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The elusive mechanotransduction machinery of hair cells

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Hair cells in the mammalian cochlea are specialized sensory cells that convert mechanical signals evoked by sound waves into electrochemical signals. Several integral membrane proteins have recently been identified that are closely linked to the mechanotransduction process. Efforts are under way to determine the extent to which they are subunits of the long thought-after mechanotransduction channel. Recent findings also suggest that mechanotransduction may have a role in fine tuning the length of the stereocilia and thus in the regulation of morphological features of hair cells that are inherently linked to the mechanotransduction process.

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Introduction

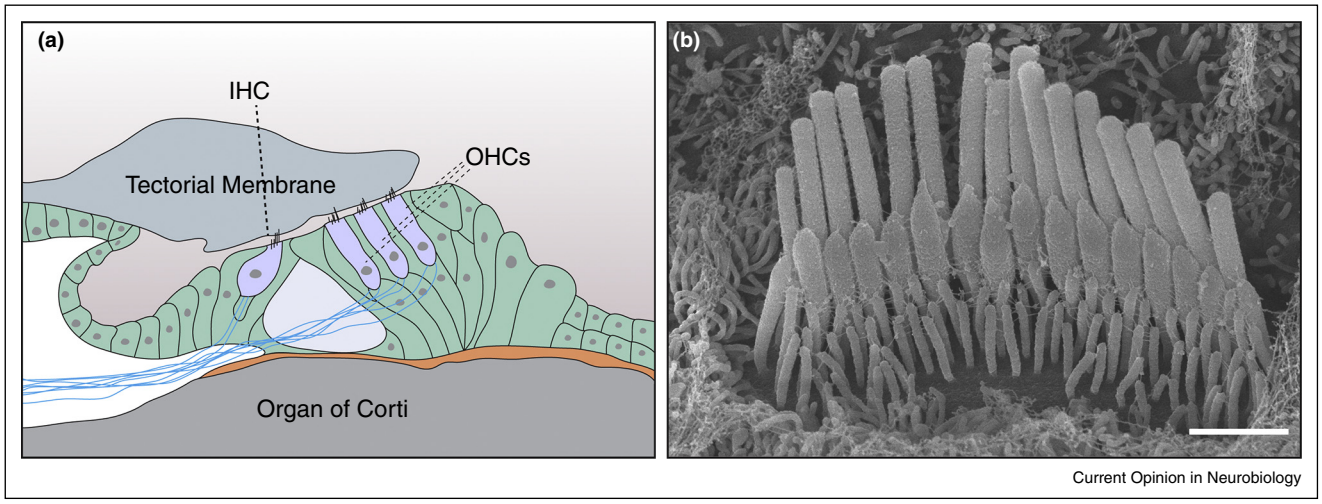
Hair cells of the mammalian cochlea (Figure 1a) are mechanosensory cells that convert sound induced vibration into electrochemical signals that are transmitted to the nervous system. Mechanically gated ion channels are located in the stereocilia that emanate from the apical hair cell surface (Figure 1b). These stereocilia are connected by tip links, extracellular filaments that are thought to gate the transduction channels (Figure 2). Several components of the mechanotransduction machinery have been identified. These include the proteins that form the tip links (Figure 2) [1–7]. Four additional integral membrane proteins, LHFPL5 (also named TMHS), TMIE, TMC1 and TMC2 have been closely linked to the transduction channel (Figure 2) [8,9,10••,11••,12••,13,14••]. Although significant progress has been made in defining their role in transduction, none of the four proteins has been shown to be a pore-forming subunit of the channel [8,9,10••,11••,12••,13,14••]. It has also remained a mystery why so many different proteins are required to build the transduction machinery. Recent findings suggest that this complexity is crucial to determine the properties of the transduction channels along the tonotopic axis of the cochlea and thus for frequency discrimination. Here we will review these findings and their implication for hair cell development and function.

The tip link: a stiff mechanical lever or a gating spring?

The tip link model of transduction channel gating was born in 1984 when ultrastructural studies identified fine extracellular filaments that connect the stereocilia of hair cells near their tips in the direction of their mechanical sensitivity [15]. These filaments were named tip links but the proteins that form the tip links remained elusive until the revolution in genome research allowed for the rapid identification of gene mutations that cause deafness. These studies identified mutations in six genes that cause Usher Syndrome Type I (USH1, deaf-blindness). USH1 genes encode the cell adhesion molecules cadherin 23 (CDH23) and protocadherin 15 (PCDH15) [16–20], the adaptor proteins harmonin and SANS [21–23], the calcium and integrin binding protein CIB2 [24], and the molecular motor myosin7a (Myo7a) [25]. Subsequent studies demonstrated that parallel homodimers of CDH23 interact with parallel homodimers of PCDH15 to form the upper and lower parts of tip links (Figure 2) [4]. Harmonin, SANS and Myo7a interact with each other, bind to CDH23, and are concentrated in the region where CDH23 inserts into the stereociliary membrane (Figure 2) [26]. CIB2 is also present in stereocilia and binds directly or indirectly to Myo7a [24], although its precise localization still needs to be defined. However, none of the identified USH1 proteins encodes a protein that resembles an ion channel.

