Mechanotransduction in Mammalian Sensory Hair Cells

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Abstract

In the inner ear, the auditory and vestibular systems detect and translate sensory information regarding sound and balance. The sensory cells that transform mechanical input into an electrical signal in these systems are called hair cells. A specialized organelle on the apical surface of the hair cells called the hair bundle detects the mechanical signals. Displacement of the hair bundle causes mechanotransduction channels to open. The morphology and organization of the hair bundle, as well as the properties and characteristics of the mechanotransduction process, differ between the different hair cell types in the auditory and vestibular systems. These differences likely contribute to maximizing the transduction of specific signals in each system. This review will discuss the molecules essential for mechanotransduction and the properties of the mechanotransduction process, focusing our attention on recent data and differences between the auditory and vestibular systems.

Keywords

Introduction

The auditory and vestibular systems detect and process sensory information regarding sound and balance. Hair cells are the sensors of these stimuli. On the apical surface of hair cells protrudes a hair bundle composed of specialized actin-filled microvilli, termed stereocilia. Stereocilia are unique from microvilli in that they are thicker and organized in rows of ascending height (Figure 1A). The sensory inputs are mechanical in nature, but the brain processes information electrically. The hair bundle is responsible for converting the mechanical inputs into an electrical signal through the process termed mechanotransduction (also referred to as mechano-electric transduction, MT, or MET). Defects in the hair cell mechanotransduction process cause deafness and balance disorders (Petit and Richardson,

2009), demonstrating the critical role of mechanotransduction. In fact, many mouse models of deafness relating to mechanotransduction dysfunction are initially identified through an easily assessed behavioral vestibular phenotype of circling and head bobbing.

Hair cell mechanotransduction is extremely sensitive. At the threshold of hearing, the auditory system is able to detect sound vibrations as small as the diameter of an atom (Hudspeth, 1997). This sensitivity relies on hair cells detecting and amplifying sound vibrations through the cochlear amplification process, which is a mechanical amplification in the cochlea (Hudspeth, 2008; Peng and Ricci, 2011). The hair bundle has a very intricate structure to transmit deflection of the hair bundle into a force that is detected by the mechanotransduction complex. At the core of this complex is the mechanotransduction channel itself, a mechanosensitive ion channel, which opens and closes in response to hair bundle deflection (Figure 1B). The channel complex modulates the current response depending on the stimulus size with larger current responses for larger hair bundle deflections. Adjacent rows of stereocilia are connected with extracellular proteins that form the tip links (Figure 1B). When stereocilia are deflected towards the tallest stereocilia, tension increases in tip links and is transmitted to the mechanotransduction channel complex to gate the channel. *In vivo*, the ions that flow through the mechanotransduction channel are K^+ and Ca^{2+} due to the special composition of the fluid surrounding the hair bundle called endolympth (Figure 1B) (Bosher and Warren, 1971, 1978). This process is mostly studied using electrophysiology, where the hair cell is voltage-clamped, and mechanical stimuli are delivered to the hair bundle to elicit the mechanotransduction current. Studies of the mechanotransduction process have identified several properties, including activation, adaptation, and mechanics. These properties exist in both auditory and vestibular hair cells, but they differ quantitatively. The differences likely contribute to maximizing the transduction of system-specific signals.

Although there have been considerable developments in understanding hair bundle development and stereocilia structure (Pacentine et al., 2020; Richardson and Petit, 2019; Tadenev et al., 2019; Tarchini and Lu, 2019), these topics lay outside the scope of this review. In this review, we will discuss the proteins associated with the mechanotransduction channel complex and the properties of the mechanotransduction process, including the multiple timescales of adaptation. In addition, we will make an effort to highlight the differences observed between auditory and vestibular hair cells.

The molecular composition of the mechanotransduction complex

The vertebrate inner ear comprises the auditory and vestibular systems. In both systems, there are specialized hair cells able to transduce appropriate stimuli into a receptor current. Even though the complete molecular composition and structure of the mechanotransduction complex are still unknown, there has been notable progress in discovering and understanding the essential proteins for mechanotransduction in the last decade. In this section, we introduce the proteins essential for the mechanotransduction process and the differences between the auditory and vestibular systems (Table 1).

CDH23 and PCDH15

Tip links were first discovered by electron microscopy in the mid- 1980s and hypothesized to be important for mechanotransduction channel gating (Pickles et al., 1984). Tip links rely on calcium ions to stabilize their structure and are severed by exposure to calcium chelators, causing a loss of mechanotransduction current (Assad et al., 1991; Indzhykulian et al., 2013). The tip links connect stereocilia in adjacent rows and are obliquely inclined and oriented along the hair bundle's axis of mechanosensitivity (Figure 1B) (Pickles et al., 1984; Shotwell et al., 1981). High-resolution electron micrographs suggest that the tip link is composed of a helical dimer (Kachar et al., 2000). Cadherin 23 (CDH23) and protocadherin 15 (PCDH15) are the two proposed components of the tip links where a dimer of PCDH15 at the lower end of the tip link is suggested to interact with a dimer of CDH23 at the upper end of the tip link (Ahmed et al., 2006; Goodyear and Richardson, 2003; Kazmierczak et al., 2007; Siemens et al., 2004; Sollner et al., 2004). CDH23 and PCDH15 interact via their amino termini, and Ca^{2+} stabilizes this binding in a handshake-like interaction (Sotomayor et al., 2012). CDH23 and PCDH15 are important for cochlear and vestibular function; mutations in genes encoding CDH23 and PCDH15 cause Usher syndrome 1D and 1F, respectively, which is associated with hearing, vestibular, and visual dysfunction (Alagramam et al., 2001; Bolz et al., 2001; Bork et al., 2001; Di Palma et al., 2001). PCDH15 has three isoforms expressed in hair bundles (PCDH15-CD1, PCDH15-CD2, PCDH15-CD3) that differ in the C-terminal cytoplasmic domains (Ahmed et al., 2006). Mice lacking PCDH15-CD1 or PCDH15-CD3 have no auditory defects or circling behavior, whereas mice lacking PCDH15-CD2 are profoundly deaf but have normal vestibular function (Webb et al., 2011). In mice lacking PCDH15-CD2, tip links are present, MET can be recorded in immature hair cells, but hair bundles are abnormally polarized (Webb et al., 2011). During the molecular maturation process of the auditory MET machinery, there is a switch from a developmental form in which the PCDH15 isoforms are functionally redundant to a fully mature form in which PCDH15-CD2 is critical (Pepermans et al., 2014; Webb et al., 2011).

TMC1 and TMC2

The identity of the mechanotransduction channel remained elusive for many years. After first being proposed as a channel candidate, the role of transmembrane channel-like protein (TMC) in mechanotransduction continued to be debated for many years. TMC1 is associated with dominant (Beethoven – *Bth* mice) and recessive (Deafness – *dn* mice) forms of deafness (Kurima et al., 2002; Vreugde et al., 2002). Early studies in TMC1^{dn} and TMC1^{Bth} mice suggested a role of TMC1 in functional maturation of the hair cell, but not for mechanotransduction since mechanotransduction currents were normal at early postnatal ages (P8) (Marcotti et al., 2006). Later work showed that this was due to a switch in expression from TMC2 to TMC1 around P3 in the cochlea, and TMC2 could at least partially compensate for TMC1 deficiency (Kawashima et al., 2011). On the other hand, mice with a deletion in TMC2 are phenotypically normal (Kawashima et al., 2011). TMC1 is expressed in the auditory and vestibular hair cells, but mutations in TMC1 do not affect vestibular function (de Heer et al., 2011; Kurima et al., 2002), likely due to the fact that TMC2 expression is transient in the cochlea but persists in vestibular hair cells (Kawashima et al., 2011). TMC1 and TMC2 are both localized near the stereocilia tips of shorter-row

stereocilia (Kawashima et al., 2011; Kurima et al., 2015), where transduction channels are localized (Beurg et al., 2009).

