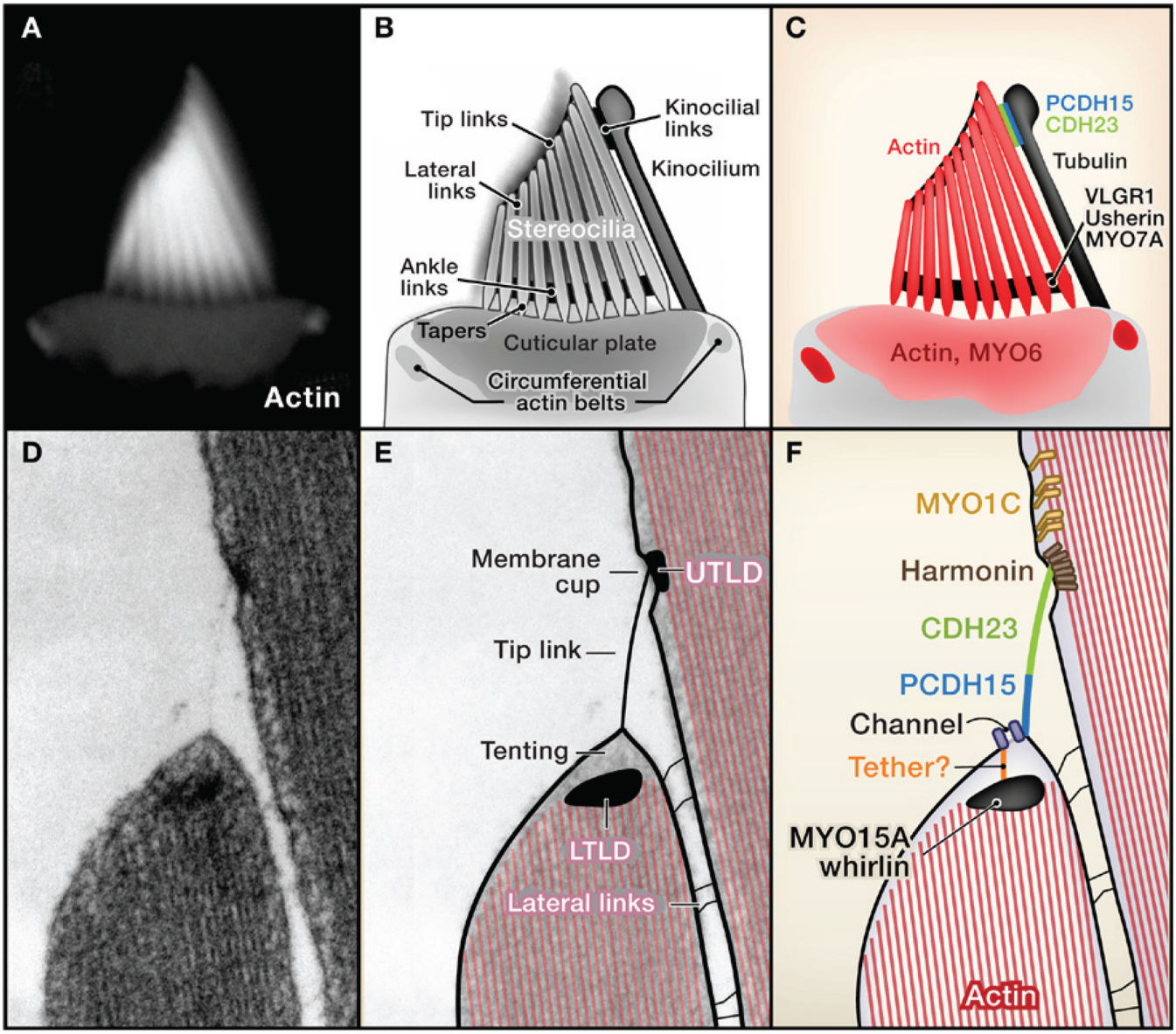

Figure 1. Anatomy of the Hair Bundle and the Transduction Apparatus

(A) Hair bundle of an isolated bullfrog hair cell, labeled with phalloidin to highlight F-actin.

