Neuron, Vol. 48, 403–415, November 3, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.neuron.2005.10.017

Hair-Cell Mechanotransduction and Cochlear Amplification

Review

Meredith LeMasurier and Peter G. Gillespie*
Oregon Hearing Research Center and
Vollum Institute
Oregon Health & Science University
3181 SW Sam Jackson Park Road
Portland, Oregon 97239

In the inner ear, sensory hair cells not only detect but also amplify the softest sounds, allowing us to hear over an extraordinarily wide intensity range. This amplification is frequency specific, giving rise to exquisite frequency discrimination. Hair cells detect sounds with their mechanotransduction apparatus, which is only now being dissected molecularly. Signal detection is not the only role of this molecular network; amplification of low-amplitude signals by hair bundles seems to be universal in hair cells. "Fast adaptation," the rapid closure of transduction channels following a mechanical stimulus, appears to be intimately involved in bundle-based amplification.

The performance of the mammalian auditory system is awe inspiring. The cochlea, the organ responsible for auditory signal transduction, responds to sound-induced vibrations and converts these mechanical signals into electrical impulses, a process known as mechanoelectrical transduction. In turn, these impulses are decoded by the auditory centers of the central nervous system. Cochlear signal transduction involves the decomposition of complex auditory stimuli into their component frequencies, while faithfully transmitting their relative intensities and temporal distribution. The hallmarks of cochlear transduction are incredible sensitivity, versatility, and speed.

At threshold, we can detect signals with intensities less than one-billionth that of atmospheric pressure. Remarkably, to detect these minute signals, the cochlea amplifies them (Dallos, 1996). Amplification endows the human inner ear with a tremendous dynamic range; we respond to sound pressures spanning seven orders of magnitude. The frequency range of human hearing is also impressive; a healthy ear can detect sounds of 20–20,000 Hz. Across this span, the cochlea is a precise frequency analyzer, effectively discriminating closely related tones. Transduction is rapid, occurring within tens of microseconds; the minimal latency between stimulation and an electrical response is critical for the detection and encoding of high-frequency sounds. In this review, we focus on the role of the mechanotransduction machinery not only in sound detection but in signal amplification as well.

Anatomy and Excitation in the Inner Ear

The mammalian ear (Figure 1) is composed of the outer, middle, and inner ears (see Slepecky, 1996, for a review). The outer ear collects sound and funnels it via the ear canal to the tympanic membrane, commonly known as the eardrum. As oscillations in air pressure impinge upon the eardrum, its vibrations are faithfully transmitted to the middle ear's ossicles—the malleus, incus, and stapes. These tiny bones, the smallest in the human body, are directly coupled to the oval window, and so the ossicles' vibrations are conveyed to the inner ear. The pressure gain of the tympanic membrane-to-oval window transmission matches impedances of the compressible air in the ear canal and incompressible fluid in the inner ear, preventing loss from signal reflection.

The inner ear consists of three fluid-filled compartments—the scala vestibuli, the scala media, and the scala tympani (Slepecky, 1996). The scala vestibuli and scala tympani form a continuous cochlear duct with two windows opening on the middle ear, the oval and round windows. When vibrations are transmitted to the cochlear fluids, a pressure wave travels down the scala vestibuli from base to apex, through to the scala tympani and ending at the round window, which freely vibrates into the air-filled space of the middle ear. In the center of the cochlea is the scala media (Figure 1), separated from the scala vestibuli by Reissner's membrane and from the scala tympani by the basilar membrane. Although the scala media is not continuous with the scala vestibuli and scala tympani, they are acoustically coupled, and the pressure wave passing through Reissner's membrane is applied to the basilar membrane (Figure 3A). The basilar membrane is not a membrane per se, but rather is composed largely of extracellular matrix materials. The basilar membrane's mechanical properties vary with position along the cochlea; it is narrow and stiff at the base, then widens and increases in compliance toward the apex. These mechanical characteristics have a profound effect on the basilar membrane's response to vibrations; the amplitude of a pressure wave changes as it travels down the basilar membrane, with the peak amplitude's position dictated by the stimulus frequency (von Békésy, 1960). Accordingly, mechanical responses to auditory stimuli of high frequency peak at the base, while lower frequencies induce peak responses near the apex (Figure 3A).

Upon the basilar membrane sits the organ of Corti (Figure 1), containing the sensory cells of the auditory system, the hair cells, named for the tuft of stereocilia protruding from their apical surfaces (Slepecky, 1996). Humans have ~15,000–20,000 hair cells organized into one row of inner hair cells and three rows of outer hair cells (Figure 2A). The less abundant inner hair cells are the primary sensory cells of the auditory system, with 90%–95% of the cochlear afferent innervation; they provide the major input to the auditory centers of the central nervous system. The outer hair cells are endowed with many efferent synapses and play a central role in signal amplification, as discussed in detail below.

Hair cells are stimulated by basilar-membrane vibration, initiating mechanotransduction. The tectorial membrane, a gelatinous and fibrous extracellular matrix, overlies the hair cells, enclosing their apical surfaces in the subtectorial space, a small fluid-filled compartment.

*Correspondence: gillespp@ohsu.edu

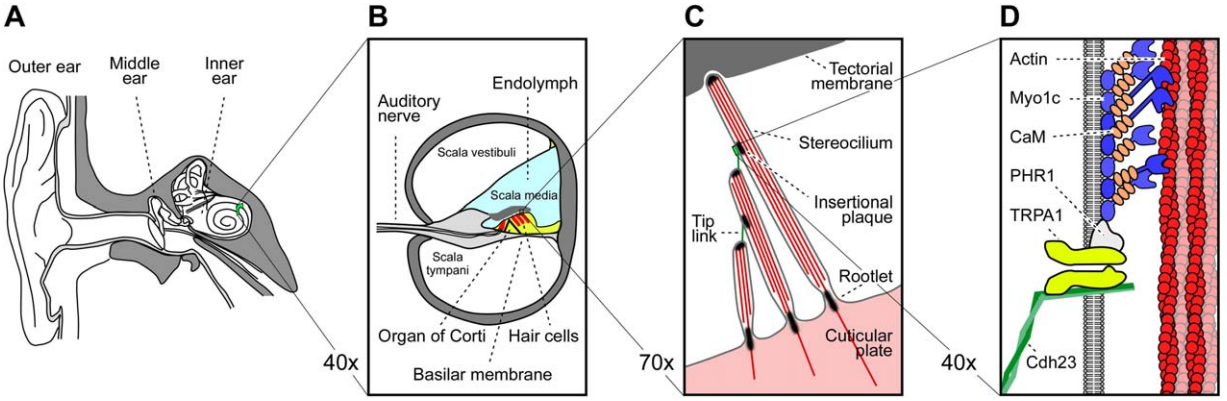

Figure 1. Anatomy of the Inner Ear

(A) Representation of the human auditory system and the relative positions of the outer, middle, and inner ears.

(B) Cross-section of the cochlear duct, indicating the auditory nerve, the three scala, with the scala media highlighted by the blue shading of its extracellular solution (the endolymph), and the organ of Corti (yellow), containing the sensory hair cells (red).

(C) Ultrastructural representation of the hair bundle. Three stereocilia are shown, with their actin filaments shaded red. At their proximal ends, the stereocilia taper and insert via their rootlets into the cuticular plate. The tip links connecting the tips of the shorter stereocilia to the sides of their taller neighbors are highlighted; the other stereociliary links have been omitted for simplicity. The tallest stereocilium is embedded in the overlying tectorial membrane.

(D) Representation of the molecular mechanotransduction machinery in a single stereocilium, highlighting candidates for key molecules: Cdh23 as the tip link, TRPA1 as the transduction channel, Myo1c as the adaptation motor with its associated calmodulin (CaM) light chains, and PHR1 as a crosslinking protein. The actin filaments are shaded red.

Outer hair cells are directly coupled to the tectorial membrane via their hair bundles and are stimulated by shear between the vibrating basilar membrane and the overlying tectorial membrane (Figure 3B). Inner hair-cell bundles are not embedded in the tectorial membrane and instead respond to fluid motion at their apical surfaces. Since traveling waves of different frequencies peak at different positions along the basilar membrane (Figure 3A), specific frequencies excite a certain subset of hair cells; hair cells at the base are stimulated by high frequencies, while those at the apex respond to low-frequency stimuli. Indeed, hair cells have a characteristic frequency to which they are most responsive, dictated by the mechanics of the basilar membrane and enhanced by the mechanical and electrical properties of the hair cells themselves.

Hair cells are not exclusively found in the cochlea but are also found in the adjacent vestibular organs. While auditory hair cells are stimulated by sound, vestibular hair cells are central to balance and the detection of movement. The mammalian vestibular system is composed of three semicircular canals, responsible for detection of angular accelerations, and the saccule and utricle, which detect linear accelerations. Although these hair cells differ in specialization and morphology, the overall mechanism of mechanotransduction appears to be well conserved. Thus, studies of mechanotransduction in vestibular hair cells have been useful in illuminating auditory hair-cell function.

Mechanotransduction Occurs in Hair Bundles

The hair bundle (Figures 1 and 2), the defining feature of all hair cells, is the mechanoreceptive organelle and is directly responsible for mechanotransduction (Hudspeth and Jacobs, 1979). The bundle is extremely sensitive to mechanical stimuli; deflections of less than the diameter of an atom are sufficient to initiate mechanotransduction. The "hairs" are finger-like projections termed stereocilia, although they are not true cilia derived from tubulin. Instead, they are composed of parallel actin filaments, cross-linked into rigid paracrystalline arrays by the actin-bundling proteins fimbrin and espin. Hair cells have 20 to 300 stereocilia, and many hair cells have a true cilium, the kinocilium, behind the tallest row of stereocilia. Mature mammalian cochlear hair cells lack kinocilia, and even in vestibular hair cells, the kinocilium is not essential for mechanotransduction (Hudspeth and Jacobs, 1979), suggesting a developmental role.