The key experiments that propelled TMCs as channel candidates were done with mutant mice lacking both TMC1 and TMC2 (Kawashima et al., 2011; Pan et al., 2013). Use of single and double knockout animals demonstrated that TMC1 and TMC2 can at least partially compensate for each other and are necessary for hair cell mechanotransduction (Kawashima et al., 2011). TMC1 point mutations resulted in altered channel properties, indicating that TMC1 was closely associated with the mechanotransduction channel, if not the mechanotransduction channel itself (Beurg et al., 2019; Beurg et al., 2015a; Beurg et al., 2021b; Corns et al., 2016; Pan et al., 2018; Pan et al., 2013).

In 2013, a second mechanosensitive current was discovered in TMC1 and TMC2 double knockout animals and after severing tip links with calcium-chelator exposure in wild-type hair cells (Kim et al., 2013; Kim and Fettiplace, 2013). This led to the possibility that TMC1 and TMC2 were not pore-forming components but rather required for the natural stimulation modality. This second mechanosensitive current was localized near the base of the hair bundle and not towards the tips of the hair bundle, and it was elicited by suction of the hair bundle regardless of direction (Beurg et al., 2016). In 2017, it was reported that this current was abolished in PIEZO2 knockout animals, whereas normal hair cell mechanotransduction was intact, indicating that the suction induced current originated from a separate channel in addition to the normal mechanotransduction channels (Wu et al., 2017). This helped to reinforce that TMC proteins were a component of the mechanotransduction channel.

Recent ideas likened TMC1 to the calcium-activated chloride channel (CaCC) TMEM16A (Hartzell et al., 2005) and facilitated a homology model of the TMC1 structure (Ballesteros et al., 2018; Pan et al., 2018). Experiments with modified amino acids to allow covalent attachment of bulky compounds showed reduced channel conductance, suggesting these amino acids are in the pore conducting pathway (Pan et al., 2018). In addition to its function in mechanotransduction, TMC1 is also suggested to have an additional role as a leak channel to modulate hair cell electrical properties (Liu et al., 2019). A further confirmation that TMCs are the pore-forming subunits of the mechanotransduction channel came from a demonstration, in a reduced system with purified proteins in liposomes, that green sea turtle TMC1 and budgerigar TMC2 are mechanically sensitive ion channels (Jia et al., 2020). To date, others have not been able to express TMC1/2 in any expression systems to demonstrate mechanosensitivity, and there are no reports of mammalian TMC1/2 in expression systems demonstrating ion channel activity. Thus, the current data indicate that TMC1 and TMC2 are pore-forming components of the mechanotransduction channel, but further work is still warranted to study the structure and mechanism of mechanosensitivity (for a more extensive review, see (Zheng and Holt, 2021)).

TMIE

In the last two decades, transmembrane inner ear (TMIE) protein has received interest in the hearing field because of its connection with hereditary deafness in humans. In particular, mutations in the TMIE gene are associated with DFNB6 characterized by a severe- to-profound non-syndromic sensorineural hearing loss. Initially, there were two mouse lines

with a spontaneous deletion used as a model for the human DFNB6: spinner (*sr*) and circling (*cir*) mice. *Sr* and *cir* mice manifest with deafness, head tossing, circling, and hyperactivity (Cho et al., 2006; Deol and Robins, 1962). The TMIE gene is the only common deleted gene in the genomic deletions of *sr* and *cir* mice, suggesting TMIE is necessary for proper function in both auditory and vestibular systems. TMIE is expressed in inner hair cells (IHCs) and outer hair cells (OHCs) near the tips of the shorter rows of stereocilia (Zhao et al., 2014). Evidence showed that TMIE directly binds to the PCDH15- CD2 and can indirectly attach to LHFPL5 through PCDH15- CD1 and PCDH15- CD3 (Zhao et al., 2014). Studies in zebrafish and mice have shown that TMIE is critical for TMC1/2 localization and mechanotransduction function (Cunningham et al., 2020; Pacentine and Nicolson, 2019). Interestingly, recent studies in cochlear hair cells have shown that TMIE is an essential subunit of the mechanotransduction complex and that TMC1/2 cannot form a channel complex without TMIE (Cunningham et al., 2020). Although much work has been done regarding cochlear hair cells, very little work has approached the mammalian vestibular hair cell besides showing expression in the cells (Zhao et al., 2014).

LHFPL5/TMHS

Lipoma HMGIC fusion partner-like 5 protein (LHFPL5), also known as tetraspan membrane protein of hair cell stereocilia (TMHS), is a protein with four predicted transmembrane domains, and mutations in LHFPL5 cause deafness in humans and mice (Kalay et al., 2006; Longo-Guess et al., 2005; Shabbir et al., 2006). Mutations in LHFPL5 cause reduced macroscopic current, reduced single-channel conductance, slowed channel activation, and impaired fast adaptation (Beurg et al., 2015b; Xiong et al., 2012). LHFPL5 can bind PCDH15 and TMIE *in vitro* (Xiong et al., 2012; Zhao et al., 2014), and it can physically interact with and stabilize TMC1 in both heterologous expression systems and the soma and hair bundle of hair cells (Yu et al., 2020). Based on these functional and expression experiments, LHFPL5 is hypothesized to stabilize the expression of TMC1 (Beurg et al., 2015b; Yu et al., 2020). LHFPL5 mutant mice exhibit circling and head tossing, and LHFPL5 is expressed in vestibular hair cells and localized strongly to the stereocilia (Gyorgy et al., 2017; Longo-Guess et al., 2005; Longo-Guess et al., 2007; Xiong et al., 2012). Studies in zebrafish have also shown auditory and vestibular functional deficits when LHFPL5 is disrupted (Erickson et al., 2019).

CIB2 and CIB3

Ca^{2+}- and integrin-binding (CIB) proteins are putative auxiliary mechanotransduction channel subunits. CIB2 mutations are associated with non-syndromic deafness and Usher Syndrome 1J (Patel et al., 2015; Riazuddin et al., 2012; Seco et al., 2016), although the association with Usher Syndrome 1J is questioned by another study (Booth et al., 2018). Cochlear hair cells predominantly express CIB2, whereas both CIB2 and CIB3 are expressed in vestibular hair cells (Giese et al., 2017; Liang et al., 2021). CIB2 mouse mutants mirror the non-syndromic deafness and present with hearing loss without vestibular deficits (Giese et al., 2017; Liang et al., 2021; Michel et al., 2017). The lack of a vestibular deficit is likely due to CIB3 compensating for the loss of CIB2 in vestibular hair cells (Giese et al., 2017; Liang et al., 2021). In cochlear hair cells, CIB2 and CIB3 are functionally interchangeable, and they regulate both the assembly and function of the

mechanotransduction channel complex (Liang et al., 2021). Also, the expression pattern of CIB2 fits with a role in mechanotransduction, where CIB2 is localized along the stereocilia length with an accumulation toward the tip of the shorter stereocilia in IHCs, OHCs, and vestibular hair cells (Giese et al., 2017; Michel et al., 2017; Riazuddin et al., 2012).

TOMT

Transmembrane O-methyltransferase (TOMT/LRTOMT) is responsible for non-syndromic deafness DFNB63. TOMT and the TMCs interact within the secretory pathway to traffic TMC proteins to the hair bundle (Erickson et al., 2017). TOMT is critical for assembling the mechanotransduction machinery of hair cells because it is required for TMC1/2 localization in the stereocilia, but TOMT itself is not localized to stereocilia (Cunningham et al., 2017; Erickson et al., 2017). No studies have tested the importance of TOMT in the mammalian vestibular hair cells; however, a vestibular phenotype is present in zebrafish due to a lack of mechanotransduction (Erickson et al., 2017).

