CHAPTER 12

Hair Cell Mechanotransduction: The Dynamic Interplay Between Structure and Function

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I. Overview
II. Auditory System
III. Hair Bundle Structure
IV. MET Involves Mechanically Gated Channels
V. Where Are These Channels?
VI. The Gating Spring Theory
VII. How Are the Channels Activated?
VIII. To be or Not to be Tethered
IX. Characterizing Channel Properties?
X. MET Channel Pore
XI. Adaptation
A. Motor Adaptation
B. Multiple Components of Adaptation
C. Fast Adaptation
D. Functional Role of Adaptation
XII. The Dynamic Hair Bundle
XIII. Summary and Future Directions
References

I. OVERVIEW

Hair cells are capable of detecting mechanical vibrations of molecular dimensions and to do this at frequencies in the 10s to 100s of kHz. This remarkable feat is accomplished by the interplay of mechanically gated ion

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channels located near the top of a complex and dynamic sensory hair bundle. The hair bundle is composed of a series of actin filled stereocilia that has both active and passive mechanical components as well as a highly active turnover process whereby the components of the hair bundle are rapidly and continually recycled. Hair bundle mechanical properties will have significant impact on the gating of the mechanically activated channels and delineating between attributes intrinsic to the ion channel and those imposed by the channel's microenvironment is often difficult. The goal of this chapter is to delineate between what is known and accepted regarding hair cell mechanotransduction and what remains to be explored, particularly in relation to the interplay between hair bundle properties and mechanotransducer channel response. In addition, the interplay between hair bundle dynamics and mechanotransduction will be discussed.

II. AUDITORY SYSTEM

The peripheral auditory system is a remarkable feat of evolutionary biological engineering with a threshold of sensitivity at molecular dimensions; where stimulus energy levels are below energy levels associated with the Brownian motion of the sensory organelle, the hair bundle (Denk and Webb, 1992; Jaramillo and Wiesenfeld, 1998). The dynamic range encompasses more than six orders of magnitude without damage or saturation. The frequency range is from 10s to 100,000s of Hertz and frequency discrimination is less than 1 Hz. For a system that is thermodynamically challenged these characteristics are remarkable (Bialek, 1987). Although hair cells come in a variety of "flavors," with different innervation patterns and different complements of ion channels, the one commonality to all hair cells is the presence of an apical hair bundle that when deflected activates a mechanically gated ion channel.

That activation of mechanically gated channels in the sensory hair bundle underlies sensory processing in the auditory and vestibular system has been known for almost 30 years (Hudspeth and Corey, 1977). Many fundamental principles regarding this mechanoelectric transduction (MET) process, such as the gating spring theory, and the presence of adaptation have been elucidated and are consistent across a variety of species and end organs. Multiple functional consequences, such as providing mechanical amplification and filtering, extending the dynamic range of the hair cell, and setting the hair cell resting potential, have been ascribed to the adaptation and activation process. Much like the Hodgkin–Huxley model of the action potential, the gating spring model of mechanotransduction established a framework from which to investigate the properties of hair cell transduction.

Similarly, this model provides a microscopic interpretation of macroscopic data that although consistently supported by results across species and end organs remain to be tested at the mechanistic level. The purpose of this chapter is to discuss MET and the dynamic properties of the sensory hair bundle including channel gating and adaptation; addressed in relation to the complex structure of the sensory hair bundle.

III. HAIR BUNDLE STRUCTURE

Hair bundles come in a variety of shapes and sizes; however, there are some fundamental commonalities to them. Each hair bundle consists of rows of stereocilia, ordered in height, that form a staircase pattern (Fig. 1B and C). The hair bundle resides on the apical surface of the hair cell and is positioned between the hair cell body and the overlying tectorial membrane (Fig. 1A), optimally located to sense any shearing motion between structures. Stereocilia are actin-filled membrane protrusions of up to 100 μm in length (in vestibular hair bundles), where parallel and uniformly polarized actin filaments are tightly cross-linked to form a paracrystalline structure. This paracrystalline actin core gives the stereocilia a rigidity that allows them to rotate about their base, rather than bend when stimulated (Crawford and Fettiplace, 1985). A variety of extracellular filaments connect stereocilia of adjacent rows. Ankle links, horizontal top connectors, shaft connectors, and the tip-link (Bashtanov *et al*., 2004) together anchor the stereocilia so that they move as a unit when stimulated (Crawford and Fettiplace, 1985).

IV. MET INVOLVES MECHANICALLY GATED CHANNELS

The hair bundle is the site of MET (Hudspeth and Corey, 1977; Tilney and Saunders, 1983). Initial work in frog saccule demonstrated that hair bundle deflection toward its tall edge reduced input resistance while movement toward the short cilia increased resistance, suggesting that an ion channel was being opened and closed in response to hair bundle deflection (Hudspeth and Corey, 1977). Measurements from a mammalian cochlea preparation similarly demonstrated an electrical response from outer hair cells (OHC) in response to sound pressure changes (Russell and Sellick, 1978). Directional sensitivity of the hair bundle was more quantitatively assessed (Shotwell *et al*., 1981) and results demonstrated a specific axis of sensitivity that aligned with the graded change in hair bundle height; sensitivity falling off as a cosine function of the angle of rotation in either direction away from the most sensitive position. Directional sensitivity of the uniquely organized OHC

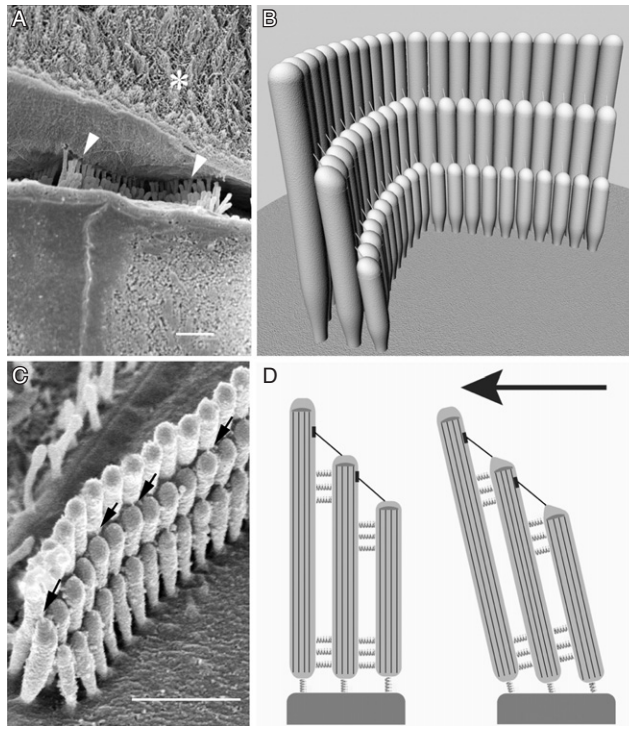

FIGURE 1 Hair bundle structure. (A) Freeze fracture image of OHC with sensory hair bundle embedded in overlying tectorial membrane; arrows indicate tallest row of stereocilia in contact with tectorial membrane. (B) Scanning electron micrograph of OHC stereocilia showing the three rows of increasing height. Arrows indicate tip-link connecting adjacent stereocilia and thought to provide directional sensitivity to the hair bundle. (C) Schematic drawing of OHC bundle showing the typical orientation of stereociliary rows. (D) Schematic of three stereocilia at rest and during stimulation. The stereocilia rotate about their base creating a shearing force near the tips where the tip-link creates tension between adjacent rows. Arrow indicates movement that opens channels.

bundle has not been directly investigated, though evidence suggests that it would be consistent with what has been found in other hair cell types. The orientation of the OHC bundle (Fig. 1B) is significantly curved so that stimulation toward the tall edge of the hair bundle can encompass up to 180° of rotation. How this might effect response properties remains to be determined.

A direct activation of an ion channel was further supported by field recordings of microphonic potentials from an isolated sensory epithelial preparation; here the kinetics of the response excluded a multistepped signal transduction process (Corey and Hudspeth, 1979b). A modification of this

preparation allowed voltage clamping of the epithelium, further demonstrating the rapid nature of mechanical sensitivity and illustrating a Ca^{2+} dependence to the activation kinetics (Corey and Hudspeth, 1983). A comparable set of experiments in mouse cochlea culture demonstrated an electrical response from hair bundle deflection consistent with ion channel gating (Russell *et al.*, 1986). Direct measurements of activation kinetics in hair cells from turtle auditory papilla (Crawford *et al.*, 1989) further validated the presence of mechanically gated ion channels in the sensory hair bundle. Single-channel recordings of the MET channel (Ohmori, 1984; Crawford *et al.*, 1991; Kros *et al.*, 1992; Ricci, 2002) unequivocally demonstrated that hair bundle deflection activated MET channels in hair cells.