The most striking feature of the hair bundle is the organization of the stereocilia, which are arrayed in multiple rows of increasing height, collectively forming a bilaterally symmetric structure. The stereocilia taper at their bases, with the hundreds of actin filaments found along most of the length of a stereocilium diminishing to only a few dozen at the base. These remaining filaments form the rootlet, which extends into the cuticular plate, a dense meshwork of actin filaments at the apical surface of the cell. Several classes of crosslinks tether the individual stereocilia together to form a cohesive unit (Goodyear et al., 2005): ankle links connect the stereociliary bases, lateral links couple the shafts of the stereocilia, and tip links extend from the tip of a shorter stereocilium to the lateral wall of its taller neighbor (Figure 2C). As discussed below, the tip link is central to mechanotransduction. When the hair bundle is deflected by a mechanical stimulus, it responds as a unit; stereocilia move as rigid rods, pivoting about their insertion points, with no flexion (Crawford and Fettiplace, 1985; Howard and Ashmore, 1986). Such movement is a consequence of the mechanical effects of the basal tapering of the stereocilia and the extensive cross-linking of the bundle; bundles move as a unit because stereocilia pivot at their bases and touch at their tips. These structural features also influence bundle mechanics in

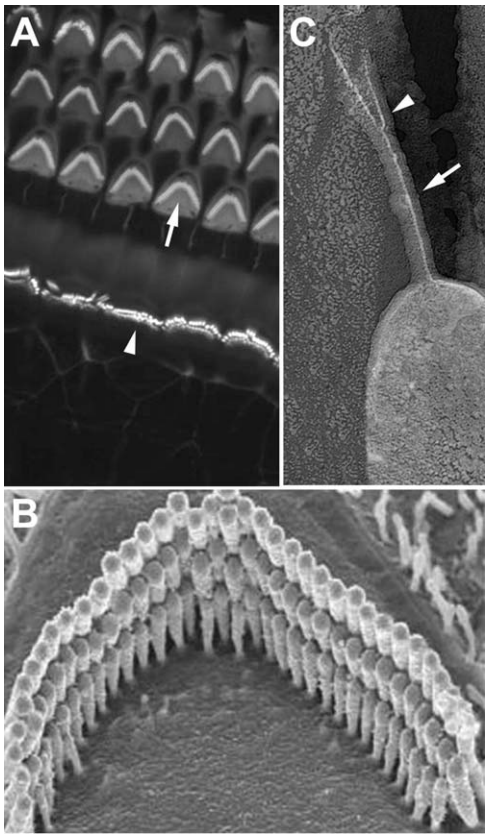

Figure 2. Cochlear Hair Bundles

(A) Rhodamine-phalloidin image of hair bundles from an early postnatal rat cochlea. Note one row of inner hair cells (arrowhead) and three rows of outer hair cells (arrow). Image courtesy of Rachel Dumont.

(B) Scanning electron micrograph of a hair bundle from an outer hair cell. Note three rows of stereocilia. Image courtesy of Bechara Kachar.

(C) Rapid-freeze, deep-etch image of a single tip link from a cochlear hair cell (arrow). Note the forking at the upper end (arrowhead). Image from Kachar et al. (2000) (Copyright 2000, National Academy of Sciences).

the absence of stimulation, allowing a significant resting tension to be developed within the bundle.

The shape of the hair bundle varies with the type of hair cell, and the bundle's mechanical properties are matched to the cell's physiological role. Mammalian cochlear hair cells each have a characteristic bundle shape: inner hair cells are crowned by flat or U-shaped bundles, while outer hair cells are capped with V- or W-shaped bundles (Figures 2A and 2B). The structure of cochlear bundles varies with tonotopic position. The height of the bundles increases from base to apex; in outer hair cells, this lengthening is accompanied by a decrease in the number of stereocilia (Lim, 1986).

The mechanism by which the elaborate architecture of the hair bundle is achieved has begun to be elucidated (Lin et al., 2005). Turnover of the bundle's actin core is coordinated; all actin subunits are replaced within 48 hr (Schneider et al., 2002). Flux rates of actin filaments vary with the height of the stereocilia; the tallest stereocilia have the fastest rates, and proportionally lower rates in shorter stereocilia ensure coordinated replacement. Espin is essential for the cross-linking of the actin filaments, and its expression correlates with bundle formation in auditory and vestibular hair cells (Li et al., 2004). Espin treadmills at the same rate as actin, indicating a synchronous turnover of the stereocilium's core (Rzadzinska et al., 2004).

Since the stereocilia are packed with actin, it is unsurprising that many unconventional myosin isoforms are essential for proper auditory function and hair-bundle formation (Libby and Steel, 2000). Of particular interest for bundle development and maintenance is myosin-15a (Myo15a), which is located at the tips of the stereocilia, the site of actin subunit incorporation (Belyantseva et al., 2003). Myo15a expression is coordinated with the beginning of the bundle's staircase formation (Belyantseva et al., 2003), and its level of expression is proportional to stereocilium length (Rzadzinska et al., 2004). Myo15a plays a critical role in bundle development, perhaps by transporting protein cargo to the stereociliary tips. For example, an interaction between Myo15a and the bundle protein whirlin has been demonstrated (Belyantseva et al., 2005; Delprat et al., 2005). As with Myo15a, whirlin is located at the tips of the stereocilia (Kikkawa et al., 2005; Delprat et al., 2005), and loss of this protein results in deafness and abnormally short stereocilia (Mburu et al., 2003). In Myo15a null mice, whirlin is mislocalized (Kikkawa et al., 2005; Belyantseva et al., 2003); introduction of functional Myo15a into these hair cells results in transport of endogenous whirlin to stereociliary tips (Belyantseva et al., 2003). Coordination of the expression and activity of these two proteins thus appears to be essential for bundle development and maintenance. Other myosins are also essential for hair-cell function, as described below.

Mechanotransduction Converts Sound Energy into Neural Impulses

Mechanoelectrical transduction begins with hair-bundle deflection, which is elicited by basilar-membrane oscillation (Figure 3). Deflection of the bundle triggers opening of a nonselective cation channel, the transduction channel (Corey and Hudspeth, 1979a). The direction of bundle displacement profoundly influences channel gating; movement toward the tallest stereocilia opens channels, movement toward the shortest stereocilia closes channels, and sideways movement has no effect (Shotwell et al., 1981). Although a fraction of the receptor current is carried by Ca^{2+} ions (Lumpkin et al., 1997; Ricci and Fettiplace, 1998), the lion's share is due to K^+ influx (Corey and Hudspeth, 1979a; Ohmori, 1985). This inward K^+ current is a manifestation of the unusually high K^+ concentration ($\sim$150 mM) in the endolymph, the extracellular solution that bathes the hair cell's apical surface. Moreover, the receptor current is enhanced by the endolymph's extracellular potential of +80 mV (the endolymphatic potential), which gives rise to a +150 mV electrical driving force for K^+ and Ca^{2+} entry. As hair cells depolarize, voltage-dependent Ca^{2+} channels near basolateral synapses open; elevated Ca^{2+} levels stimulate the neurotransmitter release at the glutamatergic synapses, initiating signal propagation to afferent neurons (Ottersen et al., 1998).

The latency between mechanical stimulation and channel gating is exceedingly brief, under 50 μs (Corey

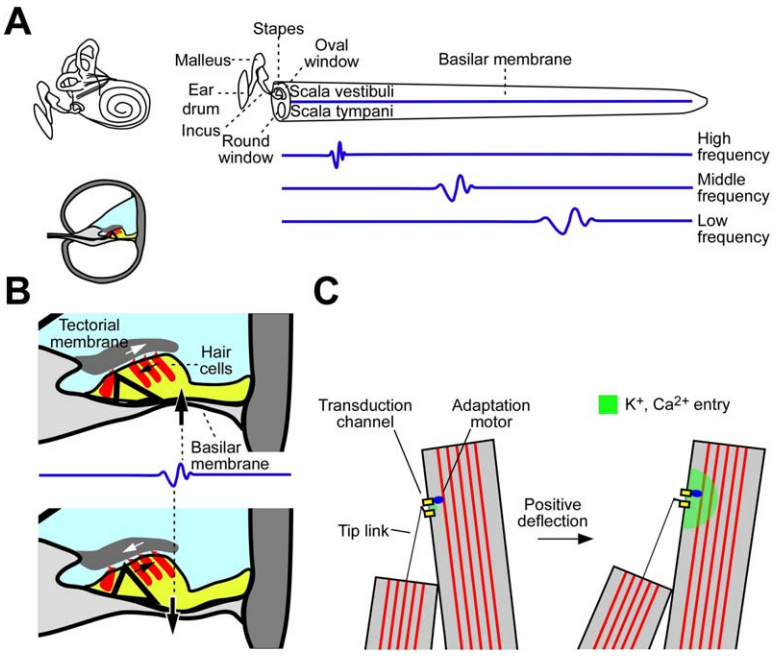

Figure 3. Sound Excitation of the Cochlea

(A) Frequency decomposition by the cochlea. The cochlea is shown here unrolled, with the scala media and organ of Corti—located above the basilar membrane—omitted for clarity. Sounds of different frequencies excite different regions of the cochlea, with high frequencies causing basilar-membrane vibration at the base of the cochlea.

(B) Movement of the basilar membrane, organ of Corti, and hair cells during sound stimulation. Movement of the basilar membrane toward the scala media causes shear between the tectorial membrane and organ of Corti that excites hair cells. By contrast, movement of the basilar membrane away from the scala media causes shear between the tectorial membrane and organ of Corti that inhibits hair cells.

(C) Deflection of hair bundles toward the taller stereocilia imparts tension into tip links and other components of transduction apparatus, causing channel opening. Open channels admit K^+ and Ca^{2+}. Transduction channels are only depicted at the upper end of the tip link; channels must be located there in order to regulate the slow-adaptation motor with entering Ca^{2+}.

and Hudspeth, 1979b, 1983; Crawford et al., 1989; Ricci et al., 2005), precluding the involvement of an enzymatic cascade. Transduction is direct: bundle movements directly open or close transduction channels.

How is the force of the mechanical stimulus transmitted to the channel? An important clue came from localization of the channel at the top of the hair bundle, identifying the site of mechanotransduction (Hudspeth, 1982; Jaramillo and Hudspeth, 1991; Denk et al., 1995; Lumpkin and Hudspeth, 1995). The orientation of tip links along the bundle's axis of sensitivity suggested that they participate in force transmission to the channel (Pickles et al., 1984). Upon bundle stimulation, Ca^{2+} rises in both the tallest and the shortest stereocilia, suggesting that channels may be located at each end of the tip link (Denk et al., 1995). The mechanical properties of the bundle dictate that deflections will cause sliding of adjacent stereocilia, and the tip link is positioned to feel strain due to these movements. If the tip link is mechanically in series with the transduction channel, alterations in tip-link tension from bundle deflection will gate the mechanically sensitive channel. In this way, the tip link could act as a gating spring (Corey and Hudspeth, 1983), reporting bundle position by modulating the open probability of the channel. Indeed, tip links are central to channel gating; destruction of the tip links by treatment with BAPTA, a Ca^{2+} chelator, abolishes transduction (Assad et al., 1991). Moreover, if tip links are allowed to regenerate, transduction returns with a time course compatible with that of tip-link regeneration (Zhao et al., 1996).