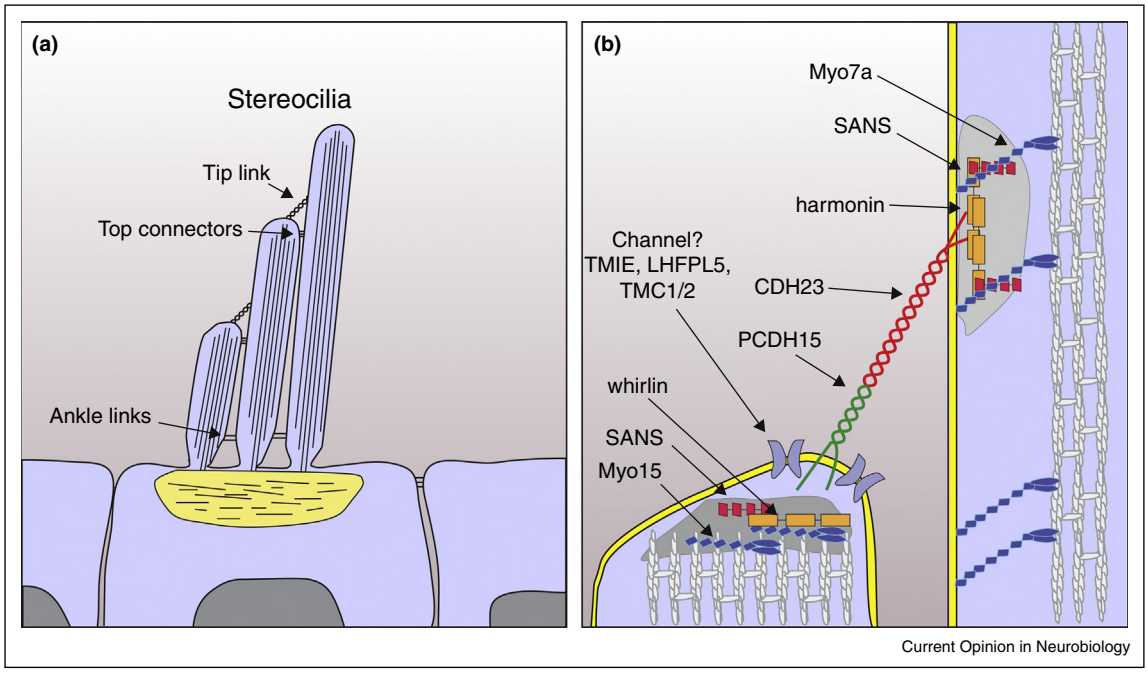
The identification of components that form tip links has facilitated new approaches to study tip-link functions in mechanotransduction. Notably, tip links have been proposed to be the gating spring of the transduction channel, which is stretched in response to mechanical stimuli, ultimately leading to channel opening [27]. Consistent with a role for tip links in transduction, transducer currents are affected in mice with mutations in *Cdh23* and *Pcdh15* [28,29]. Furthermore, tip-link regeneration in wild-type hair cells following BAPTA treatment is prevented in the presence of recombinant extracellular fragments of CDH23 and PCDH15 [30]. Interestingly,

Figure 1



Anatomy of mammalian organ of corti. (**a**) The sensory epithelia in the organ of Corti in the mammalian cochlea contain three rows of outer hair cells (OHCs) and one row of inner hair cells (IHCs). OHCs are important for the amplification of sound signals and receive efferent innervation, while IHCs are innervated by afferent neurons that carry sound information to the nervous system. Other structural features of the organ of Corti such as the basilar membrane and the tectorial membrane are indicated. (**b**) Scanning electron micrograph showing the stereocilia of an IHC that are organized in rows of decreasing heights (picture courtesy of Nicolas Grillet). Scale bar: 1 μm.

Figure 2



Model of the tip-link complex in hair cells. (**a**) Diagram of a hair cell highlighting the hair bundle and the tip-link filaments that connect the stereocilia in the direction of their mechanical sensitivity. Top-connectors and ankle-links are additional filaments that connect the stereocilia. (**b**) Key molecules associated with tip links. Parallel homodimers of CDH23 interact with parallel homodimers of PCDH15 to form the upper and lower parts of tip links. The USH1 proteins harmonin, Myo7a and SANS form a complex that localizes near CDH23 at the upper tip-link region. LHFPL5, TMIE, and TMC1/2 localize at the lower end of tip links near PCDH15 where transduction channels are located. Whirlin and Myo15 are also concentrated near tip links but are not discussed in the text because their link to transduction is unclear.

a proportion of nascent regenerating tip links consists entirely of PCDH15 at both ends of the filament [31••]. Transducer currents can be evoked in hair cells early during regeneration, but their kinetic properties and Ca^{2+} dependence are changed compared to hair cells with mature tip links [31••], arguing that tip links with different molecular compositions affect transduction in distinct ways and thus that tip links are inherently linked to the channel gating process.

The extent to which tip links serve as gating springs is less clear. Crystallographic studies have shown that the first and second extracellular cadherin repeats of CDH23 and PCDH15 adopt the typical cadherin folds and mediate trans-interactions between the two cadherins [32–34]. Based on molecular dynamics simulations it has been proposed that the trans-bond is sufficiently strong to resist the forces in hair cells and that tip links are too stiff to be the gating spring [34]. However, molecular modeling was based on monomeric fragments of CDH23 and PCDH15 containing only two cadherin repeats each. The native extracellular domains of CDH23 and PCDH15 consist of 27 and 11 cadherin repeats, respectively, and they interact with each other as dimers [4]. Thus, before final conclusions regarding the gating spring can be reached, it will be important to experimentally measure adhesive strength and stiffness using full-length dimeric CDH23 and PCDH15 molecules.

Mechanisms of adaptation: quo vadis?

Mechanotransduction channels adapt to a stimulus to maintain their sensitivity. During the initial fast adaption step that takes a few milliseconds, Ca^{2+} that enters stereocilia is thought to bind to the transduction channel or an element near the channel, which leads to channel reclosure. The subsequent slow adaptation process that takes tens of milliseconds has been proposed to depend on an adaptation motor that consist of a cluster of myosin motors that is attached to the upper tip-link end. The motor is thought to generate resting tension within the gating spring, which increases during activation and is conveyed to the motor in a Ca^{2+}-dependent manner; the motor slides down the actin filaments, relaxing tension and leading to channel closure. Subsequently, the motor complex climbs up the stereocilium and restores tension [35–38].

