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# CHAPTER 14

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## Models of Hair Cell Mechanotransduction

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- I. Overview
- II. Introduction
- III. Transduction Channel Properties
  - A. Localization and Number of Transduction Channels in Stereocilia
  - B. Pore Properties
  - C. Molecular Identity of the Transduction Channel
- IV. Gating
  - A. Transduction Channel Kinetics and Thermodynamics
  - B. Biophysical Concept of the Gating Spring
  - C. Molecular Representation of the Gating Spring
- V. Active Hair Bundle Motility
  - A. Adaptation
  - B. Spontaneous Oscillations
- VI. Conclusions
  - References

### I. OVERVIEW

Hair cell mechanotransduction is based on a finely tuned machinery residing in the hair bundle, the hair cell's receptive organelle. The machinery consists of a transduction channel, an adaptation motor, the tip link, and many other components that reside in the stereocilia. The transduction channel is connected to and opened by a gating spring for which there are several molecular candidates. The interplay between the motor, the spring, the channel, and the tip link assures that the channel is always working at

its most sensitive point of this machine, allowing very fast responses to a force stimulus. This chapter addresses the mechanisms and molecular components underlying mechanotransduction, adaptation, and motility in the hair bundle.

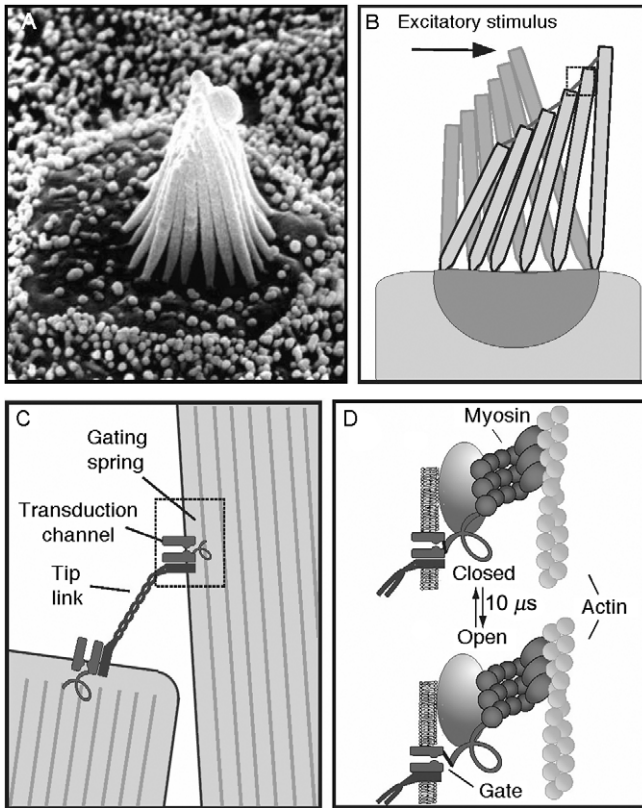
## II. INTRODUCTION

Hair cells are specialized receptor cells that transduce mechanical force (e.g., from sound waves, gravity, or vibrations) into an electrical signal. The receiving subcellular organelle, the hair bundle, exhibits a delicate and unique architecture (Fig. 1A). Rows of stereocilia, actin-filled protrusions emerging from the apical surface of the hair cell, show a staircase-like arrangement in height. In nonmammalian and vestibular hair cells, the kinocilium, a true cilium with an arrangement of nine concentric doublet microtubules surrounding two singlet microtubules, is found adjacent to the tallest stereocilia. Deflection of the hair bundle toward the largest stereocilium (Fig. 1B) gives rise to an excitatory receptor potential. In addition to many lateral links, the stereocilia are also interconnected by an apical tip link (Fig. 1C).

Bundle deflection in the excitatory direction is thought to increase tension in the tip link, which leads to opening of the mechanoelectrical transduction channel (Fig. 1C and D), located close to the insertion site of the tip link. The channel must be directly gated by force because the gating time of about  $10 \mu\text{s}$  is too short for second messenger signaling. The tension is thought to be conveyed to the channel via an elastic element termed the gating spring. The compliance of the gating spring allows the channel to rapidly fluctuate between open and closed positions even when the bundle is fixed, thereby allowing small displacements of the hair bundle to be detected as small changes of the probability of the channel being open.

Following a large excitatory (or inhibitory) stimulus, the transduction machinery is able to adapt so that it can again respond sensitively to small hair bundle deflections. Adaptation is thought to be an active process, driven by myosin motors interacting with actin filaments that form the core of the stereocilia. The active process is also thought to lead to spontaneous oscillation, which may play a role in increasing the sensitivity of hair cells to sounds of particular frequencies.

In this chapter, we review the electrophysiological, mechanical, and biochemical mechanisms underlying mechanoelectrical transduction. Of great current interest is the molecular identification of the transducer components—the channel, tip link, gating spring, and adaptation motor—and we discuss evidence for and against recently proposed candidates.



**FIGURE 1** (A) Scanning electron micrograph of the bullfrog's sacculus hair bundle (A. J. Hudspeth and R. A. Jacobs). (B) Deflection of the hair bundle causes the stereocilia to slide with respect to each other. (C) The tip link is tensed by the stereocilia-sliding motion, which conveys the tension to the transduction channel. (B and C drawn after Fig. S1 from [Sotomayor et al., 2005](#)). (D) According to the gating spring hypothesis, the gate of the channel is coupled to an elastic element, which allows the channel to open and close rapidly without moving the whole bundle. The open question is where the gating spring resides.

### III. TRANSDUCTION CHANNEL PROPERTIES

#### A. Localization and Number of Transduction Channels in Stereocilia

Many different studies locate the transduction channel near the tip of the stereocilium ([Hudspeth, 1982](#); [Jaramillo and Hudspeth, 1991](#); [Denk et al., 1995](#); [Lumpkin and Hudspeth, 1995](#)). Potential locations are near the end of the tip link in the shorter or the taller stereocilium, or at both ends ([Fig. 1C](#)).