V. WHERE ARE THESE CHANNELS?

Where in the hair bundle are these elusive channels located (Fig. 2)? Extracellular recordings first suggested that ion channels were located near the tops of the stereocilia (Hudspeth, 1982). This finding was supported by

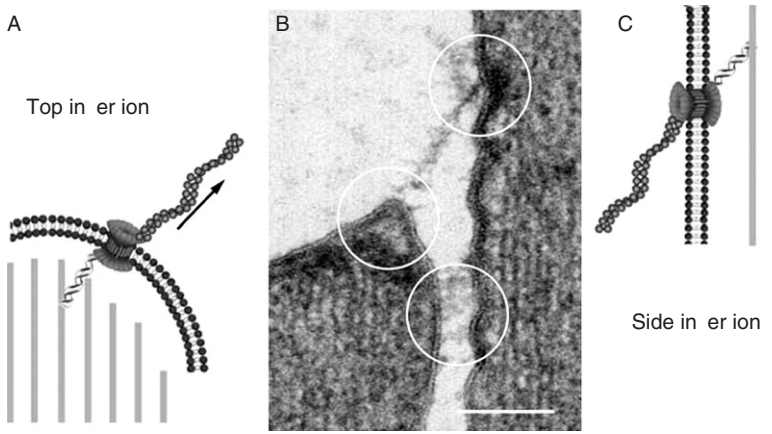

FIGURE 2 Tip-link and possible locations for the MET channel. Tip-link connects rows of adjacent stereocilia (B). Mechanically gated channels are hypothesized to be located at either (A, C) or both ends of the tip-link. A third possibility is at a dense region where the stereocilia come close together. Possible channel locations are indicated by white circles in panel (B). Also shown in the transmission electron micrograph of (B) is the tenting of the membrane at the top of the stereocilia, thought to be created by the tip-link pulling on this membrane. (A, C) show possible configurations of the MET channel tethered to the tip-link and cytoskeleton when located at either end of this link.

iontophoretic mapping of the location based on sensitivity to the channel blocker gentamycin (Jaramillo and Hudspeth, 1991), by the appearance of a Ca^{2+} "blush" near to the tops of the stereocilia, presumably created by Ca^{2+} entry through the channel (Lumpkin and Hudspeth, 1995), and antibody labeling to a putative MET channel-binding site also suggested a location near to the tops of the stereocilia (Furness *et al.*, 1996). Ca^{2+} gradients in the stereocilia, presumably created by entry through MET channels, also support a location near the tops of the stereocilia (Lumpkin and Hudspeth, 1998). Although existing data consistently puts the channels near to the top of the stereocilia, the precise location and relationship to accessory structures (like the stereociliary links) remains to be determined. Reports also exist for a channel located near the base of the stereocilia, a conclusion based on Ca^{2+} imaging experiments (Ohmori, 1988).

VI. THE GATING SPRING THEORY

The recognition that mechanotransduction involved the gating of an ion channel driven by shearing forces created by hair bundle deflection led to the initial gating spring hypothesis (Corey and Hudspeth, 1983). In its most simple form, this hypothesis suggests that a tensed elastic element exists between stereocilia and the channel such that force is exerted onto the channel with stereocilia shearing (Fig. 3). Concomitant with the identification of a channel-driven mechanism and the localization of the ion channel to the sensory hair bundle apical surface was the evaluation of hair bundle mechanics. Work in turtle auditory papilla demonstrated that the hair bundle bends as a unit, pivoting about its base, creating a shearing between stereocilia of adjacent rows (Crawford and Fettiplace, 1985; Fig. 1D). This work also provided the first evidence of active bundle movements—oscillations that might provide a mechanical filter to incoming sound waves. A hypothesis generated from the gating spring theory was that channel opening might alter hair bundle compliance (Hudspeth, 1982). The total hair bundle stiffness at a minimum is the sum of the passive stiffness and the gating compliance (Fig. 3). Estimates of gating compliance suggest that gating provides more than half of the hair bundle stiffness (Marquis and Hudspeth, 1997). The channel gate might be in series with the gating spring so that when channels open there is an increase in overall length, equivalent to the length of the gate, that momentarily slackens the gating spring reducing stiffness (increasing compliance; Fig. 3). A change in hair bundle compliance associated with MET channel gating was first reported in frog saccule (Howard and Hudspeth, 1988). A comparable change in compliance has also been

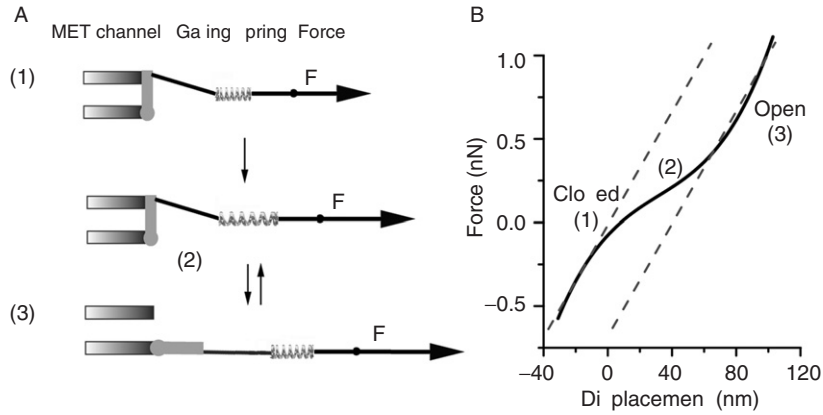

FIGURE 3 Gating spring theory. (A) Gating spring theory posits the presence of a spring through which force is applied to the channel gate. The channel gate opens in series with this spring, thus transiently reducing the force onto this spring and increasing compliance. The force displacement plot of (B) supports this simple model, illustrating a linear (Hookean) component of the plot when channels are either closed (1) or open (3), the slopes of which are the same given that the spring constant remains the same. The nonlinear component (2) represents the transition between closed and open where the channel gate (probability of opening of the channel) is increasing. This region can be described by the Boltzmann function that also describes the activation curve for the MET channel.

identified in mouse cochlea (Russell *et al.*, 1992), turtle auditory papilla (Ricci *et al.*, 2002), and rat cochlea (Kennedy *et al.*, 2005), giving strong support to a common gating mechanism across hair cells of different species and end organs. The gating spring theory has been formalized in a variety of ways (Markin and Hudspeth, 1995a; van Netten and Kros, 2000; Ricci *et al.*, 2002) incorporating two and three state models. In its simplest form, the model suggests that the difference in energy for a given stimulus is equivalent to the difference in energy between the open and closed states of the channel, which in turn is equivalent to the displacement difference times a constant (z) that is composed of the gating spring constant (k_{gs}) times the gating swing, (d) and so takes the form:

$$\Delta A = A_c - A_o = z(x - x_o) \tag{1}$$

where A is energy associated with the closed (c) or open (o) state of the channel, x is the displacement, usually referring to movement at the top of the stereocilia. More general formulations can be found that do not require the assumption of two states (Howard and Hudspeth, 1988; van Netten and Kros, 2000; van Netten *et al.*, 2003). From this simple equation it is clear

that no distinct mechanism is implied and that caution must be taken when ascribing physical components to the hypothesis. A practical example of this problem comes from the consistent estimate of *d*, the gating swing, at values in excess of 7 nm, a value much larger than would be predicted for the mechanical gate of a channel or of a conformational change in the channel as it goes from closed to open state (Howard and Hudspeth, 1988; Markin and Hudspeth, 1995a; van Netten *et al*., 2003; see schematic in Fig. 3). Similarly, estimates of the number of channels per hair bundle based on the single-channel gating force estimates are more than an order of magnitude greater than estimates made using single-channel conductance values (Ricci *et al*., 2002). These discrepancies do not negate the theory but do question the underlying mechanistic interpretation. It is possible to account for these discrepancies in several ways. First, the assumption in the gating spring model for the whole bundle is that the springs are in parallel, an assumption unlikely to be absolutely true on the microscopic level given the complex interconnections between stereocilia (Howard and Hudspeth, 1988; Markin and Hudspeth, 1995a; van Netten and Kros, 2000; Ricci *et al*., 2002). Channels in series, a likely outcome of a bundle organization with multiple rows of stereocilia, would mean summing of the gating springs (Fig. 4). At an extreme, the movement of the gating spring would be proportional to the number of rows of stereocilia and therefore might significantly reduce the length of the gating spring (Fig. 4).