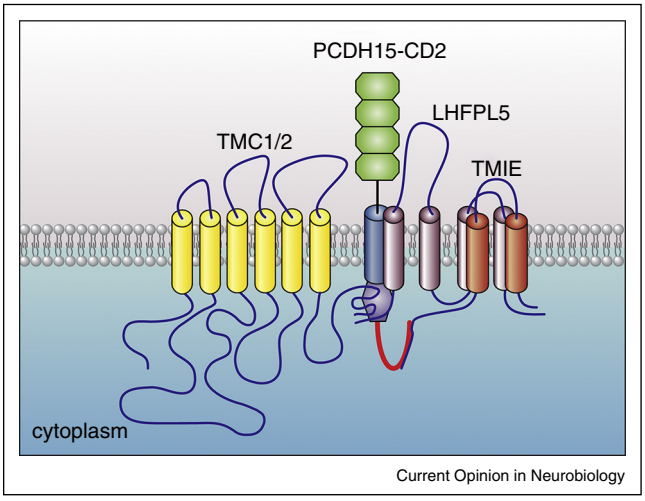
The classical model of channel adaptation is largely based on the study of vestibular hair cells in non-mammalian species and is difficult to reconcile with recent findings in mammalian cochlear hair cells. Initial studies suggested that transducer channels are located at the upper and lower end of tip links [39]. Myo1c was localized to regions near the upper and lower tip-link insertion site in vestibular hair cells [40,41]. Functional studies suggest that Myo1c acts as an adaptation motor in vestibular hair cells [42,43]. However, in cochlear hair cells Myo1c appears to be distributed along the length of stereocilia [44]. In addition, taking advantage of high-speed Ca^{2+} imaging, the transduction channel was recently localized only to the lower end of tip links near PCDH15 and far away from the proposed location of the adaptation motor [45••]. Adaptation in cochlear hair cells is also an order of magnitude faster than in vestibular hair cells and on a time scale that is difficult to reconcile with a myosin motor driven process [46,47]. At least one study found that adaptation in cochlear hair cells is Ca^{2+} independent [48], although a second study reached a different conclusion [49]. Nevertheless, these studies suggest that the classical model of channel adaptation might not apply to cochlear hair cells. Notably, cochlear hair cells operate at far higher frequencies than vestibular hair cells and perhaps fast adaptation might be most crucial for cochlear hair cells within the physiologically relevant range.

What then is the function of the USH1 proteins harmonin, SANS, and Myo7a at the upper tip-link insertion site? USH1 proteins may form a protein complex containing Myo7a that climbs up the actin filaments to adjust tip-link tension. Harmonin, and SANS might help to cluster molecules and to stabilized interactions between the tip-link protein CDH23 and Myo7a. Consistent with this model, the mechanical sensitivity of transducer channels is decreased in the absence of harmonin [7,50]. Tip link integrity and transduction are also affected in the absence of SANS, and Myo7a has been proposed to be important for establishing resting tension [5,51]. However, recent findings suggest that the original data supporting a role for Myo7a need to be re-interpreted [52••]. Thus, further studies are necessary to explore the function of USH1 proteins and Myo1c in hair cells and in particular for adaptation.

TMC1 and TMC2: a solution to the transduction channel mystery?

Properties of the mechanotransduction channel in hair cells have been investigated for decades, including its kinetic behavior, ion selectivity, and single channel conductance [53]. Many candidate genes have been proposed to encode subunits of the transduction channel but were subsequently excluded to play a role in transduction. New tantalizing insights have finally been obtained from the study of genes that are linked to deafness. These studies implicate four integral membrane proteins in the regulation of channel function. These are TMC1, TMC2, LHFPL5 and TMIE (Figure 3). All four proteins interact in heterologous systems with PCDH15 [13,14••,54••,55••]. TMIE and LHFPL5 are present near PCDH15 at the lower end of tip links where transduction channels reside [13,14••]. TMC1 is present near the tips of stereocilia, but may also be present in the longest stereocilia that do not contain active transduction channels [54••]. The localization of endogenous TMC2 protein in hair cells is unknown,

Figure 3



Model of the transduction channel complex of hair cells. TMC1/2 and LHFPL5 interact with the common region of PCDH15, while TMIE interact with the unique C-terminus of the PCDH15-CD2 isoform as well as with LHFPL5.

but when TMC2 is overexpressed in hair cells, it localizes near stereociliary tips in proximity to PCDH15 [9,54••].

The first evidence linking TMC1/2 to transduction came from the analysis of mutant mice lacking both *Tmc1* and *Tmc2*. The mutant mice are deaf and transduction currents are abolished in hair cells. Transduction could be rescued by re-expression of TMC1 or TMC2, suggesting that the two proteins can function independently of each other to support transduction [9]. Interestingly, mutations in *TMC1* but not *TMC2* have been shown to cause deafness [56–59], which can likely be explained by the fact that *Tmc2* is expressed only transiently in developing cochlear hair cells while *Tmc1* expression is maintained into adulthood [9].

The co-expression of TMC1 and TMC2 in early developing hair cells has prompted investigation into their respective functions in transduction. In rodents, the current amplitude in OHCs increases and the relative Ca^{2+} permeability decreases from the apex to base of the cochlea [11••,60]. The tonotopic gradient in channel conductance is no longer observed in the cochlea of mice lacking *Tmc1* [8,11••]. Transduction channels in hair cells expressing only *Tmc2* have a high permeability for Ca^{2+} and a large single channel conductance, while cells expressing only *Tmc1* have reduced Ca^{2+} permeability and single-channel conductance [8,10••,11••,12••]. The reduction in Ca^{2+} permeability is more pronounced in hair cells expressing a TMC1 protein that carries a mutation linked to deafness [12••]. It has therefore been proposed that TMC1/2 proteins are pore-forming subunits of the transduction channel, where differences in TMC1/2 stoichiometry explain tonotopic conductance differences [12••].

Consistent with a role in transduction, TMC1/2 interacts in heterologous cells with PCDH15 [54••,55••]. One caveat to the biochemical data is that only minor amounts of TMC1/2 reach the cell surface suggesting that interactions with PCDH15 occur in an intracellular compartment [9,54••]. Nevertheless, overexpression of a protein fragment consisting of the first 117 amino acids of TMC2, which is thought to disrupt interactions between TMC1/2 and PCDH15, changes the localization of PCDH15 and decreases the mechanosensitivity of hair cells in zebrafish [55••].