The concept of the tip link acting as the gating spring was challenged by higher-resolution images, which revealed that tip links are coiled double filaments (Kachar et al., 2000). The helical tip link is connected to the tip of the shorter stereocilium by several fine filaments and bifurcates prior to contacting the lateral wall of the taller stereocilium (Figure 2C). The tip-link structure suggests that its stiffness far exceeds the measured stiffness (~1 mN/m) of the gating spring (Howard and Hudspeth, 1988); this conclusion is supported by the hypothesis that cadherin 23 (Cdh23) is a component of the tip link (Siemens et al., 2004; Söllner et al., 2004), as the mechanical properties of Cdh23 are not consistent with those of the gating spring. Although Cdh23 has not been definitively proven to be the tip link (Gillespie et al., 2005), it is an attractive candidate. Ca^{2+}-dependent dimerization of cadherins mediates cellular adhesion, and the numerous extracellular cadherin repeats (27 in Cdh23) may be central to this process (Patel et al., 2003). Additionally, Cdh23 is a deafness gene (Bolz et al., 2001; Bork et al., 2001; Di Palma et al., 2001) and is essential for proper hair bundle morphology (Di Palma et al., 2001; Boeda et al., 2002; Holme and Steel, 2002). The localization of Cdh23 in mature hair cells is controversial; although Cdh23 immunoreactivity at the tips of adult stereocilia has been reported (Siemens et al., 2004; Rzadzinska et al., 2005), others have challenged these observations (Boeda et al., 2002; Michel et al., 2005; Lagziel et al., 2005). Regardless of the molecular identity of the tip link, it seems most likely that it does not function as the gating spring, but instead transmits force to the elastic gating spring, influencing transduction-channel open probability.

Several elements influence hair-bundle mechanics. In the resting bundles of bullfrog saccular hair cells, the summed gating springs contribute half or more of the bundle's stiffness (Jaramillo and Hudspeth, 1993). Moreover, transduction-channel gating has a profound effect on bundle stiffness: bundles have a minimum stiffness when the open probability of the transduction channels is ~0.5 (Howard and Hudspeth, 1988; Russell et al., 1992; van Netten and Kros, 2000; Ricci et al., 2002). This "gating compliance" represents the opening and closing of channels at equilibrium, reducing the overall stiffness by an amount that depends on the number of

channels, the gating-spring stiffness, and the swing of the channel's gate (Howard and Hudspeth, 1988).

The conductance of the transduction channel is fairly large, ~100 pS in high Ca^{2+}, as measured by single-channel recording (Crawford et al., 1991; Geleoc et al., 1997; Ricci et al., 2003). In auditory organs, the magnitude of the peak transduction current varies with tonotopic position, with the largest currents at the high-frequency positions (Ricci and Fettiplace, 1997; He et al., 2004). Hair cells at the base of the cochlea have greater numbers of stereocilia; their larger currents arise in part from the greater numbers of channels in these cells (He et al., 2004). In addition, the transduction channel's unitary conductance also varies with tonotopic position, so that channels in basal hair cells pass more current than those in apical cells (Ricci et al., 2003). At present, we do not know how this tonotopic variation in single-channel conductance arises, just as we do not know what factors control bundle morphology across the tonotopic map.

The prevailing hypothesis is that the transduction channel is a member of the transient receptor potential (TRP) family (Corey, 2003). TRP channels are nonselective cation channels whose diverse gating mechanisms underscore their myriad physiological roles. An original contender for the transduction channel, TRPN1, is not found in mammalian genomes (Nicolson, 2005). Another member of this family, TRPA1, has been presented as a candidate for the mammalian transduction channel based on in situ hybridization, immunocytochemistry, and knockdown experiments in both zebrafish and mice (Corey et al., 2004). Comparison of the pore properties of heterologously expressed TRPA1 channels with those of native hair-cell transduction channels, albeit under significantly different ionic conditions, reveals some similarities, in support of a role of TRPA1 in hair-cell mechanotransduction (Nagata et al., 2005). Moreover, TRPA1's 17 ankyrin repeats (protein interaction domains) have been proposed to constitute the hair cell's gating spring (Corey et al., 2004). Steered molecular dynamics simulations suggest that these repeats could coil into a structure with a spring constant of ~5 mN/m (Sotomayor et al., 2005). Four such springs attached to each of four presumed TRPA1 subunits would create a stiffness (20 mN/m) far higher than the measured hair-cell gating spring stiffness of ~1 mN/m (Howard and Hudspeth, 1988). On the other hand, if only one TRPA1 molecule contributed to a transduction-channel heteromultimer and similar channels were located at each end of a tip link (Denk et al., 1995), the stiffness contributed by TRPA1 could be as low as 2 mN/m, within the range expected for the gating spring. As with the tip link, we await definitive experiments in support of a role for TRPA1 as the mechanotransduction channel and gating spring, as discussed elsewhere (Gillespie et al., 2005).

Hair Cells Adapt on Fast and Slow Time Scales

A prominent feature of the transduction channel's response to mechanical stimulation is decay of the receptor current despite the persistence of an excitatory stimulus. Under the appropriate conditions, a bimodal decay is observed. The current decline is thus fit by two time constants, one of milliseconds or less and one about

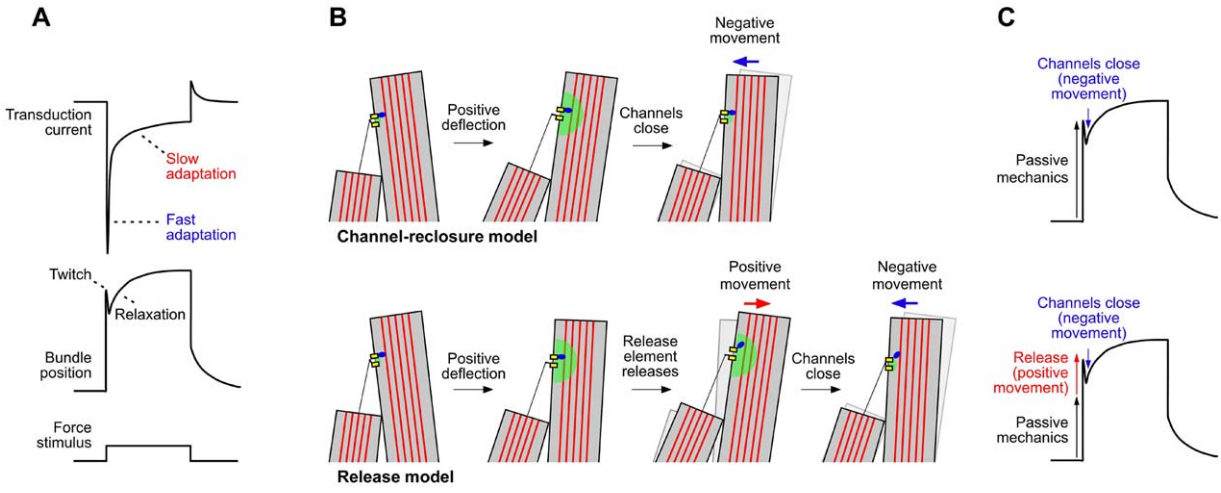

Figure 4. Fast Adaptation

(A) Simulated transduction current and mechanical behavior in response to a force stimulus. Fast and slow adaptation, apparent in the transduction-current record, are respectively associated with the rapid, negative-going bundle "twitch" and the slow positive relaxation.

(B) Models for fast adaptation. (Top) Channel-reclosure model. A positive deflection of the hair bundle opens channels and lets in K^+ and Ca^{2+}; Ca^{2+} binds to a site on or near the transduction channel, rapidly stimulating its closure. Channel closure increases gating-spring tension, resulting in negative movement of the bundle to relieve this tension. (Bottom) Release model. A positive deflection of the hair bundle increases gating-spring tension, leading to channel opening and influx of K^+ and Ca^{2+}. Ca^{2+} binds to a site on the release element, which rapidly elongates and thus reduces tension in the gating spring, allowing the bundle to relax forward. Subsequent channel closure increases gating-spring tension, resulting in negative movement of the bundle to relieve this tension.

(C) Interpretation of bundle movements for the two models. (Top) The twitch is biphasic in the channel-reclosure model; positive bundle movement due to the static stiffness of the bundle is followed by a negative movement due to channel closure. (Bottom) The twitch is triphasic in the release model. Following the initial movement due to static stiffness, the release causes a further positive movement, which is then followed by the negative movement due to channel closure.

10-fold slower, each reflecting a distinct molecular phenomenon with the same conclusion, closure of transduction channels. Owing to their different kinetics, these two processes are commonly referred to as fast and slow adaptation (Figure 4). We begin with a discussion of slow adaptation, as it is better understood at the molecular level and its physiological relevance is less controversial.

Analysis of current decay due to slow adaptation has revealed that this process represents a shift of the hair cell's displacement-response curve in the direction of the stimulus (Eatock et al., 1987; Crawford et al., 1989; Assad and Corey, 1992). The channels are not inactivated, but rather their operating range is shifted and can be reopened to the same maximal amplitude when a second stimulus is presented. The physiological utility is clear: by adapting to prolonged stimuli, hair cells remain sensitive to new stimuli and no information is missed. Current decline is synchronized with bundle relaxation in the positive direction (Figure 4A), which suggests that this process reduces gating-spring tension (Howard and Hudspeth, 1987).

Compelling evidence suggests that slow adaptation is mediated by myosin-1c (Myo1c), which decreases or increases gating-spring tension by slipping or climbing along actin filaments (Gillespie and Cyr, 2004). In the slipping phase, Ca^{2+} influx stimulates Myo1c detachment from actin, and the reduced tension closes channels. As Ca^{2+} influx ceases, the motors climb up the stereocilia to restore resting tension and the native sensitivity of the bundle. The asymmetric response of slow adaptation to positive and negative deflections reflects the fixed rate of motor climbing and displacement-dependence of the motor's slipping rate (Assad and Corey, 1992). Definitive evidence for the role of Myo1c in slow adaptation has come from analysis of mice expressing a mutant Myo1c that was sensitized to a modified ADP analog (Holt et al., 2002; Stauffer et al., 2005). In these experiments, robust inhibition of slow adaptation by the analog was seen only in the mutant mice, confirming the central role of Myo1c in slow adaptation.

