Biophysical Journal
Biophysical Perspective

Biophysical Society

Is TMC1 the Hair Cell Mechanotransducer Channel?

Robert Fettiplace[1,*]
[1]Department of Neuroscience, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin

ABSTRACT Transmembrane channel-like protein isoform-1 (TMC1) has emerged over the past five years as a prime contender for the mechano-electrical transducer (MET) channel in hair cells of the inner ear. TMC1 is thought to have a six-transmembrane domain structure reminiscent of some other ion-channel subunits, and is targeted to the tips of the stereocilia in the sensory hair bundle, where the MET channel is located. Moreover, there are *TMC1* mutations linked to human deafness causing loss of conventional MET currents, hair cell degeneration, and deafness in mice. Finally, mutations of *Tmc1* can alter the conductance and Ca^{2+} selectivity of the MET channels. For several reasons though, it is unclear that TMC1 is indeed the MET channel pore: 1) in other animals or tissues, mutations of TMC family members do not directly affect cellular mechanosensitivity; 2) there are residual manifestations of mechanosensitivity in hair cells of mouse *Tmc1:Tmc2* double knockouts; 3) there is so far no evidence that expression of mammalian *Tmc1* generates a mechanically sensitive ion channel in the plasma membrane when expressed in heterologous cells; and 4) there are other proteins, such as TMIE and LHFPL5, which behave similarly to TMC1, their mutation also leading to loss of MET current and deafness. This review will present these disparate lines of evidence and describes recent work that addresses the role of TMC1.

Ion channels sensitive to mechanical deformation of the cell membrane are widely distributed in vertebrates and are central to the function of specialized mechanoreceptors, such as those in the sensory neurons of the skin or the inner ear. The molecular structure of these mechanically gated channels has been the subject of much research and speculation (1,2). Although considerable progress was achieved by identifying PIEZO2 as a mechanosensitive channel underlying transduction in mammalian touch receptors (2,3), the composition of the mechano-electrical transducer (MET) channel in hair cells of the inner ear is still unclear (4,5). Transduction in auditory hair cells is distinguished by sensitivity to submicron displacements of the mechanosensory hair bundle composed of multiple rows of stereocilia in a staircase projecting from the top of the cell (Fig. 1 *A*). MET channels are opened by deflection of the bundle toward its taller edge, so increasing tension in extracellular tip links bridging adjacent stereocilia, and thereby activating channels at the lower ends of the links (4). Many proteins comprising the tip links and their attachments (Fig. 1 *B*) have been discovered by the effects of specific mutations, such as those arising in Usher syndrome (6), but the identity of the channel is still controversial. In cochlear hair cells, the MET channel is cation selective with high permeability for Ca^{2+} (4,7). Its most notable biophysical properties are its large single-channel conductance of 100 pS or more (4,8), and ultrafast activation kinetics (9,10), probably in the microsecond range (11). Such fast activation is essential for faithfully encoding sound stimuli at high frequencies that in some mammals extend up to 100 kHz.

The identity of the hair cell MET channel has been pursued for more than 20 years, with several candidates, including analogs of the epithelial Na^{+} channel and multiple transient receptor potential (TRP) channels, proposed and rejected (4,12). Most recently, a protein family of previously unknown function, the transmembrane channel-like (TMC) proteins (13,14), was put forward based on loss of the MET current and deafness by null mutations in *Tmc1* (15,16). TMC1 is one product of a family of eight genes encoding transmembrane proteins that are classified into three subfamilies: TMC1, TMC2, and TMC 3; TMC5 and TMC 6; and TMC4, TMC 7, and TMC 8 (14,17). TMC1 is highly expressed and TMC4 moderately expressed in adult cochlear hair cells, whereas TMC2 is expressed during early neonatal development (15,18,19). The effects of *Tmc1* mutations on MET function will be described and evidence supporting TMC1 as a pore-forming module of the transduction channel will be assessed.

Mutations in TMC1 and TMC2

The small number of hair cells per cochlea (mouse <5,000) and the expected complement of MET channels per cell

Submitted April 12, 2016, and accepted for publication May 19, 2016.