(B) Key hair-bundle structures overlaid on the image from (A). Hair bundles consist of actin-rich steroecilia and a microtubule-based kinocilium, not visible in (A). The kinocilium is not required for mechanotransuction and absent in mature cochlear hair cells.

(C) Key molecules of the hair bundle. Protocadherin 15 (PCDH15) and cadherin 23 (CDH23) form kinociliary links between the kinocilium and the longest stereocilia, as well as the tip links that connect stereocilia. The very large G protein-coupled receptor 1 (VLGR1) and usherin are localized at the base of stereocilia, where they are thought to form ankle links. Ankle links are present in vestibular hair cells and transiently during development in mammalian auditory hair cells; because they lose the kinocilium, mammalian auditory hair cells also lose their kinocilial links. Myosin 6 (MYO6) is highly concentrated in the cuticular plate at the apical hair cells surface but is also localized to stereocilia. MYO7A is expressed throughout stereocilia and, in some auditory and vestibular epithelia, is enriched at ankle links.

(D) Transmission electron micrograph of a stereocilia pair showing a single tip link. Image courtesy of R.A. Jacobs and A.J. Hudspeth.
(E) Features of the tip link and its anchor points overlaid on image from (D).
(F) Key molecules associated with the tip link. Note that MYO15A and whirlin extend beyond the lower tip-link density (LTLD), as they localize near the ends of all stereocilia actin filaments.