LOXHD1

LOXHD1 is associated with hearing loss DFNB77, and as part of a mutagenesis screen, LOXHD1 was identified as causing hearing loss in the *samba* mouse line (Grillet et al., 2009). LOXHD1 is localized along the plasma membrane of stereocilia in hair cells. Lack of LOXHD does not affect the development of the hair bundle but causes degeneration of hair cells with age (Grillet et al., 2009). LOXHD1 is important for mechanotransduction, but its precise role is unclear (Trouillet et al., 2021). At P11, inner hair cells have severely diminished mechanotransduction current amplitudes without showing a morphological hair bundle defect or reduction in tip-link number. Vestibular hair cells express reduced amounts of LOXHD1, but vestibular function is not affected in samba mice (Grillet et al., 2009).

Different hair cells, different hair bundle morphology

Hair cells differ in the cochlear and vestibular system (Figure 1A), but they all detect mechanical motions through the hair bundle. The two systems process different types of information, likely leading to the different hair cell types. A major property that can differentiate hair cell types is the hair bundle morphology (Figure 1A). In this section, we discuss the mode of stimulation in different sensory organs, the types of hair cells present in the inner ear, the morphological hair bundle differences, and how the morphology can affect the detection and processing of stimuli.

The cochlea is a spiral-shaped organ that detects high frequencies at the base and low frequencies at the apex. In the cochlea, there are two types of hair cells: inner hair cells (IHCs) and outer hair cells (OHCs), organized as one row of IHCs and three rows of OHCs that sit atop a basilar membrane running along the entire spiral shape. A gelatinous tectorial membrane lays atop the hair cells and is coupled to the OHC stereocilia, so the motion of the basilar membrane in relation to the tectorial membrane causes shearing of the OHC hair bundle and activation of the mechanotransduction channels to drive the cochlear amplifier (Peng and Ricci, 2011). IHCs are considered to play a purely sensory role, encoding and transmitting information via the cochlear nerve fibers to auditory nuclei

in the brainstem. IHC stereocilia are generally thought to be free-standing (i.e., not attached to the tectorial membrane) and thought to be deflected by the sound-induced local motion of the endolymphatic fluid (Guinan, 2012; Hoshino, 1976, 1977; Kimura, 1966; Lenoir et al., 1987; Lim, 1972; Von Békésy, 1960). However, there has long been evidence of an attachment of the IHC stereocilia to the tectorial membrane in the base of the cochlea (Hoshino, 1976; Lenoir et al., 1987; Lim, 1986), and more recently, filamentous structures have been postulated to connect IHC stereocilia to the tectorial membrane in the apex of the cochlea (Hakizimana and Fridberger, 2021). These findings raise doubts about the stimulation mode of IHC stereocilia *in vivo*. The tectorial membrane is also hypothesized to act as a calcium source near stereocilia, where it can regulate calcium levels surrounding the stereocilia and affect the hair bundles' mechanical and mechanotransduction properties (Hakizimana and Fridberger, 2021; Strimbu et al., 2019). Stereocilia attachments to structures like the tectorial membrane affect the loading and coordination of stereocilia, which determine how stereocilia move when stimulated.

The peripheral vestibular system consists of three semicircular canals that detect angular acceleration and two otolithic organs, the utricle and saccule, that detect linear acceleration. At the end of each semicircular canal, we find the ampulla containing a cluster of hair cells with stereocilia that project up into a gelatinous cupula. Rotation of the head induces inertial pressure by the fluid within the canal against the gelatinous cupula, which in turn causes displacement of the hair bundle (Goldberg, 2012). The utricle detects linear motion primarily in the horizontal plane, while the saccule detects motion primarily in the vertical plane. Atop the hair bundles of otolithic organs lies the otolithic membrane, a gelatinous material with overlying calcium carbonate crystals called otoconia. Thus, hair bundles are displaced from the inertia of the otoconia, and in this way, the hair cells can sense postural changes of head position using inertia and gravity (Goldberg, 2012). Some of the hair bundles in the utricle are tall enough to be embedded within the otolithic membrane, but other hair bundles are too short (Lim, 1976). These shorter hair bundles may be connected to the otolithic membrane via their kinocilium, which could provide a different stimulus type from embedded hair bundles; however, shorter hair bundles may also be stimulated by a meshwork that exists below the otolithic membrane or by fluid motion itself (Lim, 1984; Takumida et al., 1992). Although we have good hypotheses surrounding how hair bundles are stimulated, some uncertainties still exist which can affect the type of stimuli that various hair bundles receive.

In all five organs of the vestibular system, there are two types of hair cells: type I and II. Type II hair bundles are generally shorter and smaller (Li et al., 2008). Bundle heights affect the hair cell's operating range and sensitivity Longer stereocilia have a greater operating range since larger displacements of the hair bundle tip can result in the same angular stimulation; therefore, a larger range of displacements can be encoded. The number of stereocilia in a hair bundle will dictate the amplitude of the mechanotransduction current since more stereocilia will generally mean a greater number of channels are available to be activated. Longer stereocilia will also have a lower hair bundle stiffness (if force is applied to the stereocilia tip). Therefore, the structural properties of the hair bundle have functional implications on stimulus encoding in the hair cell.

Cochlear hair bundles differ from vestibular bundles in a few ways. IHC and OHC bundles are organized in three rows of stereocilia in an open "V" or "W" shape, while vestibular hair bundles have more than 3 rows of stereocilia and are more cohesive (Figure 1A) (Peng et al., 2011). Vestibular hair bundles are taller than cochlear hair bundles and are specialized to detect lower frequencies. During development, the kinocilium is lost in the cochlear hair bundle, but it persists in vestibular hair bundles. The kinocilium is important during development for cell polarity; it is resorbed in mouse cochlear hair cells around ten days after birth (Kikuchi and Hilding, 1965; Kimura, 1966), however, it re-forms after mechanical injury (Sobkowicz et al., 1995). The kinocilium remains in vestibular hair bundles after development, where it could play a role during stimulus detection (e.g., being embedded in the otolithic membrane). The structural differences between cochlear and vestibular hair bundles likely reflect the differences in the type of stimuli they are tuned towards.

Mechanotransduction process: properties and kinetics

Frequency selectivity

Tonotopy in the cochlea refers to the organization of detecting decreasing frequencies from base to apex of the cochlea. The OHCs are the cochlear amplifiers, as they selectively amplify specific frequencies along the cochlear length; thus, different OHCs have become specialized to detect different frequencies. Multiple properties likely contribute to the hair cells' ability to detect a specific frequency (Figure 2). The structure of the cochlea and the mechanical properties of the basilar membrane on which the hair cells sit have a large contribution to the hair cells' frequency selectivity and the characteristic traveling wave of the cochlea (Altoe and Shera, 2020; Olson et al., 2012). In addition to the mechanical properties of the basilar membrane and shape of the cochlea, the morphology and the mechanical properties of the hair bundle, as well as the intrinsic properties of the mechanotransduction process, vary tonotopically. These variations can contribute to the hair cells' ability to maximize the signal of specific frequencies.