More quantitative information regarding linkages between stereocilia and the relative movements between stereocilia are needed to resolve this issue. Interestingly, estimates of gating swing in mammalian cochlear hair cells are closer to 2 nm per channel—values more attuned with the molecular dimensions of a channel. Interestingly, such lower values can be obtained by taking into account bundle organization that includes both series and parallel components (van Netten and Kros, 2000). A second possibility is that the gating swing does not solely represent a channel gate but includes elements in series with the channel conformational change. These elements could be protein or lipid. A third possibility is that the scaling value (often termed γ) that converts the bundle motion at the top of the stereocilia to that at the channel could be wrong. As we do not know where the channels are located, there are assumptions associated with the estimates. In saccular hair cells, γ is about 0.1–0.15 (Markin and Hudspeth, 1995a) while in mammalian hair cells more widely ranging values have been estimated (OHC's: 0.05–1.0; vestibular hair cells: 0.02–0.04; Geisler, 1993; Pickles, 1993; Markin and Hudspeth, 1995a; Furness *et al*., 1997; van Netten and Kros, 2000). And finally, it is possible, though somewhat unlikely that the compliance change measured is not actually associated with the MET channel but represents an additional compliant component of the hair bundle.

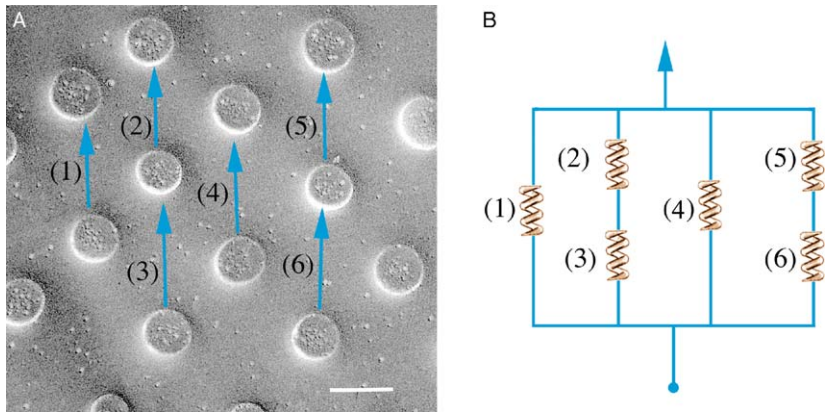

FIGURE 4 Stereociliary organization suggests both series and parallel contributions to the gating spring. Freeze fracture (A) of the apical surface of an OHC showing the insertion points of the stereocilia. Blue arrows indicate directional sensitivity and the portion of the bundle diagrammed in (B). (B) is a schematic representation of the circuit presumed to be generated by the orientation of the stereocilia, demonstrating that although most models have considered the gating springs to be in parallel, there is a significant series component to the structure.

VII. HOW ARE THE CHANNELS ACTIVATED?

The identification of a link between stereocilia, located near to the tops and connecting stereocilia of adjacent rows and aligned along the axis of sensitivity of the hair bundle, added a morphological correlate to the gating spring theory (Pickles *et al.*, 1984; Fig. 2B). This tip-link appeared to be localized appropriately and to have properties associated with a putative gating spring. Deflection of the hair bundle would stretch the link, increasing tension onto the channel. Loss of the tip-link results in loss of transduction (Assad *et al.*, 1991); recovery of this link restored transduction in chick (Zhao *et al.*, 1996). For the tip-link to be the gating spring it needs to be inherently elastic (like a spring). Ultrastructural investigations revealed a coiled filamentous structure very unlikely to have the appropriate elastic properties (Kachar *et al.*, 2000). Findings have implicated cadherin 23 as a component of the tip-link complex (Siemens *et al.*, 2004; Sollner *et al.*, 2004). Other investigations have demonstrated cadherin 23 as a component of side-links and questioned their role as the tip-link, in particular because detection of cadherin 23 in mature hair bundles is limited (Gillespie *et al.*, 2005; Michel *et al.*, 2005). Modeling of the elastic properties of cadherin 23 suggests it

cannot be the gating spring because it is too stiff (Sotomayor *et al*., 2005). Here too the model must be interpreted cautiously because it models only a portion of the molecule and makes assumptions regarding the structural organization of the hair bundle, that is, that macroscopic gating force estimates can be accurately interpreted at the single-channel level. Together these findings suggest that perhaps the tip-link serves as a tether, translating the force associated with hair bundle deflection to the ion channel, as some element is required to translate the shearing motion of the stereocilia into a force exerted onto the channel. Determining the role of the tip-link in transduction requires revisiting the site of the MET channels. Figure 2 depicts two stereocilia with the tip-link in between. The channel being located near to the top of the stereocilia suggests it might be located at either end or both ends of the tip-link (Hudspeth, 1989; Hudspeth and Gillespie, 1994; Corey, 2003). Another argument considers the tip-link simply as a structural element serving to keep the stereocilia in close approximation and places the channels at a location, based on immunocytochemistry, below the tip-link, where the stereocilia are juxtaposed (Furness *et al*., 1996, 2002). A third possibility is that the tip-link serves as a tether but does not directly couple to the MET channels, rather it serves to stretch the membrane and the membrane exerts force onto the channel (Kachar *et al*., 2000; Fig. 5). Schematic representations of these possibilities are given in Figs. 2 and 4. The only experimental argument suggesting that MET channels exist at both ends of the tip-link is from Ca^{2+} imaging data (Denk *et al*., 1995). The argument states that the geometry of the bundle requires MET channels be located on the side of the tall stereocilia (top end of the tip-link) if there is a Ca^{2+} signal present in the tallest row of stereocilia. If a Ca^{2+} signal were detected in the shortest row of stereocilia, then channels must be located at the tops of the stereocilia as well. Both results were observed. Unfortunately, the quantification of the links near the top of tallest stereocilia is lacking. Horizontal links may provide additional stimulation between tall stereocilia thus making interpretations regarding the channel location at the side questionable. In addition, the argument that channels need be near the sterocilia top (base of the tip-link) because of a signal in the lowest rank of stereocilia does not require that the channel be directly coupled to the tip-link. Other than these Ca^{2+} imaging data, there is no direct evidence to delineate between channel locations at the microscopic level. The gating spring model can accommodate all of these possibilities. The tenting observed in the top of the stereocilia (Fig. 2B) is suggestive of an increased membrane tension (Kachar *et al*., 2000). This tenting is lost when the tip-link is disrupted (Rzadzinska *et al*., 2005). As will be discussed further below, identifying the precise location of the MET channel is critical to unraveling some of the mechanical constraints on how the system might operate at the molecular level.

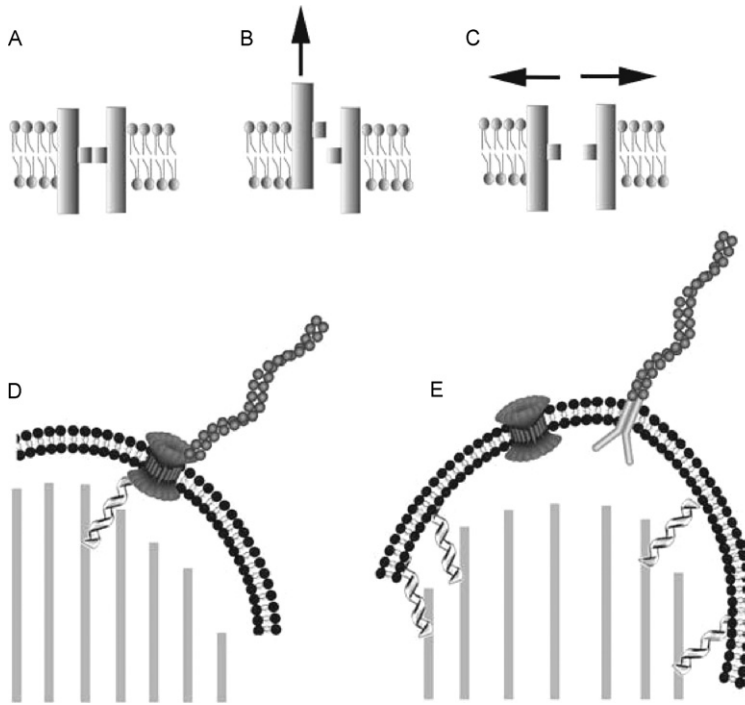

FIGURE 5 How is the MET channel gated? (A–C) The direction that force is sensed by these mechanically gated channels is unknown. Force can be represented either perpendicular (B) or parallel to the membrane (C). Mechanically gated channels of other systems have been shown to be sensitive to either so both are real possibilities for hair cells. (D) illustrates the classical hypothesis that the MET channel is directly tethered to the tip-link; however, direct evidence for this tethering does not exist and the possibility that the channel is nontethered remains to be rigorously explored (E). The tip-link can exert force onto the channel either directly or indirectly by tenting the membrane.