The attachment site at the lower stereocilium is characterized by electron dense plaques at the membrane and the cytoskeleton, a potentially stretched membrane and filaments that run from the membrane toward the actin cytoskeleton. At the taller stereocilium, the site of tip link contact is also characterized by electron-dense material, called the insertional plaque (Kachar *et al.*, 2000). Denk *et al.* (1995) observed calcium entry into the shortest as well as into the tallest stereocilium on bundle deflection in frog saccular hair cells, supporting the model of hair cell transducer channels at both ends of the tip link. Localization of components that are associated with the transduction channel support that theory as well: Myosin 1c, a candidate for the adaptation motor, as well as calmodulin, which may confer calcium sensitivity to the motor, show immunolocalization at both sides of the tip link (García *et al.*, 1998). However, the significant ultrastructural differences at the two sides of insertion of the tip links as well as the different geometry with respect to the actin cytoskeleton argue against the same molecular channel complex acting on both sides. Channels on both sides connected by the tip link also imply a negative cooperativity between the two channels. Such negative cooperativity would prevent the channel apparatus from displaying the observed negative stiffness (Martin *et al.*, 2000). Localization at the sites of calcium entry in other hair cells, especially from mammalian cochlea is necessary to resolve the issue of whether there are channels at both ends of the tip link.

The number of transduction channels opened by hair bundle deflection is rather low. It has been estimated that there are around 50–100 functional channels per bundle, translating into 1–2 channels per stereocilium only, from the fluctuation analysis of the transduction current (Holton and Hudspeth, 1986), from the mechanical compliance of the hair bundle (Howard and Hudspeth, 1988), and from the relative size of the single channel current (Crawford *et al.*, 1991; Ricci *et al.*, 2003). This number is consistent with localization at either or both ends of the tip links.

### B. Pore Properties

The hair cell transduction channel is a nonselective cation channel that allows calcium and potassium, as well as other small mono- and divalent cations, to pass (Corey and Hudspeth, 1979a; Ohmori, 1985).

The conductance of  $\sim 100$  pS is quite large (Ohmori, 1985; Crawford *et al.*, 1991; Denk *et al.*, 1995; Geleoc *et al.*, 1997; van Netten and Kros, 2000; Ricci *et al.*, 2003). The unusually large single-channel conductance suggests a wide pore and this is supported by the fact that large organic compounds such as choline, tetraethylammonium (TEA), and dihydrostreptomycin (DHS) are

able to permeate the transduction channel (Marcotti *et al.*, 2005). The conductivity is reduced to about 50% by high extracellular calcium and nearly doubled when lowering extracellular  $\text{Ca}^{2+}$  from 2.8 mM to 50  $\mu\text{M}$  (Ricci *et al.*, 2003); these results indicate that calcium interacts strongly with the pore. Ricci *et al.* (2003) also found that the conductance is positively correlated with the hair cell's characteristic frequency: transduction channel properties such as conductance, activation, and adaptation vary with respect to the tonotopic organization of the sensory epithelia.

No high affinity blockers for the hair cell transduction channel have been found. However, there are several low affinity blockers. These include inorganic cations such as  $\text{Ca}^{2+}$  ( $K_D = 1 \text{ mM}$ ),  $\text{Mg}^{2+}$ ,  $\text{La}^{3+}$ , and  $\text{Gd}^{3+}$  ( $K_D = 10 \mu\text{M}$ ) (Ohmori, 1985; Crawford *et al.*, 1991; Kimitsuki *et al.*, 1996; Ricci and Fettiplace, 1998). Transduction channels are also blocked by several aminoglycoside antibiotics including gentamicin and DHS at  $\geq 1 \mu\text{M}$  concentrations (Kroese *et al.*, 1989; Kimitsuki *et al.*, 1996), as well as amiloride with  $K_D = 50 \mu\text{M}$  (Jorgensen and Ohmori, 1988; Rusch *et al.*, 1994). The interaction site with the channel probably lies at the negatively charged selectivity filter: the polycationic aminoglycosides only block the receptor current at negative potentials and have only little effect at positive potentials (Ohmori, 1985; Kroese *et al.*, 1989).

The electrical and pharmacological properties of the transduction channel—nonspecific selectivity to cations, large conductance, and weak block by polycations—are not sufficiently unique to place it into any specific channel family (Hille, 2001).

### C. Molecular Identity of the Transduction Channel

#### 1. Candidate Families

A number of channels from different channel families have been suspected to comprise the transduction channel in hair cells.

Members of the ENaC/DEG/ASIC family are known to be involved in many different types of mechanotransduction. ENaC channels are involved in baroreception (Drummond *et al.*, 1998, 2001) and the DEG/ENaC family members MEC-4 and MEC-10 have been implicated in touch reception in *Caenorhabditis elegans*. MEC-4 has been proven to be part of the mechanotransducer channel in touch receptor cells in *C. elegans*: *in vivo* whole-cell patch clamp recordings of *C. elegans* touch neurons showed that the MEC-4 channel complex is directly activated by mechanical stimuli (O'Hagan *et al.*, 2005). UNC-8 in *C. elegans* (Tavernarakis *et al.*, 1997) and pickpocket in *Drosophila melanogaster* (Ainsley *et al.*, 2003) detect locomotion and body stretch.

An anti-ENaC antibody was found to label stereocilia tips (Hackney *et al.*, 1992), but no further proof in that direction was found since then. Interestingly, DEG/ENaC channels are blocked by amiloride (Benos *et al.*, 1995; Garty and Palmer, 1997). Taken together, this evidence makes the DEG/ENaC family a possible candidate for being the transduction channel.

On the other hand, arguments against DEG/ENaC channels are that they have neither a large conductance nor the calcium permeability that are characteristic to the hair cell transduction channel. Furthermore, the amiloride block shows a much higher affinity and has a different mechanism (Rusch *et al.*, 1994; Benos *et al.*, 1995). Finally, *in situ* localization studies showed ENaC expression in the cochlea, but never in hair cells (Couloigner *et al.*, 2001; Grunder *et al.*, 2001). Thus, DEG/ENaC channels are unlikely to be the hair cells mechanotransduction channel.

A member of the P2X family of ATP-gated channels, P2X(2), is expressed in hair cells and localizes to the apical region of stereocilia (Housley *et al.*, 1999). P2X(2) shows similar behavior with respect to  $\text{Ca}^{2+}$  ions as the transduction channel (Evans *et al.*, 1996; Virginio *et al.*, 1998; Ding and Sachs, 1999). There are a few thousands active P2X(2) channels in hair cells (Raybould and Housley, 1997), although they are not gated by mechanical stimulation. Because P2X(2) is upregulated after sustained loud noise, leading to a measurable increase of ATP-gated inward current, it has been suggested that P2X(2) receptors have a regulatory role in hair cells (Wang *et al.*, 2003).