Despite these interesting findings, the extent to which TMC1 and TMC2 are pore-forming subunits of the transduction channel remains unclear. Adult cochlear hair cells only express *Tmc1*. Thus, variations in the stoichiometry of TMC1/2 cannot explain tonotopic variations in transducer currents of the adult cochlea. Furthermore, robust transducer currents can be evoked in hair cells from *Tmc1/2* double mutant mice by deflection of the stereocilia in the opposite from normal direction [8,10••]. Although it is unclear whether the same channel carries the normal and reverse polarity current, the two share many features such as block by high concentration of extracellular Ca^{2+} and by standard pharmacological channel inhibitors (albeit with somewhat different efficacy) [8,10••,52••]. Activation of the reverse polarity current in *Tmc1/2* mutant hair cells does not depend on tip links [10••], and a similar reverse polarity current is observed when tip links are disrupted by mutations in *Cdh23* and *Pcdh15* or by application of BAPTA to wild-type hair cells [10••,28,52••]. Interestingly, in *Tmc1* and *Tmc2* single mutants, less tip links are occupied with channels compared to *Tmc2* deficient hair cells expressing mutant TMC1 [12••]. Thus it seems possible that TMC1/2 function to transport channels into stereocilia and/or to stabilize interactions with tip links [9]. Alternatively, it has been proposed that TMC1/2 might form a vestibule of the transduction channel that shuttles ions towards the pore [8,52••].

Ultimately, it will be important to analyze the properties of TMC1 and TMC2 in heterologous cells. So far, attempts have failed to express TMC1 and TMC2 at the cell surface of heterologous cells or to demonstrate that the proteins encode ion channels [9,14••]. A TMC ortholog in worm has been shown to be necessary for salt sensation and to generate a sodium-sensitive channel when expressed in heterologous cells [61]. Independent replication of these results is eagerly awaited.

LHFPL5 and TARPs: regulating transducer channels and tonotopia

In a screen that was designed to identify components of the mechanotransduction machinery of hair cells, Xiong *et al.* (2012) demonstrated that deaf mice with mutations in *Lhfpl5*, a protein with four transmembrane domains,

have transducer currents with an amplitude that is reduced by ~90%. When expressed in heterologous cells, LHFPL5 and PCDH15 colocalize at the cell membrane and interact with each other. Subsequent studies demonstrated that LHFPL5 regulates the transports of PCDH15 into stereocilia thereby controlling the efficiency of tip-link formation [13]. In addition, LHFPL5 directly affects the properties of the transduction channel. Single channel recordings demonstrated that in the absence of LHFPL5, the conductance of the transduction channel is reduced and adaptation is severely impaired [13]. These findings suggest that LHFPL5 is an accessory subunit of the transduction channel. Interestingly, LHFPL5 shares certain features with the TARP subunits of AMPA receptor. Like LHFPL5, TARPs have four transmembrane domains and regulate both the transport of the pore forming subunits of AMPA receptors and channel conductance [62]. In analogy, it has been proposed that LHFPL5 acts as a TARP-like protein for the transduction channel [13]. Alternatively, LHFPL5 might affect membrane properties locally, which is a function attributed to some tetraspans [63].

A recent study has established a functional link between LHFPL5 and TMC1. In mice lacking *Lhfpl5* as well as in mice lacking *Tmc1*, the tonotopic gradient in the conductance of the transducer channels is similarly blunted [54••]. Immunolocalization studies demonstrated that TMC1 is no longer localized to the stereocilia of *Lhflp5*-deficient hair cells, suggesting that TMC1 and LHFPL5 act in a common molecular complex [54••]. Variations in levels of LHFPL5 and/or TMC1 along the cochlear axis could explain tonotopic differences in the conductance properties of the transducer channel. Notably, LHFPL5 is a member of a branch of the tetraspan family that consists in mammals of six members [64]. An interesting hypothesis is that different LHFPL family members might cooperate to regulate properties of transduction channels in hair cells.

TMIE: adding to the transduction channel mystery

In a yeast two-hybrid screen that was tailored towards the identification of interactions between transmembrane proteins, TMIE was identified as a binding partner for PCDH15 and LHFPL5 [14••]. TMIE is a protein with two predicted transmembrane domains and is linked to deafness in both humans and mice [65,66]. Studies in zebrafish have shown that *Tmie*-deficient hair cells show degenerative changes and lack microphonic potential in response to vibratory stimulation, indicating that transduction is affected [67]. Recent studies in mice demonstrate that TMIE is localized in the tip-link region and essential for normal mechanotransduction but not for tip-link formation. As in mice lacking tip links or TMC1/2, reverse polarity currents can still be evoked in *Tmie*-deficient hair cells. The transduction defect can be rescued be re-expression of TMIE, but not by overexpression of either LHFPL5 or TMC1/2 [14••]. What then is the function of TMIE in transduction? And what is its relation to tip links, LHFPL5 and TMC1/2? The localization of TMIE in hair cells and its binding to PCDH15 and LHFPL5 suggests that TMIE is an integral component of the transduction machinery. Biochemical studies provide additional clues. PCDH15 is expressed in three major alternative spliced variants (PCDH15-CD1, PCDH15-CD2, PCDH15-CD3) that are identical in their extracellular and transmembrane domains as well as in a short membrane proximal cytoplasmic domain that mediates interactions with TMC1/2 and LHFPL5 (Figure 3) [1,13,54••,55••]. The splice variants differ in the remaining C-terminal part of their cytoplasmic domains [1]. Remarkably, PCDH15-CD2 is the PCDH15 isoform crucial for mechanotransduction in mature hair cells [68] and TMIE binds to the unique C-terminus of PCDH15-CD2 (Figure 3) [14••]. TMIE also interacts with all three PCDH15 isoforms in a ternary complex with LHFPL5 (Figure 3) [14••]. Overexpression of a TMIE fragment consisting of the C-terminal half that disrupts interactions both with PCDH15 and LHFPL5 affects transduction in wild-type hair cells. Transduction is also disrupted by overexpressing the CD2 domain of PCDH15, a protein fragment that affects interactions between TMIE and PCDH15-CD2 [14••]. Taken together, the findings suggest that TMIE is part of a protein complex containing LHFPL5 and TMIE that connects the tip link to the transduction channel. Notably, proteins with two transmembrane domains such as ENac/DEG in *Caenorhabditis elegans* oligomerize to form a channel pore [69]. However, it has so far not been demonstrated that TMIE has ion channel properties, either alone or together with other proteins such as LHFPL5 and TMC1/2 [14••]. Thus further studies are important to define the precise function of TMIE for transduction.

