



Distinct functions of TMC channels: a comparative overview

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Received: 14 February 2019 / Revised: 25 June 2019 / Accepted: 28 June 2019 / Published online: 4 October 2019
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Abstract

In the past two decades, transmembrane channel-like (TMC) proteins have attracted a significant amount of research interest, because mutations of *Tmc1* lead to hereditary deafness. As evolutionarily conserved membrane proteins, TMC proteins are widely involved in diverse sensorimotor functions of many species, such as hearing, chemosensation, egg laying, and food texture detection. Interestingly, recent structural and physiological studies suggest that TMC channels may share a similar membrane topology with the Ca²⁺-activated Cl⁻ channel TMEM16 and the mechanically activated OSCA1.2/TMEM63 channel. Namely, these channels form dimers and each subunit consists of ten transmembrane segments. Despite this important structural insight, a key question remains: what is the gating mechanism of TMC channels? The major technical hurdle to answer this question is that the reconstitution of TMC proteins as functional ion channels has been challenging in mammalian heterologous systems. Since TMC channels are conserved across taxa, genetic studies of TMC channels in model organisms such as *C. elegans*, *Drosophila*, and zebrafish may provide us critical information on the physiological function and regulation of TMCs. Here, we present a comparative overview on the diverse functions of TMC channels in different species.

Keywords TMC channels · *C. elegans* · *Drosophila* · Zebrafish · Membrane excitability · Hearing · Mechanosensation

Introduction

Transmembrane channel-like (TMC) proteins are a novel family of ion channel-like proteins that are conserved from *C. elegans* to humans [1, 2]. The discovery of TMC1, the founding member of TMC family proteins, benefited tremendously from genetic studies of deafness in mice and humans. In 1958, Deol and Kocher first reported a deafness (dn) mouse strain with a recessive inheritance [3].

More than 20 years later, Steel and Bock examined these deafness mice and found that they lacked cochlear microphonics [4]. Within a few years, human siblings with recessive deafness were identified and its locus was linked to 9q11–q21 (DFNB7) region [5, 6]. Remarkably, this region of the human genome is syntenic to the mouse deafness (dn) locus, implying that mutations of a common gene are likely responsible for the deafness in both humans and mice. In 2002, using positional cloning, Kurima et al. identified eight mutations of a novel gene, *Tmc1*, in a dominant deafness locus DFNA36 which maps to the human chromosome 9q13–21 in a region overlapping the DFNB7/B11 locus for recessive deafness [7]. They also showed that a 1.6 kb deletion in *Tmc1* was causative disruption in the deafness (dn) mouse [7]. Coincidentally, an independent study from Vreugde et al. reported that the Beethoven (Bth) mutant mouse, which carried a methionine to lysine substitution of residue 412 in TMC1, is a new mouse model for dominant, progressive hearing loss DFNA36 [8]. Collectively, these exciting studies have paved the way for the discovery of TMC family proteins.

Through sequence homology search, seven additional TMC channels (TMC2–TMC8) were identified in humans and mice (Fig. 1a) [2, 7]. These TMC channels were strongly