The transmembrane cochlear-expressed gene TMC1 encodes a novel transmembrane protein that does not belong into any known channel family. Recessive and dominant mutations in TMC1 lead to congenital (DFNB7/B11) and progressive hearing loss (DFNA36) in mice (Kurima *et al.*, 2002; Vreugde *et al.*, 2002). TMC1 is expressed in both inner and outer hair cells from an early stage in development. These lines of evidence make TMC1 a possible candidate for being the hair cell transduction channel, although there is no evidence that TMC1 actually forms a pore. Rather than being the transduction channel, it has been proposed that TMC1 is involved in trafficking of molecules to the plasma membrane or that it serves as an intracellular regulatory signal for differentiation of immature hair cells into fully functional auditory receptors (Marcotti *et al.*, 2006).

## 2. TRP Channel Family

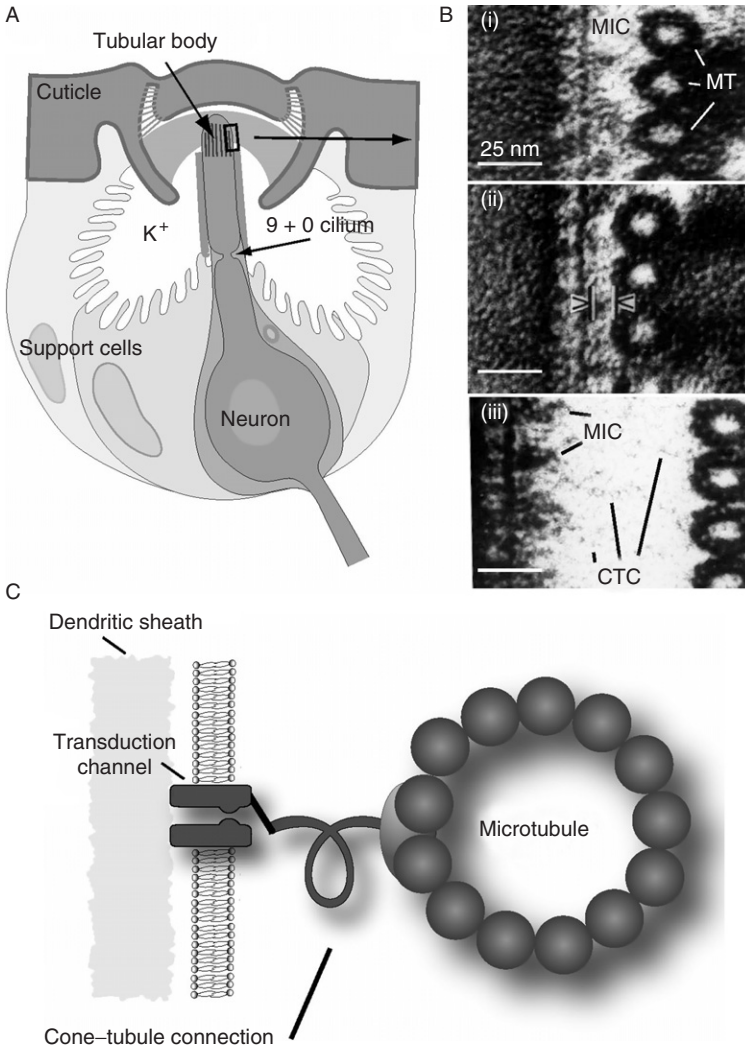
The TRP channel family is the biggest and most divergent family of ion channels involved in sensory transduction (Clapham *et al.*, 2001). Members of this family sense light, pain, stretch, fluid flow, heat, cold, pheromones, capsaicin as well as sweet, bitter, and umami taste. Besides acting as sensory channels, they are involved in a wide variety of cellular functions from  $\text{Ca}^{2+}$

and  $Mg^{2+}$  homeostasis to cell-cycle control (Reuter *et al.*, 1992; Clapham *et al.*, 2001; Nilius and Voets, 2005; Ramsey *et al.*, 2006). The huge variability in function is accompanied by a similar diversity in selectivity, gating mechanisms, and other channel properties. The involvement of TRPs in mechanotransduction processes ranging from stretch and touch in invertebrates to kidney fluid flow in vertebrates as well as the variability within the family, make TRP channels an attractive group in which to search for the hair cell transducer channel.

*a. NompC.* The first evidence for TRP channels involved in mechanotransduction was a mechanoreception defective mutant in *Drosophila* called NompC, which also showed defects in hearing (Walker *et al.*, 2000). NompC is also expressed in hair cells in zebrafish (*Danio rerio*) and *Xenopus* (Sidi *et al.*, 2003, 2005). It localizes to stereocilia and most prominently to the kinocilial bulb in *Xenopus* (Shin *et al.*, 2005). Morpholino-mediated knock-down and exon-deletion in zebrafish give phenotypes such as missing acoustic startle reflex and tilted or circular swim behavior (Sidi *et al.*, 2003). Furthermore, both the uptake of FM1-43 into neuromasts and microphonic potentials were abolished in morphants, indicating a role for NompC in auditory and vestibular function. The results point toward NompC being the transduction channel.

There are several questions that arise when thinking about NompC as the transduction channel. First, it has not been found in higher vertebrates, leading to speculation that the gene has been lost from the genome (Corey, 2003). Second, in the fly NompC works in microtubule-based mechanoreceptors (Fig. 2A–C), in contrast to the actin-based stereocilia found in hair bundles. Third, NompC in fly mechanoreceptors is opened by compression of the dendrite at the site of mechanotransduction (Thurm *et al.*, 1983), in contrast to the hair cell transduction channel which is opened by tension conveyed by the tip link. Although the latter difference could potentially be overcome by the presence of different cytoskeleton adaptor molecules in different types of mechanoreceptors, these discrepancies argue against NompC being the hair cell transducer channel.

What then is the role of NompC in the hair cells of lower vertebrates? If NompC never made the transition from microtubule- to actin-based mechanotransducers, then it is likely that NompC plays a role in kinocilium function, which is in good agreement with its localization to kinocilia in *Xenopus* (Shin *et al.*, 2005). Perhaps the kinocilium of lower vertebrate hair cells is mechanically sensitive, providing a second mechanosensory system in these cells. But a role in higher vertebrates is unlikely, especially in the cochlea where the hair cells lose their kinocilium during development.