Mechanotransduction: a role in hair bundle morphogenesis?

Hair bundles of mammalian hair cell consist of three rows of stereocilia of decreasing heights. The structure and staircase pattern of the hair bundle is crucial for hair cell function since deflection only in the direction of the longest stereocilia leads to an increase in the open probability of mechanotransduction channels. It has therefore long been speculated that tip links and transduction channels regulate hair bundle morphogenesis. The most conclusive evidence for this model stems from the recent analysis of genetically modified mice, where the *Sans* and *Cdh23* genes were inactivated at postnatal ages. In these mice, rows of stereocilia of graded heights have already developed. Following gene inactivation, tip links are disrupted and the shortest and second rows of stereocilia, which contain transduction channels near their tips, shrink [5]. Tip-link mediated tension, possibly via the SANS/harmonin/Myo7a complex, may be crucial to

maintain the stereociliary staircase. However, it is currently unclear whether this protein complex acts on the actin cytoskeleton of stereocilia by tension forces and/or by activating transduction channels. One way to address this question is to analyze morphological changes in the hair bundles from *Tmc1/2*-deficient or *Tmie*-deficient mice. Although hair bundles with a staircase pattern develop in these mutant mice [9,14••], a morphometric analysis of stereociliary length has not been carried out. Clearly, hair bundles eventually degenerate in the mutant mice. It is therefore tempting to speculate that mechanically evoked currents are crucial to regulate the F-actin assembly process at the tips of stereocilia.

Summary

Recent studies have revealed an unexpected complexity in the transduction machinery of hair cells. These findings have shown that the transduction machinery of hair cells has an inherent asymmetry that manifests in the composition of tip links and the localization of distinct protein complexes to the upper and lower ends of tip links. Several USH1 proteins form a protein complex at the upper end of tip links that appears to establish tension in the tip link. This protein complex might be involved in setting resting tension and controlling adaptation. Several integral transmembrane proteins that bind to PCDH15 are appropriately localized near the lower end of tip links to be subunits of the transduction channel. Current data suggest that the transduction channel consists of pore forming and accessory subunits and that variation in the stoichiometry of these components is crucial establishing tonotopia. However, the extent to which LHFPL5, TMIE, TMC1/2 contribute to the channel pore is unclear. Ultimately, it will be important to reconstitute the mechanically gated ion channel in a heterologous system. Given the molecular complexity in the transduction machinery and structural requirements that might depend on a highly organized cell shape, this might be a difficult but ultimately crucial task.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Ahmed ZM, Goodyear R, Riazuddin S, Lagziel A, Legan PK, Behra M, Burgess SM, Lilley KS, Wilcox ER, Riazuddin S *et al.*: **The tip-link antigen, a protein associated with the transduction complex of sensory hair cells, is protocadherin-15**. *J Neurosci* 2006, **26**:7022-7034.

2. Siemens J, Lillo C, Dumont RA, Reynolds A, Williams DS, Gillespie PG, Muller U: **Cadherin 23 is a component of the tip link in hair-cell stereocilia**. *Nature* 2004, **428**:950-955.

3. Sollner C, Rauch GJ, Siemens J, Geisler R, Schuster SC, Muller U, Nicolson T, Tubingen Screen C: **Mutations in cadherin 23 affect tip links in zebrafish sensory hair cells**. *Nature* 2004, **428**:955-959.

4. Kazmierczak P, Sakaguchi H, Tokita J, Wilson-Kubalek EM, Milligan RA, Muller U, Kachar B: **Cadherin 23 and protocadherin 15 interact to form tip-link filaments in sensory hair cells**. *Nature* 2007, **449**:87-91.

5. Caberlotto E, Michel V, Foucher I, Bahloul A, Goodyear RJ, Pepermans E, Michalski N, Perfettini I, Alegria-Prevot O, Chardenoux S *et al.*: **Usher type 1G protein sans is a critical component of the tip-link complex, a structure controlling actin polymerization in stereocilia**. *Proc Natl Acad Sci U S A* 2011, **108**:5825-5830.

6. Grati M, Kachar B: **Myosin VIIa and sans localization at stereocilia upper tip-link density implicates these Usher syndrome proteins in mechanotransduction**. *Proc Natl Acad Sci U S A* 2011, **108**:11476-11481.

7. Grillet N, Xiong W, Reynolds A, Kazmierczak P, Sato T, Lillo C, Dumont RA, Hintermann E, Sczaniecka A, Schwander M *et al.*: **Harmonin mutations cause mechanotransduction defects in cochlear hair cells**. *Neuron* 2009, **62**:375-387.

8. Beurg M, Kim KX, Fettiplace R: **Conductance and block of hair-cell mechanotransducer channels in transmembrane channel-like protein mutants**. *J Gen Physiol* 2014, **144**:55-69.

9. Kawashima Y, Geleoc GS, Kurima K, Labay V, Lelli A, Asai Y, Makishima T, Wu DK, Della Santina CC, Holt JR *et al.*: **Mechanotransduction in mouse inner ear hair cells requires transmembrane channel-like genes**. *J Clin Invest* 2011, **121**:4796-4809.

10. •• Kim KX, Beurg M, Hackney CM, Furness DN, Mahendrasingam S, Fettiplace R: **The role of transmembrane channel-like proteins in the operation of hair cell mechanotransducer channels**. *J Gen Physiol* 2013, **142**:493-505.
The authors observe that in hair cells lacking TMC1 and TMC2, mechanotransduction currents can still be evoked by stimulating hair bundles in the opposite from normal excitatory direction. Similar currents are observed after disruption of tip links by sustained perfusion of BAPTA. The reverse polarity currents share many features with normal polarity currents such as sensitivity to the extracellular Ca^{2+} concentration and sensitivity to pharmacological channel blockers.