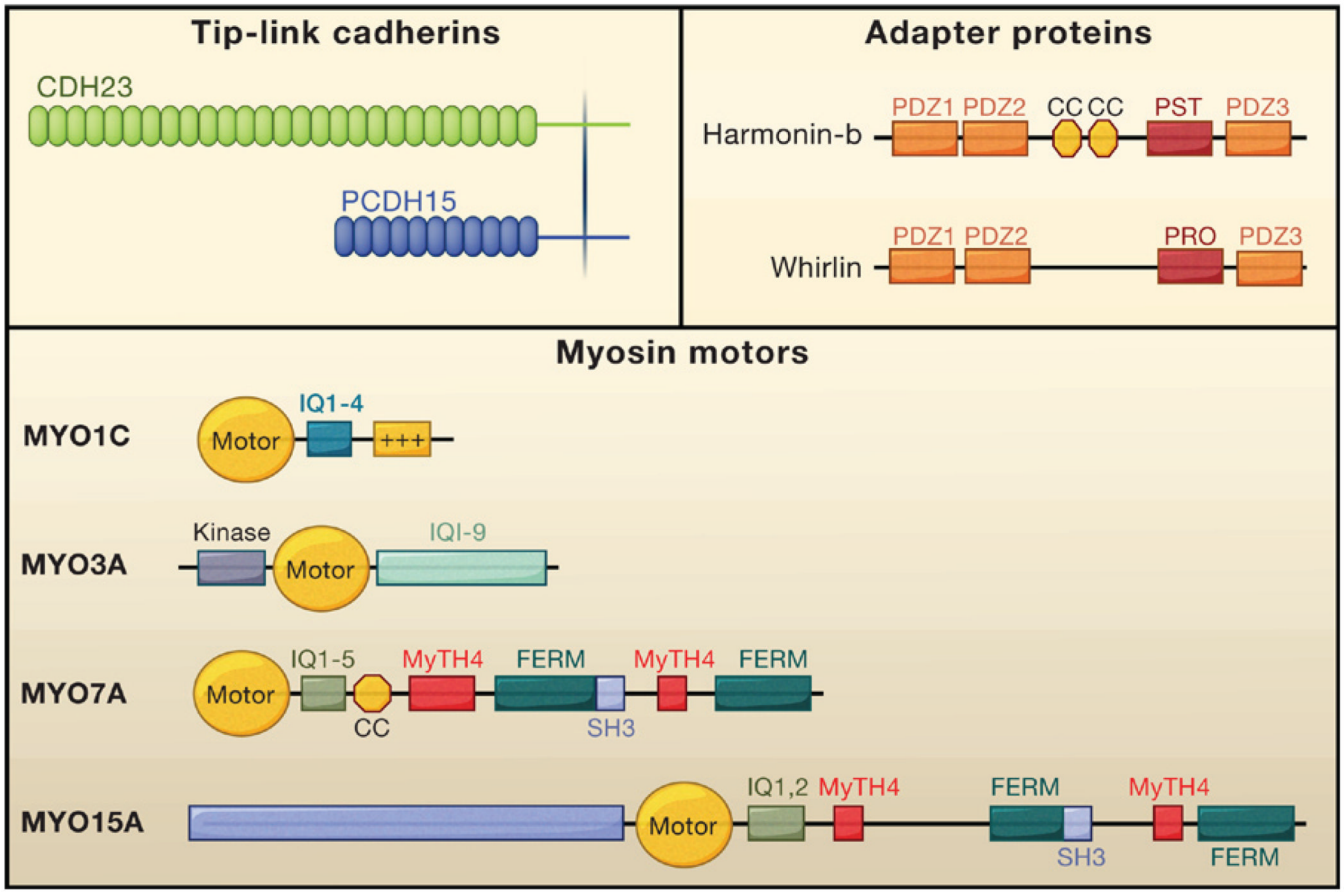

Figure 2. Structures of Key Hair-Bundle Proteins

The domain structure of molecules discussed in the Review is indicated. Abbreviations are as follows: 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.

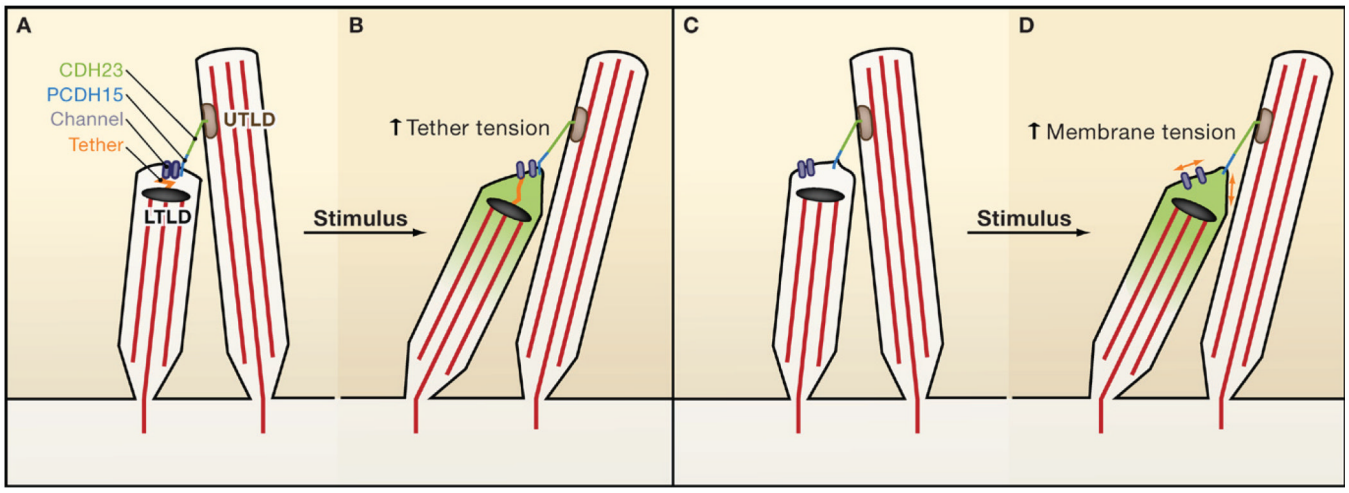

Figure 3. Transduction-Channel Gating Mechanisms

(A and B) Tether model. Here, the transduction channel binds directly to protocadherin 15 (PCDH15) and to the tether, presumably by strong interactions. Green fill indicates ion flux as channels open.

(C and D) Membrane-tension model. By contrast, the transduction channel is not attached to PCDH15 and instead feels lateral membrane tension, elevated by bundle deflection.