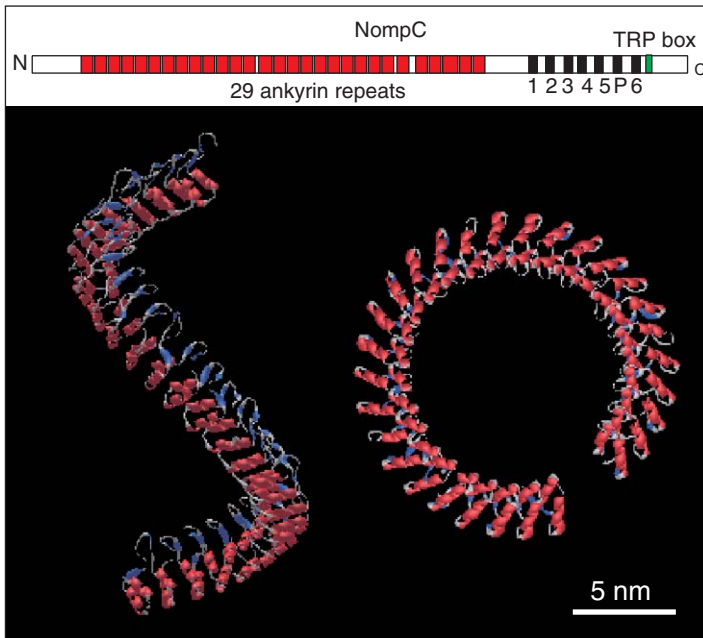


**FIGURE 2** (A) Schematic drawing of the campaniform receptor, a bristle type receptor in the fly. A bipolar neuron extends its single dendrite into the cuticle. The distal part of the dendrite is separated by a cilium (9 + 0) from the cell body. The dendrite is filled with a very regular arrangement of microtubules called the tubular body. (B) A detailed view of the region between the membrane and microtubules in the tubular body. In the membrane, electron dense structures (MIC, membrane-integrated cones) are visible. They connect via cone-tubule connections (CTCs) to microtubules (MTs). Panel (ii) was taken after bending the cuticle in the excitatory direction (excitatory stimulus). Clearly the distance between the membrane and the microtubule is shortening. The lower panel was taken after unphysiological bending of the cuticle. This situation nicely illustrates the cone-tubule connection (CTC). An extendable filamentous-like protein spans the distance between the membrane and the microtubule cytoskeleton.



*b. TRPA1.* TRPA1 has been a hot candidate for the hair cell transducer channel. TRPA1, also known as ANKTM1 in *Drosophila*, was first found in nociceptive neurons and described as a cold-sensing channel (Story *et al.*, 2003). Later, the channel was reported to have a more general role in nociception and to be gated by pungent chemicals such as mustard oils and isothiocyanates (Bandell *et al.*, 2004; Jordt *et al.*, 2004). Like NompC, the channel possesses a large number (17) of intracellular ankyrin domains (Fig. 3).

Many lines of evidence—localization, RNAi, and morpholino-mediated knockdowns in mice and zebrafish, as well as the channel's electrophysiological signature—support the role of TRPA1 as the transduction channel (Corey *et al.*, 2004; Nagata *et al.*, 2005). However, TRPA1 knockout mice (Bautista *et al.*, 2006; Kwan *et al.*, 2006) do not have any auditory or vestibular defects. Instead mice lacking TRPA1 show reduced sensitivity to mustard oil and bradykinin, as well as to painful cold and mechanical cutaneous stimuli.



**FIGURE 3** Upper panel: schematic representation of the NompC channel structure. A large N-terminus consists of 29 ankyrin repeats, followed by the six transmembrane domains and a short C-terminus, containing the TRP box. Lower panels: ankyrin spring structure deduced from 12 ankyrin repeat structure (IN11; Michaely *et al.*, 2002). Left panel: side view showing the spring-like conformation of a 29 ankyrin repeat structure. Right panel: top view showing an almost perfect full turn of the 29 ankyrin structure.

These results prove the suggested role of TRPA1 as a pain sensor in DRG neurons, but give no evidence of an auditory function for TRPA1.

There is no hint of a transduction channel gene among the inherited deafness genes, even though a large gene necessary to encode a channel should be hit by mutagenesis with a relatively high frequency. Perhaps any interference with hair cell transduction channel function leads to early lethality, implying a second important function for the channel.

Where do we go from here? The hunt for the transduction channel is wide open.

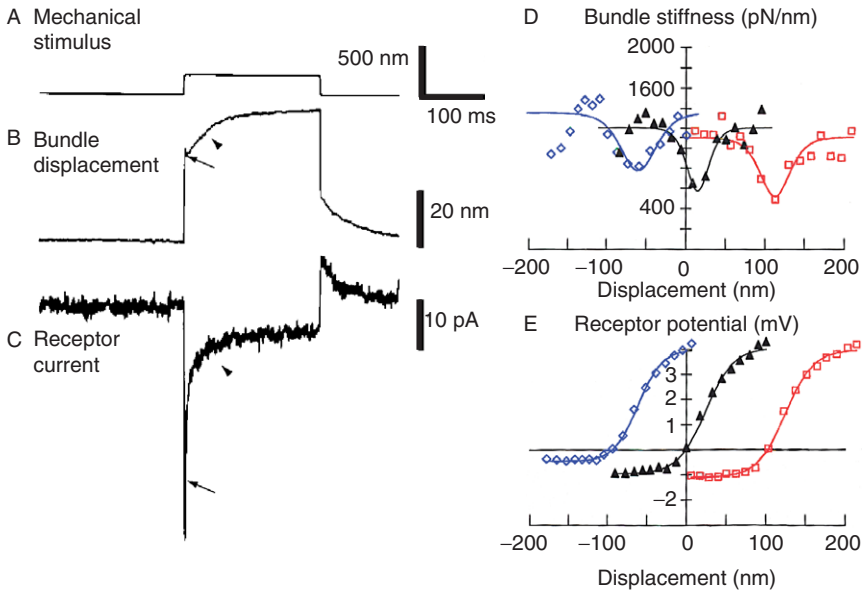
#### IV. GATING

##### *A. Transduction Channel Kinetics and Thermodynamics*

Transduction channel gating is fast and direct (Corey and Hudspeth, 1979b, 1983b). The extremely short delay, estimated to be only 13  $\mu$ s at 37°C, excludes any diffusible second messenger involvement in channel gating. Instead, the channel has to be directly gated by mechanical forces acting on the hair bundle.