Fast adaptation is less well understood (Figure 4). As with slow adaptation, fast adaptation is a decline in the transduction current that requires Ca^{2+} influx; the molecular mechanism of this process, however, is unresolved. Owing to its kinetics and insensitivity to the myosin inhibitors vanadate and butanedione monoxime, fast adaptation does not appear to involve ATPase activity of myosin motors and could arise from direct binding of Ca^{2+} to the transduction channel, inducing its closure (Wu et al., 1999). Moreover, the physiological role of fast adaptation remains controversial; although it may be central for the sensitivity and discrimination of the auditory system, this assertion has not been robustly proven. In subsequent sections, we explore the mechanism and relevance of fast adaptation in greater depth.

Cochlear Amplification: The Role of Prestin

Mammals are unique in their ability to efficiently detect high-frequency sounds, despite the severe dissipation of sound energy by viscous damping in the cochlea (Gold, 1948). The mammalian ear counters this attenuation with a "cochlear amplifier," an active process that converts cellular energy into hair-cell movements, thus overcoming hydrodynamic drag and tuning hair cells to precise frequencies (Robles and Ruggero, 2001). If energy is added to the system at the frequency being detected, i.e., at the level of the hair cells, mechanical amplification could account for enhanced tuning seen in the cochlea relative to a passive system. The compressive nonlinearity of the cochlear amplifier, where the amplification of basilar-membrane displacement is a highly nonlinear function of sound pressure, ensures the preferential amplification of the softest sounds. Two primary mechanisms have been proposed for cochlear amplification: membrane electromotility and active hair-bundle motion; in the mammalian cochlea, these two mechanisms may act in concert.

Outer hair cells are essential for cochlear amplification, and observations of voltage-dependent longitudinal movements in these cells suggested that basilar membrane motion could be augmented if such movements were stimulated by the receptor potential (Brownell et al., 1985). The voltage-sensitive membrane protein prestin is the outer hair cell's somatic molecular motor (Zheng et al., 2000); prestin apparently responds to changes in membrane potential by changing its cross-sectional profile in the membrane, generating large axial forces. Models incorporating prestin force production in response to a receptor potential can account for most features of the cochlear amplifier (e.g., Geisler, 1993; Geisler and Sang, 1995).

A significant limitation of the somatic electromotility mechanism, however, is that the membrane time constant of the hair cell will attenuate membrane-potential oscillations at high frequencies (above a few kilohertz). Possible solutions to this time-constant problem have been proposed, including prestin activation by extracellular receptor potentials (Dallos and Evans, 1995; Fridberger et al., 2004) or through a mechanically activated Cl^- conductance (Rybalchenko and Santos-Sacchi, 2003). The lack of outer hair-cell electromotility and loss of cochlear sensitivity in prestin knockout mice (Liberman et al., 2002), as well as its identification as a human deafness gene (Liu et al., 2003), demonstrate prestin's importance for proper cochlear function. Whether prestin serves as a cycle-by-cycle force generator or serves some other purpose in cochlear amplification remains to be determined (Dallos and Fakler, 2002; Santos-Sacchi, 2003; Geleoc and Holt, 2003). Regardless, prestin plays an essential role in amplification of high-frequency sounds by the mammalian cochlea.

Fast Adaptation May Allow Mechanical Amplification of Soft Sounds

The second amplification mechanism invokes the transduction machinery itself, where active hair-bundle motions correlated with transduction-channel gating resonate with the stimulus and enhance basilar-membrane movement. The remainder of this review focuses on this process.

Active hair-bundle motion has been studied extensively in nonmammalian vertebrates (Hudspeth et al., 2000; Fettiplace et al., 2001). Under appropriate conditions, frog and turtle bundles spontaneously oscillate, indicative of an active process (Crawford and Fettiplace, 1985; Martin and Hudspeth, 1999). Stimulation of frog saccular bundles with a flexible fiber at a frequency matching that of its spontaneous oscillations reveals

that the bundle can move greater distances than the stimulus, unequivocally amplifying the motion (Martin and Hudspeth, 1999). The bundle is most sensitive to stimulation at its spontaneous oscillation frequency; this amplification has inherent frequency selectivity (Martin and Hudspeth, 2001).

As with slow adaptation, fast adaptation has a mechanical effect on the hair bundle and has been directly correlated with active bundle motions (Figure 4). In the bullfrog saccule, the two phases of current decline associated with fast and slow adaptation are correlated with two phases of bundle motion—a twitch in the negative direction, followed by a positive relaxation toward the kinocilium (Howard and Hudspeth, 1987). In turtle cochlear hair cells, negative bundle movement occurs on the timescale of current decay due to fast adaptation (Ricci et al., 2000), and the kinetics of this recoil vary with the resonant frequency of the cell, as does the fast-adaptation time constant (Ricci et al., 1998). In mammalian outer hair cells, the kinetics of fast adaptation are sufficiently rapid that associated bundle motions could drive amplification at auditory frequencies (Kennedy et al., 2003).

Evidence has recently accumulated implicating stereocilia mechanisms in mammalian cochlear amplification (Chan and Hudspeth, 2005b; Kennedy et al., 2005). With a novel in vitro cochlear preparation from the gerbil that maintains the ionic separation of the intact cochlea, Chan and Hudspeth (2005b) demonstrated that bundle movements of inner hair cells, resulting from acoustically stimulated basilar-membrane motion, displayed compressive nonlinearity, a hallmark of cochlear amplification. Furthermore, bundle movements depended on Ca^{2+} entry into hair cells, not on depolarization, implicating a bundle-based mechanism in cochlear amplification (Chan and Hudspeth, 2005b). Although the net gain—the enhancement of bundle motion—was small, adjustment of the preparation conditions may well enhance the observed amplification, allowing for a detailed in vitro study of a correlate of cochlear amplification.

By demonstrating large hair-bundle movements evoked by membrane-potential stimuli in voltage-clamped mammalian outer hair cells, Jia and He (2005) recently challenged the interpretation of the Chan and Hudspeth results. These voltage-evoked movements were not blocked by inhibitors of the transduction channel, nor were they present in hair cells of mice that lacked prestin (Jia and He, 2005). Partially consistent with these results, a more in-depth analysis of the Chan and Hudspeth preparation confirmed that bundle movement in response to transepithelial voltage stimuli arose in part from somatic electromotility (Chan and Hudspeth, 2005a). Nevertheless, the observation of channel-dependent bundle movements in response to acoustic stimuli (Chan and Hudspeth, 2005a, 2005b) cannot be reconciled with the Jia and He results. Why the latter authors did not see channel-dependent bundle movements remains unclear; their cells have large transduction currents, so these movements are expected. A possible explanation is that their cells became Ca^{2+} loaded due to the prolonged exposure to high-Ca^{2+} perilymph, and Ca^{2+}-dependent channel properties were lost.

Hair bundles of rat outer hair cells unequivocally generate force during fast adaptation (Kennedy et al., 2005). Displacement of bundles with flexible glass fibers generated remarkably nonlinear bundle movements; under the appropriate conditions, the bundle could move the tip of the fiber even farther than its base was moved by the piezoelectric stimulator, a direct consequence of force generation by the bundle. The true force generation of bundles may be even greater; these studies were limited significantly by the relatively slow rise time of the mechanical stimulation, which is affected by viscous drag on the bundle and stimulating fiber. This effect distorted and slowed both fast adaptation and the mechanical responses, as channel closing and bundle movements occurred during the rising phase of the stimulus.

Two specific models have been proposed for fast adaptation (Figure 4B). The channel-reclosure model suggests that entering Ca^{2+} binds to an open transduction channel, forcing it to close and making it harder to reopen (Howard and Hudspeth, 1988; Ricci et al., 2002; Cheung and Corey, 2005). A second model, the release model, suggests that entering Ca^{2+} triggers the release of a mechanical element in series with the transduction apparatus (Bozovic and Hudspeth, 2003; Martin et al., 2003). This release slackens the gating spring, leading to closure of the channel due to the reduced tension. It should be emphasized that the release model does not correspond to detachment of the transduction apparatus from the cytoskeleton, but rather to a lengthening of the mechanical chain incorporating the transduction channel. These two models predict dissimilar mechanical consequences for the hair bundle (Figure 4C). In the channel-reclosure mechanism, the increased tension from the gating of the channel predicts a negative movement of the bundle to offset this force. By contrast, the release model predicts two phases of movement: movement in the positive direction due to mechanical relaxation of the bundle, followed by a negative movement as gating-spring tension is restored. Strong evidence for each model has been recently put forward (Cheung and Corey, 2005; Stauffer et al., 2005), raising the possibility that both mechanisms might be operational in the same hair cell.

The Release Model Predicts Generation of Large Forces by Bundles

In order to incorporate consequences of mechanical release, we would like to suggest a new and simple model for hair bundle mechanics. We previously suggested that the large forces generated by rat outer hair-cell bundles (Kennedy et al., 2005) are predicted by the release model for fast adaptation (Stauffer et al., 2005). The energy source for bundle force generation can be derived from the resting tension in the transduction apparatus, which stores elastic potential energy. As previously suggested (Hudspeth, 1992), if we envision a bundle as being strung like a bow (Figures 5A and 5B), then the generator of resting tension, the molecular motor exerting resting force F_m, can be assumed to extend both the gating spring (of stiffness K_g) and the stereocilia spring (K_s). The stereocilia spring may correspond to the basal insertions of the stereocilia, crosslinks between stereocilia, or both. If a large release occurred instantaneously in a bundle, equivalent to cutting the gating spring, stored energy in the extended stereocilia spring would