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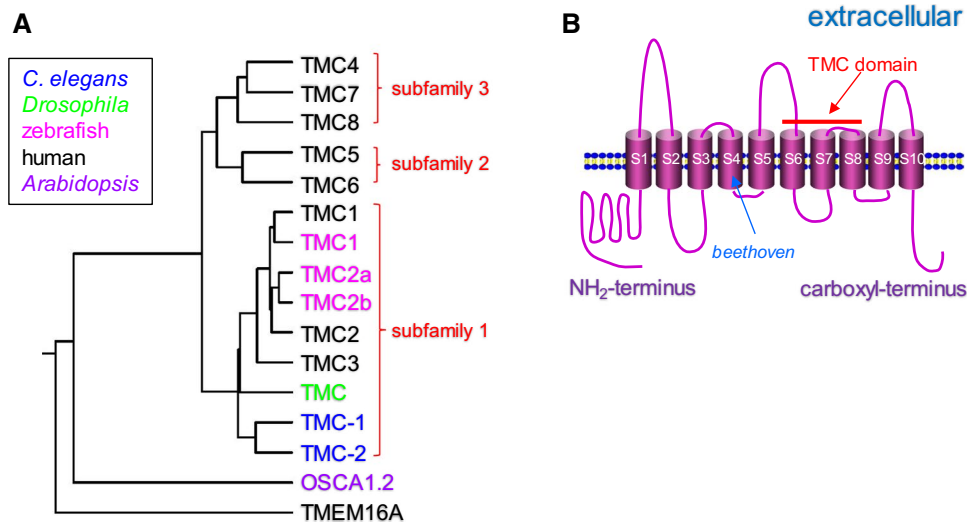


Fig. 1 TMC channels and their predicted membrane topology. **a** Protein sequences of TMC channels from *C. elegans* (in blue), *Drosophila* (in green), zebrafish (in magenta), and human (in black), OSCA1.2 channel from *Arabidopsis* (in purple), and TMEM16A channel from human (in black) are aligned up using ClustalW (<https://www.genome.jp/tools-bin/clustalw>). All these ion channels are predicted to share a similar membrane topology. Based on sequence homology, TMC channels form three subfamilies: TMCs1–3 (subfamily 1), TMCs5–6 (subfamily 2), and TMCs4, 7 and 8 (subfamily

3). **b** Predicted membrane topology of TMC1 channel. In general, TMC1 protein contains ten transmembrane segments (S1–S10) and distinct mutations of TMC1 can cause both dominant and recessive deafness in mammals. Shown is the beethoven missense mutation of TMC1 (M412K) in the fourth transmembrane segment, which causes dominant deafness in mice. The most conserved region among all TMC proteins is the TMC domain which is formed by the S6–S8 segments

predicted to encode proteins with 6–10 transmembrane domains and they all contain a novel conserved 120-amino-acid sequence termed TMC domain (Fig. 1b). Based on their amino-acid sequence similarities, TMC channels can be further grouped into three subfamilies that include TMCs 1–3, TMCs 5 and 6, and TMCs 4, 7 and 8 [2]. In addition to mammals, homologues of TMC channels were identified in other species such as *C. elegans*, *Drosophila*, and zebrafish, indicating that TMC proteins might have evolutionarily conserved functions. Here, we present an overview of the diverse functions of TMC channels in different species.

TMC channels in *C. elegans*

Chemosensation

The *C. elegans* genome encodes two TMC proteins, TMC-1 and TMC-2, which are most similar to mammalian TMC3 [9]. The *C. elegans* TMC-1 is widely expressed in multiple types of neurons (e.g., ASH, ADF, ASE, ADL, AQR, PQR, URX, and PHA), body wall muscle, and vulval muscle, while TMC-2 is mainly expressed in the body wall muscle and vulval muscle [9–11]. TMC-1 was originally reported as a sodium-sensitive ion channel and it seemed to be required for the ASH neuron-mediated high salt chemosensation [10]. However, Wang et al. later reported that, acting in

the ASH neuron, TMC-1 actually mediated the nociceptive response to high pH, but not sodium, allowing worms to avoid strongly alkaline environments, where most animals cannot survive [12].

The *C. elegans* ASH neuron is a polymodal sensory neuron that is sensitive to multiple nociceptive stimuli such as high osmolarity, alkaline pH, and aversive odorants [13]. Intriguingly, although acid sensation has been well characterized before, we know little about how animals sense alkali in the environment. By screening a collection of ion channel (e.g., TRP, ENaC/DEG, TMC, and CNG channels) mutants for defects in the alkali-induced avoidance behavior, Wang et al. found that both *osm-9* (a TRPV channel) and *tmc-1* mutants were severely defective in this avoidance behavior [12]. Furthermore, transgenic expression of *tmc-1* or *osm-9* cDNA in ASH was sufficient to rescue the behavioral phenotype, suggesting that they act in ASH to mediate the alkaline pH-induced avoidance behavior.