Figure 4A–C shows an early experiment from Howard and Hudspeth (1987) in which the hair bundle is stimulated with a glass fiber. The fast onset of receptor current (Fig. 4C) is followed by a decline in current called adaptation. A fast and a slow adaptation phase can be distinguished (arrow and arrowhead, respectively). The position of the hair bundle shows a fast rebound (twitch, Fig. 4B arrow) in the opposite direction of the initial stimulus and then a slower relaxation in the stimulatory direction (Fig. 4B, arrowhead). These movements are mechanical correlates of fast and slow adaptation, respectively. The transducer is sensitive over a range of about 100 nm (Fig. 4E), corresponding to  $\sim 1^\circ$  of angular rotation of the stereocilia (Corey and Hudspeth, 1983a; Holton and Hudspeth, 1986; Ohmori, 1987; Howard and Hudspeth, 1988). At the perceptual threshold of hearing, stereocilia bundles are deflected by about 0.1–1 nm (Rhode and Geisler, 1967; Rhode, 1984), a stimulus corresponding to a current response of about 1 pA in the hair cell.

The open probability at the bundle's resting position is not zero. In experimental setups, it has been estimated that about 10–20% of the transduction channels are open (Corey and Hudspeth, 1983a; Ohmori, 1987). The system is already under tension and set near its point of maximal responsiveness such that a small deflection at the resting position give a large change in receptor response.



**FIGURE 4** (A) Mechanical stimulus exerted on a bullfrog's sacculus hair bundle with an elastic glass fiber. (B) Bundle displacement due to the stimulus given in A. (C) Subsequent receptor current response (A–C from [Howard and Hudspeth, 1987](#)). (D) Bundle stiffness determined from experiments similar to A–C. The bundle's stiffness is lowest in the region of a natural stimulus (few nanometers in the excitatory direction). (E) Receptor potential as a function of displacement determined by experiments as in A–C. For D and E, the black curve resembles experiments done without prior manipulation of the bundle, while the red curves show responses after a sustained stimulus in the excitatory direction and the blue curves show bundle responses after sustained deflection in the opposite direction. It is clear that bundle responses are the same each time just shifted by the amount of prior sustained deflection. This demonstrates the action of slow adaptation, mediated by myosin motors that are climbing or slipping along the actin cytoskeleton during the adaptation process, setting the bundle to its most sensitive point according to the new stimulus. D and E from [Howard and Hudspeth \(1988\)](#).

Transduction channel activation involves two steps, a fast one and a slow one ([Corey and Hudspeth, 1983b](#); [Crawford \*et al.\*, 1989](#); [van Netten and Kros, 2000](#)). Gating kinetics are dependent on the magnitude of hair bundle deflection ([Corey and Hudspeth, 1983b](#); [Crawford \*et al.\*, 1989](#)). In response to large stimuli, the activation time constant is a few microseconds in mammals ([Ricci \*et al.\*, 2005](#)), whereas time constants are a couple of hundred microseconds for small stimuli. Activation time constants also depend on the calcium concentration. Calcium ions also affect adaptation: under low

external  $\text{Ca}^{2+}$  (0.05 mM instead of 2.8 mM), the time constant of fast adaptation doubles (Fettiplace *et al.*, 2003). This result might indicate another direct interaction of  $\text{Ca}^{2+}$  with the transducer channel in addition to its interaction with the pore or another component of the transduction machinery that is connected mechanically to the channel.

The tuning of hair cells to different frequencies is thought to involve different kinetics of channel activation and fast adaptation (Ricci, 2002; Ricci *et al.*, 2005). Activation and adaptation time courses depend on the frequency range of hearing of the species. For example, they are slower in turtle than in rat. In mammals, kinetics of channel activation (and adaptation) are more than one order of magnitude faster than in nonmammals, consistent with the need for higher frequency detection. The mammalian cochlea is tonotopically organized with hair cells tuned to higher frequency at the base and cells tuned to lower frequency at the apex. The variation in characteristic frequency of hair cells along the cochlea cannot be exclusively explained by the variation of mechanical properties of the cochlea tissue (e.g., basilar membrane). Frequency tuning appears to be augmented by intrinsic properties of the hair cells: for example, basal hair cells display faster kinetics than apical ones (Ricci *et al.*, 2005). What causes these differences in kinetics between morphologically similar hair bundles? The answer probably lies in the transduction channel complex itself. Splice variants, different accessory subunits, or alternative channel composition could provide a toolbox for building the kinetic gradient in the cochlea. In order to make channel kinetics faster the gating spring has to become stiffer. In Section IV.C.3, we discuss the myosin light-chain-binding domain as a potential molecular representation of the gating spring. Myosin has the attractive feature that the light chains can be readily exchanged on multiple binding sites within the light-chain-binding domain. If different hair cells have different mixtures of light chains, and if different light chains confer different stiffness to the domain (Howard and Spudich, 1996), then gradual tonotopic organization could be achieved.

### *B. Biophysical Concept of the Gating Spring*

Fluid movement in the inner ear leads to bundle deflection, which has to be translated into a change of open probability of the transduction channel.

As we noted earlier, the very short latency of opening of these channels implies that external mechanical forces must directly couple to the channel without involving a second messenger. To match the mechanical impedance of the channel molecule with that of the hair bundle, Corey and Hudspeth (1983b) postulated the existence of a gating spring, an elastic structure that transmits forces generated by the shearing of the stereocilia (Fig. 1B) to the molecular

gate of the channel (Fig. 1C and D). By compressing and extending the gating spring, the channel can flicker rapidly between its open and closed states without necessitating the much slower movements of the whole hair bundle (Fig. 1D). The advantage of such a mechanism is that because the current is proportional to the open probability, even one channel can convey graded information about the stimulus strength (Holton and Hudspeth, 1986). Sensitive mechanical measurements confirmed the existence of the gating spring. The gating spring postulate predicts that the open probability,  $p$ , depends approximately on the displacement of the hair bundle  $X$  (in the positive excitatory direction) according to

$$p = \frac{1}{1 + e^{-z(X-X_0)/kT}} \quad (1)$$

where  $z$  is the single-channel gating force,  $X_0$  is the displacement at which half the channels are open, and  $kT$  the Boltzmann constant times absolute temperature (Corey and Hudspeth, 1983a). The equation provides an approximal fit to the experimental data, yielding  $z \sim 0.6$  pN (Howard and Hudspeth, 1988; Hudspeth *et al.*, 2000; Martin *et al.*, 2000).