Hair bundle morphology varies along the length of the cochlea. Cochlear hair cells in various mammals can encode a frequency range from tens of Hz to over 100 kHz (Peng and Ricci, 2011). Differences in hair bundle morphology affect the mechanical properties of the hair bundle and likely help to enhance the cell's frequency selectivity (Figure 2). Stereocilia length changes along the cochlea, decreasing from apex to base in both IHCs and OHCs (Garfinkle and Saunders, 1983; Kaltenbach et al., 1994). Longer stereocilia would exhibit less stiffness, therefore, would be sensitive to lower frequencies. Quantitative analysis of stereocilia number per hair cell shows a decrease toward the apex (Garfinkle and Saunders, 1983; Lim, 1986). Lower stereocilia numbers can also decrease the hair bundle stiffness, with fewer stereocilia contributing to the overall stiffness, although attachment to the tectorial membrane and hair bundle cohesiveness will affect this. Mammalian hair bundles have a characteristic "V" or "W" formation. Basal hair cells have a wider shape angle, while apical hair cells have a narrower shape (Lim, 1986). Some hypothesize that the characteristic shape may reduce the fluid dynamic resistance of fluid movement between the reticular lamina (i.e., the surface of the hair cell epithelium) and the tectorial membrane (Ciganovic et al., 2017; Pau and Pau, 2006), however, the reasoning for the tonotopic

variations has not been explored. All these properties affect the mechanical properties of the hair bundle, which would change the type of signals maximally detected by the hair bundle.

The differences in bundle morphology discussed thus far influence the mechanical proprieties of the stereocilia; however, mechanical properties are also imparted by micro-scale structures of the mechanotransduction apparatus. The gating spring is a mechanical construct connected to mechanotransduction channels and gates the channel (i.e., opens and closes the channel) (Corey and Hudspeth, 1983b; Howard and Hudspeth, 1987). When tip links are broken, the gating spring is lost, therefore, the tip link was originally thought to be the gating spring itself (Assad et al., 1991; Gillespie and Corey, 1997). Later work suggested that the tip link itself may be too rigid based on its structure (Kachar et al., 2000; Sotomayor et al., 2012). More recently, the idea that the tip link could be the gating spring has resurfaced based on mechanical models and single-molecule force experiments demonstrating tip-link elasticity (Araya-Secchi et al., 2016; Bartsch et al., 2019; Choudhary et al., 2020; Dionne et al., 2018; Mulhall et al., 2021). However, any component connected to the tip link to convey the force from shearing/bending of the stereocilia to the mechanotransduction channels can contribute to the gating spring, including any connections to the stereocilia core or any interaction partners of tip-link components. The tip link acts as a link in the chain to transmit force to the channel, such that if a middle link in the chain is broken (e.g., the bond between Pcdh15 and Cdh23), then the whole chain no longer transmits force; therefore, any component of the chain could be contributing to the gating spring stiffness, and the total stiffness of the gating spring will be dominated by the most compliant link in the chain. The gating spring is also under resting tension, which is demonstrated by the movement of the hair bundle upon tip-link breakage (Assad et al., 1991; Tobin et al., 2019). This resting tension is critical for maintaining the sensitivity of the mechanotransduction channel and is hypothesized to be generated by myosin VIIa (Li et al., 2020). The resting tension and the gating spring stiffness are also parameters that vary tonotopically (Figure 2) (Tobin et al., 2019). It is unclear whether the gating spring itself acts as a non-linear spring where increased tension will change its stiffness. Definitively identifying the molecular components of the gating spring will allow a better understanding of the mechanotransduction process.

In addition to the mechanical properties of the hair bundle, there are intrinsic properties of the mechanotransduction process that contribute to the hair cell response. The maximum mechanotransduction current helps to determine how much the hair cell depolarizes to a given stimulus. The size of depolarization is dictated by the number of channels present in the cell, the conductance of each individual channel (i.e., the amount of current that passes through a single ion channel), and the input resistance of the cell (i.e., how leaky the membrane of the cell is since lower input resistance will require more current to depolarize the cell). The number of stereocilia and tip links in a hair bundle will determine the number of channels. It is generally thought that two channels exist per tip link based on the MET current amplitude, tip link number, and measured single-channel conductance (Beurg et al., 2006; Beurg et al., 2009). For OHCs, but not IHCs, the maximum current is greater in the base than the apex (Figure 2) (Kim and Fettiplace, 2013). This is partly contributed by the greater number of stereocilia in basal OHCs as compared to apical OHCs (Lim, 1986),

partly by the gradient in single-channel conductance (Beurg et al., 2018; Beurg et al., 2006), and partly by differences in calcium permeability (Kim and Fettiplace, 2013).

There are two predominant methods used to measure single-channel conductance in hair cells. The first uses calcium chelators (e.g., EGTA or BAPTA) to break tip links such that there is only one remaining tip link in the hair bundle (Ricci et al., 2003). With this method, measured single-channel conductance is greater in basal OHCs than in apical OHCs (Beurg et al., 2018; Beurg et al., 2006). There has been an increased interest in synchronous gating of channels, potentially mediated by the lipid bilayer, which could lead to a larger apparent single-channel conductance by having more channels per tip link in the base of the cochlea that are activated synchronously (Beurg et al., 2018; Gianoli et al., 2017). While this idea is attractive, definitive proof is still lacking. The second method for determining single-channel conductance uses noise or fluctuation analysis of the macroscopic mechanotransduction current (Holton and Hudspeth, 1986). However, recent data have shown that this method, when applied to mammalian cochlear hair cells, can create artifacts and underestimate the conductance value due to the fast activation speed of the mechanotransduction channel, raising the question as to whether values determined by this method are accurate (Beurg et al., 2021a).

When analyzing the maximum mechanotransduction current and the single-channel conductance of the cell, the calcium permeability of the channel needs to be considered. In some instances, the single-channel conductance values vary across different studies (Beurg et al., 2018; Beurg et al., 2006; Beurg et al., 2015a; Pan et al., 2018; Pan et al., 2013). Calcium permeability is important to consider because calcium ions act as permeable blockers to the mechanotransduction channel (Peng et al., 2016; Ricci and Fettiplace, 1998). We state it as "calcium" permeability since this is generally what is referred to in the field, but it is important to note that other divalents also act as permeable blockers (Crawford et al., 1991; Hacohen et al., 1989; Peng et al., 2016), where magnesium is the most common other divalent ion used in experiments. Therefore, the concentration of divalent ions used in the extracellular solution in various experiments needs to be accounted for when making comparisons. As the calcium permeability of the channel changes with different TMC mutations, isoforms, and tonotopic position (Beurg et al., 2019; Beurg et al., 2018; Beurg et al., 2015a; Kim and Fettiplace, 2013; Pan et al., 2013), it could change the divalent permeable block of the channel, making it more difficult to compare single-channel conductance measurements. The best way to disentangle single-channel conductance and calcium permeability may be to record single-channel conductances in low calcium, zero magnesium extracellular solution (at least 150 μM Ca^{2+}, since at this concentration, all of the calcium permeable block of the channel was released (Peng et al., 2016), however, this value may change based on tonotopic position and channel modifications) and measure the calcium permeability ratios as currently performed.

Vestibular epithelia are divided into striola/central and extrastriola/peripheral zones. The orientation of hair bundles varies in the utricle, making the organ sensitive to various directions. Mammalian utricular hair cells primarily detect nonperiodic signals and have a low-frequency capability, detecting a frequency range of 0 (DC, direct current) to 2 Hz (Goldberg et al., 1990). Nerve fibers that innervate hair cells in the striola are more

often irregular firing afferents and transmit more phasic signals (Eatock, 2018; Eatock and Songer, 2011). Nerve fibers that innervate hair cells in the extrastriola are generally regular firing afferents and transmit tonic signals. A general organizing principle of the vestibular epithelia like the tonotopy observed in the cochlea is lacking, but recent work suggests that tuning for fast and slow responses and developmental sequence may be an organizing principle of the larval zebrafish utricle (Liu et al., 2021). Little data exist on the quantification of mammalian vestibular hair bundle ultrastructure (Bagger-Sjoback and Takumida, 1988; Lapeyre et al., 1992; Lim, 1976). One study in guinea pigs found that vestibular hair bundles have 70–100 stereocilia (Bagger-Sjoback and Takumida, 1988), similar to the number of stereocilia found in cochlear hair cells (Lim, 1986). Single-channel conductance measurements in vestibular hair cells indicate similar conductances to auditory hair cells when using similar calcium concentrations, but maximum mechanotransduction currents are less than half the amplitude in vestibular hair cells as compared to auditory hair cells (Figure 3A and C) (Beurg et al., 2006; Caprara et al., 2020; Michalski et al., 2009; Pan et al., 2013). These discrepancies warrant further investigations. Additionally, whether mechanotransduction properties differ in different regions of the utricle has not been thoroughly explored. One study of mechanotransduction properties in striolar and extrastriolar hair cells found no significant difference, but this study also had not accounted for the later modified definitions of striola and extrastriola in the mouse (Eatock and Songer, 2011; Vollrath and Eatock, 2003). More studies of vestibular ultrastructure and mechanotransduction are clearly needed.