Using whole-cell patch clamp recordings, Wang et al. next recorded from the *tmc-1;osm-9* double mutant and found that the double mutant abolished the alkali-evoked current in ASH. Moreover, the *tmc-1* single mutant has a significantly stronger defect in the alkali-evoked current than does the *osm-9* mutant, indicating that TMC-1, but not OSM-9, might be the major contributor of alkali sensing in ASH [12]. Interestingly, while OSM-9 is sensitive to both acidic and basic pH, TMC-1 exhibits a specificity

toward alkali, as it is required for the alkali- but not acid-triggered behavioral response and electrical current in ASH [12]. Based on these findings, a working model has been

established on how the nociceptive ASH neuron transduces environmental stimuli (Fig. 2a) [14].

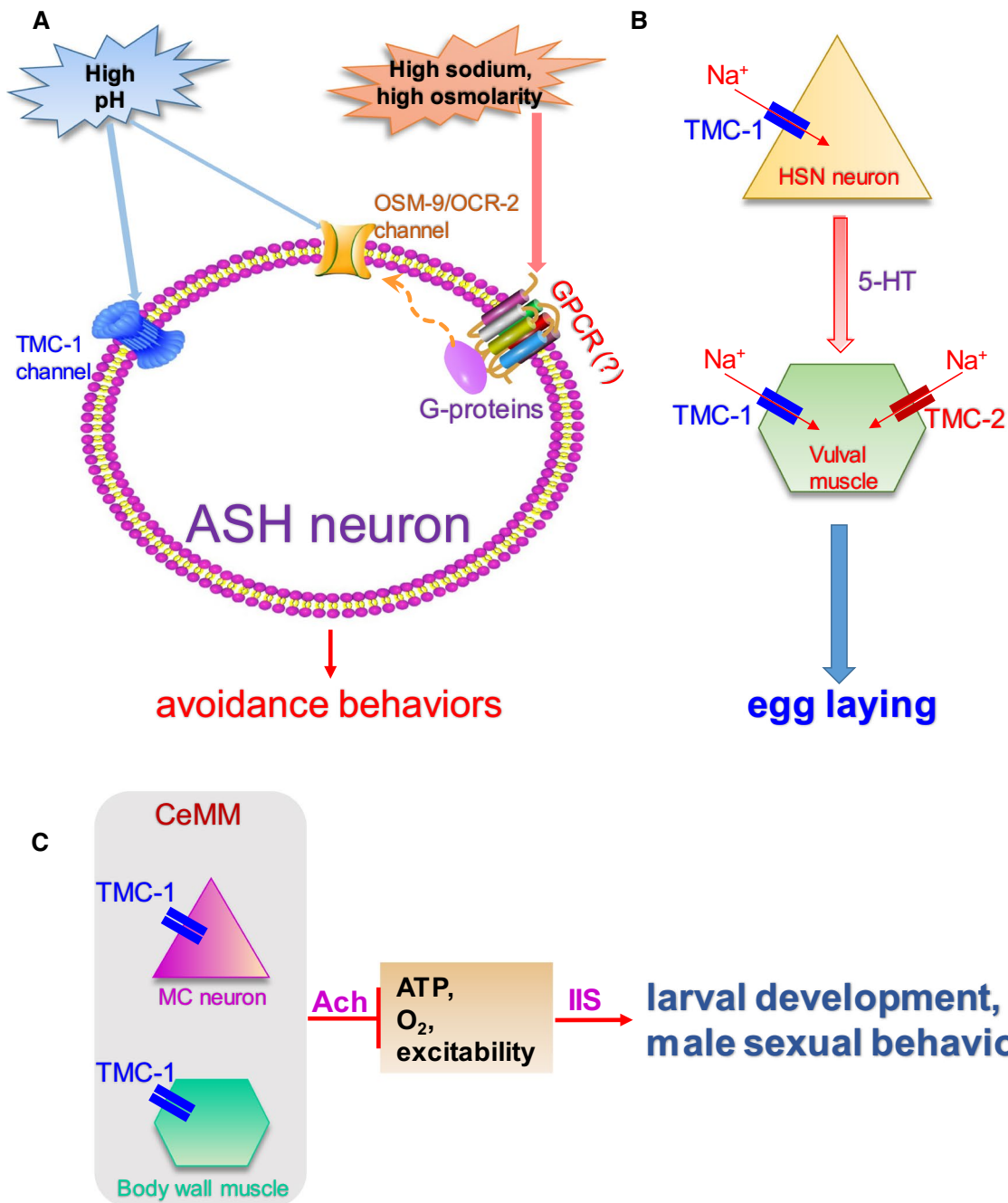


Fig. 2 Functions of TMC channels in *C. elegans*. **a** TMC-1 acts in the nociceptive ASH neuron to mediate the alkaline sensation. Both TMC-1 and TPRV (OSM-9, OCR-2) channels are expressed in the ASH neuron. TMC-1 is a major player in sensing noxious alkaline environment independent of G protein signaling. By contrast, high sodium, high osmolarity seem to activate the *C. elegans* TRPV channels through G protein signaling and unknown G protein-coupled receptors (GPCRs) [14]. **b** TMC channels promote *C. elegans* egg laying by modulating membrane excitability. TMC channels are

expressed in the egg-laying circuit of *C. elegans* (HSN neurons and vulval muscle), where they modulate membrane excitability through a background Na^+ -leak conductance [9]. The HSN motor neuron secretes serotonin (5HT) to innervate vulval muscle cells. **c** Synthetic CeMM suppresses larval development and male sexual behaviors in *C. elegans*, which is mediated by TMC-1 in the pharyngeal MC neurons and body wall muscle cells through cholinergic signaling and insulin/insulin-like growth factor signaling (IIS) [11]

Promoting membrane excitability