The molecular interpretation of the gating force is

$$z = \gamma\kappa d \quad (2)$$

where  $\gamma = 0.14$  is the geometrical gain between hair bundle displacement and gating spring extension (Howard *et al.*, 1988),  $\kappa$  is the stiffness of a single gating spring, and  $d$  is the distance by which a gating spring shortens as a channel opens. Without addition of mechanical or structural data it is not possible to determine  $\kappa$  or  $d$ .

According to the gating spring postulate, the opening and closing of the channel make the bundle less stiff within the range of displacements that the channels are most sensitive. This additional compliance is observed (Fig. 4D, black curve) and allows one to estimate the stiffness of the gating spring as well as providing another independent estimate of channel number. The number of channels agrees with that estimated from electrophysiology with about 1–2 channels per stereocilium, and the stiffness of each gating spring is estimated at 0.5 pN/nm. This also allows an estimate of the swing of the gate  $d$  to be 4–8 nm (Howard and Hudspeth, 1988; Martin *et al.*, 2000). This distance implies that a force of 1 pN acting on the channel does  $1-2kT$  ( $kT \approx 4 \times 10^{-21}$  J at room temperature) work during the closed to open transition. The swing of the gate is large compared to the size of the structural change associated with the opening of potassium channels,  $\sim 1$  nm from structural studies (Jiang *et al.*, 2002). This indicates that the hair cell transduction channel may possess or be connected to a rigid lever that, by increasing the effective swing of the gate, makes the opening more sensitive to force.

Several experimental observations deviate from the two-state model described by Eq. (1). For example, some studies (Corey and Hudspeth, 1983b; Kros *et al.*, 2002) indicate that the open probability of the channels is not well described by the Boltzmann curve [Eq. (1)] and the gating compliance deviates from that predicted by the two-state model (Jaramillo *et al.*, 1993; Kros *et al.*, 2002). These results fit better to a three-state model with two distinct closed channel states ( $C1 \rightarrow C2 \rightarrow O$ ) (Corey and Hudspeth, 1983b; Markin and Hudspeth, 1995).

### C. Molecular Representation of the Gating Spring

The tip link, the transduction channel itself, the lipid bilayer, or any accessory protein that attaches the channel to the actin cytoskeleton are potential molecular candidates for the gating spring. In the following sections, three possible candidates are discussed.

#### 1. Cadherins

Because transmission electron micrographs show the tip link to be a thin, delicate strand (Pickles *et al.*, 1984), it was hypothesized that the tip link might have sufficient compliance to form the gating spring (Howard and Hudspeth, 1988). This hypothesis has been called into question by higher resolution microscopy showing that the tip link consists of two extended polypeptide filaments that form a helically coiled rope-like structure that does not appear to possess sufficient compliance to form the gating spring (Kachar *et al.*, 2000).

Two different cadherins have been suggested to be tip link components. Cadherin 23, also called otocadherin, is a calcium-dependent cell adhesion molecule, containing a single transmembrane domain and 27 cadherin domains. It has been linked genetically to hearing and was suggested to be the tip link (Siemens *et al.*, 2004; Sollner *et al.*, 2004). However, a study by Michel *et al.* (2005) reports cadherin 23 as a component of transient lateral links during development, with no detection of cadherin 23 in the mature cochlea. A recent paper provides evidence that protocadherin-15 might be a component of the tip link (Ahmed *et al.*, 2006). For both cadherins the following argument applies: if the tip link is composed of cadherin, then the tip link is almost certainly not the gating spring, because the cadherin domains are expected to be almost inextensible in response to piconewton-scale forces typical of physiological stimulation (Sotomayor *et al.*, 2005).

Thus, the molecular identity of the tip link is still uncertain. But irrespective of whether the tip link is cadherin 23 or protocadherin-15, it is unlikely that cadherins constitute the gating spring.

## 2. Ankyrin Repeat Domain in TRP Channels

Recently, the channel itself has come into focus in the search for the gating spring. In invertebrate mechanoreceptors, as well as in zebrafish hair cells, the candidate transduction channel is NompC, a member of the TRP channel family. In higher vertebrates another TRP channel family member, TRPA1, has been identified as a candidate transduction channel as discussed above (Corey *et al.*, 2004). Both channel proteins share a large cytosolic domain containing 29 (NompC) or 17 (TRPA1) ankyrin domains (Fig. 3). What is a huge N-terminal ankyrin repeat doing on a potential mechanotransducer channel? One possibility is that it targets the channel to its correct location, as shown for other membrane proteins (Bennett and Chen, 2001). Alternatively, it could be a protein-interaction domain. Another possibility, however, is that it transmits mechanical forces to the channel's gate. Extrapolating from a 12 ankyrin repeat protein (Michaely *et al.*, 2002), 29 ankyrin repeats are expected to form approximately one turn of a helix with a pitch of about 20 nm (Howard and Bechstetd, 2004). Such a helical geometry is expected to confer compliance to the structure, even if the protein itself is quite rigid: a Young's modulus of 1 GPa for the protein, typical for structural proteins such as actin and tubulin (Howard, 2001), would yield a stiffness of the helix of  $\sim 1$  pN/nm, similar to the gating spring. This order of stiffness has also been inferred from molecular dynamics simulations (Sotomayor *et al.*, 2005). Indeed, direct mechanical measurements on ankyrin repeat proteins of various lengths by atomic force microscopy (AFM) confirm the stiffness to be on the order of 1 pN/nm (Lee *et al.*, 2006). Thus, the ankyrin repeat domain is a good candidate for the gating spring (Corey and Sotomayor, 2004; Howard and Bechstetd, 2004).

The actual stiffness of the channel complex may be two or four times larger than that of a single ankyrin repeat domain depending on whether a heterotetrameric or a homotetrameric channel architecture is assumed.