VIII. TO BE OR NOT TO BE TETHERED

As the molecular identity of the MET channel remains unknown, the mechanism by which the channel is activated is also a point of much speculation. Illustrations like those shown in Fig. 2 have reinforced the concept that the MET channel must be tethered extracellularly as well as to the cytoskeleton. In addition, the ubiquitous presence of the tip-link in different hair cell types furthered the argument that the tip-link is involved in channel gating and that the channel must be tethered. In fact, adjacent rows of stereocilia need to be tethered in order for force to be translated to the

channel; no data exists suggesting that the channel requires a direct tethering. A mechanically gated channel can be activated by forces exerted either parallel or perpendicular to the lipid bilayer. Many mechanoreceptors work without tethers and sense force via the plasma membrane (Sukharev *et al.*, 1997; Kung, 2005). The bacterial mechanogated channels, the MscL family, are activated by forces generated by the hydrophobic interactions between the lipid and the channel protein—forces that are applied parallel to the membrane (Sukharev *et al.*, 1997; Kung, 2005). On the other hand, in *C. elegans* touch receptors, evidence exists for tethering from both extracellular and intracellular sites at least in part via microtubules (Huang *et al.*, 1995; Du *et al.*, 1996; O'Hagan and Chalfie, 2006). However, evidence has begun to question the requirement of intracellular tethers, particularly the larger microtubules (microtubules composed of 15 protofilaments; O'Hagan and Chalfie, 2006). Both TREK and TRAAK are two mechano-gated potassium channels that are sensitive to membrane stretch and do not require cytoskeletal or extracellular tethering (Maingret *et al.*, 1999; Patel *et al.*, 2001). In hair cells, direct evidence regarding gating or tethering is sparse. Schematic representations of tethered versus nontethered activation of the hair cell MET channel are given in Fig. 5. In the tethered version, the tip-link is shown directly coupled to the channel so that force is exerted perpendicular to the membrane while in the nontethered version the tip-link is shown serving as a lever pulling on the membrane, exerting force parallel to the bilayer. The two examples represent the extreme cases (despite the tethered version being the current model), the two additional possibilities, tethered only externally or tethered only internally are not shown but could equally explain existing data. Interestingly, the gating spring theory can account for any of these configurations, perhaps slightly better for the nontethered version where the gating swing would now include a lipid bilayer component being pulled and thus may be predicted to be larger (Fig. 5). Hypotheses have suggested that a series of ankyrin repeats at the terminal region of a channel could have spring-like properties that might represent the molecular correlate of the gating spring (Howard and Bechstedt, 2004; Sotomayor *et al.*, 2005). Without more accurate data regarding single-channel gating forces and without molecular identification of the channel, these hypotheses, though novel and exciting, remain to be tested.

Presently, data does not exist to determine whether the MET channel senses force exerted perpendicular or parallel to the membrane. The two possibilities are depicted in Fig. 5 and MET channels sensitive to either force direction have been identified (see other chapters). Limiting the ability to determine the mechanism of channel activation are several factors including identifying channel location, the channel molecular nature, and the mechanism of stimulation. Hair bundle stimulation has taken a variety of forms, fluid jet,

stiff or flexible fibers attached to piezo-driven actuators, or optical tweezers (Corey and Hudspeth, 1980; Crawford and Fettiplace, 1985; Ohmori, 1985, 1987; Howard and Hudspeth, 1987; Crawford *et al*., 1989; Kros *et al*., 1992; Benser *et al*., 1993; Jaramillo and Hudspeth, 1993; Holt *et al*., 1997; Ricci *et al*., 2000; Kros *et al*., 2002; Kennedy *et al*., 2003; Vollrath and Eatock, 2003; Ricci *et al*., 2005; Cheung and Corey, 2006). The major limitation to these methods is that the stimulus to the channel is filtered via hair bundle mechanics, mechanics that are not being controlled or monitored at the molecular level. The most obvious example of this is adaptation, a process that resets the molecular orientation of the channel with reference to the hair bundle position occurs when stiff probes, meant to be a displacement clamp are used (Fig. 8). If the bundle were clamped at the molecular level, adaptation would not occur. That is, adaptation requires tension on the MET channel to be relieved, this can only occur if there is a physical movement within the hair bundle, a movement that should be eliminated by a true displacement clamp. The problem is akin to trying to interpret voltage clamp data that is not properly space clamped. Development of new methodologies is needed for investigations of MET channel and mechanics at the single (or paired) stereocilium level in order to more directly investigate gating mechanisms.

IX. CHARACTERIZING CHANNEL PROPERTIES?

Separating properties intrinsic to the MET channel from those imposed onto the channel from accessory proteins and hair bundle mechanics is difficult largely due to the problems described above. Not having absolute control over the micromechanics of the hair bundle limits the ability to directly probe molecular mechanisms. A clear example of this problem is the investigations of channel kinetics. Activation kinetics has been inferred in frog from macroscopic measurements (Corey and Hudspeth, 1983) and has been measured directly in turtle (Crawford *et al*., 1989; Ricci *et al*., 2005) and rat (Crawford *et al*., 1989; Ricci *et al*., 2005). In both frog and turtle, the kinetics were Ca^{2+}-dependent (Corey and Hudspeth, 1983; Ricci *et al*., 2005). In turtle, kinetics varied with characteristic frequency of the hair cell, suggesting variations in channel structure. In rat, the kinetics are too fast to accurately measure, implying that they are at least an order of magnitude faster than in turtle or frog (Crawford *et al*., 1989; Ricci *et al*., 2005), further suggesting variations in channel structure. However, not having a direct measure of the force exerted onto the channel makes a determination regarding the nature and the underlying mechanism of the kinetic difference difficult. It is as likely that hair bundle mechanics vary allowing a faster force

application to the channel in mammals and in higher frequency hair cells as it is that the intrinsic channel properties vary tonotopically. Without knowing the rate-limiting step, conclusions regarding mechanism are speculation. So even channel properties normally considered intrinsic to the structure of the channel protein, like activation kinetics, must be evaluated carefully for the hair cell MET channel.

X. MET CHANNEL PORE

What channel properties might be considered intrinsic to the channel? Given that the channel identity remains elusive, creating a profile of properties serves as an important tool for correctly identifying the channel protein. Most likely properties associated with the channel pore, like permeation, rectification, and pharmacology, will be intrinsic to the channel protein. The MET channel is a nonspecific cation channel (Corey and Hudspeth, 1979a; Ohmori, 1985, 1989; Crawford *et al.*, 1989; Kros *et al.*, 1992; Farris *et al.*, 2004). MET channels show little rectification in high extracellular Ca^{2+} solutions but a slight inward rectification appears when Ca^{2+} is lowered (Crawford *et al.*, 1989; Kros *et al.*, 1992; Farris *et al.*, 2004). The channels have a high Ca^{2+} permeability (Ohmori, 1985; Crawford *et al.*, 1991; Lumpkin *et al.*, 1997; Ricci and Fettiplace, 1998). Ca^{2+} both permeates and blocks the channel with a half blocking [Ca^{2+}] of 1 mM (Crawford *et al.*, 1991; Kros *et al.*, 1992; Lumpkin *et al.*, 1997; Ricci and Fettiplace, 1998; Gale *et al.*, 2001), likely as a function of ion interactions within the channel pore (Lumpkin *et al.*, 1997). Ca^{2+} binds within the pore at a distance equivalent to about 0.5 of the distance into the electric field (Kros *et al.*, 1992; Gale *et al.*, 2001; Farris *et al.*, 2004).

The pharmacology of the MET channel is unusual in that many compounds serve as open channel blockers (Farris *et al.*, 2004). The major properties of molecules thought to be MET channel blockers are their being positively charged so as to be driven into the channel electrochemically and the molecule being of sufficient size to plug the pore (Farris *et al.*, 2004). Aminoglycosides have long been known to block hair cell MET channels (Kroese *et al.*, 1989; Kimitsuki and Ohmori, 1993; Glowatzki *et al.*, 1997; Ricci, 2002; Marcotti *et al.*, 2005; Waguespack and Ricci, 2005). The block appears to hold the channel in its open state (Kroese *et al.*, 1989; Denk *et al.*, 1992; Jaramillo and Hudspeth, 1993;) and the efficacy of block is directly related to the probability of opening of the channel (Ricci, 2002). Evidence suggests that aminoglycosides are permeable blockers of the channel but also report the unusual finding that the permeability works with external application but not with internal application (Marcotti *et al.*, 2005). This unusual finding was also observed with the permeable blocker FM1–43 (Gale *et al.*, 2001).

