CHAPTER 12

Hair Cell Mechanotransduction:
The Dynamic Interplay Between Structure and Function

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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
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 mechanolectric 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
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 multisteped 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; Ohmori, 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](image-url)  
**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 $\text{Ca}^{2+}$ “blush” near to the tops of the stereocilia, presumably created by $\text{Ca}^{2+}$ 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). $\text{Ca}^{2+}$ 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 $\text{Ca}^{2+}$ 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
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:

$$\Delta A = A_c - A_o = z(x - x_o)$$

(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

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.
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 \( \gamma \)) 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, \( \gamma \) 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.
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 stereocilia 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.
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, adapta-
tion 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 dis-
placement 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
closer than in turtle or frog (Crawford et al., 1989; Ricci et al., 2005), further
suggesting variations in channel structure. However, not having a direct
measurement 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\textsuperscript{2+} solutions but a slight inward rectification appears when Ca\textsuperscript{2+} is lowered (Crawford et al., 1989; Kros et al., 1992; Farris et al., 2004). The channels have a high Ca\textsuperscript{2+} permeability (Ohmori, 1985; Crawford et al., 1991; Lumpkin et al., 1997; Ricci and Fettiplace, 1998). Ca\textsuperscript{2+} both permeates and blocks the channel with a half blocking [Ca\textsuperscript{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\textsuperscript{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
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. (See Color Insert.)
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; 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 isoymes in the hair bundle and its immunolocalization at the tip-link insertion sites also implicated this isoyme 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
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.B). In addition, immunocytochemistry (Kachar, in preparation) 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., 2002). 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.
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
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 Goiff 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 curves
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 stereocilia 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.
Polymerization and cross-linking of actin

Treadmilling

Actin depolymerization

Myosin 15a
Myosin 1c, 7

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. (See Color Insert.)
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.
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References


12. Hair Cell Mechanotransduction


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