Activation curve and Operating Range

The activation curve of the mechanotransduction channel describes the sensitivity of the channel to displacements of the hair bundle. The curve takes on a sigmoidal shape and is often fit by a double Boltzmann equation (Figure 3B), which represents a two closed and one open state channel model (Corey and Hudspeth, 1983b). The operating range describes the displacement amplitudes that result in a measurable change in the mechanotransduction current, and the size of the range can be quantified as the width of the activation curve. The wider the activation curve, the less sensitive because the slope of the curve is shallower, but the channel can encode a greater range of stimuli. On the other hand, a narrower activation curve is more sensitive but encodes a smaller range of stimuli. IHCs and OHCs have different activation curve properties. IHCs have wider activation curves than OHCs (Figure 3B) (Peng et al., 2013). The resting open probability of the mechanotransduction channel quantifies the fraction of channels in the open state when no stimulus is presented to the hair bundle. The resting open probability, often expressed as a percentage, helps to describe the set point of the channel and indicates the cell's position along the activation curve when no stimulation is present. *In vivo*, the resting open probability of IHCs is also lower than OHCs. OHCs have about 50% resting open probability (Beurg et al., 2010; Corns et al., 2014; Johnson et al., 2011; Peng et al., 2013), such that they are at the steepest part of the activation curve, which makes them most sensitive to small movements of the hair bundle. In contrast, IHCs have an estimated 20% resting open probability *in vivo* (Corns et al., 2014; Johnson et al., 2011). These differences in the activation curve may have arisen from the different functions that IHCs (detectors) and OHCs (amplifiers) have in the cochlea.

Vestibular hair cells also appear to be specialized for detecting a wide range of stimuli. The activation curve of vestibular hair cells is wider than the activation curve in OHCs, and the resting open probability is more comparable to IHCs than OHCs when recorded with similar conditions (Figure 3B) (Caprara et al., 2020; Peng et al., 2013). The sensitivity of the vestibular hair cell is also lower than OHCs. It was proposed that the activation curve was similar between OHCs and vestibular hair cells when accounting for the angular stimulation of the hair bundle (Geleoc et al., 1997), however, that is not our experience and may be a result of the previous report using neonatal hair cells where mechanotransduction properties have not fully matured (Lelli et al., 2009; Waguespack et al., 2007). We find that, on average, mouse vestibular type II cells have an activation curve nine times wider than mouse OHCs (Figure 3C, VHC: 0.77 ± 0.29 μm, OHC: 0.083 ± 0.023 μm). When compared to IHCs, the width of the activation curve is similar quantitatively, but qualitatively, the shapes of the curves are different, with IHCs having a steep initial rise, followed by a shallow roll-off (Figure 3B). The vestibular hair bundle is taller than the cochlear hair bundle, which can account for some of the difference, but not all of it. When comparing similarly processed tissue to account for artifacts of dehydration (Miller et al., 2021), mouse vestibular type II hair bundles are on the order of 6–7 μm tall, and cochlear OHC bundles are about 3 μm tall (Carlton et al., 2021; Li et al., 2008). Taller hair bundles will exhibit greater motion for the same angular movement. However, with the nine times difference in width, the vestibular hair bundles would need to be 27 μm tall to account for the sensitivity difference. Another way to measure the effect of bundle height differences is to estimate the stretch of tip links using the geometrical gain, estimated as 0.047 for vestibular hair cells and 0.12 for OHCs (Holt et al., 1997; Tobin et al., 2019). The 2–3 fold difference in geometrical gain also cannot fully account for the nine times difference in the width of the activation curve. Therefore, the mechanotransduction machinery of vestibular hair bundles must be inherently less sensitive than that of OHC hair bundles, and the mechanistic molecular differences for this are yet to be discovered.

Activation

The activation kinetics of the mammalian cochlear hair cell mechanotransduction channel is faster than most ion channels and has not been directly measured to date. The fast speed of channel activation is used to argue for a direct tethering of the tip link to the mechanotransduction channel, however, little to no data currently exist to substantiate this hypothesis (Effertz et al., 2015; Peng et al., 2011). Recently, more attention has been paid to the lipid interactions of the mechanotransduction channel, raising the possibility that channel activation may occur through the lipid bilayer like many other mechanosensors (Beurg et al., 2018; Cunningham et al., 2020; Effertz et al., 2017; Gianoli et al., 2017; Jia et al., 2020; Peng et al., 2016; Zheng and Holt, 2021). The time constant of OHC mechanotransduction activation has also been estimated recently to be as fast as a 4 μs and 10 μs time constant for basal and apical hair cells at room temperature, respectively (Beurg et al., 2021a). This would yield 10–90 rise times (i.e., time for the response to go from 10% to 90% of the maximum value) of 8.8 μs and 22 μs. With rise times this fast, direct measurements of the activation kinetics will be difficult with even the best traditional stimulation probes that have rise times of 11 μs (Peng et al., 2013). Ideally, probes would be more than two times faster than the response rise time. The use of nanomechanical force probes that have rise times

of 5 μs would provide sufficient speed of stimulation for many regions of the cochlea (Doll et al., 2012), however, patch-clamp recordings are limited by clamp speed which is also required to be more than two times faster than the activation kinetics. Typically hair cells have a cell capacitance of 5–10 pF, which would require that compensated series resistance be less than 0.5 MΩ to be at least two times faster than the channel's activation time constant Additionally when using *in vitro* stimulation techniques, the stimulus probe often only contacts a subset of stereocilia (Nam et al., 2015), which causes uneven stimulation of stereocilia and can result in desynchronizing channel activation across the hair bundle and slow the measured activation kinetics. Fluid jet stimuli also can cause uneven stimulation of hair bundles, requiring other stimulation methods to be developed (Peng et al., 2021). Some recent studies have developed other stimulations techniques, but more data is required to fully understand their limits (Abeytunge et al., 2021; Azimzadeh et al., 2018). These technical hurdles limit the ability to measure mechanotransduction channel activation time constants in mammalian OHCs directly.

The activation kinetics of mammalian vestibular hair cells is likely slower than cochlear hair cells since the detected frequency range is much lower. Activation kinetics of 380–750 μs have been measured in the turtle auditory hair cells (Ricci et al., 2005), and we would expect vestibular hair cells to have activation kinetics of this order or slower, which are well within the stimulation and clamp speed limitations. Vestibular hair cells are also not limited by uneven stimulation issues since the hair bundle structure is more cohesive (Figure 1A). These measurements are currently theoretically attainable with some effort.