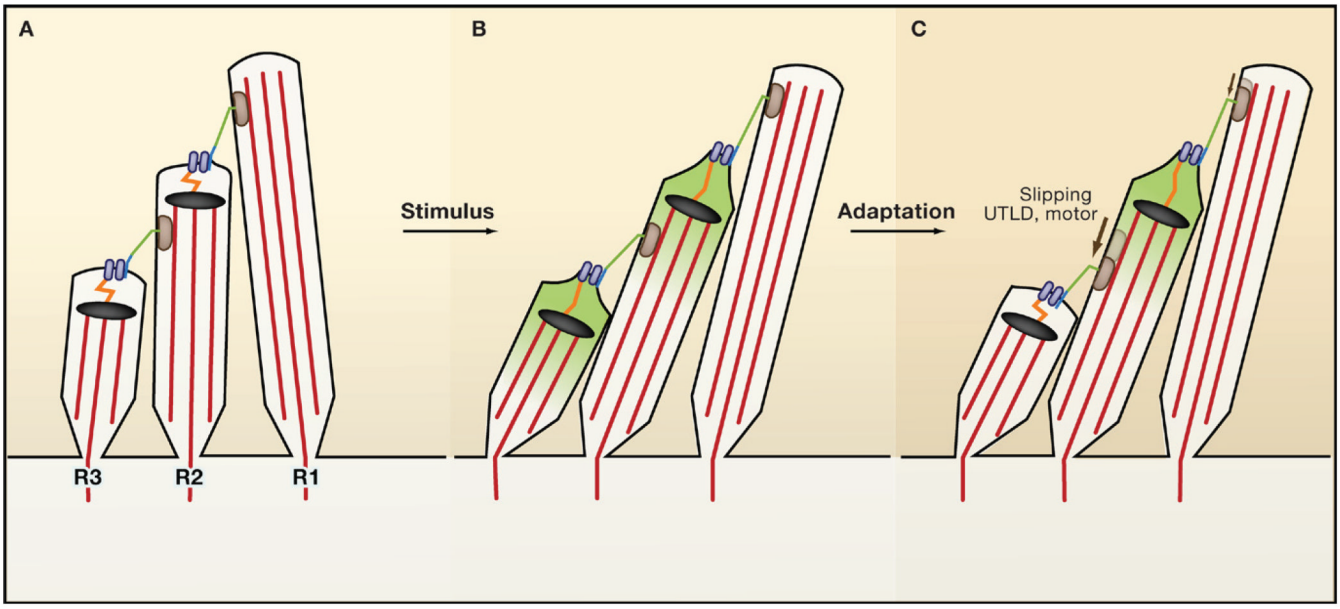

Figure 4. Slow Adaptation

(A) Hair bundle at rest. Components and colors are the same as in Figure 3. R1–R3 indicate stereocilia rows from tallest to shortest.

(B) Stimulated bundle. Channels in R2 and R3 both open as gating-spring tension rises.

(C) Post-adaptation bundle. The upper tip-link density (UTLD) and myosin 1C (MYO1C), considered together to be the adaptation motor, slip down the cytoskeleton to relieve gating-spring tension. Because the motor's rate is accelerated 5-fold by Ca^{2+}, the motor in R2 slips farther than that in R1. Consequentially, the R3 channel closes but the R2 channel does not.