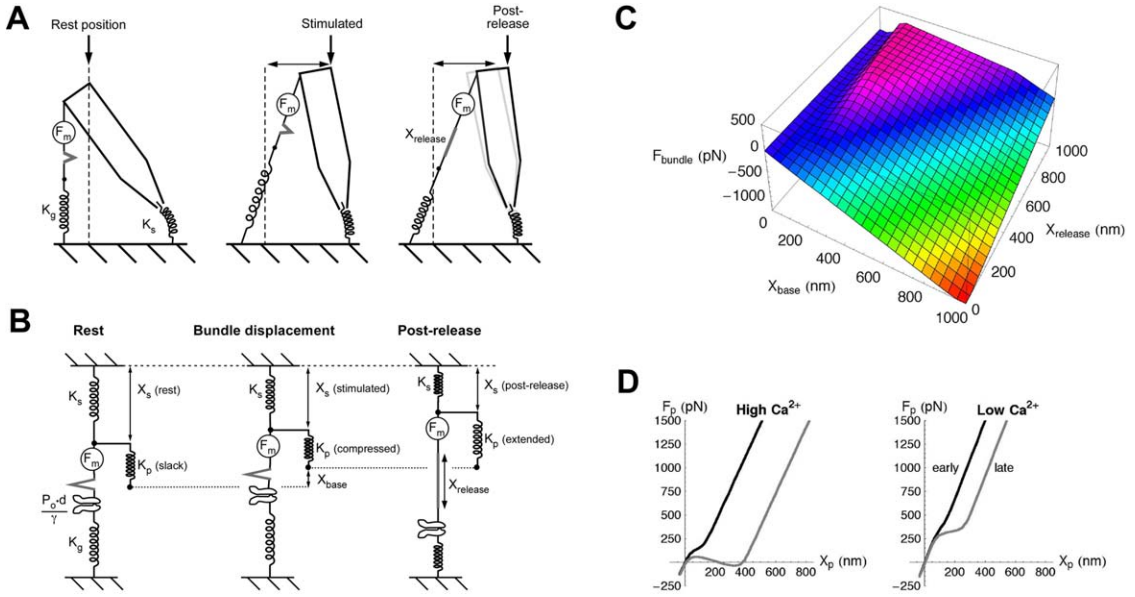

Figure 5. Mechanical Description of Release Model for Fast Adaptation

(A) Depiction of force generation by release mechanism. (Left) Force generator (F_m) extends both K_s, diagrammed as a pivot spring, and K_g, the gating spring. Release element (with length $X_{release}$; gray) is short. (Middle) Stimulation in the positive direction increases tension in K_g and decreases it in K_s. (Right) Lengthening of $X_{release}$ allows the bundle to move farther in the positive direction, exerting force on the stimulus probe (not depicted).

(B) Mechanical model for the arrangement in (A), with the addition of a stimulus probe (K_p) and transduction channel (indicated by $P_o \bullet d/\gamma$). A displacement of the base of K_p will compress it, generating forces in all three springs; a sufficiently large release will allow stored potential energy in K_s to move K_p positive.

(C) Output of bundle-mechanics model based on arrangement in (B). The force exerted by the hair bundle (F_{bundle}) is plotted as a function of the stimulus applied to the base of the stimulus probe (X_{base}) and $X_{release}$. Details of the model are provided in the Supplemental Data. Parameter values were as follows: K_g, 3 mN/m; K_s, 0.5 mN/m; K_p, 2 mN/m; N (number of channels per bundle), 80; γ (geometric gain), 0.1; d (swing of channel's gate), 1 nm; F_m, 500 pN.

(D) Force in the stimulus probe (F_p) as a function of displacement for $X_{release}$ of 300 nm (tip coordinates). We assumed that at the "early" time, $X_{release}$ is already extended by 20%, allowing replication of the displacement-stiffness curves seen in Kennedy et al. (2005). Their stimulus rise-time was close to the fast-adaptation time constant, so substantial release should have occurred before the peak of the transduction current.

be dissipated or redistributed between that spring and the spring of an attached elastic stimulus probe (K_p). We therefore suggest that the force the bundle exerts on the probe can be obtained by

$$F_{bundle} = \frac{F_m \bullet K_p}{K_p + K_s} \quad (1)$$

If $K_p >> K_s$, a force equal and opposite to the resting tension F_m is exerted on the stimulus probe. The experimental manifestation of this effect is the movement of the probe *by* the hair bundle (Kennedy et al., 2005).

The resting tension, and hence the potential energy, in outer hair-cell gating springs is high. The forces generated by rat outer hair cells, as well as an estimate of resting tension from bundle movement following gating-spring cleavage, suggest that F_m is as large as 500 pN, expressed in tip coordinates (Meyer et al., 1998; Kennedy et al., 2005). To approximate the resting tension generated by an individual force generator on a single tip link, we can express the force in channel coordinates as $f = F_m/N\gamma$. We estimate the number of generators (N) to be 80 and the geometrical gain (γ – the bundle height divided by distance between stereocilia) to be 0.1 (Figure 5), giving a force on an individual tip link of ~60 pN, considerably larger than the ~10 pN estimated for vestibular hair cells (Hudspeth and Gillespie, 1994; Gillespie and Cyr, 2004). Interestingly, the sizes of the insertional plaques, believed to house the mechanotransduction apparatus and the motors that set the resting tension, are similar in auditory and vestibular hair cells (Hudspeth and Gillespie, 1994; Furness and Hackney, 1985). It therefore seems that either the composition of the force generators in the plaque differs between the two cell types or that the generators are located outside the plaque.

What motor is responsible for setting the hair bundle's significant resting tension? Although it could be Myo1c, as proposed in vestibular hair cells (Gillespie and Cyr, 2004), another promising candidate is myosin-7a (Myo7a), a motor that is critical for proper auditory function (Weil et al., 1995). The lack of resting tension in gating springs of Myo7a null mice supports a role for this motor in force generation (Kros et al., 2002). There is no evidence suggesting that Myo7a is present in the insertional plaque, but it is found throughout cochlear stereocilia (Hasson et al., 1997), as is Myo1c (Dumont et al., 2002). While Myo1c is clearly central to adaptation (Holt et al., 2002; Stauffer et al., 2005), Myo7a appears to be an unlikely physiological choice to act as an adaptation motor. Myo7a's high K_m for ATP (>2 mM) in motility assays (Inoue and Ikebe, 2003) suggests that it will remain tightly bound to actin and move relatively slowly under physiological ATP concentrations. We speculate that both motors are central to bundle mechanics, with Myo7a generating

a large resting tension and Myo1c mediating the adaptive response to stimuli. Interestingly, the PH-domain protein PHR1 has been reported to bind to both Myo1c and Myo7a (Etournay et al., 2005), suggesting the two motors might be functionally coupled.

Quantitation of Release Amplitude—A New Model

To quantify the dependence of hair-bundle force on displacement size, we expanded on Equation 1 and developed a comprehensive model that employs the mechanical arrangement portrayed in Figures 5A and 5B (described in the Supplemental Data). Our model uses static solutions to determine bundle force before and after a mechanical release and incorporates the mechanical features of the hair bundle, as well as the ramifications of Ca^{2+} influx through the transduction channel. Notably, the model effectively replicates the Kennedy et al. (2005) data (Figure 5D). Generation of large forces requires increased Ca^{2+} influx to trigger the release; because ion entry will occur as the bundle is displaced, the release amplitude will be a function of the channel open probability (P_{open}), the affinity and Hill coefficient of the binding site for Ca^{2+}, and the stereocilia Ca^{2+} concentration. Force generation should only occur over a narrow range of displacements where channel opening is promoted, yet the stereocilia spring remains extended in the negative direction. Excitatory bundle displacements reduce tension in K_s, which diminish the maximum force production possible. Moreover, less force is generated if the release is not large enough to slacken K_g. Note that this model predicts apparent negative bundle stiffness (Supplemental Data), which has also been found in vestibular hair cells investigated under physiological Ca^{2+} conditions (Martin et al., 2000).

What is the physiological basis of the proposed release element? In our model, we have replicated the Kennedy et al. (2005) data with an $X_{release}$, the distance the release element lengthens by, equal to 300 nm. In channel coordinates, this value corresponds to a lengthening of ~30 nm, much larger than the swing of the channel's gate, measured at 2–4 nm (Howard and Hudspeth, 1988). The adaptation-motor myosins indeed might represent the release element, particularly if the consequence of Ca^{2+} binding to their IQ domains was to dramatically reduce the stiffness of the Myo1c lever arm (Bozovic and Hudspeth, 2003). Because each IQ domain contributes ~4 nm to a myosin lever arm (Moore et al., 2004), a full 180° rotation of the three-IQ lever arm of Myo1c could generate a movement of 24 nm. If the helical IQ domains deformed further under tension, the movement could be yet larger. Thus, Myo1c is a plausible candidate for the release element (Stauffer et al., 2005), as is any other molecule located in series with the gating spring. Indeed, a combined arrangement of Myo1c and Myo7a could account for the observed behavior of outer hair cells. In this model, Myo7a generates a large resting tension; if the effective frictional coefficient for Myo1c is much lower than that for Myo7a, adaptation—fast and slow—could occur when Myo1c relaxes in response to a stimulus.

How do we reconcile the mechanical observations predicted by the release model with documented hair-bundle motions? In the release model, a biphasic mechanical response is expected for moderately sized stimuli. As the release element lets go, the reduced tension in the gating spring moves the bundle forward positively, while simultaneously promoting channel closure (Figure 3C). As the transduction channels close in response to the slackening of the gating spring, the bundle will move in the negative direction. Negative bundle movements during the mechanical responses of mammalian bundles have not been observed; the forces exerted by rat cochlear hair bundles are in the positive direction (Kennedy et al., 2005).

Why was the obligatory negative force exerted by closing channels not detected in these experiments? As noted previously, the relatively slow mechanical stimulation distorted the bundle's mechanical responses, as the rising phase of the stimulus overlapped with channel closure and bundle movements. Because our model predicts a release magnitude much greater than the swing of the channel's gate, the associated positive motion dominated the mechanical effect under these experimental conditions. In bullfrog hair cells, positive forces preceding negative bundle motions are of a lesser magnitude than the negative forces, suggesting a different relative contribution of release and channel closure to hair-bundle mechanics than seen in mammals (Benser et al., 1996). More experiments are required to illuminate the mechanism of bundle force production and its contribution to cochlear signal amplification.

Stereocilia Force Generation and Tuning

One of the defining features of the cochlear amplifier is its tuning; for a given hair cell, amplification only occurs at a precise frequency, permitting the ear to discriminate sounds differing in frequency by less than 0.2% (Dallos, 1996). In the release mechanism, the generated forces will assist the positive phase of hair-bundle movement by an external stimulus, i.e., force production would be in phase with the stimulus or slightly lead it. Tuning will also be sharpened by the enhancement of the negative phase of bundle movement due to negatively directed forces ~180° out of phase with the stimulus resulting from channel closure. Direct Ca^{2+}-dependent reclosure of transduction channels could drive negatively directed bundle movements, a mechanism which has been proposed to underlie fast adaptation (Howard and Hudspeth, 1988; Cheung and Corey, 2005). Restiffening of the release element could also contribute to negative bundle motion; like myosin after inorganic phosphate release, a conformational change in the release element, occurring when Ca^{2+} dissociates, would store elastic energy that could drive further negative motion (Howard, 2001). If the kinetics of the release and channel closure are matched to the stimulus frequency, bundle motions arising from this force generation will amplify the stimulus in a frequency-specific manner and contribute to cochlear tuning.