*Correspondence: fettiplace@wisc.edu

Editor: Brian Salzberg

http://dx.doi.org/10.1016/j.bpj.2016.05.032

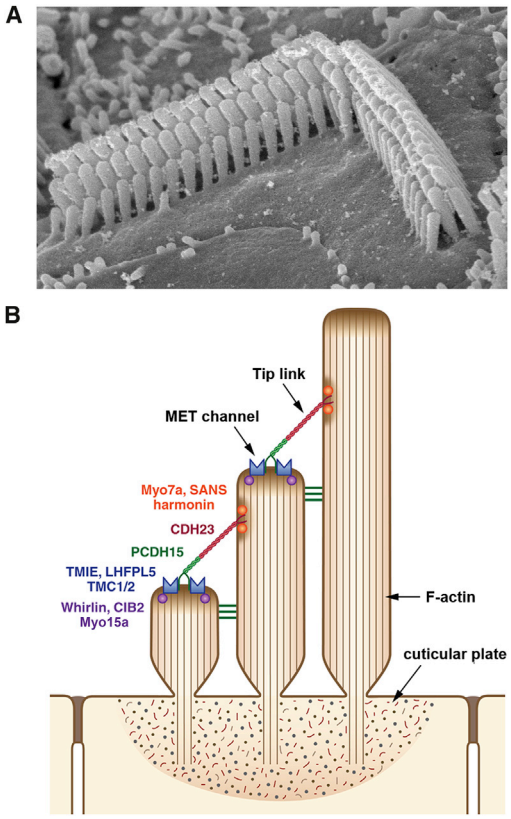

FIGURE 1 The hair cell mechano-transduction apparatus. (*A*) Scanning electron micrograph of the V-shaped hair bundle of an OHC depicts three rows of stereocilia stepping in height across the bundle from 2 to 4 μm (4). (*B*) Some key molecular components of the transduction apparatus associated with the tip link through which force is applied to the MET channel. Tip links comprise homodimers of cadherin 23 (CDH23) at the upper end and protocadherin 15 (PCDH15) at the lower end. CDH23 is anchored to the stereociliary actin cytoskeleton by the Usher protein complex Myo7a, SANS, and harmonin-b (56). PCDH15 interacts with the MET channel via putative channel components LHFPL5 (50), TMIE (51), and TMC1 (53,54). The MET channel may be connected to the actin cytoskeleton by Usher proteins whirlin and CIB2 (57) and Myo15a.

(~100) have precluded biochemical approaches that were successfully used to isolate components of the transduction machinery in other sensory systems such as photoreceptors. Isolating the hair-cell transduction complex has largely relied on the analysis of mutations underlying various types of hereditary deafness (http://hereditaryhearingloss.org). The most severe form of Usher syndrome (type 1) has revealed many components of the transduction complex (6), but the MET channel was not among them. TMC1 is noteworthy in that there are more than 35 mutations causing human hereditary hearing loss (autosomal dominant deafness DFNA36 and recessive deafness DFNB7/11) (16), making it, in terms of the number of different mutations, one of the most common deafness proteins. There are equivalent TMC1 mutations in mice (20), two of which are the recessive mutation *deafness* (*dn*) (13,21) and the dominant mutation *Beethoven* (*Bth*) (22); these cause profound deafness at birth and slower progressive hearing loss, respectively, and therefore have effects comparable with DFNB7/11 and DFNA36 in humans. Despite an early claim that the hair cell MET current was unaffected in *Tmc1*$^{dn/dn}$ mice (23), subsequent work showed that *Tmc1* knockouts are associated with abolition of the MET current in both outer hair cells (OHCs) and inner hair cells (IHCs), but this loss of current is not complete until the second postnatal week (15,24). A significant MET current in *Tmc1* knockouts at an earlier age has been attributed to the additional presence of TMC2 that was proposed to compensate for lack of TMC1 (15). *Tmc2* is down-regulated in the cochlea after postnatal day (P)6 (15) and TMC2 protein disappears from the stereocilia by P10 (25) and, consequently, double knockouts of *Tmc1* and *Tmc2* cause complete loss of the normal MET current even in early neonates (15,24). In *Tmc2* nulls, there is no hereditary deafness or defective transduction in the cochlea, but there is reduction in the MET current of type II hair cells of the utricle and resulting vestibular defects (15), thus arguing for the persistence of TMC2 in adult vestibular hair cells. Loss of utricular hair cell transduction in *Tmc2* nulls was not mitigated by the presence of TMC1.

More detailed investigation of *Tmc* mutants indicated that although the normal MET current is absent in *Tmc1:Tmc2* double mutants, it is replaced by an atypical mechanically sensitive current evoked by reversed-polarity displacement of the hair bundle (26,27); this current is activated by deflections of the bundle toward its shorter edge. The connection between the normal and reverse-polarity MET currents is presently not understood, nor is it clear that they are manifestations of the same mechanosensitive channel. The reverse-polarity currents appear under conditions where normal transduction is lost, such as in mutations of the tip-link proteins CDH23 and PCDH15 (28), and after tip link destruction with 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) (26,27,29). Reverse-polarity currents have been reported in some other mutants (*Myo15* $^{Sh2/Sh2}$; (30)), and also during embryonic and early postnatal development before the appearance of the normal MET current (26,31). One conclusion might be that the reverse-polarity current reflects residual mechanosensitivity, indicating that TMC1 is not the MET channel. The channels underlying the reverse-polarity current are thought to be located on the hair-cell apical surface, where they are manifested as stretch-sensitive channels gated by deformation of the plasma membrane overlying the hair bundle or the cell apex around the base of the bundle (31). Use of the "reverse-polarity" epithet is therefore inaccurate, and they should be more properly referred to as unconventional MET channels. In assessing the significance of this phenomenon, concern was raised that the *Tmc1*$^{dn/dn}$ mutants are not true nulls and that some TMC1 function persists to account for the residual mechanosensitive current; the *deafness* mutant results from deletion of exon 14 and flanking

intronic sequences, but mRNA might still be made by splicing exon 13 sequence in frame to exon 15 sequence. *Tmc1* and *Tmc2* knockouts were originally achieved (15) by exon replacements with green fluorescent protein (GFP), and were subsequently referred to as $Tmc1^{\Delta/\Delta}$ and $Tmc2^{\Delta/\Delta}$. However, reverse-polarity currents could be recorded when *Tmc1:Tmc2* double knockouts were created with $Tmc1^{\Delta/\Delta}$ rather than $Tmc1^{dn/dn}$ (27) suggesting that the unconventional MET current was not attributable to expression of a modified isoform of TMC1.