11. •• Kim KX, Fettiplace R: **Developmental changes in the cochlear hair cell mechanotransducer channel and their regulation by transmembrane channel-like proteins**. *J Gen Physiol* 2013, **141**:141-148.
The authors characterize the properties of mechanically gated currents in cochlear hair cells lacking either TMC1 or TMC2. They demonstrate that TMC1 is crucial to establish the tonotopic gradient in the conductance properties of the transduction channel in hair cells where TMC2 affects both ion selectivity and current size.

12. •• Pan B, Geleoc GS, Asai Y, Horwitz GC, Kurima K, Ishikawa K, Kawashima Y, Griffith AJ, Holt JR: **TMC1 and TMC2 are components of the mechanotransduction channel in hair cells of the mammalian inner ear**. *Neuron* 2013, **79**:504-515.
The authors have generated genetically modified mice expressing only TMC1, TMC2, or a mutant TMC1 protein in hair cells. The authors demonstrate that channels containing TMC1 only, TMC2 only, or mutant TMC1 only have altered conductance properties relative to wild-type hair cells. The authors conclude that TMC1 and TMC2 are subunits of the transduction channel.

13. Xiong W, Grillet N, Elledge HM, Wagner TF, Zhao B, Johnson KR, Kazmierczak P, Muller U: **TMHS is an integral component of the mechanotransduction machinery of cochlear hair cells**. *Cell* 2012, **151**:1283-1295.

14. •• Zhao B, Wu Z, Grillet N, Yan L, Xiong W, Harkins-Perry S, Muller U: **TMIE is an essential component of the mechanotransduction machinery of cochlear hair cells**. *Neuron* 2014, **84**:954-967.
The study shows that TMIE, a protein with two predicted transmembrane domains, is essential for mechanotransduction of hair cells. TMIE localizes to the tip-link region near PCDH15 and binds both to PCDH15 and

LHFPL5. Interactions between TMIE with PCDH15 and LHFPL5 are crucial for mechanotransduction, suggesting that the proteins are part of a molecular complex

15. Pickles JO, Comis SD, Osborne MP: **Cross-links between stereocilia in the guinea pig organ of Corti, and their possible relation to sensory transduction**. *Hear Res* 1984, **15**:103-112.

16. Ahmed ZM, Riazuddin S, Bernstein SL, Ahmed Z, Khan S, Griffith AJ, Morell RJ, Friedman TB, Riazuddin S, Wilcox ER: **Mutations of the protocadherin gene PCDH15 cause Usher syndrome type 1F**. *Am J Hum Genet* 2001, **69**:25-34.

17. Alagramam KN, Yuan H, Kuehn MH, Murcia CL, Wayne S, Srisailpathy CR, Lowry RB, Knaus R, Van Laer L, Bernier FP *et al.*: **Mutations in the novel protocadherin PCDH15 cause Usher syndrome type 1F**. *Hum Mol Genet* 2001, **10**:1709-1718.

18. Bolz H, von Brederlow B, Ramirez A, Bryda EC, Kutsche K, Nothwang HG, Seeliger M, del CSCM, Vila MC, Molina OP *et al.*: **Mutation of CDH23, encoding a new member of the cadherin gene family, causes Usher syndrome type 1D**. *Nat Genet* 2001, **27**:108-112.

19. Bork JM, Peters LM, Riazuddin S, Bernstein SL, Ahmed ZM, Ness SL, Polomeno R, Ramesh A, Schloss M, Srisailpathy CR *et al.*: **Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene CDH23**. *Am J Hum Genet* 2001, **68**:26-37.

20. Di Palma F, Holme RH, Bryda EC, Belyantseva IA, Pellegrino R, Kachar B, Steel KP, Noben-Trauth K: **Mutations in Cdh23, encoding a new type of cadherin, cause stereocilia disorganization in waltzer, the mouse model for Usher syndrome type 1D**. *Nat Genet* 2001, **27**:103-107.

21. Bitner-Glindzicz M, Lindley KJ, Rutland P, Blaydon D, Smith VV, Milla PJ, Hussain K, Furth-Lavi J, Cosgrove KE, Shepherd RM *et al.*: **A recessive contiguous gene deletion causing infantile hyperinsulinism, enteropathy and deafness identifies the Usher type 1C gene**. *Nat Genet* 2000, **26**:56-60.

22. Verpy E, Leibovici M, Zwaenepoel I, Liu XZ, Gal A, Salem N, Mansour A, Blanchard S, Kobayashi I, Keats BJ *et al.*: **A defect in harmonin, a PDZ domain-containing protein expressed in the inner ear sensory hair cells, underlies Usher syndrome type 1C**. *Nat Genet* 2000, **26**:51-55.

23. Weil D, El-Amraoui A, Masmoudi S, Mustapha M, Kikkawa Y, Laine S, Delmaghani S, Adato A, Nadifi S, Zina ZB *et al.*: **Usher syndrome type I G (USH1G) is caused by mutations in the gene encoding SANS, a protein that associates with the USH1C protein, harmonin**. *Hum Mol Genet* 2003, **12**:463-471.

24. Riazuddin S, Belyantseva IA, Giese AP, Lee K, Indzhykulian AA, Nandamuri SP, Yousaf R, Sinha GP, Lee S, Terrell D *et al.*: **Alterations of the CIB2 calcium- and integrin-binding protein cause Usher syndrome type 1J and nonsyndromic deafness DFNB48**. *Nat Genet* 2012, **44**:1265-1271.

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

26. Kazmierczak P, Muller U: **Sensing sound: molecules that orchestrate mechanotransduction by hair cells**. *Trends Neurosci* 2012, **35**:220-229.

27. Corey DP, Hudspeth AJ: **Kinetics of the receptor current in bullfrog saccular hair cells**. *J Neurosci* 1983, **3**:962-976.

28. Alagramam KN, Goodyear RJ, Geng R, Furness DN, van Aken AF, Marcotti W, Kros CJ, Richardson GP: **Mutations in protocadherin 15 and cadherin 23 affect tip links and mechanotransduction in mammalian sensory hair cells**. *PLoS One* 2011, **6**:e19183.