Together these data suggest that the channel may have an open state at positive potentials that is different than that at negative potentials. This hypothesis was first suggested by the complex blocking effects of amiloride on hair cell MET channels (Jorgensen and Ohmori, 1988; Rusch *et al.*, 1994) and also in oocyte mechanosensitive channels (Lane *et al.*, 1993). The potential for an additional open state is indirectly supported by evidence suggesting channel rectification when the hair bundle is placed in lowered Ca^{2+} solutions (Crawford *et al.*, 1989) and by single-channel measurements that support this rectification (Ricci *et al.*, 2003). The pharmacological profile established for the MET channel overlaps with that of several classes of channels including cyclic nucleotide gated channels, transient receptor potential channels, Ca^{2+} channels, and nicotinic receptor channels (Farris *et al.*, 2004). Using techniques established for investigating nicotinic pore properties (Adams *et al.*, 1980) and applied to other mechanosensitive channels (Cruickshank *et al.*, 1997), sodium channels (Hille, 1971), and NMDA receptor channels (Zarei and Dani, 1994), the hair cell pore dimensions were estimated from the external face. A summary schematic of the MET channel is presented in Fig. 6 that depicts relative pore dimensions (Farris *et al.*, 2004). These estimates suggest a large pore size, befitting the large single-channel conductance measurements (Ricci *et al.*, 2003). Unusually, there was no difference observed in pore dimensions between frequency location despite there being a difference in single-channel

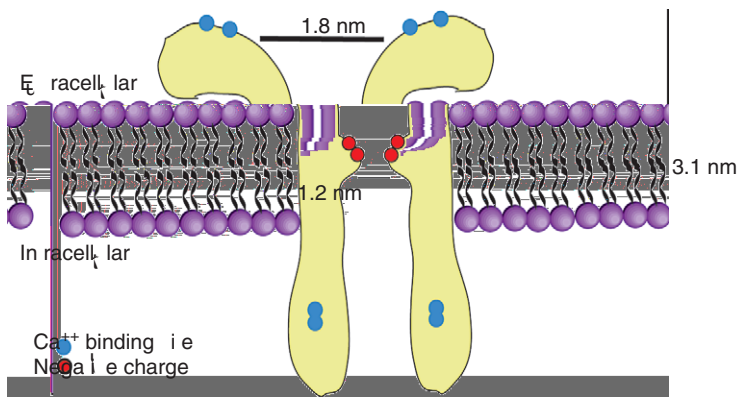

FIGURE 6 Illustration representation of the pore of the MET channel with dimensions estimated from ionic permeabilities (Farris *et al.*, 2004). Width of the external face was estimated based on pharmacological antagonists. Ca^{2+}-binding sites both intracellular and extracellular are posited based on changes in channel kinetics (Ricci *et al.*, 2005). The negative charges near the central region of the channel have been estimated based on the current–voltage responses.

conductance (Ricci *et al.*, 2003; Farris *et al.*, 2004). Similarly, no difference in Ca^{2+} permeation has been identified between frequency locations (Ricci, 2002).

Single-channel properties have been measured in turtle (Crawford *et al.*, 1991; Ricci *et al.*, 2003), chick (Ohmori, 1984), and mouse (Kros *et al.*, 1992). The single-channel conductance increases with characteristic frequency (Ricci *et al.*, 2003)—a property thought to in part underlie tonotopic differences in adaptation kinetics (see below; Ricci and Fettiplace, 1997). The single-channel conductance was also sensitive to external Ca^{2+}, increasing as Ca^{2+} was lowered (Ricci *et al.*, 2003). All of the single-channel data must be evaluated carefully in part because of the unusual manner in which the measurements are made. Whole-cell recordings of single-channel properties are limited in resolution due to membrane noise and filtering imposed by having the entire cell membrane in the electrical circuit. Obtaining single channels by disrupting the hair bundle with BAPTA may have unrecognized consequences. An example of single-channel recordings is given in Fig. 7. These results demonstrate the presence of a mechanically sensitive channel in the stereocilia that has an apparent large single-channel conductance. Because of the filtering and signal:noise difficulties arise resolving subconductance or flickering behavior so that it is possible that other states exist that have not yet been characterized. Measurements using noise analysis obtained much smaller values for this conductance, values likely difficult to observe with direct measurements (Holton and Hudspeth, 1986). Although noise analysis typically underestimates conductance values, the difference (about an order of magnitude) is greater than predicted by the error associated with the technique and may suggest that the sensitivity of the single-channel recordings is limited. The single-channel conductance estimates when compared with macroscopic maximal current responses suggest one or at most two channels per stereocilia (Crawford *et al.*, 1991; Kros *et al.*, 1992; Ricci *et al.*, 2003).

XI. ADAPTATION

To this point, a general overview of transduction and the gating spring theory has been presented that depicts the hair bundle as a passive element and channel gating as the active element. These assumptions will be further elucidated and challenged below, but first the third major component to mechanotransduction, adaptation, need be formally introduced. Figure 8 presents an example of MET currents elicited from mechanical deflection of a hair bundle in the rat cochlea and turtle auditory papilla. The currents activate rapidly and then, despite a constant stimulus, decay with a time course that is dependent on the stimulus intensity and typically has two

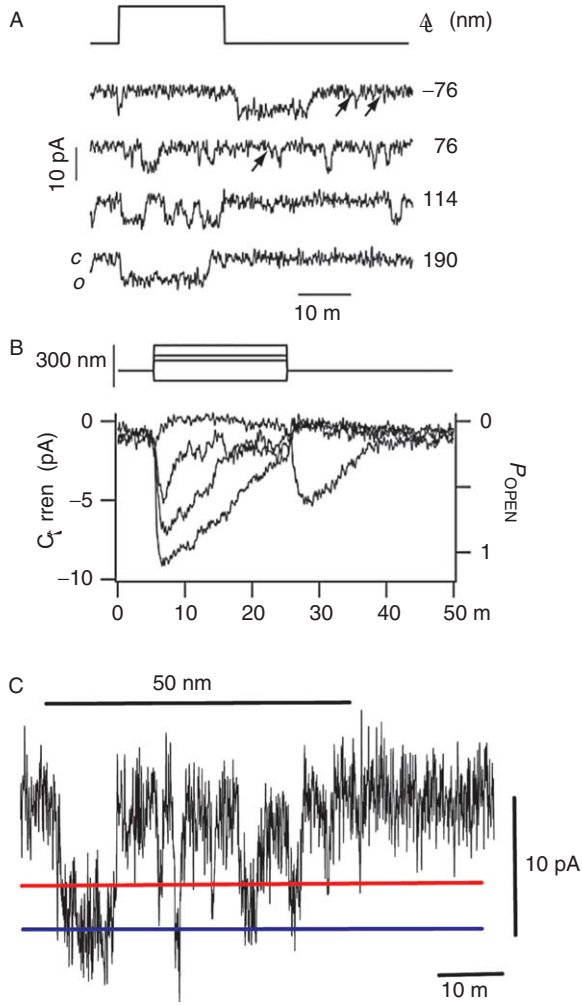

FIGURE 7 (A) Single-channel recordings of MET currents from turtle auditory hair cells (Ricci *et al.*, 2003). (B) Averages of single-channel recordings from increasing stimuli demonstrating that the single-channel behavior is similar to the macroscopic behavior (that is open time increases with increased stimulus). (C) is an enlarged example of single-channel recording meant to demonstrate the limitation of recording single channels in the whole-cell mode. Flickering behavior was observed (red line) that could indicate additional conductance state or could simply reflect a limited voltage clamp speed. Blue line indicates level used to estimate single-channel conductance.

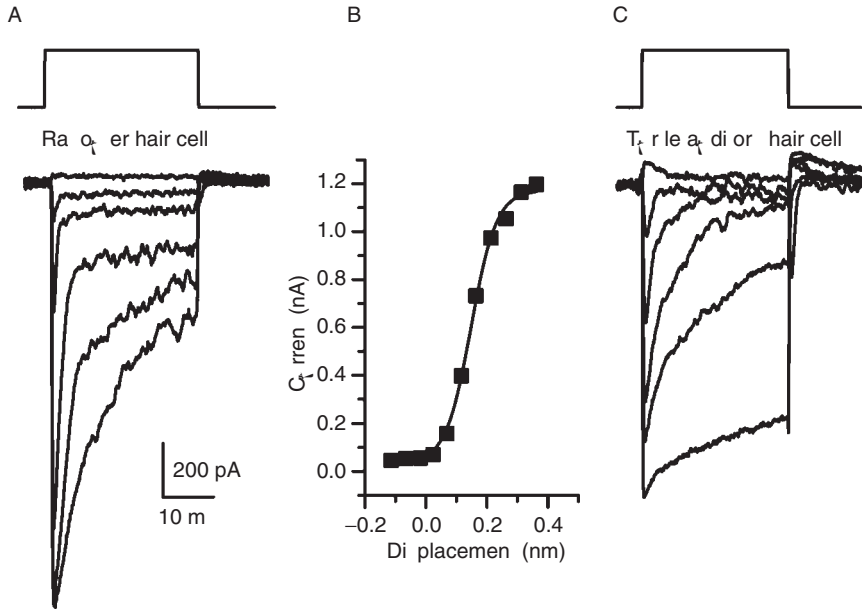

FIGURE 8 Transduction and adaptation are similar between species. Voltage clamp recordings of MET currents from a rat OHC preparation (A) and a turtle auditory papilla hair cell (C). Qualitatively these responses are quite similar. The peak current vs voltage plot for the OHC response is shown in (B) and fit with a double Boltzmann function.