Evidence for TMC1 as a channel

When investigating a novel membrane protein, a convincing argument for its role as a prospective ion channel is that its mutation influences ion fluxes across the membrane. For example, with proteins of unconventional structure, such as the mechanoreceptor, PIEZO1, mutations of a crucial glutamate residue can alter the Ca^{2+} selectivity and unitary conductance of the channel (32,33). Such observations strengthen the case for it being an ion channel, and identify a likely pore region. Mouse TMC1 is composed of 757 amino-acid residues and does not resemble other recognized ion channels. The topology of the protein was studied by introducing Hemagglutinin (HA) tags at seven places along the molecule followed by heterologous expression of the construct. The expressed TMC1 was confined to the endoplasmic reticulum, but determining the HA epitope accessibility indicated six transmembrane domains, cytoplasmic N- and C termini, and a long intracellular loop between transmembrane domains S4 and S5 (Fig. 2 *A*; (34)). In several respects, TMC1 resembles a K^+ channel α-subunit in its transmembrane arrangement, but the site of the pore is not established. In $Tmc1^{dn/dn}$, profound deafness results from a 1656 base-pair deletion encoding amino-acid residues 463–519 in this intracellular loop, which suggests this region may be functionally important.

An obstacle to systematic study of TMC1, as compared with PIEZO1, is that it has not so far been possible to express it in heterologous cell lines and have the protein trafficked to the plasma membrane (but see (35)). In contrast, GFP-tagged TMC1 is targeted to the hair-cell plasma membrane, localizing near the tips of the stereocilia (25), where the MET channels are thought to reside (36). If plasma-membrane targeting were achievable in cell lines, it might be possible to record whole-cell currents in response to mechanical indentation of the cell, thus demonstrating it to be a mechanically sensitive ion channel as was done with PIEZO1 (37) and no mechanoreceptor potential C (NOMPC) (38). Nevertheless, investigation of the MET channel in both IHCs and OHCs demonstrates that knockout or mutations of *Tmc1* or *Tmc2* affect the single-channel conductance and Ca^{2+} selectivity (24,26,27,39). The results of *Tmc1* knockout on MET-channel conductance are clearest, revealing a reduction from wild-type to mutant of 63 to 49 pS at the cochlear apex and 102 to 60 pS at the base in OHCs. Consequently, the well-established increase in OHC channel conductance along the tonotopic axis (4) was virtually abolished (26,27). In contrast, *Tmc2*