Adaptation

Hair cells are able to adapt during sustained stimuli to shift the range of their sensitivity (Corey and Hudspeth, 1983a; Crawford et al., 1989; Eatock et al., 1987). This adaptation allows cells to maintain a high sensitivity (i.e., narrow width of the activation curve) while also encoding a larger range of stimuli effectively increasing the cell's dynamic range Experimentally, adaptation is observed in two ways: (1) a decline in the mechanotransduction current in response to a sustained hair bundle displacement, and (2) a parallel shift in the set point of the activation curve without a change in activation curve width. In hair cells of lower vertebrates such as the frog and turtle, at least two adaptation processes have been described that differ in their kinetics (i.e., the rate that they occur), termed fast and slow adaptation with time constants of several milliseconds and tens of milliseconds, respectively (Vollrath and Eatock, 2003; Wu et al., 1999). Tonotopic variations of fast adaptation have been described, where fast adaptation is faster in the base than the apex of the cochlea (Figure 2) (Ricci et al., 2005; Waguespack et al., 2007). The gradient in adaptation time constants is hypothesized to contribute to the mechanical filtering properties of the mechanotransduction process (Ricci et al., 2005). Previously, both fast and slow adaptation processes were thought to require calcium to enter the cell to mediate the adaptation process (Assad and Corey, 1992; Crawford et al., 1991; Eatock et al., 1987; Kennedy et al., 2003; Ricci and Fettiplace, 1997; Ricci et al., 1998). For fast adaptation, it was thought that calcium bound to the channel itself, or a location very close to the channel due to the faster kinetics (Choe et al., 1998; Crawford et al., 1989; Howard and Hudspeth, 1988; Stauffer et al., 2005). For slow adaptation, it was discovered that this

mechanism required myosin motor activity, and it was hypothesized that calcium bound to a myosin motor, proposed to be myosin Ic, to cause the myosin motors to slip down the side of stereocilia to release tension in the tip link; this hypothesized mechanism is often referred to as the motor model of adaptation (Assad and Corey, 1992; Holt et al., 2002; Howard and Hudspeth, 1987; Walker and Hudspeth, 1996; Yamoah and Gillespie, 1996). The motor requirement of slow adaptation led it to be sometimes referred to as slow motor adaptation. Contrary to these proposed mechanisms, fast adaptation in cochlear hair cells has been more recently shown not to require calcium entry, and this idea fits with observations that calcium permeability and adaptation rates can change in TMC variants in a manner opposite to that which would support fast adaptation relying on calcium (Caprara et al., 2019; Goldring et al., 2019; Peng et al., 2013). In cochlear hair cells, slow adaptation was confirmed to require calcium entry and myosin motor function. However, the proposed mechanism of slipping down the sides of stereocilia to release tension has been challenged as well as the specific requirement of myosin Ic function, where another myosin motor may compensate (Caprara et al., 2020). Fewer studies have been performed in mammalian vestibular hair cells on slow adaptation, but what we do know is that some mechanistic properties are the same, including the calcium reliance and lack of support for the motor model of adaptation, but others differ, where myosin Ic function is required for slow adaptation in vestibular hair cells (Caprara et al., 2020; Holt et al., 2002; Stauffer et al., 2005).

For decades, it was thought that fast and slow adaptation were the only mechanisms for regulating the set point of the mechanotransduction channel; therefore, when the set point was altered, it was assumed that adaptation was involved. However, data now indicate that set point regulation and adaptation can be decoupled, where there is an effect on either set point or adaptation but not the other (Caprara et al., 2020; Liang et al., 2021; Peng et al., 2016). These results suggest that there are other mechanisms to control the set point of the channel other than fast and slow adaptation. For example, increases in resting open probability from lowering extracellular calcium and depolarization were thought to be driven by effects of fast and slow adaptation; however, use of the tarantula toxin GsMTx4 could block the modulation without affecting the fast adaptation process, but effects on slow adaptation were not explicitly tested (Peng et al., 2016). Additionally, the rate of the resting open probability change with depolarization was on the order of 100 ms, which is slower than fast or slow adaptation, suggesting a different mechanism. Another mode of mechanotransduction channel modulation during a prolonged depolarization was found to decrease resting open probability on the order of seconds (Peng et al., 2013; Peng et al., 2016). One could argue that these are still adaptation mechanisms since it adjusts the set point, but regardless of what they are called, they are separate mechanisms from the classically thought fast and slow adaptation since they operate on timescales different from fast and slow adaptation. Other mechanisms also exist to modulate the mechanotransduction channel set point independent of adaptation like cyclic adenosine monophosphate (cAMP) (Ricci and Fettiplace, 1997). Further work is needed to elucidate these additional mechanisms.

The type of stimulation used *in vitro* for mechanotransduction experiments can lead to differences in the manifestation of adaptation (Caprara et al., 2019). There are two common types of stimulators: (1) stiff probes that directly couple to stereocilia and deliver step-like

displacement and (2) fluid jets that deliver force stimuli by ejecting extracellular solution onto hair bundles from a pipette tip (Caprara et al., 2019; Dinklo et al., 2007; Kros et al., 1992; Peng et al., 2013; Peng and Ricci, 2016). Previous experiments showed that in rat OHCs, stiff probes with a rise time as short as 11 μs provided finer temporal resolution of the adaptation processes. Fast adaptation resulting from these fast probes during a short 5 ms duration stimulus could be fitted with two exponential decays with average time constants of about 0.2 ms and 1 ms (Peng et al., 2013). Delivering longer stimuli of 50 ms duration with the stiff probe required three time constants, with the third time constant ranging between 8 and 50 ms. We classify the two faster time constants as fast adaptation and could represent two separate fast processes, and the slowest time constant represents the slow adaptation process that requires myosin motors (i.e., slow motor adaptation).

In contrast, mechanotransduction currents elicited by a fluid jet do not show fast-current decays (with an exponential decay time constant < 5 ms) and only exhibit slow-current decays with about 20 ms time constants (Caprara et al., 2020; Caprara et al., 2019). The fluid jet stimulus uses a jet of fluid to stimulate the hair bundle, therefore delivers a force stimulus rather than a displacement stimulus delivered by the stiff probe. Imaging of the bundle motion during a step-like force stimulation using a fluid jet reveals a mechanical creep, which is a continued movement in the direction of the applied force (Caprara et al., 2019). This creep indicates an increasing effective hair bundle compliance as observed by others (Howard and Hudspeth, 1987; Kennedy et al., 2005; Kros et al., 2002; Russell et al., 1989). The creep was previously correlated with the adaptation process, therefore suggested that the myosin motors would slip down the sides of the stereocilia to release tension in tip links to mediate slow adaptation (Assad and Corey, 1992; Howard and Hudspeth, 1987). The hair bundle creep appears to mask the fast adaptation process. When the fluid-jet stimulus is shaped to deliver a step-like displacement stimulus, a fast-current decay is unmasked (Caprara et al., 2019). This result suggests that the hair bundle creep could be a manifestation of fast adaptation. The masking of fast adaptation is also advantageous when studying slow adaptation, since visualization of the slow adaptation in the current decay is much easier when fast adaptation is masked (Caprara et al., 2020; Caprara et al., 2019). Studies conducted in this way confirmed that slow adaptation requires myosin motor function, depends on calcium entry, is not correlated with the creep, and requires new models of the slow adaptation mechanism (Caprara et al., 2020).

In search of a new mechanism for slow adaptation, we have turned to the essential role of phosphatidylinositol 4,5-bisphosphate (PIP_2) in slow adaptation (Hirono et al., 2004). The role of lipid constituents on ion channel properties has been increasing, and mechano-sensitive ion channels naturally interact with the lipid membrane. The most well-known signaling lipid is PIP_2, and it has been shown to regulate multiple ion channels and bind to the channels themselves (Harraz et al., 2020; Hille et al., 2015; Robinson et al., 2019). PIP_2 has been shown to affect mammalian hair cell mechanotransduction properties, including set point and single-channel conductance, suggesting it may also bind the mechanotransduction channel or components of the channel (Cunningham et al., 2020; Effertz et al., 2017). Studying the function of PIP_2 in slow adaptation could lead to a new mechanistic understanding of the slow adaptation process.