components (Wu *et al.*, 1999). The decay in current is termed adaptation. Adaptation was first described in frog saccule (Eatock *et al.*, 1987). The process is Ca^{2+}-dependent, underlies several important physiological processes, and involves multiple mechanisms (Eatock, 2000; Hudspeth, 2005; Lemasurier and Gillespie, 2005; Fettiplace and Hackney, 2006). Generally, adaptation is a resetting of the relationship between hair bundle position and force sensed by the MET channel. Deflection of the hair bundle toward the tallest rows increases tension in the hair bundle opening channels, Ca^{2+} enters driving a reduction in tension sensed by the MET channel resulting in channel closure. A reduction in tension elicited by hair bundle deflection away from the tallest rows results in an opposite phenomenon where channels initially close, reducing Ca^{2+} in the stereocilia leading to an increase in hair bundle tension that reopens channels. The adaptation processes create a Ca^{2+}-dependent feedback that sets the resting open probability of the channel (Ricci *et al.*, 1998). This feedback system, integrated with other stereociliary Ca^{2+} homeostatic mechanisms (like Ca^{2+} ATPases and buffers) serve to

maintain Ca^{2+} at a constant steady-state level. A consequence of adaptation then is the shifting of the current displacement plot depending on whether intraciliary Ca^{2+} is increased (rightward shift) or decreased (leftward shift). A variety of pieces of data support this basic description of adaptation. The classical experiment compares the current–displacement (*I–X*) plot about the hair bundle resting position against the plot elicited when the bundle is biased by a known amount (Crawford *et al.*, 1989). Measurements of hair bundle compliance show an increased compliance that correlates with slow adaptation rates (Howard and Hudspeth, 1987; Assad *et al.*, 1989; Ricci *et al.*, 2000; Cheung and Corey, 2006). Disruption of tip-links results in a bundle "relaxation" movement indicating a standing tension in the bundle, presumably established by adaptive forces (Assad *et al.*, 1991). Voltage-dependent hair bundle movements also support this basic description (Assad *et al.*, 1989; Cheung and Corey, 2006; Ricci *et al.*, 2002).

A. Motor Adaptation

Adaptation was first suggested to be a myosin-driven process based on its Ca^{2+} sensitivity and change in hair bundle compliance (Eatock *et al.*, 1987; Howard and Hudspeth, 1987; Assad and Corey, 1992). The premise of the model suggests that the MET channels are tethered to the actin cytoskeleton by myosin, Ca^{2+} entry triggers the release of myosin from the actin resulting in a slippage of the channel down the stereocilia, reducing tension in the gating spring closing channels. When channels are closed, the myosin climbs the actin restoring tension to the gating spring. Implicit with the classical view of adaptation is that the channels are located along the side insertion of the tip-link so that myosin can move up and down the actin and also that the channel is tethered to the cytoskeleton. Effects on channels located near the top of the stereocilia would be indirect via translation through the tip-link. A variety of evidence exists supporting the basic hypothesis that myosin is involved in adaptation. The process is Ca^{2+}-dependent (Corey and Hudspeth, 1983; Crawford *et al.*, 1989, 1991; Hudspeth and Gillespie, 1994; Benser *et al.*, 1996; Walker and Hudspeth, 1996; Ricci and Fettiplace, 1998; Ricci *et al.*, 1998). Interfering with the myosin cycle alters adaptation (Gillespie and Hudspeth, 1993; Wu *et al.*, 1999). Identification of myosin 1C isozymes in the hair bundle and its immunolocalization at the tip-link insertion sites also implicated this isozyme in adaptation (Gillespie *et al.*, 1993; Metcalf *et al.*, 1994). The calmodulin-dependence of adaptation (Walker and Hudspeth, 1996) indirectly implicated myosin 1C as direct interactions between calmodulin and myosin 1C have been observed (Cyr *et al.*, 2002). A novel chemical-genetic strategy provides the most direct evidence implicating myosin 1C

(Holt *et al.*, 2002). The ATP-binding site of myosin 1C was altered making it selectively vulnerable to a modified ATP analogue. Incorporation of this modified myosin into hair cells allowed evaluation of its role in adaptation, where adaptation was reduced when the myosin cycle was interrupted (Holt *et al.*, 2002). Recent construction of a mouse permanently modified with this construct has confirmed these initial findings in vestibular hair cells (Stauffer *et al.*, 2005). Confirmation of these results in the auditory system remains as does the direct link to hair bundle mechanics that this mouse should provide. Several elegant experiments have investigated myosin 1C force-generating properties at the single molecule level; this work argues that the myosin properties are ideal for an adaptation motor in that they have a strain-sensing ADP release mechanism and two movements associated with the head group (Batters *et al.*, 2004a,b).

Although a variety of evidence supports a role for myosin 1C in hair cell adaptation, this mechanism is by no means solved. Whether motor adaptation exists in mammalian OHCs may be questioned by the current recordings which show little slow decay in current, being largely the fast component of adaptation (Kennedy *et al.*, 2003; Section XI.C). In addition, immunocytochemistry (Schneider *et al.*, 2006) shows a much more diffuse pattern of labeling along the stereocilia as compared to originally reported (Garcia *et al.*, 1998; Steyger *et al.*, 1998) not necessarily consistent with the conventional interpretation of its role in adaptation. Given that myosins have many roles in cellular function and maintenance care must be taken when ascribing a particular function to these ubiquitous proteins (Hasson and Mooseker, 1997; Friedman *et al.*, 1999; Krendel and Mooseker, 2005).

B. Multiple Components of Adaptation

From the early work investigating adaptation, a discrepancy existed, where data from frog implicated a motor mechanism with adaptation rates in the tens of milliseconds (Eatock *et al.*, 1987; Howard and Hudspeth, 1987; Assad *et al.*, 1989; Assad and Corey, 1992), whereas data from turtle auditory papilla suggested millisecond time courses and initially did not find a mechanical correlate of adaptation thereby implicating a channel mechanism (Crawford and Fettiplace, 1985; Crawford *et al.*, 1989). The discrepancy was furthered when the mechanical response of turtle hair bundles was shown to be in the opposite direction to that reported in frog (Assad *et al.*, 1989; Ricci *et al.*, 2000). However, this work also demonstrated that a second mechanical response could be obtained depending on hair bundle resting position (Ricci *et al.*, 2002). In addition, the kinetics of adaptation as well as pharmacological sensitivities suggested perhaps two components of adaptation might exist

(Wu *et al.*, 1999). More light was shed onto the discrepancy when methods of hair bundle stimulation were compared (Holt *et al.*, 1997). It was shown that stimulus rise-time altered the rates of adaptation (Wu *et al.*, 1999; Vollrath and Eatock, 2003). When comparable stimuli were used comparable results between turtle and frog were obtained (Eatock, 2000; Vollrath and Eatock, 2003). And finally, evidence suggests that hair bundle mechanics from frog are comparable to those of turtle when the time frame of imaging and recording are comparable (Ricci *et al.*, 2000; Cheung and Corey, 2006). Ultimately, a resolution to the discrepancy may be that multiple forms of adaptation exist and that each form can be found in both auditory and vestibular hair cells but that the apparent contribution of each depends strongly on experimental design (Wu *et al.*, 1999; Eatock, 2000; Holt and Corey, 2000; Vollrath and Eatock, 2003). The conventional form of adaptation (described above) called slow or motor adaptation and a fast adaptation (or Ca^{2+}-dependent channel closure) coexist; whether the underlying mechanisms are independent remains to be determined.