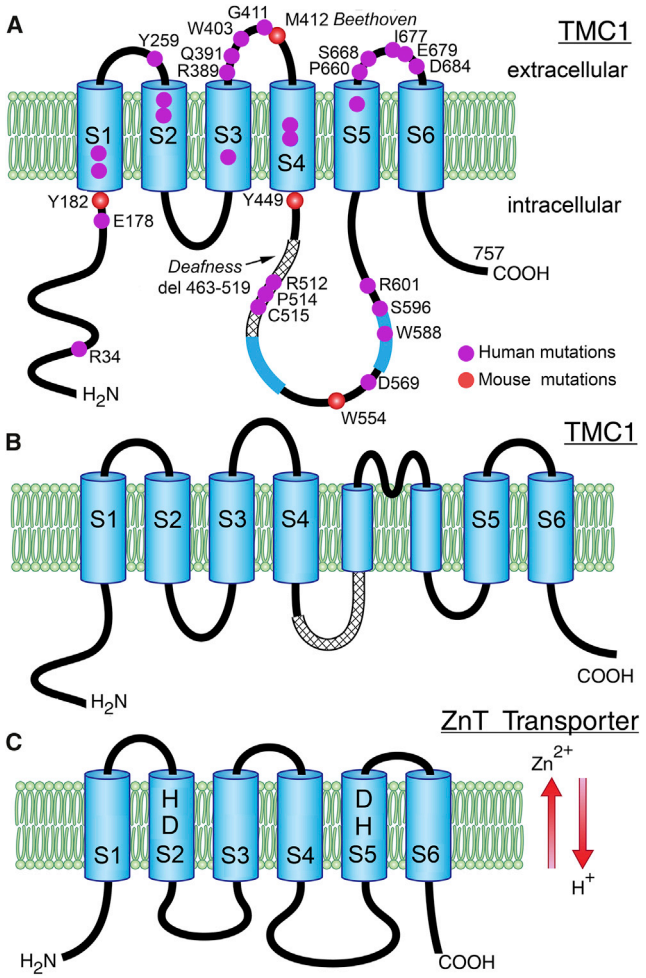

FIGURE 2 Structure of TMC1. (*A*) Putative transmembrane configuration based on tags and hydropathy plots indicate six transmembrane domains (S1 to S6), with intracellular N- and C termini, and a long intracellular loop between domains S4 and S5 (34). Point mutations causing progressive deafness in mice including *Beethoven* are denoted by red circles (20), and the hatched segment indicates amino acids 463–519 deleted in *Tmc1 deafness* (13), with numbering of residues referring to the mouse sequence. Point mutations causing deafness in humans are indicated by pink circles (16,58–60), with residue numbering referring to the human sequence, which differs by a few residues from mouse; only those mutations causing amino-acid substitutions rather than truncations are noted. The mutated residues in the transmembrane domains are S1, G197, and N199; S2, P274, and Y277; S3 and V372; S4, G444, and R445; S5 and M654. The putative intracellular loop between S4 and S5 also includes two other potential transmembrane domains shown in blue (34). (*B*) Alternative structure is shown that incorporates the two additional transmembrane regions with connecting section forming a reentrant loop and pore that will include mutation sites W554 and D569. N-terminus shortened to save space. (*C*) A subunit of the dimeric ZnT-1 zinc transporter is shown that displays six transmembrane domains like some ion channels; the transporting region is built from S2 and S5, which are adjacent in the three-dimensional structure, with each containing crucial histidine and aspartate residues (49).

knockouts gave no significant reduction in OHC single MET-channel conductance at either apex or base (26). *Tmc1* knockouts were reported to reduce single MET-channel conductance in IHCs too (39), though the conclusion was obscured by the wild-type displaying an unexplained range of conductance levels that may reflect the superposition of multiple TMC channel events (27).

The wild-type MET channel is a cation channel with high selectivity for Ca^{2+}, which is several times more permeant than monovalent cations Na^+, K^+, or Cs^+ (7,8). Owing to the developmental changes in Ca^{2+} selectivity occurring during the first two neonatal weeks (24), the effects of knockouts on this channel property have been less easy to interpret. A reduction in Ca^{2+} selectivity has been reported in *Tmc2* knockouts for both apical OHCs and IHCs (24,39), though little or no change was seen in *Tmc1* knockouts (24,27). This is puzzling since *Tmc1* knockouts cause a change in channel conductance but not in Ca^{2+} selectivity, whereas with *Tmc2* knockouts the reverse is true, with a reduction in Ca^{2+} selectivity but not in channel conductance. Thus, although some changes in MET channel unitary conductance and Ca^{2+} selectivity are reported with *Tmc* knockouts, the results are neither straightforward nor consistent. One hypothesis on the contributions of TMC1 and TMC2 to the Ca^{2+} permeability of the MET channel is as follows. In neonates younger than P6, the Ca^{2+} permeability of the channel relative to Cs^+ (P_{Ca}/P_{Cs}) of apical OHCs is greater than the P_{Ca}/P_{Cs} in basal OHCs, but declines during the second week to be identical to that of the base (~4.6; (24)). If the change is due to loss of TMC2, this result suggests that channels containing TMC2 have larger P_{Ca}/P_{Cs} compared with those with TMC1; consistent with this notion, TMC1 knockout increases P_{Ca}/P_{Cs} at the base, indicating for a limited time TMC2 can compensate. In contrast, *Tmc1* knockout has little effect at the apex, perhaps because in the first postnatal week, the apical OHC MET channel is composed mainly of TMC2. Despite these effects, one should be cautious in concluding that the results of *Tmc* mutations on P_{Ca}/P_{Cs} imply that the TMC proteins are part of the pore. A useful comparison is the CRAC channel, composed of Orai1 and activated by STIM1 (40). Although acidic residues (E106) in the Orai1 contribute to the Ca^{2+} selectivity of the channel, the selectivity can under some circumstances be modulated by binding of the activator STIM1 (40), indicating the gating process can influence ionic selectivity.

Further insights have been derived from study of *Beethoven*, an allele with a point mutation in *Tmc1* causing a methionine to lysine substitution at residue 412 (M412K; Fig. 2 *A*). This is a dominant mutation, and $Tmc1^{Bth/+}$ heterozygous mice display progressive hearing loss associated with postnatal hair cell degeneration. In contrast, $Tmc1^{Bth/Bth}$ homozygous mice are profoundly deaf at birth (22). There is consensus that the *Beethoven* mutation results in a reduction in Ca^{2+} selectivity of the MET channel (39,41,42). However, the reported effect on single-channel conductance differed between IHCs and OHCs, with a reduction in IHCs (39) but no effect on either apical or basal OHCs (41). There is also uncertainty over the location of the M412K mutation in the ion conduction pathway. In one case, the mutation is depicted from the putative channel structure as being in the extracellular loop between the S3 and S4 domains (16,34) (Fig. 2); in the other case, based on the differential intracellular or extracellular block of the channel by the antibiotic dihydrostreptomycin, the mutated residue is posited to reside on the internal face of the pore (42). These different conclusions might be partially reconciled if the S3/S4 linker were to form the wall of an external vestibule of the pore. However, the point mutation is unlike that employed in establishing function in other ion channels, for which a glutamate, providing negative charge in the pore to promote cation fluxes, is neutralized (32,38,40). For *Beethoven*, a neutral residue is replaced by a positively charged one. If the MET channel contains a vestibule at the external face of the pore (8,27), perhaps formed by the S3/S4 loop, introduction of a positively charged lysine within the vestibule may repel Ca^{2+} ions thus decreasing their local concentration and diminishing Ca^{2+} permeability.