29. Senften M, Schwander M, Kazmierczak P, Lillo C, Shin JB, Hasson T, Geleoc GS, Gillespie PG, Williams D, Holt JR *et al.*: **Physical and functional interaction between protocadherin 15 and myosin VIIa in mechanosensory hair cells**. *J Neurosci* 2006, **26**:2060-2071.

30. Lelli A, Kazmierczak P, Kawashima Y, Muller U, Holt JR: **Development and regeneration of sensory transduction in auditory hair cells requires functional interaction between cadherin-23 and protocadherin-15**. *J Neurosci* 2010, **30**:11259-11269.

31. •• Indzhykulian AA, Stepanyan R, Nelina A, Spinelli KJ, Ahmed ZM, Belyantseva IA, Friedman TB, Barr-Gillespie PG, Frolenkov GI: **Molecular remodeling of tip links underlies mechanosensory regeneration in auditory hair cells**. *PLoS Biol* 2013, **11**:e1001583.

High resolution immunolocalization studies are used to determine the molecular composition of tip links in regenerating hair cells. At least a fraction of tip links in regenerating hair cells consists of PCDH15 only. Electrophysiological recordings suggest that the properties of transducer currents in the regeneration hair cells differ from those in functioally mature hair cells, where tip links are formed by PCDH15 and CDH23.

32. Elledge HM, Kazmierczak P, Clark P, Joseph JS, Kolatkar A, Kuhn P, Muller U: **Structure of the N terminus of cadherin 23 reveals a new adhesion mechanism for a subset of cadherin superfamily members**. *Proc Natl Acad Sci U S A* 2010, **107**:10708-10712.

33. Sotomayor M, Weihofen WA, Gaudet R, Corey DP: **Structural determinants of cadherin-23 function in hearing and deafness**. *Neuron* 2010, **66**:85-100.

34. Sotomayor M, Weihofen WA, Gaudet R, Corey DP: **Structure of a force-conveying cadherin bond essential for inner-ear mechanotransduction**. *Nature* 2012, **492**:128-132.

35. LeMasurier M, Gillespie PG: **Hair-cell mechanotransduction and cochlear amplification**. *Neuron* 2005, **48**:403-415.

36. Farris HE, Wells GB, Ricci AJ: **Steady-state adaptation of mechanotransduction modulates the resting potential of auditory hair cells, providing an assay for endolymph [Ca2+]**. *J Neurosci* 2006, **26**:12526-12536.

37. Jia S, Dallos P, He DZ: **Mechanoelectric transduction of adult inner hair cells**. *J Neurosci* 2007, **27**:1006-1014.

38. Gillespie PG, Muller U: **Mechanotransduction by hair cells: models, molecules, and mechanisms**. *Cell* 2009, **139**:33-44.

39. Denk W, Holt JR, Shepherd GM, Corey DP: **Calcium imaging of single stereocilia in hair cells: localization of transduction channels at both ends of tip links**. *Neuron* 1995, **15**:1311-1321.

40. Garcia JA, Yee AG, Gillespie PG, Corey DP: **Localization of myosin-Ibeta near both ends of tip links in frog saccular hair cells**. *J Neurosci* 1998, **18**:8637-8647.

41. Steyger PS, Gillespie PG, Baird RA: **Myosin Ibeta is located at tip link anchors in vestibular hair bundles**. *J Neurosci* 1998, **18**:4603-4615.

42. Holt JR, Gillespie SK, Provance DW, Shah K, Shokat KM, Corey DP, Mercer JA, Gillespie PG: **A chemical-genetic strategy implicates myosin-1c in adaptation by hair cells**. *Cell* 2002, **108**:371-381.

43. Stauffer EA, Scarborough JD, Hirono M, Miller ED, Shah K, Mercer JA, Holt JR, Gillespie PG: **Fast adaptation in vestibular hair cells requires myosin-1c activity**. *Neuron* 2005, **47**:541-553.

44. Schneider ME, Dose AC, Salles FT, Chang W, Erickson FL, Burnside B, Kachar B: **A new compartment at stereocilia tips defined by spatial and temporal patterns of myosin IIIa expression**. *J Neurosci* 2006, **26**:10243-10252.

45. •• Beurg M, Fettiplace R, Nam JH, Ricci AJ: **Localization of inner hair cell mechanotransducer channels using high-speed calcium imaging**. *Nat Neurosci* 2009, **12**:553-558.

Using high speed Ca^{2+} imaging, the authors demonstrate that mechanotransduction channels in hair cells are localized near the lower end of tip links.

46. Kennedy HJ, Evans MG, Crawford AC, Fettiplace R: **Fast adaptation of mechanoelectrical transducer channels in mammalian cochlear hair cells**. *Nat Neurosci* 2003, **6**:832-836.

47. Ricci AJ, Kennedy HJ, Crawford AC, Fettiplace R: **The transduction channel filter in auditory hair cells**. *J Neurosci* 2005, **25**:7831-7839.

48. Peng AW, Effertz T, Ricci AJ: **Adaptation of mammalian auditory hair cell mechanotransduction is independent of calcium entry**. *Neuron* 2013, **80**:960-972.

49. Corns LF, Johnson SL, Kros CJ, Marcotti W: **Calcium entry into stereocilia drives adaptation of the mechanoelectrical transducer current of mammalian cochlear hair cells**. *Proc Natl Acad Sci U S A* 2014, **111**:14918-14923.

50. Michalski N, Michel V, Caberlotto E, Lefevre GM, van Aken AF, Tinevez JY, Bizard E, Houbron C, Weil D, Hardelin JP *et al.*: **Harmonin-b, an actin-binding scaffold protein, is involved in the adaptation of mechanoelectrical transduction by sensory hair cells**. *Pflugers Arch* 2009, **459**:115-130.

51. Kros CJ, Marcotti W, van Netten SM, Self TJ, Libby RT, Brown SD, Richardson GP, Steel KP: **Reduced climbing and increased slipping adaptation in cochlear hair cells of mice with Myo7a mutations**. *Nat Neurosci* 2002, **5**:41-47.