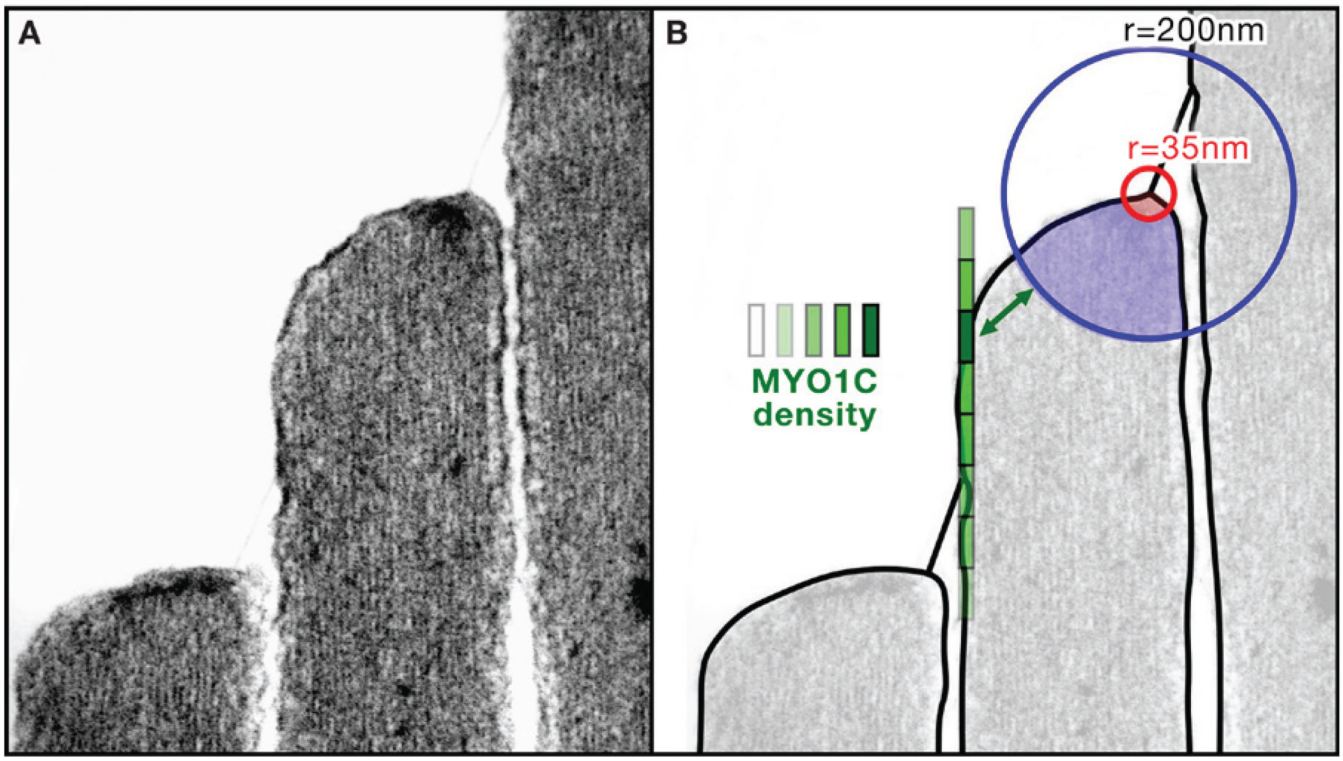

Figure 5. Diffusion of Ca^{2+} from Transduction Channels to Adaptation Sites

Although the distance from the transduction channel to the fast-adaptation site is very short, suggesting it is close to the tip link, the distance from the channel to the site regulating channel conductance at rest and slow adaptation is much greater. Thus it is plausible that the slow-adaptation motor could be regulated by Ca^{2+} diffusing from the very top of a stereocilium down to the upper end of a tip link.

(A) Transmission electron micrograph of a turtle cochlea hair cell showing two tip links. For distance calibration, stereocilia are ~400 nm in diameter and tip links are ~150 nm in length. Image courtesy of C. Hackney and R. Fettiplace.

(B) Image from (A) overlaid with outlines of key structures. The circle of radius 35 nm indicates the distance Ca^{2+} diffuses to influence fast adaptation (Ricci et al., 1998; Wu et al., 1999). The circle of radius 200 nm indicates the distance Ca^{2+} diffuses to influence channel conductance at rest (Ricci et al., 1998) and slow adaptation (Wu et al., 1999). In addition, the density of gold immunolabeling for myosin 1C (MYO1C) for frog saccular stereocilia is indicated; units are gold particles per μm^2 (Garcia et al., 1998). Because the distance from the upper end of a tip link to the top of the stereocilium is shorter in turtle cochlear hair cells than in frog saccular hair cells, the plotted MYO1C distribution extends beyond the top of the stereocilium.