Adaptation in mammalian vestibular hair cells has different properties as compared to cochlear hair cells. Fast adaptation in vestibular hair cells occurs with a time constant on the order of 5 ms (Songer and Eatock, 2013; Vollrath and Eatock, 2003). This time constant is more similar to the slower fast adaptation time constant observed in the cochlea and may indicate the absence of the fastest time constant on the order of 0.2 ms observed in the cochlea (Peng et al., 2013; Waguespack et al., 2007). The slow adaptation time constant observed in vestibular hair cells is on the order of 50 ms and may be comparable to the slow motor adaptation time constant in the cochlea, although, in the cochlea, it appears to be closer to about 20 ms (Caprara et al., 2020; Songer and Eatock, 2013; Stauffer and Holt, 2007; Stauffer et al., 2005; Vollrath and Eatock, 2003). Further studies in different vestibular hair cell types are required for measuring adaptation properties to help determine the types and frequency of signals that the cells are tuned towards and how vestibular hair cells process balance signals.

Concluding remarks

Although the study of hair cell mechanotransduction has occurred for many decades, some of the mechanisms still remain elusive. The discovery of the molecular components of the mechanotransduction machinery will surely fuel a better understanding of these mechanisms. Classically only two forms of adaptation were recognized, but now more mechanisms of modulating the mechanotransduction channel on different time scales are becoming apparent and require further study.

While many mechanotransduction studies have been done in cochlear hair cells, mammalian vestibular hair cells appear to have different characteristics that may be more specific to the information they encode. While it is often that findings in vestibular type hair cells are applied to mammalian cochlear hair cells, these two types of hair cells also have distinct properties and are very likely different in their implementation of specific processes on the molecular level. Studies in vestibular hair cells are an underappreciated line of research that deserves further efforts to understand how our balance system encodes signals to serve its functions.

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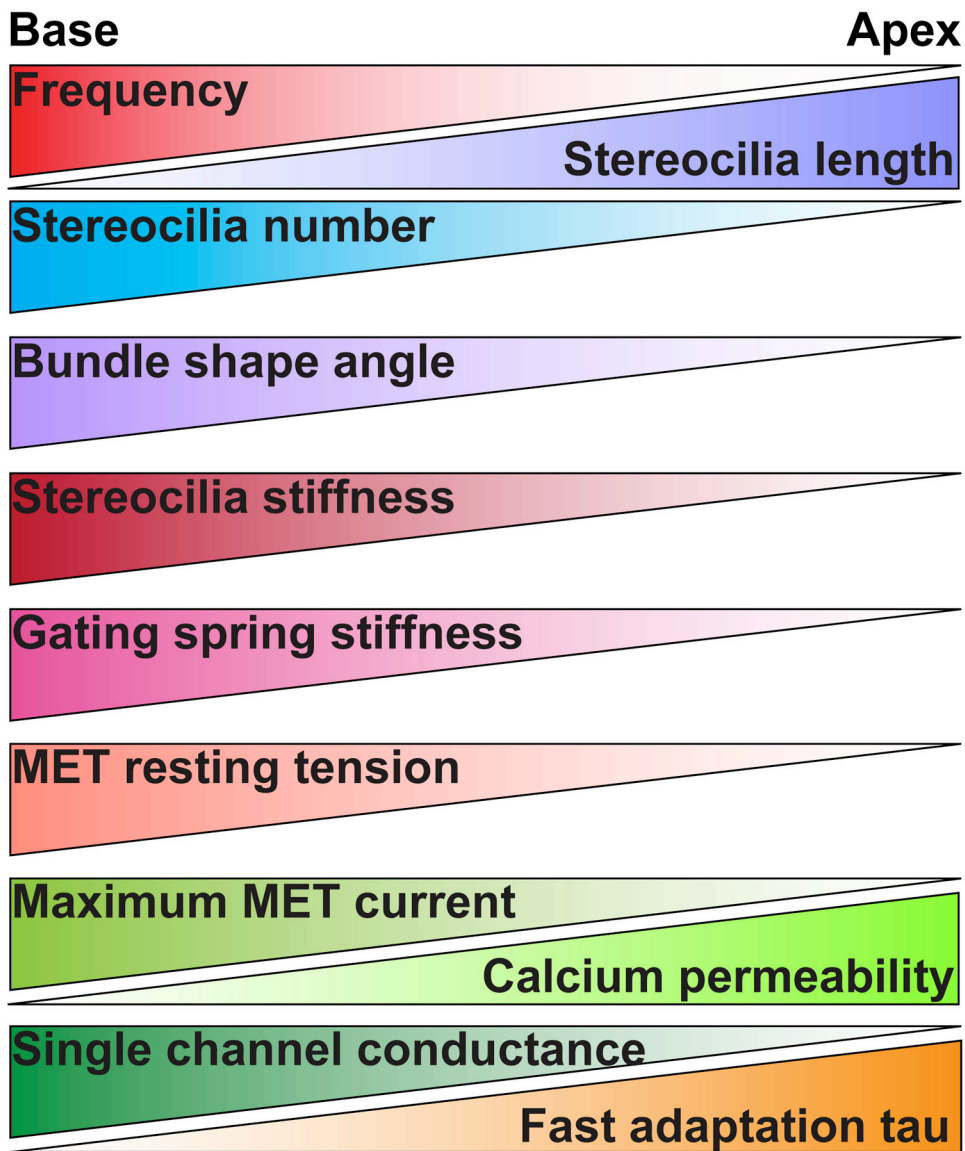
Highlights

- Substantial progress has been made in identifying the components of the mechanotransduction machinery.

- New data have revised hypotheses of how hair cell mechanotransduction mechanisms work