Other clues to the structure are the sites of the *TMC1* mutations conferring deleterious effects on hearing. The human mutations (Fig. 2 *A*) are distributed throughout the protein, but the majority occurs in the central portion of the protein. The mutated residues are concentrated in the putative extracellular loops S3/S4 and S5/S6, and in the large intracellular region connecting S4 and S5 (16,34). It might be speculated that the two extracellular domains line the vestibule of the MET channel. The intracellular region includes the extended sequence that is deleted in the mouse deafness mutation. This region also contains two sequences predicted at lower probability to be transmembrane domains (34), in which case the segment linking these two candidate transmembrane domains might form a reentrant loop that acts as the MET channel pore (Fig. 2 *B*). Such a hypothesis for the TMC1 protein structure might account for the large number of lethal point mutations over the S4/S5 region, which would most likely affect ion conduction through the MET channel.

TMC1 in other cell types

Is there any evidence for mechanoreceptor function in other members of the TMC family? Although the *Tmc* gene name implies that these proteins are ion channels, there is no direct evidence for their having channel activity and their molecular function remains unknown. Neither of the vertebrate TMC1 or TMC2 isoforms has been shown to confer channel activity or a mechanical response in a heterologous expression system. In the nematode worm, *Caenorhabditis elegans*, which possesses two tmc genes, one of the gene

products, TMC-1, was reported to be required for Na^+ chemosensation in ASH neurons, and to behave as a Na^+ sensor in the worm olfactory neurons and in cultured mammalian cells (35). Thus it may make the ASH sensory neurons detect stressful levels of external Na^+ and trigger an aversive behavioral reflex. However, these observations have not been repeated. Other work (43) has implied that the function of TMC-1 is more complex and involved in metabolism. Slow development and poor reproduction in *C. elegans* is evoked by nutritional stress or restriction of optimal food, and it has been hypothesized that the worm uses TMC-1 as part of its stress-sensing system to delay the rate of development and reproduction until the animal finds the best food. Consistent with this notion, *tmc-1* mutation promotes growth and reproductive success. Experiments showed that, in response to nutritional stress, TMC-1 enhanced the excitability of some motoneurons and body-wall musculature. The increased excitability might occur via Na^+ influx, which would be consistent with experiments demonstrating TMC-1–mediated Na^+ conduction. However, raising environmental Na^+ had no obvious effect on development for either wild-type or *tmc-1* mutants (43). These findings argue that TMC-1 might have other functions to regulate the cellular physiology, in addition to aversive environmental Na^+ sensation. There is no evidence that TMC-1 can operate as a mechanoreceptor in *C. elegans*, and indeed there are a number of other membrane proteins that fulfill this role, including TRP-4, OSM-9, and DEG/ENaC channels (44).

Two members of the *TMC* gene family have a recognized role in humans. Mutations in *TMC6* or *TMC8* genes (also known as *EVER1* and *EVER2*, respectively) cause epidermodysplasia verruciformis, a rare autosomal recessive disorder characterized by abnormal susceptibility to certain human cutaneous papillomaviruses (HPVs) and risk of skin cancer (14,45). Uncontrolled HPV infections result in the growth of scaly macules and plaques, especially on the hands and feet, from which it is known colloquially as tree man illness. The precise function of the *TMC6* and *TMC8* gene-products are not fully understood, but one hypothesis is that they play a role in regulating the distribution of Zn^{2+} among cytoplasmic organelles. TMC6 and TMC8 are localized to the endoplasmic reticulum membrane (45,46), and, based on yeast two-hybrid screening and co-immunoprecipitation experiments, these proteins are thought to interact with the zinc transporter, ZnT-1. The TMC proteins promote Zn^{2+} influx into the endoplasmic reticulum in keratinocytes, and regulate Zn^{2+} homeostasis in lymphocytes (46,47). TMC6 and TMC8 might either modulate the activity of ZnT-1, or serve as Zn^{2+} transporters themselves. Zn^{2+} is a necessary cofactor for many viral proteins, and the activity of the TMC6/TMC8 complex may restrict the access of viral proteins to cellular zinc stores so limiting their growth (46). It has also been found that TMC8, when expressed in HEK293 cells, is targeted to the endoplasmic reticulum membrane, and there controls receptor-mediated release of Ca^{2+} and Zn^{2+} into the cytoplasm following ATP stimulation (48). All three proteins, TMC6, TMC8, and ZnT-1, are of comparable size (TMC6, 810 amino-acid residues; TMC8, 723 residues; and ZnT1, 503 residues) and, with six transmembrane domains (49), the ZnT-1 is reminiscent of the structure of an ion channel subunit (Fig. 2). Is it possible that ZnT-1, which functions as dimer (49), in association with TMC6 and TMC8 forms a tetrameric exchanger or channel complex in the endoplasmic reticulum membrane? If a similar structural arrangement were to occur in the stereocilia, could two TMC1 molecules create a channel complex along with a pair of other, as yet unknown, channel proteins?