A critical, unresolved question about stereocilia mechanisms for amplification is whether they can be fast enough. Prestin can drive hair-cell force production at auditory frequencies (>50 kHz) if supplied with a membrane-potential stimulus (Frank et al., 1999). Unlike the prestin-based mechanism, however, stereocilia mechanisms that rely on Ca^{2+} entry are not constrained by the membrane time constant of the cell. Instead, the kinetics of Ca^{2+} association and dissociation from the site that

influences bundle mechanics, as well as the time required for conformational changes in those binding sites, will limit the speed at which the mechanism can operate. Although fast-adaptation time constants as low as 50 μs have been measured (Ricci et al., 2005), that rate corresponds to a characteristic frequency of only ~3 kHz, well below the maximal frequencies of cochlear amplification. Those measurements were limited by the speed at which the bundle could be stimulated experimentally; consideration of other factors (temperature, endolymphatic potential, K^+ as the current-carrying ion) suggests that the time constant may be an order of magnitude smaller. Nevertheless, the mechanical movements underlying fast adaptation have not yet been shown to operate at tens of kilohertz, nor have Ca^{2+}-triggered conformational changes been demonstrated at such high rates in other systems. A critical test of the significance of bundle mechanisms for cochlear amplification is thus a clear demonstration that the mechanism can act fast enough.

What mechanisms might underlie the inherent tuning in fast adaptation? Tonotopic variations in the kinetics of Ca^{2+} binding to the release element could contribute. These modifications could arise from direct modulation of the association rate of Ca^{2+} and the release element (Putkey et al., 2003) or alterations in the single-channel conductance of the transduction channel (Ricci et al., 2003), which would influence the Ca^{2+} concentration in the bundle. At present it is unclear how these changes in the conductance of the transduction channel arise; differential expression of splice variants or differences in subunit composition across the tonotopic map could mediate this phenomenon. The lipid environment adjacent to the Ca^{2+}-dependent regulatory site could also affect Ca^{2+} kinetics, with highly charged lipids like phosphatidylinositol-4,5-bisphosphate (PIP_2) concentrating Ca^{2+} nearby (Hirono et al., 2004).

Could splicing, post-translational modification, changes in subunit composition, or lipid environment modify Ca^{2+} binding over a 100-fold range in frequency? The kinetics of fast adaptation could also be influenced by how fast Ca^{2+} rises in stereocilia, which will be affected by the Ca^{2+} concentration outside the hair bundles. For example, elevated extracellular Ca^{2+} at the base of the cochlea would speed fast adaptation in these cells tuned to high frequencies. We have hypothesized that the local Ca^{2+} concentration is elevated by the plasma membrane Ca^{2+}-ATPase (PMCA) of hair bundles (Yamoah et al., 1998), which is known to be PMCA2a (Dumont et al., 2001). This hypothesis has garnered experimental support: mice with a hypomorphic mutation in PMCA2 have substantially reduced endolymphatic Ca^{2+} concentrations (Wood et al., 2004). In wild-type guinea pigs, although the bulk concentration of Ca^{2+} is lower in the basal turn of the cochlea, the concentration is substantially more than expected given the higher endocochlear potential here relative to the apex (Salt et al., 1989). This result suggests that a source of endolymph Ca^{2+}, likely the hair cells, extrudes the ion at a higher rate in the base than the apex. If PMCA2a activity results in a substantially elevated Ca^{2+} concentration close to the bundle (Yamoah et al., 1998), tonotopic variations in PMCA2 expression would contribute to tuning by establishing a gradient in local Ca^{2+} concentration along the cochlea. Although technically challenging, direct measurement of endolymph Ca^{2+} in the subtectorial space—without perturbing standing gradients—would resolve this issue.

Summary

The mammalian cochlear amplifier is an extraordinary mechanism, allowing detection of soft sounds of a wide range of frequencies. Although the molecular mechanism for cochlear amplification remains unknown, the mechanical behavior that arises during fast adaptation in hair cells may well contribute substantially. The mechanism of fast adaptation itself remains controversial, with some evidence implicating Ca^{2+}-dependent direct channel reclosure and other experiments suggesting that a molecule like Myo1c extends to allow channels to close. Both mechanisms might be involved, particularly if their intrinsic kinetics were sufficiently resolved to allow the release to be followed by the induced channel reclosure. In so doing, the hair cell would exert force during both phases of a periodic mechanical stimulus. If two temporally segregated, Ca^{2+}-dependent mechanisms are involved, tuning could occur simply by changing the amount of Ca^{2+} entering during a stimulus, either by modulation of the Ca^{2+} conductance of the transduction channel or by regulating the extracellular Ca^{2+} concentration. Regardless of the role of bundle-based amplification, mammals also require prestin, a remarkable molecule whose expression correlates with the ability to amplify high-frequency sounds; how bundle amplification and prestin interact is a crucial issue that has not yet been explored significantly. The fundamental importance of cochlear amplification to hearing ensures that considerable attention will be paid to these problems in the coming years.

Supplemental Data

The Supplemental Data for this article can be found at http://www.neuron.org/cgi/content/full/48/3/403/DC1/.

Acknowledgments

We thank Rachel Dumont and Tianying Ren for comments on the manuscript, and David Corey and Joe Howard for discussions on the modeling. Work from the Gillespie lab is supported by the National Institute on Deafness and Other Communication Disorders grants DC002368, DC003279, DC004571, and DC005983.

References

Assad, J.A., and Corey, D.P. (1992). An active motor model for adaptation by vertebrate hair cells. J. Neurosci. *12*, 3291–3309.

Assad, J.A., Shepherd, G.M.G., and Corey, D.P. (1991). Tip-link integrity and mechanical transduction in vertebrate hair cells. Neuron *7*, 985–994.

Belyantseva, I.A., Boger, E.T., and Friedman, T.B. (2003). Myosin XVa localizes to the tips of inner ear sensory cell stereocilia and is essential for staircase formation of the hair bundle. Proc. Natl. Acad. Sci. USA *100*, 13958–13963.

Belyantseva, I.A., Boger, E.T., Naz, S., Frolenkov, G.I., Sellers, J.R., Ahmed, Z.M., Griffith, A.J., and Friedman, T.B. (2005). Myosin-XVa is required for tip localization of whirlin and differential elongation of hair-cell stereocilia. Nat. Cell Biol. *7*, 148–156.

Benser, M.E., Marquis, R.E., and Hudspeth, A.J. (1996). Rapid, active hair bundle movements in hair cells from the bullfrog's sacculus. J. Neurosci. *16*, 5629–5643.

Boeda, B., El-Amraoui, A., Bahloul, A., Goodyear, R., Daviet, L., Blanchard, S., Perfettini, I., Fath, K.R., Shorte, S., Reiners, J., et al. (2002). Myosin VIIa, harmonin and cadherin *23*, three Usher I gene products that cooperate to shape the sensory hair cell bundle. EMBO J. *21*, 6689–6699.

Bolz, H., von Brederlow, B., Ramirez, A., Bryda, E.C., Kutsche, K., Nothwang, H.G., Seeliger, M., Cabrera, M., Vila, M.C., Molina, O.P., et al. (2001). Mutation of CDH23, encoding a new member of the cadherin gene family, causes Usher syndrome type 1D. Nat. Genet. *27*, 108–112.

Bork, J.M., Peters, L.M., Riazuddin, S., Bernstein, S.L., Ahmed, Z.M., Ness, S.L., Polomeno, R., Ramesh, A., Schloss, M., Srisailpathy, C.R., et al. (2001). Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene CDH23. Am. J. Hum. Genet. *68*, 26–37.

Bozovic, D., and Hudspeth, A.J. (2003). Hair-bundle movements elicited by transepithelial electrical stimulation of hair cells in the sacculus of the bullfrog. Proc. Natl. Acad. Sci. USA *100*, 958–963.

Brownell, W.E., Bader, C.R., Bertrand, D., and de Ribaupierre, Y. (1985). Evoked mechanical responses of isolated cochlear outer hair cells. Science *227*, 194–196.

Chan, D.K., and Hudspeth, A.J. (2005a). Mechanical responses of the organ of Corti to acoustic and electrical stimulation in vitro. Biophys. J. *89*, in press.

Chan, D.K., and Hudspeth, A.J. (2005b). Ca^{2+} current-driven nonlinear amplification by the mammalian cochlea in vitro. Nat. Neurosci. *8*, 149–155.

Cheung, E.L., and Corey, D.P. (2005). Ca^{2+} changes the force sensitivity of the hair-cell transduction channel. Biophys. J., in press.

Corey, D.P. (2003). New TRP channels in hearing and mechanosensation. Neuron *39*, 585–588.

Corey, D.P., and Hudspeth, A.J. (1979a). Ionic basis of the receptor potential in a vertebrate hair cell. Nature *281*, 675–677.

Corey, D.P., and Hudspeth, A.J. (1979b). Response latency of vertebrate hair cells. Biophys. J. *26*, 499–506.

Corey, D.P., and Hudspeth, A.J. (1983). Kinetics of the receptor current in bullfrog saccular hair cells. J. Neurosci. *3*, 962–976.

Corey, D.P., Garcia-Anoveros, J., Holt, J.R., Kwan, K.Y., Lin, S.Y., Vollrath, M.A., Amalfitano, A., Cheung, E.L., Derfler, B.H., Duggan, A., et al. (2004). TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. Nature *432*, 723–730.

Crawford, A.C., and Fettiplace, R. (1985). The mechanical properties of ciliary bundles of turtle cochlear hair cells. J. Physiol. *364*, 359–379.

Crawford, A.C., Evans, M.G., and Fettiplace, R. (1989). Activation and adaptation of transducer currents in turtle hair cells. J. Physiol. *419*, 405–434.

Crawford, A.C., Evans, M.G., and Fettiplace, R. (1991). The actions of calcium on the mechano-electrical transducer current of turtle hair cells. J. Physiol. *434*, 369–398.

Dallos, P. (1996). Overview: Cochlear Neurobiology. In The Cochlea, P. Dallos, A.N. Popper, and R.R. Fay, eds. (New York: Springer-Verlag), pp. 1–43.

Dallos, P., and Evans, B.N. (1995). High-frequency motility of outer hair cells and the cochlear amplifier. Science *267*, 2006–2009.

Dallos, P., and Fakler, B. (2002). Prestin, a new type of motor protein. Nat. Rev. Mol. Cell Biol. *3*, 104–111.