This stiffness is in good agreement with the stiffness of  $\sim 3$  pN/nm measured for fly bristle receptors (Thurm *et al.*, 1983). An appealing feature of the ankyrin spring hypothesis for invertebrate mechanoreceptors is that it can account for the filament-like connection between the membrane, where the fly transduction channels are located, and the microtubule cytoskeleton (Fig. 2). The connection, called the membrane-integrated cone, has a dimension of about 20 nm in a resting campaniform receptor dendrite (Thurm *et al.*, 1983). The structure can be compressed such that the gap between the microtubules and the membrane becomes narrower (Fig. 2Bii). This is thought to occur during excitatory stimulation (Thurm, 1983). Bending of the cuticle in the inhibitory direction leads to stretching of the microtubule-membrane connection up to 65 nm (Völker, 1982). The observed length for the membrane-microtubule connection in the unstimulated, stimulated, and

stretched situation (Fig. 2Bi–iii) matches very well the size of the ankyrin spring. The large elongation could be accommodated by unfolding of the ankyrin repeats, which is found to occur at forces  $>10$  pN (Lee *et al.*, 2006; Li *et al.*, 2006). Thus, the case that the ankyrin helix forms the gating spring is strong in fly mechanoreceptors.

The case that the gating spring in hair cells is an ankyrin helix is less strong than it is in flies. The main problem is that it is unclear whether the hair cell transduction channel is a member of the TRP family of channel proteins that contain ankyrin repeats. On the other hand, there are many similarities between the microtubule-based mechanoreceptors found in insect hearing and touch organs and the actin-based mechanoreceptors found in vertebrate hair cells. For example, fast transduction kinetics and adaptation have been found for the fly bristle receptor (Walker *et al.*, 2000). Active amplification and spontaneous oscillations are also found in the fly's ear, the Johnston's organ (Gopfert and Robert, 2002, 2003). The potassium-rich receptorlymph in fly bristle-type receptors (Grunert and Gnatzy, 1987) is similar to the endolymph in the vertebrate inner ear (Wangemann and Schacht, 1996). The same molecules atonal (*math1*), delta, and notch are required for mechanoreceptor development in flies and mice. Thus, it seems that general biophysical and developmental principles are conserved from insects to mammals. Considering the morphological presence of a spring-like molecule in fly mechanoreceptors, an ankyrin spring is still an attractive, though unproven hypothesis, for the vertebrate hair cell.

### 3. Myosin Lever Arm

As mentioned before, any compliant protein in series with the gate of the transduction channel and the actin cytoskeleton could in principle act as the gating spring. One potential candidate is the motor protein myosin 1c. It consists of a head, a long light-chain-binding domain called the neck or lever domain, and a small C-terminus. The neck region is formed by an  $\alpha$ -helix that contains three calmodulin-binding IQ motifs. The neck can act as a lever arm to amplify small movements in the motor domains associated with changes in the nucleotide state (Uyeda *et al.*, 1996; Ruff *et al.*, 2001). Of particular interest is that myosin II, which binds two calmodulin-like light chains, has a stiffness of 0.7–2 pN/nm (Veigel *et al.*, 1998) and myosin V, which has six light chains, has a stiffness of 0.2 pN/nm. Thus, myosins have sufficient compliance to act as the gating spring. Calculations suggest that the bending of the neck might contribute some or perhaps most of the compliance (Howard and Spudich, 1996). Myosin 1c in addition to being the adaptation motor (Holt *et al.*, 2002; Stauffer *et al.*, 2005), might therefore also act as the gating spring. Thus, offers an alternative to the tip link and the ankyrin helix as a compliant element in the transduction complex.



## V. ACTIVE HAIR BUNDLE MOTILITY

### A. Adaptation

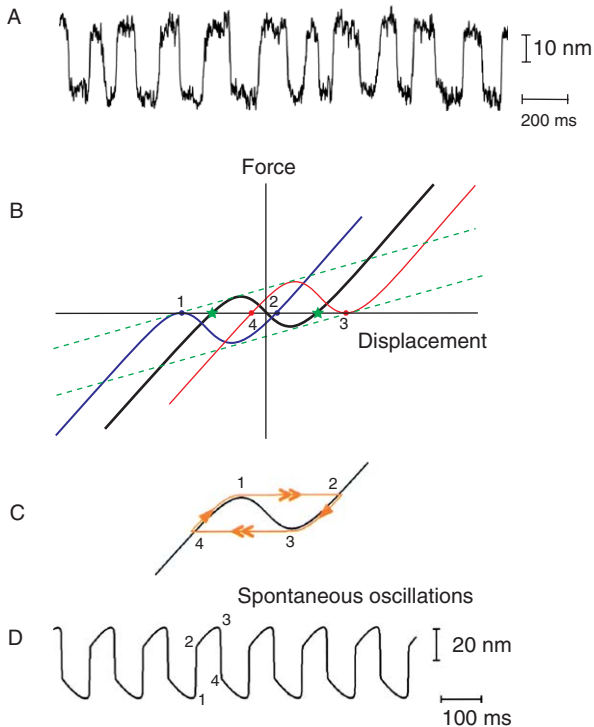
As discussed earlier, there are two types of adaptation in hair cells. Fast adaptation, occurring on a timescale of 1 ms or less is thought to be due to the direct binding of  $\text{Ca}^{2+}$  to the transducer channel or to an element directly in series with the channel, causing the channel to close. Slow adaptation occurring at a timescale of tens of milliseconds is thought to be due to myosin motors that climb up or slip back along the actin cytoskeleton (Howard and Hudspeth, 1987; Eatock, 2000; LeMasurier and Gillespie, 2005). Thus, the prevailing hypothesis is that fast and slow adaptations are caused by different underlying mechanisms. These mechanisms can be distinguished by different mechanical consequences for the hair bundle. The twitch in a fast movement of the hair bundle in opposite direction to the stimulus (Fig. 4B arrow) is thought to be the mechanical correlate of fast adaptation and represents the fast reclosure of transduction channels. The slow mechanical relaxation in the same direction as the stimulus (Fig. 4B, arrowhead) is thought to be the mechanical correlate of slow adaptation and represents the slipping (or climbing for stimuli of opposite direction) of adaptation motors down the actin cytoskeleton to decrease tension in the gating spring. The best candidate for the adaptation motor is myosin 1c (Holt *et al.*, 2002; Batters *et al.*, 2004; Stauffer *et al.*, 2005). Interestingly, recent studies suggest that both types of adaptation may be due to the same underlying mechanism:  $\text{Ca}^{2+}$  acting on the adaptation motor may trigger a rapid bundle movement and channel reclosure as a result of the negative stiffness of the gating apparatus (Tinevez, 2006; Tinevez *et al.*, 2007). This is the same mechanism thought to drive spontaneous oscillations as described in the next section.