TMHS and TMIE, other proteins of the hair-cell transduction complex

Besides TMC1 and TMC2, two other deafness proteins, LHFPL5 and TMIE, have been identified as resulting in loss of mechanotransduction (50–52). Both LHFPL5 and TMIE are localized to the tips of the stereocilia near the transduction machinery, and have been shown to interact with the N-terminus of PCDH15 at the lower end of the tip link (51,53), just as TMC1 does (53,54). Compared with ion channel subunits that generate multimeric channels, such as voltage-dependent K^+ channels with ~500 residues, LHFPL5 and TMIE are small proteins, 219 and 153 amino acid residues, respectively, and their size casts doubt on them being pore-forming subunits of an ion channel. The larger LHFPL5 (originally known as TMHS for transmembrane protein of hair cell stereocilia) has four transmembrane domains with properties analogous to the structurally unrelated tetraspan TARP proteins like stargazin. TARP proteins regulate trafficking of the pore-forming subunits of AMPA receptors to the postsynaptic membrane and also influence channel conductance (55). Similarly, LHFPL5 may control the properties of the MET channel complex and its steering to the stereociliary tips (50). LHFPL5 is needed for appropriate targeting of the TMC1 to the hair bundles and the results of *Lhfpl5* mutations may at least partly reflect the absence of TMC1 in the hair bundle. Thus immunolabeling for TMC1 showed its presence in OHC hair bundles of wild-type mice but not in bundles from $Lhfpl5^{-/-}$ (53). Hair cells of zebrafish deficient in TMIE exhibit no microphonic potentials or uptake of the fluorescent dye FM1-43, both effects attesting to loss of mechanotransduction (52), and mice with *Tmie* mutations are deaf (51). Co-immunoprecipitation experiments suggest TMIE interacts with LHFPL5 and with the naturally occurring CD2 isoform of PCDH15, and TMIE and LHFPL5 have been proposed to part of the protein complex coupling the tip link to the MET channel (51). In both *Tmie* and *Lhfpl5* knockout mice, in which the normal MET current is lost, an unconventional (reverse-polarity) current can still be

recorded (51,53), indicating that this unconventional current requires neither TMIE nor LHFPL5. Unfortunately, these results provide no information about whether LHFPL5 or TMIE contribute to the MET channel pore.

CONCLUSIONS

TMC1 is likely to be intimately associated with the MET channel complex because its knockout diminishes channel conductance and the tonotopic gradient thereof; this conclusion is also supported by reduction in ionic (Ca^{2+}) selectivity in several TMC mutants. However, these effects do not unequivocally argue for TMC1 forming the pore. Additional evidence on the contributions of TMC6 and TMC8 to epidermodysplasia verruciformis, and predisposition to HPV, raise the tantalizing possibility that the TMC paralogs may form an ion channel by associating with transporters to influence the cell balance of ions such as Zn^{2+} and Ca^{2+}. It seems reasonable to conclude that TMC1 is one component of a MET channel complex, which may also include LHFPL5, TMIE, and probably other as yet unidentified proteins. The inability to express TMC1 in a heterologous cell line and have it trafficked to the plasma membrane poses a major hurdle in definitively assigning function to the protein. Nevertheless, localization of tagged TMC proteins to the stereociliary tips in mice expressing TMC1-mCherry or TMC2-AcGHFP confirms that they are capable of being targeted to the plasma membrane (25). Further progress will require either demonstration of a TMC1-based mechanosensitive channel in a heterologous expression system, or discovery of new MET channel proteins in stereocilia.

ACKNOWLEDGMENTS

Thanks to Maryline Beurg for much advice, and to the reviewers for their helpful comments.

Work in the author's lab supported by grant RO1 DC01362 from the National Institutes of Deafness and other Communication Disorders.