Delprat, B., Michel, V., Goodyear, R., Yamasaki, Y., Michalski, N., El-Amraoui, A., Perfettini, I., Legrain, P., Richardson, G., Hardelin, J.P., and Petit, C. (2005). Myosin XVa and whirlin, two deafness gene products required for hair bundle growth, are located at the stereocilia tips and interact directly. Hum. Mol. Genet. *14*, 401–410.

Denk, W., Holt, J.R., Shepherd, G.M., and Corey, D.P. (1995). Calcium imaging of single stereocilia in hair cells: localization of transduction channels at both ends of tip links. Neuron *15*, 1311–1321.

Di Palma, F., Holme, R.H., Bryda, E.C., Belyantseva, I.A., Pellegrino, R., Kachar, B., Steel, K.P., and Noben-Trauth, K. (2001). Mutations in Cdh23, encoding a new type of cadherin, cause stereocilia disorganization in waltzer, the mouse model for Usher syndrome type 1D. Nat. Genet. *27*, 103–107.

Dumont, R.A., Lins, U., Filoteo, A.G., Penniston, J.T., Kachar, B., and Gillespie, P.G. (2001). Plasma membrane Ca2+-ATPase isoform 2a is the PMCA of hair bundles. J. Neurosci. *21*, 5066–5078.

Dumont, R.A., Zhao, Y.D., Holt, J.R., Bahler, M., and Gillespie, P.G. (2002). Myosin-I isozymes in neonatal rodent auditory and vestibular epithelia. J. Assoc. Res. Otolaryngol. *3*, 375–389.

Eatock, R.A., Corey, D.P., and Hudspeth, A.J. (1987). Adaptation of mechanoelectrical transduction in hair cells of the bullfrog's sacculus. J. Neurosci. *7*, 2821–2836.

Etournay, R., El-Amraoui, A., Bahloul, A., Blanchard, S., Roux, I., Pezeron, G., Michalski, N., Daviet, L., Hardelin, J.P., Legrain, P., and Petit, C. (2005). PHR1, an integral membrane protein of the inner ear sensory cells, directly interacts with myosin 1c and myosin VIIa. J. Cell Sci. *118*, 2891–2899.

Fettiplace, R., Ricci, A.J., and Hackney, C.M. (2001). Clues to the cochlear amplifier from the turtle ear. Trends Neurosci. *24*, 169–175.

Frank, G., Hemmert, W., and Gummer, A.W. (1999). Limiting dynamics of high-frequency electromechanical transduction of outer hair cells. Proc. Natl. Acad. Sci. USA *96*, 4420–4425.

Fridberger, A., de Monvel, J.B., Zheng, J., Hu, N., Zou, Y., Ren, T., and Nuttall, A. (2004). Organ of Corti potentials and the motion of the basilar membrane. J. Neurosci. *24*, 10057–10063.

Furness, D.N., and Hackney, C.M. (1985). Cross-links between stereocilia in the guinea pig cochlea. Hear. Res. *18*, 177–188.

Geisler, C.D. (1993). A realizable cochlear model using feedback from motile outer hair cells. Hear. Res. *68*, 253–262.

Geisler, C.D., and Sang, C. (1995). A cochlear model using feedforward outer-hair-cell forces. Hear. Res. *86*, 132–146.

Geleoc, G.S., and Holt, J.R. (2003). Auditory amplification: outer hair cells press the issue. Trends Neurosci. *26*, 115–117.

Geleoc, G.S., Lennan, G.W., Richardson, G.P., and Kros, C.J. (1997). A quantitative comparison of mechanoelectrical transduction in vestibular and auditory hair cells of neonatal mice. Proc. Biol. Sci. *264*, 611–621.

Gillespie, P.G., and Cyr, J.L. (2004). Myosin-1c, the hair cell's adaptation motor. Annu. Rev. Physiol. *66*, 521–545.

Gillespie, P.G., Dumont, R.A., and Kachar, B. (2005). Have we found the tip link, transduction channel, and gating spring of the hair cell? Curr. Opin. Neurobiol. *15*, 389–396.

Gold, T. (1948). Hearing II. The physical basis of action in the cochlea. Proc. R. Soc. Lond. B. Biol. Sci. *135*, 492–498.

Goodyear, R.J., Marcotti, W., Kros, C.J., and Richardson, G.P. (2005). Development and properties of stereociliary link types in hair cells of the mouse cochlea. J. Comp. Neurol. *485*, 75–85.

Hasson, T., Gillespie, P.G., Garcia, J.A., MacDonald, R.B., Zhao, Y., Yee, A.G., and Corey, D.P. (1997). Unconventional myosins in inner-ear sensory epithelia. J. Cell Biol. *137*, 1287–1307.

He, D.Z., Jia, S., and Dallos, P. (2004). Mechanoelectrical transduction of adult outer hair cells studied in a gerbil hemicochlea. Nature *429*, 766–770.

Hirono, M., Denis, C.S., Richardson, G.P., and Gillespie, P.G. (2004). Hair cells require phosphatidylinositol 4,5-bisphosphate for mechanical transduction and adaptation. Neuron *44*, 309–320.

Holme, R.H., and Steel, K.P. (2002). Stereocilia defects in waltzer (Cdh23), shaker1 (Myo7a) and double waltzer/shaker1 mutant mice. Hear. Res. *169*, 13–23.

Holt, J.R., Gillespie, S.K., Provance, D.W., Shah, K., Shokat, K.M., Corey, D.P., Mercer, J.A., and Gillespie, P.G. (2002). A chemical-genetic strategy implicates myosin-1c in adaptation by hair cells. Cell *108*, 371–381.

Howard, J. (2001). Mechanics of Motor Proteins and the Cytoskeleton (Sunderland, MA: Sinauer Associates).

Howard, J., and Ashmore, J.F. (1986). Stiffness of sensory hair bundles in the sacculus of the frog. Hear. Res. *23*, 93–104.

Howard, J., and Hudspeth, A.J. (1987). Mechanical relaxation of the hair bundle mediates adaptation in mechanoelectrical transduction

by the bullfrog's saccular hair cell. Proc. Natl. Acad. Sci. USA *84*, 3064–3068.

Howard, J., and Hudspeth, A.J. (1988). Compliance of the hair bundle associated with gating of mechanoelectrical transduction channels in the bullfrog's saccular hair cell. Neuron *1*, 189–199.

Hudspeth, A.J. (1982). Extracellular current flow and the site of transduction by vertebrate hair cells. J. Neurosci. *2*, 1–10.

Hudspeth, A.J. (1992). Hair-bundle mechanics and a model for mechanoelectrical transduction by hair cells. Soc. Gen. Physiol. Ser. *47*, 357–370.

Hudspeth, A.J., and Jacobs, R. (1979). Stereocilia mediate transduction in vertebrate hair cells (auditory system/cilium/vestibular system). Proc. Natl. Acad. Sci. USA *76*, 1506–1509.

Hudspeth, A.J., and Gillespie, P.G. (1994). Pulling springs to tune transduction: adaptation by hair cells. Neuron *12*, 1–9.

Hudspeth, A.J., Choe, Y., Mehta, A.D., and Martin, P. (2000). Putting ion channels to work: mechanoelectrical transduction, adaptation, and amplification by hair cells. Proc. Natl. Acad. Sci. USA *97*, 11765–11772.

Inoue, A., and Ikebe, M. (2003). Characterization of the motor activity of mammalian myosin VIIA. J. Biol. Chem. *278*, 5478–5487.

Jaramillo, F., and Hudspeth, A.J. (1991). Localization of the hair cell's transduction channels at the hair bundle's top by iontophoretic application of a channel blocker. Neuron *7*, 409–420.

Jaramillo, F., and Hudspeth, A.J. (1993). Displacement-clamp measurement of the forces exerted by gating springs in the hair bundle. Proc. Natl. Acad. Sci. USA *90*, 1330–1334.

Jia, S., and He, D.Z. (2005). Motility-associated hair-bundle motion in mammalian outer hair cells. Nat. Neurosci. *8*, 1028–1034.

Kachar, B., Parakkal, M., Kurc, M., Zhao, Y., and Gillespie, P.G. (2000). High-resolution structure of hair-cell tip links. Proc. Natl. Acad. Sci. USA *97*, 13336–13341.

Kennedy, H.J., Evans, M.G., Crawford, A.C., and Fettiplace, R. (2003). Fast adaptation of mechanoelectrical transducer channels in mammalian cochlear hair cells. Nat. Neurosci. *6*, 832–836.

Kennedy, H.J., Crawford, A.C., and Fettiplace, R. (2005). Force generation by mammalian hair bundles supports a role in cochlear amplification. Nature *433*, 880–883.

Kikkawa, Y., Mburu, P., Morse, S., Kominami, R., Townsend, S., and Brown, S.D. (2005). Mutant analysis reveals whirlin as a dynamic organizer in the growing hair cell stereocilium. Hum. Mol. Genet. *14*, 391–400.

Kros, C.J., Marcotti, W., van Netten, S.M., Self, T.J., Libby, R.T., Brown, S.D., Richardson, G.P., and Steel, K.P. (2002). Reduced climbing and increased slipping adaptation in cochlear hair cells of mice with Myo7a mutations. Nat. Neurosci. *5*, 41–47.

Lagziel, A., Ahmed, Z.M., Schultz, J.M., Morell, R.J., Belyantseva, I.A., and Friedman, T.B. (2005). Spatiotemporal pattern and isoforms of cadherin 23 in wild type and waltzer mice during inner ear hair cell development. Dev. Biol. *280*, 295–306.

Li, H., Liu, H., Balt, S., Mann, S., Corrales, C.E., and Heller, S. (2004). Correlation of expression of the actin filament-bundling protein espin with stereociliary bundle formation in the developing inner ear. J. Comp. Neurol. *468*, 125–134.

Libby, R.T., and Steel, K.P. (2000). The roles of unconventional myosins in hearing and deafness. Essays Biochem. *35*, 159–174.

Liberman, M.C., Gao, J., He, D.Z., Wu, X., Jia, S., and Zuo, J. (2002). Prestin is required for electromotility of the outer hair cell and for the cochlear amplifier. Nature *419*, 300–304.

Lim, D.J. (1986). Functional structure of the organ of Corti: a review. Hear. Res. *22*, 117–146.

Lin, H.W., Schneider, M.E., and Kachar, B. (2005). When size matters: the dynamic regulation of stereocilia lengths. Curr. Opin. Cell Biol. *17*, 55–61.