### B. Spontaneous Oscillations

Hair bundles have been observed to oscillate spontaneously (Crawford and Fettiplace, 1985; Howard and Hudspeth, 1987; Rusch and Thurm, 1990; Denk and Webb, 1992; Martin and Hudspeth, 1999; Martin *et al.*, 2003). The hair bundle itself contains a motor that can give rise to hair bundle movements even in the absence of stimuli. It has been hypothesized that these active movements might be involved in cochlea amplification and be the cause for otoacoustic emission (Hudspeth, 1989).

It has been shown experimentally that bullfrog hair bundles display spontaneous movements (example in Fig. 5A), which are noisy oscillations between 5 and 50 Hz (Martin *et al.*, 2003). These spontaneous oscillations are

driven by an active process (Martin and Hudspeth, 1999; Martin *et al.*, 2001). The prevailing idea is that the system consisting of the transduction channel, gating spring, and adaptation motor together with  $\text{Ca}^{2+}$ , which acts as a negative feedback signal between channel opening and motor forces, is able to undergo spontaneous oscillations. Such processes require a region of negative stiffness in the force–displacement curve (Fig. 5B) and an element, such as the adaptation motor, that forces the system into this unstable region (Martin *et al.*, 2000). Theoretical studies have shown that negative stiffness can produce the observed hair bundle movements, and that it can enhance sensitivity and frequency selectivity (Choe *et al.*, 1998;



**FIGURE 5** Spontaneous oscillations. (A) Example for spontaneous oscillation in the bullfrog's sacculus (from Martin *et al.*, 2003). (B) Force–displacement curve showing the region of negative stiffness. Due to the negative slope, the bundle is bistable around zero displacement. The two stable points are indicated by green stars. (C) Scheme from the force–displacement curve showing the points between the bundle moves during spontaneous oscillations. (D) Model of a spontaneous oscillation. The numbers are indicating movement according the points in the force–displacement curve in C. B–D from Martin *et al.* (2000).

Camalet *et al.*, 2000; Martin *et al.*, 2003; Vilfan and Duke, 2003; Nadrowski *et al.*, 2004).

In Fig. 5B–D, a model for hair bundle oscillation is shown. Without stimulation, the bundle should reside at the point of zero force. The force–displacement curve (Fig. 5B) shows a negative slope at this point (black curve), indicating that the bundle is bistable. The two stable points are shown by green stars in Fig. 5B. If the bundle settles at the left stable point (negative bundle displacement) the transduction channel's open probability will be zero, leading to a decrease in  $\text{Ca}^{2+}$  concentration. This causes adaptation and a shift of the force–displacement curve in the negative direction (blue curve) along the green dotted line. The stable point vanishes, and the bundle must jump (1  $\rightarrow$  2) to its positive stable point to maintain zero force conditions. At the new position, channel open probability increases and  $\text{Ca}^{2+}$  levels inside the stereocilium rise, shifting the force–displacement curve to the right (red) (slow process 2  $\rightarrow$  3). Here the opposite effect happens. When the local minimum (3) reaches the abscissa, any further movement would violate the zero force condition and the bundle jumps (3  $\rightarrow$  4) to the left stable point. From here the sequence starts again giving rise to the characteristic oscillation pattern. In summary, the hair bundle is acting as a relaxation oscillator at a point of instability close to its maximum mechanical sensitivity, which is set by the adaptation motor.

The interplay between a negative stiffness region in the force–displacement curve, the transduction channel and  $\text{Ca}^{2+}$  as a feedback signal can account for the observed hair bundle oscillations and possibly frequency-selective amplification of hair cells (Tinevez, 2006; Tinevez *et al.*, 2007). The adaptive shifts correspond to slow adaptation caused by myosins for lower frequency oscillations (Jülicher and Prost, 1997; Camalet *et al.*, 2000) and fast adaptation for higher frequencies (Hudspeth, 1997; Ricci *et al.*, 2000).

The hair bundle's active processes have been suggested to function in mammalian cochlea amplification (Chan and Hudspeth, 2005; Kennedy *et al.*, 2005; Cheung and Corey, 2006). Clearly, the bundle is able to generate force due to the activity of the channel, the gating spring, calcium-dependent channel reclosure, and the action of a myosin adaptation motor, which places the transduction channel complex at the point of maximal sensitivity. This mechanism is almost certainly involved in amplification in nonmammals that do not hear at such high frequencies as mammals. In mammals, somatic hair cell electromotility (Brownell *et al.*, 1985) mediated by prestin (Zheng *et al.*, 2000) has been suggested to be the cause of amplification (for review see Dallos and Fakler, 2002; Geleoc and Holt, 2003). As shown by Fettiplace and colleagues (Kennedy *et al.*, 2005), both somatic and hair bundle motilities can be found in outer hair cells of rats, indicating that both processes could take part in cochlea amplification.

## VI. CONCLUSIONS

Work on hair cells has provided us with extensive knowledge about mechano-electrical transduction. We know that the transduction channel has a large pore that is permeable to cations with preference for calcium, and that calcium interacts with the pore. Activation kinetics are very fast, implying that the channel is directly gated by mechanical force, which is transmitted to the channel via the gating spring. We also begin to understand how the interplay between the channel, calcium, and the adaptation motor is able to cause spontaneous hair bundle motility, a possible mechanism underlying cochlea amplification. Calcium is a key player, modulating channel activation, adaptation, and spontaneous oscillations. Calcium interacts with the transduction channel pore and may regulate myosin function by binding to its calmodulin light chains (Batters *et al.*, 2004).

Despite many years of research, we still do not know the molecular identity of many key players in the transduction complex. The transduction channel properties and evidence from invertebrates points toward the channel being a member of the TRP family. The tip link is probably at least partially formed by cadherins. How it couples to the channel and whether there is a channel at either side of the tip link is still unknown. The gating spring, a well-characterized biophysical element in hair cell channel gating, might be formed from the light-chain-binding domain of myosin or another compliant protein domain such as an ankyrin repeat domain.

Genetic approaches have proved highly effective in identifying transduction molecules, but may fail to identify essential proteins of the transduction complex. Gene expression analysis and proteomic approaches using mass spectrometry might offer alternative ways forward.

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