REFERENCES

1. Arnadóttir, J., and M. Chalfie. 2010. Eukaryotic mechanosensitive channels. *Annu. Rev. Biophys.* 39:111–137.
2. Ranade, S. S., R. Syeda, and A. Patapoutian. 2015. Mechanically activated ion channels. *Neuron.* 87:1162–1179.
3. Coste, B., J. Mathur, …, A. Patapoutian. 2010. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science.* 330:55–60.
4. Fettiplace, R., and K. X. Kim. 2014. The physiology of mechanoelectrical transduction channels in hearing. *Physiol. Rev.* 94:951–986.
5. Zhao, B., and U. Müller. 2015. The elusive mechanotransduction machinery of hair cells. *Curr. Opin. Neurobiol.* 34:172–179.
6. Richardson, G. P., J. B. de Monvel, and C. Petit. 2011. How the genetics of deafness illuminates auditory physiology. *Annu. Rev. Physiol.* 73:311–334.
7. Ohmori, H. 1985. Mechano-electrical transduction currents in isolated vestibular hair cells of the chick. *J. Physiol.* 359:189–217.
8. Beurg, M., M. G. Evans, …, R. Fettiplace. 2006. A large-conductance calcium-selective mechanotransducer channel in mammalian cochlear hair cells. *J. Neurosci.* 26:10992–11000.
9. Corey, D. P., and A. J. Hudspeth. 1983. Kinetics of the receptor current in bullfrog saccular hair cells. *J. Neurosci.* 3:962–976.
10. Ricci, A. J., H. J. Kennedy, …, R. Fettiplace. 2005. The transduction channel filter in auditory hair cells. *J. Neurosci.* 25:7831–7839.
11. Doll, J. C., A. W. Peng, …, B. L. Pruitt. 2012. Faster than the speed of hearing: nanomechanical force probes enable the electromechanical observation of cochlear hair cells. *Nano Lett.* 12:6107–6111.
12. Corey, D. P. 2006. What is the hair cell transduction channel? *J. Physiol.* 576:23–28.
13. Kurima, K., L. M. Peters, …, A. J. Griffith. 2002. Dominant and recessive deafness caused by mutations of a novel gene, TMC1, required for cochlear hair-cell function. *Nat. Genet.* 30:277–284.
14. Kurima, K., Y. Yang, …, A. J. Griffith. 2003. Characterization of the transmembrane channel-like (TMC) gene family: functional clues from hearing loss and epidermodysplasia verruciformis. *Genomics.* 82:300–308.
15. Kawashima, Y., G. S. G. Géléoc, …, A. J. Griffith. 2011. Mechanotransduction in mouse inner ear hair cells requires transmembrane channel-like genes. *J. Clin. Invest.* 121:4796–4809.
16. Kawashima, Y., K. Kurima, …, J. R. Holt. 2015. Transmembrane channel-like (TMC) genes are required for auditory and vestibular mechanosensation. *Pflugers Arch.* 467:85–94.
17. Keresztes, G., H. Mutai, and S. Heller. 2003. TMC and EVER genes belong to a larger novel family, the TMC gene family encoding transmembrane proteins. *BMC Genomics.* 4:24.
18. Liu, H., J. L. Pecka, …, D. Z. Z. He. 2014. Characterization of transcriptomes of cochlear inner and outer hair cells. *J. Neurosci.* 34:11085–11095.
19. Scheffer, D. I., J. Shen, …, Z.-Y. Chen. 2015. Gene expression by mouse inner ear hair cells during development. *J. Neurosci.* 35:6366–6380.
20. Manji, S. S. M., K. A. Miller, …, H.-H. M. Dahl. 2012. Identification of three novel hearing loss mouse strains with mutations in the Tmc1 gene. *Am. J. Pathol.* 180:1560–1569.
21. Steel, K. P., and G. R. Bock. 1980. The nature of inherited deafness in deafness mice. *Nature.* 288:159–161.
22. Vreugde, S., A. Erven, …, K. P. Steel. 2002. Beethoven, a mouse model for dominant, progressive hearing loss DFNA36. *Nat. Genet.* 30:257–258.
23. Marcotti, W., A. Erven, …, C. J. Kros. 2006. Tmc1 is necessary for normal functional maturation and survival of inner and outer hair cells in the mouse cochlea. *J. Physiol.* 574:677–698.
24. Kim, K. X., and R. Fettiplace. 2013. Developmental changes in the cochlear hair cell mechanotransducer channel and their regulation by transmembrane channel-like proteins. *J. Gen. Physiol.* 141:141–148.
25. Kurima, K., S. Ebrahim, …, B. Kachar. 2015. TMC1 and TMC2 localize at the site of mechanotransduction in mammalian inner ear hair cell stereocilia. *Cell Reports.* 12:1606–1617.
26. Kim, K. X., M. Beurg, …, R. Fettiplace. 2013. The role of transmembrane channel-like proteins in the operation of hair cell mechanotransducer channels. *J. Gen. Physiol.* 142:493–505.
27. Beurg, M., K. X. Kim, and R. Fettiplace. 2014. Conductance and block of hair-cell mechanotransducer channels in transmembrane channel-like protein mutants. *J. Gen. Physiol.* 144:55–69.
28. Alagramam, K. N., R. J. Goodyear, …, G. P. Richardson. 2011. Mutations in protocadherin 15 and cadherin 23 affect tip links and mechanotransduction in mammalian sensory hair cells. *PLoS One.* 6:e19183.
29. Marcotti, W., L. F. Corns, …, C. J. Kros. 2014. 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.* 34:5505–5514.