C. *Fast Adaptation*

Fast adaptation was observed in frog as a minor component of the hair bundle mechanical response (Howard and Hudspeth, 1987) and later characterized more carefully as a "notch" (Benser *et al.*, 1996). It has been modeled as a Ca^{2+}-dependent closed channel state (Crawford *et al.*, 1989, 1991; Choe *et al.*, 1998; Wu *et al.*, 1999). Effects of Ca^{2+} buffers suggest a site of Ca^{2+} binding very close to the channel and support the hypothesis that fast adaptation can be understood as a Ca^{2+}-dependent feedback that serves to maintain Ca^{2+} at a constant level near to its binding site within the stereocilia (Ricci *et al.*, 1998). There is a mechanical correlate to fast adaptation (Fig. 9); however, whether this change represents a change in compliance is unclear (Ricci *et al.*, 2002). As the bundle has moved less after adaptation, a decrease in compliance could be predicted. However, if the compliance curve has shifted due to adaptation, without a compliance change, a similar bundle movement would be obtained (Fig. 9). To date, the mechanism underlying fast adaptation remains controversial. A Ca^{2+}-dependent relaxation of the gating spring has been suggested (Martin *et al.*, 2003). Evidence suggests a more direct effect of Ca^{2+} onto the channel (Cheung and Corey, 2006). However, a role for myosin 1C has also been reported (Stauffer *et al.*, 2005), implicating a role for rocking of the myosin head group without unbinding from the actin and supported by the reported properties for myosin 1C (Batters *et al.*, 2004a). It may be difficult to delineate between direct mechanistic effects or indirect effects by altering a protein in series with the channel. That is, can fast and slow adaptation be

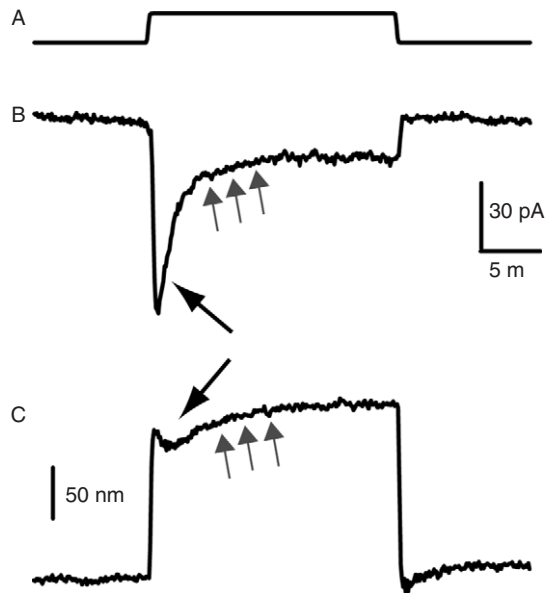

FIGURE 9 Hair bundle movements mimic MET currents. Recordings with a flexible fiber allow hair bundle mechanical responses to be imaged with a photodiode motion detector (Crawford and Fettiplace, 1985). (A) shows the stimulus, (B) the MET current response to the stimulus, and (C) the response of the hair bundle to the force stimulus. The large black arrows indicate fast adaptation responses while the three gray arrows point to the slow adaptation response. A mechanical correlate to both adaptation responses are observed in both the current and the hair bundle response.

independently modulated without indirectly altering each other? An additional question remains regarding myosin 1C as the mechanism underlying fast adaptation. The kinetics of fast adaptation vary tonotopically; however, slow adaptation kinetics have yet to be shown to have any frequency-related variations, thus if one molecule is responsible for both processes it would seem that an important point is missing. Further investigations are needed to clarify this mechanism.

D. Functional Role of Adaptation

What are the functional consequences of these complex adaptation mechanisms? Adaptation in any form prevents saturation, extending the dynamic range of the sensory cell. Here adaptation can reset the operating

range such that the MET dynamic range is extended severalfold (Eatock *et al.*, 1987; Crawford *et al.*, 1989; Fig. 10A). Adaptation also serves to maintain hair bundle sensitivity at its optimal and most linear point, that is the resting hair bundle position is kept at the steepest portion of the activation curve. Adaptation provides a mechanical filter to incoming sound (Ricci and Fettiplace, 1997). Variations in both activation and adaptation rates create a mechanical bandpass filter (Ricci and Fettiplace, 1997; Ricci *et al.*, 2005; Fig. 10B). The time course of adaptation varies by orders of magnitude across species and end organs (Ricci and Fettiplace, 1997; Kennedy *et al.*, 2003, 2005). It is unclear at this point what mechanisms underlie the tonotopic variation in adaptation rate. Differences in channel properties, numbers, and also hair bundle mechanics may contribute. Adaptation has also been posited to be part of a mechanical amplification process (Jaramillo *et al.*, 1993; Markin and Hudspeth, 1995b; Hudspeth, 1997; Choe *et al.*, 1998; Jaramillo and Wiesenfeld, 1998; Hudspeth *et al.*, 2000; Indresano *et al.*, 2003). The mechanism for amplification is thought to involve the gating spring compliance, the adaptation motors, and possibly fast adaptation (Martin *et al.*, 2000; Chan and Hudspeth, 2005; Le Goff *et al.*, 2005). Cooperative interactions between MET channels have also been implicated as a mechanism for amplification (Iwasa and Ehrenstein, 2002). As adaptation sets the resting open probability of the MET channel, it also plays an important role in setting the hair cell resting potential (Farris *et al.*, 2006; Fig. 10C). These multiple important roles for adaptation warrant a better understanding of the underlying mechanisms.

XII. THE DYNAMIC HAIR BUNDLE

A theme throughout this chapter has been attempting to delineate properties associated with the sensory hair bundle from those associated with the MET channel. Initial investigations have treated the hair bundle as an invariant structure in the transduction process; however, growing evidence suggests the hair bundle is very dynamic. A simple example is considering the number of myosin isoforms found in the hair bundle and cell. Myosin XVa is located near the tops of the stereocilia, forming a cap-like structure (Rzadzinska *et al.*, 2004). This myosin is critical for proper development of the hair bundle (Liang *et al.*, 1999; Anderson *et al.*, 2000; Liburd *et al.*, 2001; Rzadzinska *et al.*, 2004). Myosin VIIa is also localized along the length of the stereocilia (Hasson *et al.*, 1995; Rzadzinska *et al.*, 2004). Defects in myosin VIIa are associated with Usher's syndrome (el-Amraoui *et al.*, 1996; Mburu *et al.*, 1997; Todi *et al.*, 2005) and are typically associated with hair bundle defects (Rhodes *et al.*, 2004). Mice lacking myosin VIIa have MET activation curvesM80960(bunM

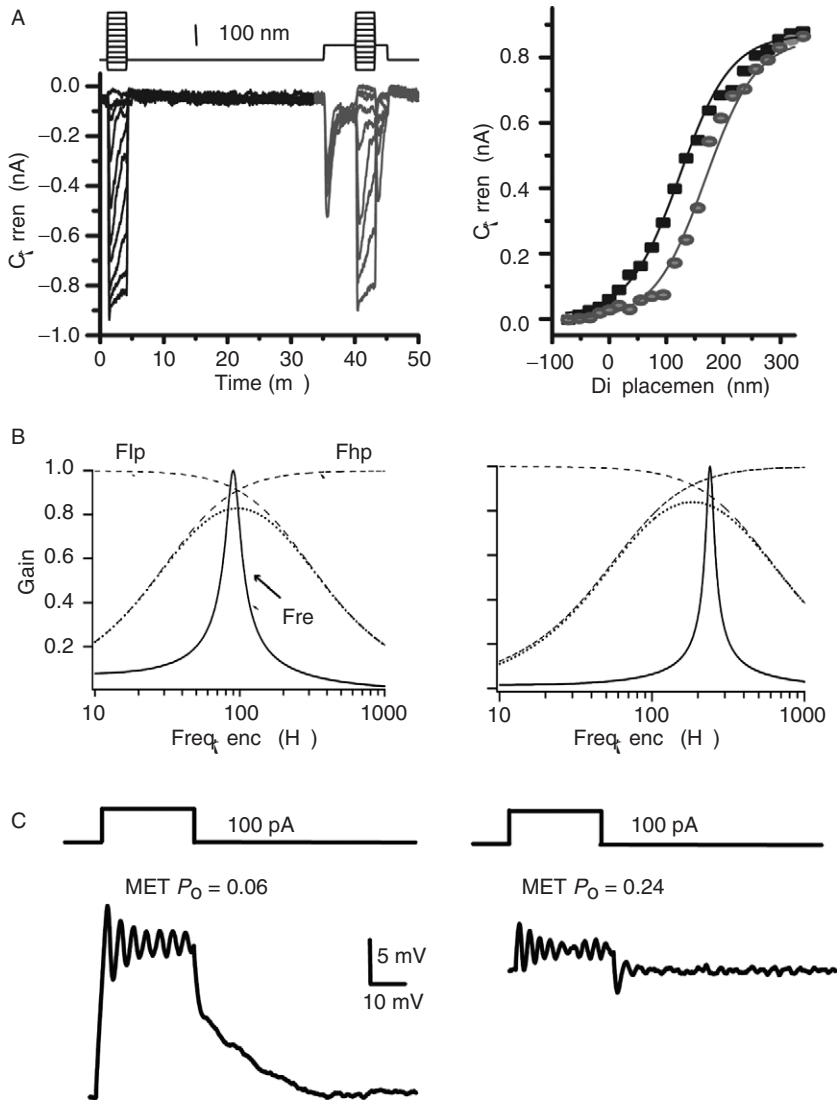

FIGURE 10 Adaptation serves multiple functional roles. (A) demonstrates a double pulse protocol used to first characterize adaptation. By generating activation curves about the hair bundles resting position and comparing it to the activation curve generated from a displaced position, the ability of adaptation to extend the dynamic range of the hair cell response is observed. (B) illustrates that the combination of activation kinetics, which generate a low pass filter (Flp) and adaptation kinetics, which generate a high pass filter (Fhp), together produces a bandpass filter with a center frequency similar to the electrical resonant frequency of the hair cells (adapted from Ricci *et al*., 2005). (C) demonstrates that changing the resting open