52. •• Marcotti W, Corns LF, Desmonds T, Kirkwood NK, Richardson GP, Kros CJ: **Transduction without tip links in cochlear hair cells is mediated by ion channels with permeation properties distinct from those of the mechano-electrical transducer channel**. *J Neurosci* 2014, **34**:5505-5514.

The authors characterize the properties of a mechanically evoked current in hair cells that is observed after breakage of tip links. This current is activated when hair bundles are deflected in the opposite from normal direction. The authors demonstrate that the current shares some but not all properties with the normal polarity current.

53. Fettiplace R, Kim KX: **The physiology of mechanoelectrical transduction channels in hearing**. *Physiol Rev* 2014, **94**:951-986.

54. •• Beurg M, Xiong W, Zhao B, Müller U, Fettiplace R: **Subunit determination of the conductance of hair-cell mechanotransducer channels**. *Proc Natl Acad Sci U S A* 2015, **112**:1589-1594.

The authors demonstrate that in the absence of LHFPL5, the tonotopic gradient in the conductance properties of the mechanotransduction channel is severely blunted. A similar phenotype is observed in Tmc1-deficient hair cells. Intriguingly, TMC1 no longer localizes to stereocilia of *Lhfpl5*-deficient hair cells, providing evidence that LHFPL5 and TMC1 act in a common molecular complex to regulate transduction.

55. •• Maeda R, Kindt KS, Mo W, Morgan CP, Erickson T, Zhao H, Clemens-Grisham R, Barr-Gillespie PG, Nicolson T: **Tip-link protein protocadherin 15 interacts with transmembrane channel-like proteins TMC1 and TMC2**. *Proc Natl Acad Sci U S A* 2014, **111**:12907-12912.

The authors provide biochemical evidence that TMC1 and TMC2 proteins can interact with PCDH15. Expression of a protein fragment that is predicted to disrupt interactions between PCDH15 and TMC affects transduction in zebrafish, suggesting that interactions of TMC with the tip link are important for transduction.

56. Kurima K, Peters LM, Yang Y, Riazuddin S, Ahmed ZM, Naz S, Arnaud D, Drury S, Mo J, Makishima T *et al.*: **Dominant and recessive deafness caused by mutations of a novel gene, TMC1, required for cochlear hair-cell function**. *Nat Genet* 2002, **30**:277-284.

57. Vreugde S, Erven A, Kros CJ, Marcotti W, Fuchs H, Kurima K, Wilcox ER, Friedman TB, Griffith AJ, Balling R *et al.*: **Beethoven, a mouse model for dominant, progressive hearing loss DFNA36**. *Nat Genet* 2002, **30**:257-258.

58. Santos RL, Wajid M, Khan MN, McArthur N, Pham TL, Bhatti A, Lee K, Irshad S, Mir A, Yan K *et al.*: **Novel sequence variants in the TMC1 gene in Pakistani families with autosomal recessive hearing impairment**. *Hum Mutat* 2005, **26**:396.

59. Tlili A, Rebeh IB, Aifa-Hmani M, Dhouib H, Moalla J, Tlili-Chouchene J, Said MB, Lahmar I, Benzina Z, Charfedine I *et al.*: **TMC1 but not TMC2 is responsible for autosomal recessive nonsyndromic hearing impairment in Tunisian families**. *Audiol Neurootol* 2008, **13**:213-218.

60. Beurg M, Evans MG, Hackney CM, Fettiplace R: **A large-conductance calcium-selective mechanotransducer channel in mammalian cochlear hair cells**. *J Neurosci* 2006, **26**:10992-11000.

61. Chatzigeorgiou M, Bang S, Hwang SW, Schafer WR: **tmc-1 encodes a sodium-sensitive channel required for salt chemosensation in *C. elegans***. *Nature* 2013, **494**:95-99.

62. Jackson AC, Nicoll RA: **The expanding social network of ionotropic glutamate receptors: TARPs and other transmembrane auxiliary subunits**. *Neuron* 2011, **70**:178-199.

63. Yanez-Mo M, Barreiro O, Gordon-Alonso M, Sala-Valdes M, Sanchez-Madrid F: **Tetraspanin-enriched microdomains: a functional unit in cell plasma membranes**. *Trends Cell Biol* 2009, **19**:434-446.

64. Longo-Guess CM, Gagnon LH, Cook SA, Wu J, Zheng QY, Johnson KR: **A missense mutation in the previously undescribed gene Tmhs underlies deafness in hurry-scurry (hscy) mice**. *Proc Natl Acad Sci U S A* 2005, **102**:7894-7899.

65. Naz S, Giguere CM, Kohrman DC, Mitchem KL, Riazuddin S, Morell RJ, Ramesh A, Srisailpathy S, Deshmukh D, Riazuddin S *et al.*: **Mutations in a novel gene, TMIE, are associated with hearing loss linked to the DFNB6 locus**. *Am J Hum Genet* 2002, **71**:632-636.

66. Mitchem KL, Hibbard E, Beyer LA, Bosom K, Dootz GA, Dolan DF, Johnson KR, Raphael Y, Kohrman DC: **Mutation of the novel gene Tmie results in sensory cell defects in the inner ear of spinner, a mouse model of human hearing loss DFNB6**. *Hum Mol Genet* 2002, **11**:1887-1898.

67. Gleason MR, Nagiel A, Jamet S, Vologodskaia M, Lopez-Schier H, Hudspeth AJ: **The transmembrane inner ear (Tmie) protein is essential for normal hearing and balance in the zebrafish**. *Proc Natl Acad Sci U S A* 2009, **106**:21347-21352.

68. Pepermans E, Michel V, Goodyear R, Bonnet C, Abdi S, Dupont T, Gherbi S, Holder M, Makrelouf M, Hardelin JP *et al.*: **The CD2 isoform of protocadherin-15 is an essential component of the tip-link complex in mature auditory hair cells**. *EMBO Mol Med* 2014, **6**:984-992.

69. Delmas P, Coste B: **Mechano-gated ion channels in sensory systems**. *Cell* 2013, **155**:278-284.