30. Stepanyan, R., and G. I. Frolenkov. 2009. Fast adaptation and Ca2+ sensitivity of the mechanotransducer require myosin-XVa in inner but not outer cochlear hair cells. *J. Neurosci.* 29:4023–4034.
31. Beurg, M., A. C. Goldring, …, R. Fettiplace. 2016. Development and localization of reverse-polarity mechanotransducer channels in cochlear hair cells. *Proc. Natl. Acad. Sci. USA.* 113:6767–6772.
32. Coste, B., S. E. Murthy, …, A. Patapoutian. 2015. Piezo1 ion channel pore properties are dictated by C-terminal region. *Nat. Commun.* 6:7223.
33. Zhao, Q., K. Wu, …, B. Xiao. 2016. Ion permeation and mechanotransduction mechanisms of mechanosensitive piezo channels. *Neuron.* 89:1248–1263.
34. Labay, V., R. M. Weichert, …, A. J. Griffith. 2010. Topology of transmembrane channel-like gene 1 protein. *Biochemistry.* 49:8592–8598.
35. Chatzigeorgiou, M., S. Bang, …, W. R. Schafer. 2013. Tmc-1 encodes a sodium-sensitive channel required for salt chemosensation in C. elegans. *Nature.* 494:95–99.
36. Beurg, M., R. Fettiplace, …, A. J. Ricci. 2009. Localization of inner hair cell mechanotransducer channels using high-speed calcium imaging. *Nat. Neurosci.* 12:553–558.
37. Coste, B., B. Xiao, …, A. Patapoutian. 2012. Piezo proteins are pore-forming subunits of mechanically activated channels. *Nature.* 483:176–181.
38. Yan, Z., W. Zhang, …, Y. N. Jan. 2013. Drosophila NOMPC is a mechanotransduction channel subunit for gentle-touch sensation. *Nature.* 493:221–225.
39. Pan, B., G. S. Géléoc, …, J. R. Holt. 2013. TMC1 and TMC2 are components of the mechanotransduction channel in hair cells of the mammalian inner ear. *Neuron.* 79:504–515.
40. Prakriya, M., and R. S. Lewis. 2015. Store-operated calcium channels. *Physiol. Rev.* 95:1383–1436.
41. Beurg, M., A. C. Goldring, and R. Fettiplace. 2015. The effects of Tmc1 Beethoven mutation on mechanotransducer channel function in cochlear hair cells. *J. Gen. Physiol.* 146:233–243.
42. Corns, L. F., S. L. Johnson, …, W. Marcotti. 2016. Tmc1 point mutation affects Ca2+ sensitivity and block by dihydrostreptomycin of the mechanoelectrical transducer current of mouse outer hair cells. *J. Neurosci.* 36:336–349.
43. Zhang, L., D. G. Gualberto, …, L. R. Garcia. 2015. TMC-1 attenuates C. elegans development and sexual behaviour in a chemically defined food environment. *Nat. Commun.* 6:6345.
44. Schafer, W. R. 2015. Mechanosensory molecules and circuits in *C. elegans*. *Pflugers Arch.* 467:39–48.
45. Ramoz, N., L.-A. Rueda, …, M. Favre. 2002. Mutations in two adjacent novel genes are associated with epidermodysplasia verruciformis. *Nat. Genet.* 32:579–581.
46. Lazarczyk, M., C. Pons, …, M. Favre. 2008. Regulation of cellular zinc balance as a potential mechanism of EVER-mediated protection against pathogenesis by cutaneous oncogenic human papillomaviruses. *J. Exp. Med.* 205:35–42.
47. Lazarczyk, M., C. Dalard, …, R. S. Liblau. 2012. EVER proteins, key elements of the natural anti-human papillomavirus barrier, are regulated upon T-cell activation. *PLoS One.* 7:e39995.
48. Sirianant, L., J. Ousingsawat, …, K. Kunzelmann. 2014. TMC8 (EVER2) attenuates intracellular signaling by Zn2+ and Ca2+ and suppresses activation of Cl- currents. *Cell. Signal.* 26:2826–2833.
49. Kambe, T., T. Tsuji, …, N. Itsumura. 2015. The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism. *Physiol. Rev.* 95:749–784.
50. Xiong, W., N. Grillet, …, U. Müller. 2012. TMHS is an integral component of the mechanotransduction machinery of cochlear hair cells. *Cell.* 151:1283–1295.
51. Zhao, B., Z. Wu, …, U. Müller. 2014. TMIE is an essential component of the mechanotransduction machinery of cochlear hair cells. *Neuron.* 84:954–967.
52. Gleason, M. R., A. Nagiel, …, A. J. Hudspeth. 2009. The transmembrane inner ear (Tmie) protein is essential for normal hearing and balance in the zebrafish. *Proc. Natl. Acad. Sci. USA.* 106:21347–21352.
53. Beurg, M., W. Xiong, …, R. Fettiplace. 2015. Subunit determination of the conductance of hair-cell mechanotransducer channels. *Proc. Natl. Acad. Sci. USA.* 112:1589–1594.
54. Maeda, R., K. S. Kindt, …, T. Nicolson. 2014. Tip-link protein protocadherin 15 interacts with transmembrane channel-like proteins TMC1 and TMC2. *Proc. Natl. Acad. Sci. USA.* 111:12907–12912.
55. Jackson, A. C., and R. A. Nicoll. 2011. The expanding social network of ionotropic glutamate receptors: TARPs and other transmembrane auxiliary subunits. *Neuron.* 70:178–199.
56. Grati, M., and B. Kachar. 2011. Myosin VIIa and sans localization at stereocilia upper tip-link density implicates these Usher syndrome proteins in mechanotransduction. *Proc. Natl. Acad. Sci. USA.* 108:11476–11481.
57. Riazuddin, S., I. A. Belyantseva, …, Z. M. Ahmed. 2012. Alterations of the CIB2 calcium- and integrin-binding protein cause Usher syndrome type 1J and nonsyndromic deafness DFNB48. *Nat. Genet.* 44:1265–1271.
58. Atik, T., H. Onay, …, F. Ozkinay. 2015. Comprehensive analysis of deafness genes in families with autosomal recessive nonsyndromic hearing loss. *PLoS One.* 10:e0142154.
59. Imtiaz, A., A. Maqsood, …, S. Naz. 2016. Recessive mutations of TMC1 associated with moderate to severe hearing loss. *Neurogenetics.* 17:115–123.
60. Hu, J., F. Liu, …, Z. Ma. 2016. Exome sequencing identifies a mutation in TMC1 as a novel cause of autosomal recessive nonsyndromic hearing loss. *J. Transl. Med.* 14:29.