- Differences exist between cochlear and vestibular hair cells in terms of morphology and response properties, therefore mechanistic differences may exist as well.
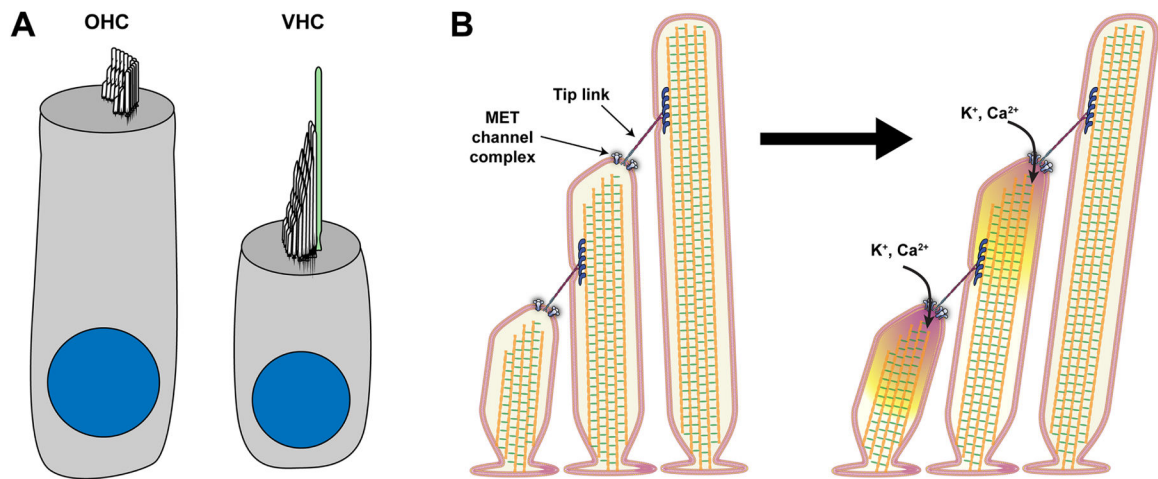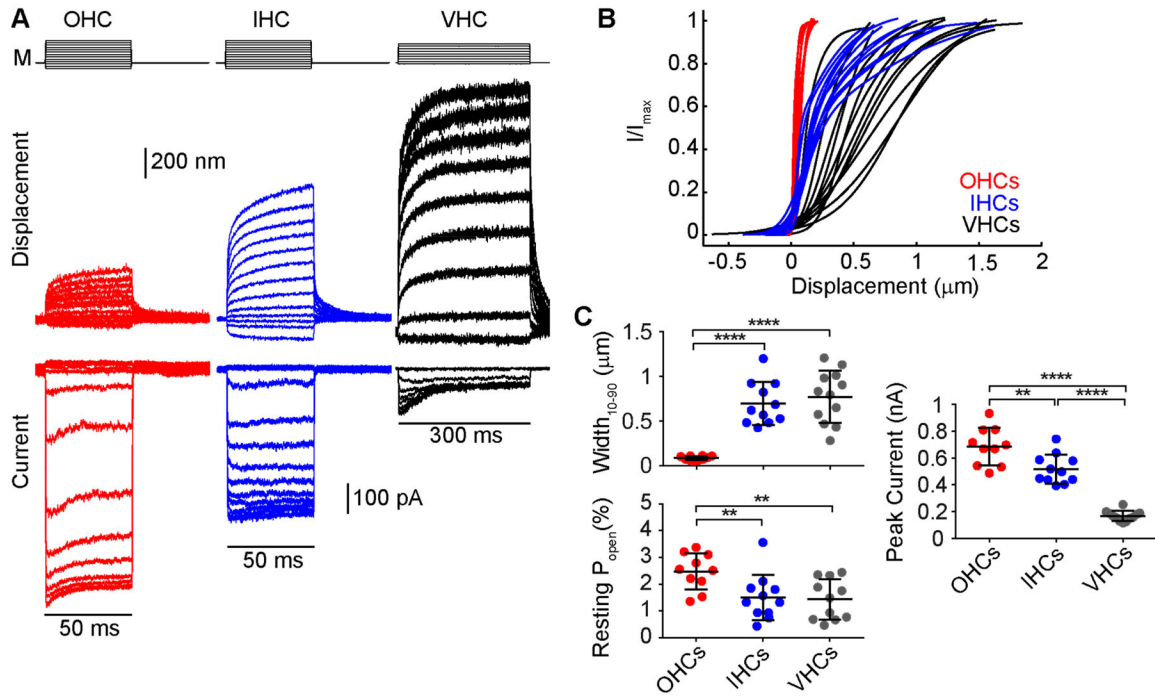
Figure 1.

(A) Depiction of an auditory outer hair cell (OHC) and a vestibular hair cell (VHC). The actin-filled stereocilia hair bundles protrude from the hair cell's apical surface. Vestibular hair cells also possess a kinocilium (green), a tubulin-based cilium, whereas, in auditory hair cells, the kinocilium disappears during development. (B) Taking a slice through the stereocilia rows depicts the mechanotransduction process. Tension within the tip link upon stereocilia deflection causes the opening of MET channels and the influx of potassium and calcium ions into Stereocilia.

Base   **Apex**

Frequency

Stereocilia length

Stereocilia number

Bundle shape angle

Stereocilia stiffness

Gating spring stiffness

MET resting tension

Maximum MET current

Calcium permeability

Single channel conductance

Fast adaptation tau

Figure 2.
Hair bundle and mechanotransduction properties that vary tonotopically in OHCs. Stereocilia length (Garfinkle and Saunders, 1983; Kaltenbach et al., 1994). Stereocilia number (Garfinkle and Saunders, 1983; Lim, 1986). Bundle shape angle (Lim, 1986). Stereocilia stiffness (Tobin et al., 2019). Gating spring stiffness (Tobin et al., 2019). MET resting tension (Tobin et al., 2019). Maximum MET current (Kim and Fettiplace, 2013). Single channel conductance (Beurg et al., 2018; Beurg et al., 2006). Calcium permeability (Kim and Fettiplace, 2013). Fast adaptation tau (Ricci et al., 2005; Waguespack et al., 2007).

Figure 3.
Activation curves in IHCs and vestibular hair cells are significantly wider than those of OHCs. (A) Example of a family of curves (bottom) in mouse cochlear hair cells (OHC-red and IHC-blue) and a mouse utricular type II hair cell (black) taken using a fluid-jet stimulus (M) and extracting the displacement of the hair bundle (middle) using high-speed imaging to generate the activation curves (Caprara et al., 2020). (B) Activation curves for multiple different cells in the same recording configuration across different cell types. Cells were recorded using an intracellular solution containing 0.1 mM BAPTA. n = 10 OHCs, P7-P9; n = 11 IHCs, P7-P8; n = 12 vestibular type II hair cells, P8-P11. (C) Summary plots of the width, resting open probability and maximum mechanotransduction current amplitude indicate a significantly lower peak current, wider activation curve, and lower resting probability in IHCs and vestibular hair cells. **** $p < 0.0001$ ** $p < 0.01$ Student's t-test.

Table 1.

Summary of auditory and vestibular phenotypes of various mouse models and whether the expression is documented in the sensory hair cells.

	Hearing phenotype	Vestibular phenotype	Cochlea expression	Vestibular expression
CDH23	Yes (Bolz et al., 2001; Bork et al., 2001; Di Palma et al., 2001)	Yes (Bolz et al., 2001; Bork et al., 2001; Di Palma et al., 2001)	Yes (Kazmierczak et al., 2007; Siemens et al., 2004)	Yes (Kazmierczak et al., 2007)
PCDH15	Yes (Alagramam et al., 2001)	Yes (Alagramam et al., 2001)	Yes (Ahmed et al., 2006; Kazmierczak et al., 2007)	Yes (Ahmed et al., 2006)
TMC1	Yes (Kurima et al., 2002; Vreugde et al., 2002)	No (de Heer et al., 2011; Kurima et al., 2002)	Yes (adulthood) (Kawashima et al., 2011; Kurima et al., 2002)	Yes (lifetime) (Kawashima et al., 2011; Kurima et al., 2002)
TMC2	No (Kawashima et al., 2011)	No (de Heer et al., 2011; Kurima et al., 2002)	Yes (only first days after birth) (Kawashima et al., 2011; Kurima et al., 2002)	Yes (lifetime) (Kawashima et al., 2011; Kurima et al., 2002)
TMIE	Yes (Naz et al., 2002)	Yes (Cho et al., 2006; Deol and Robins, 1962)	Yes (Cunningham et al., 2020; Zhao et al., 2014)	Yes (Zhao et al., 2014)
LHFPL5	Yes (Kalay et al., 2006; Longo-Guess et al., 2005; Longo-Guess et al., 2007; Shabbir et al., 2006)	Yes (Cho et al., 2006; Gyorgy et al., 2017; Longo-Guess et al., 2007)	Yes (Longo-Guess et al., 2005; Longo-Guess et al., 2007; Xiong et al., 2012)	Yes (Longo-Guess et al., 2005; Longo-Guess et al., 2007; Xiong et al., 2012)
CIB2	Yes (Giese et al., 2017; Liang et al., 2021; Michel et al., 2017; Patel et al., 2015; Riazuddin et al., 2012; Seco et al., 2016)	No (Giese et al., 2017; Liang et al., 2021; Michel et al., 2017)	Yes (Giese et al., 2017)	Yes (lifetime) (Giese et al., 2017; Liang et al., 2021)
CIB3	No (Liang et al., 2021)	No (Liang et al., 2021)	No (Liang et al., 2021)	Yes (lifetime) (Giese et al., 2017; Liang et al., 2021)
TOMT	Yes (Cunningham et al., 2017)	Unknown	Yes (Cunningham et al., 2017)	Unknown
LOXHD1	Yes (Grillet et al., 2009)	No (Grillet et al., 2009)	Yes (Grillet et al., 2009)	Yes (Grillet et al., 2009)