Liu, X.Z., Ouyang, X.M., Xia, X.J., Zheng, J., Pandya, A., Li, F., Du, L.L., Welch, K.O., Petit, C., Smith, R.J., et al. (2003). Prestin, a cochlear motor protein, is defective in non-syndromic hearing loss. Hum. Mol. Genet. *12*, 1155–1162.

Lumpkin, E.A., and Hudspeth, A.J. (1995). Detection of Ca^{2+} entry through mechanosensitive channels localizes the site of mechanoelectrical transduction in hair cells. Proc. Natl. Acad. Sci. USA *92*, 10297–10301.

Lumpkin, E.A., Marquis, R.E., and Hudspeth, A.J. (1997). The selectivity of the hair cell's mechanoelectrical-transduction channel promotes Ca^{2+} flux at low Ca^{2+} concentrations. Proc. Natl. Acad. Sci. USA *94*, 10997–11002.

Martin, P., and Hudspeth, A.J. (1999). Active hair-bundle movements can amplify a hair cell's response to oscillatory mechanical stimuli. Proc. Natl. Acad. Sci. USA *96*, 14306–14311.

Martin, P., and Hudspeth, A.J. (2001). Compressive nonlinearity in the hair bundle's active response to mechanical stimulation. Proc. Natl. Acad. Sci. USA *98*, 14386–14391.

Martin, P., Mehta, A.D., and Hudspeth, A.J. (2000). Negative hair-bundle stiffness betrays a mechanism for mechanical amplification by the hair cell. Proc. Natl. Acad. Sci. USA *97*, 12026–12031.

Martin, P., Bozovic, D., Choe, Y., and Hudspeth, A.J. (2003). Spontaneous oscillation by hair bundles of the bullfrog's sacculus. J. Neurosci. *23*, 4533–4548.

Mburu, P., Mustapha, M., Varela, A., Weil, D., El-Amraoui, A., Holme, R.H., Rump, A., Hardisty, R.E., Blanchard, S., Coimbra, R.S., et al. (2003). Defects in whirlin, a PDZ domain molecule involved in stereocilia elongation, cause deafness in the whirler mouse and families with DFNB31. Nat. Genet. *34*, 421–428.

Meyer, J., Furness, D.N., Zenner, H.P., Hackney, C.M., and Gummer, A.W. (1998). Evidence for opening of hair-cell transducer channels after tip-link loss. J. Neurosci. *18*, 6748–6756.

Michel, V., Goodyear, R.J., Weil, D., Marcotti, W., Perfettini, I., Wolfrum, U., Kros, C.J., Richardson, G.P., and Petit, C. (2005). Cadherin 23 is a component of the transient lateral links in the developing hair bundles of cochlear sensory cells. Dev. Biol. *280*, 281–294.

Moore, J.R., Krementsova, E.B., Trybus, K.M., and Warshaw, D.M. (2004). Does the myosin V neck region act as a lever? J. Muscle Res. Cell Motil. *25*, 29–35.

Nagata, K., Duggan, A., Kumar, G., and Garcia-Anoveros, J. (2005). Nociceptor and hair cell transducer properties of TRPA1, a channel for pain and hearing. J. Neurosci. *25*, 4052–4061.

Nicolson, T. (2005). Fishing for key players in mechanotransduction. Trends Neurosci. *28*, 140–144.

Ohmori, H. (1985). Mechano-electrical transduction currents in isolated vestibular hair cells of the chick. J. Physiol. *359*, 189–217.

Ottersen, O.P., Takumi, Y., Matsubara, A., Landsend, A.S., Laake, J.H., and Usami, S. (1998). Molecular organization of a type of peripheral glutamate synapse: the afferent synapses of hair cells in the inner ear. Prog. Neurobiol. *54*, 127–148.

Patel, S.D., Chen, C.P., Bahna, F., Honig, B., and Shapiro, L. (2003). Cadherin-mediated cell-cell adhesion: sticking together as a family. Curr. Opin. Struct. Biol. *13*, 690–698.

Pickles, J.O., Comis, S.D., and Osborne, M.P. (1984). Cross-links between stereocilia in the guinea pig organ of Corti, and their possible relation to sensory transduction. Hear. Res. *15*, 103–112.

Putkey, J.A., Kleerekoper, Q., Gaertner, T.R., and Waxham, M.N. (2003). A new role for IQ motif proteins in regulating calmodulin function. J. Biol. Chem. *278*, 49667–49670.

Ricci, A.J., and Fettiplace, R. (1997). The effects of calcium buffering and cyclic AMP on mechano-electrical transduction in turtle auditory hair cells. J. Physiol. *501*, 111–124.

Ricci, A.J., and Fettiplace, R. (1998). Calcium permeation of the turtle hair cell mechanotransducer channel and its relation to the composition of endolymph. J. Physiol. *506*, 159–173.

Ricci, A.J., Wu, Y.C., and Fettiplace, R. (1998). The endogenous calcium buffer and the time course of transducer adaptation in auditory hair cells. J. Neurosci. *18*, 8261–8277.

Ricci, A.J., Crawford, A.C., and Fettiplace, R. (2000). Active hair bundle motion linked to fast transducer adaptation in auditory hair cells. J. Neurosci. *20*, 7131–7142.

Ricci, A.J., Crawford, A.C., and Fettiplace, R. (2002). Mechanisms of active hair bundle motion in auditory hair cells. J. Neurosci. *22*, 44–52.

Ricci, A.J., Crawford, A.C., and Fettiplace, R. (2003). Tonotopic variation in the conductance of the hair cell mechanotransducer channel. Neuron *40*, 983–990.

Ricci, A.J., Kennedy, H.J., Crawford, A.C., and Fettiplace, R. (2005). The transduction channel filter in auditory hair cells. J. Neurosci. *25*, 7831–7839.

Robles, L., and Ruggero, M.A. (2001). Mechanics of the mammalian cochlea. Physiol. Rev. *81*, 1305–1352.

Russell, I.J., Kössl, M., and Richardson, G.P. (1992). Nonlinear mechanical response of mouse cochlear hair bundles. Proc. R. Soc. Lond. B. Biol. Sci. *250*, 217–227.

Rybalchenko, V., and Santos-Sacchi, J. (2003). Cl- flux through a non-selective, stretch-sensitive conductance influences the outer hair cell motor of the guinea-pig. J. Physiol. *547*, 873–891.

Rzadzinska, A.K., Schneider, M.E., Davies, C., Riordan, G.P., and Kachar, B. (2004). An actin molecular treadmill and myosins maintain stereocilia functional architecture and self-renewal. J. Cell Biol. *164*, 887–897.

Rzadzinska, A.K., Derr, A., Kachar, B., and Noben-Trauth, K. (2005). Sustained cadherin 23 expression in young and adult cochlea of normal and hearing-impaired mice. Hear. Res. *208*, 114–121.

Salt, A.N., Inamura, N., Thalmann, R., and Vora, A. (1989). Calcium gradients in inner ear endolymph. Am. J. Otolaryngol. *10*, 371–375.

Santos-Sacchi, J. (2003). New tunes from Corti's organ: the outer hair cell boogie rules. Curr. Opin. Neurobiol. *13*, 459–468.

Schneider, M.E., Belyantseva, I.A., Azevedo, R.B., and Kachar, B. (2002). Rapid renewal of auditory hair bundles. Nature *418*, 837–838.

Shotwell, S.L., Jacobs, R., and Hudspeth, A.J. (1981). Directional sensitivity of individual vertebrate hair cells to controlled deflection of their hair bundles. Ann. N Y Acad. Sci. *374*, 1–10.

Siemens, J., Lillo, C., Dumont, R.A., Reynolds, A., Williams, D.S., Gillespie, P.G., and Müller, U. (2004). Cadherin 23 is a component of the tip link in hair-cell stereocilia. Nature *428*, 950–955.

Slepecky, N. (1996). Structure of the Mammalian Cochlea (New York: Springer-Verlag).

Söllner, C., Rauch, G.J., Siemens, J., Geisler, R., Schuster, S.C., Müller, U., and Nicolson, T. (2004). Mutations in cadherin 23 affect tip links in zebrafish sensory hair cells. Nature *428*, 955–959.

Sotomayor, M., Corey, D.P., and Schulten, K. (2005). In search of the hair-cell gating spring elastic properties of ankyrin and cadherin repeats. Structure (Camb) *13*, 669–682.

Stauffer, E.A., Scarborough, J.D., Hirono, M., Miller, E.D., Shah, K., Mercer, J.A., Holt, J.R., and Gillespie, P.G. (2005). Fast adaptation in vestibular hair cells requires myosin-1c activity. Neuron *47*, 541–553.

van Netten, S.M., and Kros, C.J. (2000). Gating energies and forces of the mammalian hair cell transducer channel and related hair bundle mechanics. Proc. R. Soc. Lond. B. Biol. Sci. *267*, 1915–1923.

von Békésy, G. (1960). Experiments in Hearing (New York: McGraw-Hill Inc.).

Weil, D., Blanchard, S., Kaplan, J., Guilford, P., Gibson, F., Walsh, J., Mburu, P., Varela, A., Levilliers, J., Weston, M.D., et al. (1995). Defective myosin VIIA gene responsible for Usher syndrome type 1B. Nature *374*, 60–61.

Wood, J.D., Muchinsky, S.J., Filoteo, A.G., Penniston, J.T., and Tempel, B.L. (2004). Low endolymph calcium concentrations in deafwaddler2J mice suggest that PMCA2 contributes to endolymph calcium maintenance. J. Assoc. Res. Otolaryngol. *5*, 99–110.

Wu, Y.C., Ricci, A.J., and Fettiplace, R. (1999). Two components of transducer adaptation in auditory hair cells. J. Neurophysiol. *82*, 2171–2181.

Yamoah, E.N., Lumpkin, E.A., Dumont, R.A., Smith, P.J., Hudspeth, A.J., and Gillespie, P.G. (1998). Plasma membrane Ca^{2+}-ATPase extrudes Ca^{2+} from hair cell stereocilia. J. Neurosci. *18*, 610–624.

Zhao, Y., Yamoah, E.N., and Gillespie, P.G. (1996). Regeneration of broken tip links and restoration of mechanical transduction in hair cells. Proc. Natl. Acad. Sci. USA *93*, 15469–15474.

Zheng, J., Shen, W., He, D.Z., Long, K.B., Madison, L.D., and Dallos, P. (2000). Prestin is the motor protein of cochlear outer hair cells. Nature *405*, 149–155.