that are shifted to the right so that there is no MET current on at rest which prevents aminoglycoside accumulation (Richardson *et al.*, 1997, 1999; Kros *et al.*, 2002). These findings demonstrate the complexity of the hair bundle–MET channel interaction in that the myosin VIIa, which is unlikely to be directly associated with the channel, has profound effects on channel function. It is possible that myosin VIIa is involved in establishing the resting tension of the hair bundle; loss of this tension reduces the coupling between hair bundle deflection and force sensed by the MET channel. Mutations of myosin VI also lead to sensorineural hearing loss (Ahmed *et al.*, 2002, 2003; Mohiddin *et al.*, 2004). The subcellular localization of myosin VI is not well established but also thought to be in the stereocilia (Rzadzinska *et al.*, 2004). Myosin VI is known to regulate endocytosis and may play a role in apical endocytosis in hair cells (Swiatecka-Urban *et al.*, 2004). Apical endocytosis appears to be involved in the turnover and renewal of stereocilia membrane components (Kachar *et al.*, 1997; Grati *et al.*, 2006).

Not only are the elements present in the hair bundle for it to play a dynamic role in signal transduction, but the stereocilia and the hair bundle structure also appear to be constantly remodeling (Lin *et al.*, 2005). Length regulation and turnover of the streocilia actin core follows an actin treadmill mechanism (Fig. 11) that involves a variety of molecules, including some myosins and espins (Rzadzinska *et al.*, 2004, 2005). The actin treadmilling appears to work from a top-down mechanism, with actin polymerization occurring near the top of the stereocilia (Rzadzinska *et al.*, 2004). Onto this continuous turnover is placed the machinery of mechanotransduction (Fig. 11); separating the components of these different processes is an important remaining task. Determining how hair bundle shape is driven or modulated by activity of the MET channel is a question for the future. Given that Ca^{2+} entry into the stereocilia is largely through MET channels and that many of the structural proteins involved in stereocilia turnover and maintenance are Ca^{2+}-dependent, it seems likely that an interaction between these components will be identified. The number of identified proteins required for hair bundle development and function is rapidly expanding. Proteins like harmonin (Verpy *et al.*, 2000; Siemens *et al.*, 2002), whirlin (Mburu *et al.*, 2003; Belyantseva *et al.*, 2005), espins (Zheng *et al.*, 2000; Li *et al.*, 2004; Sekerkova *et al.*, 2004, 2006; Rzadzinska *et al.*, 2005), cadherins (Di Palma *et al.*, 2001; Siemens *et al.*, 2002, 2004; Sollner *et al.*, 2004; Michel *et al.*, 2005), and fimbrin (Tilney *et al.*, 1989; Zine *et al.*, 1995) have

probability of the MET channel by exposing the hair bundle to different external Ca^{2+} concentrations alters the resting potential of the hair cell and may modulate the frequency selectivity of the filter in this way.

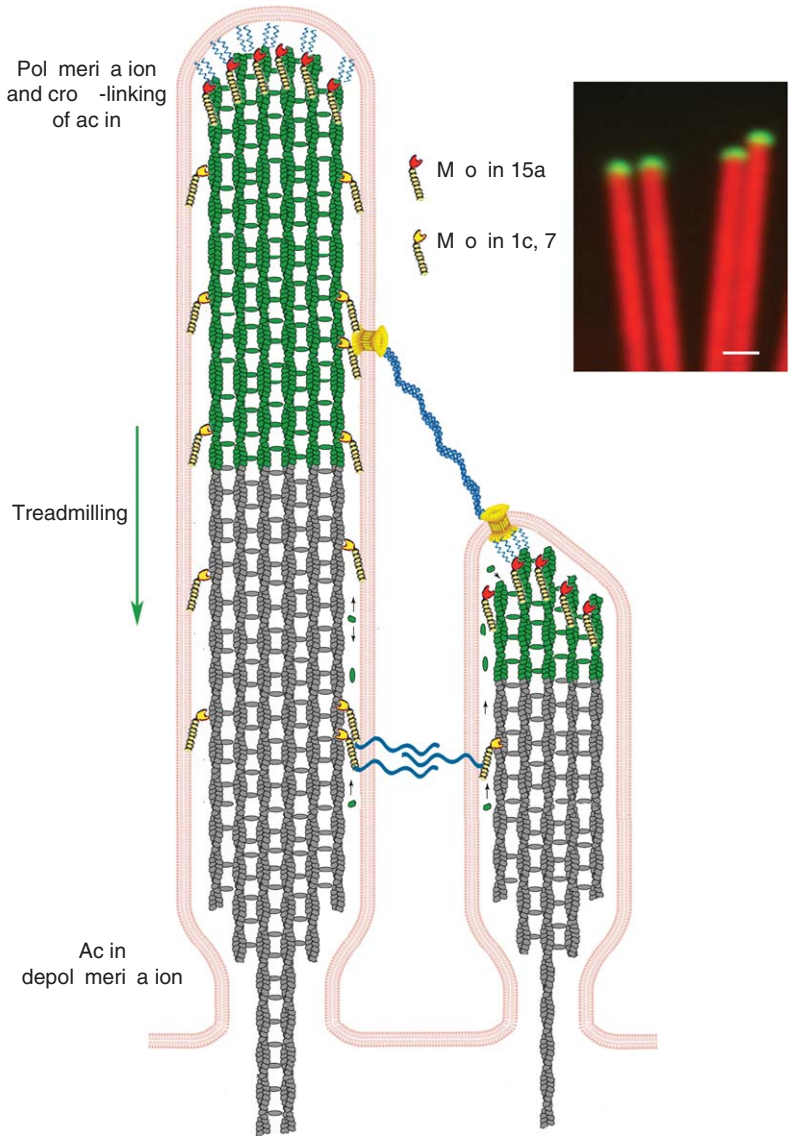

FIGURE 11 Superimposing hair bundle dynamic recycling with MET proteins illustrates the complexities of the system. Schematic representation of stereociliary pair illustrates the dynamics of actin turnover. Included are the various myosins located at specific sites along the stereocilia, the tip-links, side-links, and putative location for MET channel. Inset shows myosin 15 immunolabeling (green), actin (red, rhodamine phalloidin). Scale bar is 0.5 μm.

important roles as scaffolding and cross-linking elements, but their function in terms of hair cell mechanotransduction remain to be elucidated.

To this point, three mechanical bundle movements have been identified and related to MET currents. Gating spring compliance, thought to be associated with the opening and closing of the MET channel, slow motor adaptation, thought to be driven by myosins climbing and slipping along the actin cytoskeleton, and fast adaptation, where the underlying mechanism is less clear but may be directly associated with the channel or with myosins. Several additional hair bundle movements exist that have yet to be explored in terms of function or mechanism. One of these movements which has been called a "flick" (though it remains throughout the duration of a stimulus) is a voltage-dependent, Ca^{2+}-independent movement that does not require current through the MET channel but does require an intact hair bundle (Ricci *et al.*, 2000; Cheung and Corey, 2006). A second movement, termed a "sag" is often seen with long depolarizations and is a return (negative movement) to the hair bundle's resting position or even negative to that position during a constant depolarization. This movement has a very slow time course, yet the movement can be large (Ricci *et al.*, 2002). How these additional movements factor into our understanding of hair bundle dynamics remain to be elucidated.

XIII. SUMMARY AND FUTURE DIRECTIONS

Over the past 25 years, a great deal of information has been collected regarding the MET process in hair cells. The hair bundle structure and the component proteins that contribute to this structure are rapidly being elucidated. Exploring the functional role of these new components in the transduction process has already revealed previously unrecognized complexities. Long-standing hypotheses regarding mechanisms of activation and adaptation are being both supported and challenged. New technologies are allowing more detailed experimentation at the physiological, molecular, and protein levels. Identification of all the players will greatly aid in deciphering the mechanisms of mechanotransduction. Physiological measurements at the single molecule or at least single stereocilia level are needed to distinguish between existing models of mechanotransduction. Through all these new developments, the gating spring theory at its simplest is still capable of explaining much existing data. Care, however, must be taken when applying molecular mechanisms to this generalized gating hypothesis. Important questions remain as to how hair bundle dynamics are influenced by the MET process and which proteins are critical for transduction and which are critical for hair bundle maintenance and turnover.

Acknowledgments

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