Membrane excitability is a fundamental feature for all excitable cells (e.g., neurons, muscle cells, and certain neuroendocrine cells) by determining the responsiveness of these cells. The resting membrane potential is tightly controlled by the counteractions between hyperpolarizing leak conductances (K^+ and Cl^-) and depolarizing leak conductances (Na^+). While the two-pore-domain potassium channels are well established to function as background K^+ -leak channels and hyperpolarize the plasma membrane [15], much less is known about the depolarizing background leak channels. NCA/NALCN channels mediate a depolarizing Na^+ -leak conductance in certain types of neurons to modulate many physiological processes including basal locomotion, response to volatile anesthetics, and normal respiratory rhythms [16–20]. However, extra neuronal Na^+ -leak currents are still present in the absence of NCA/NALCN proteins and the channels mediating muscular background depolarizing leak conductance are unknown [19, 20].

Recently, we identified an unexpected role of TMC channels: both TMC-1 and TMC-2 are required for normal egg laying in *C. elegans* (Fig. 2b) [9]. The disruption of TMC proteins hyperpolarizes membrane potential and reduces the rhythmic calcium activities of neurons and muscle cells involved in egg laying. Through electrophysiological recordings, we demonstrated that TMC channels can function as another type of background leak channel to modulate membrane excitability in both neurons and muscle cells [9]. Furthermore, we found that TMC channels were non-selective cation channels, though they displayed the highest permeability to Na^+ . Finally, unlike NCA/NALCN channels that are known to be inhibited by Ca^{2+} , TMC channels appeared to be insensitive to the extracellular Ca^{2+} , supporting the idea that TMC channels and NCA/NALCN channels are distinct types of background leak channels [9].

Since TMC-1 mediates alkaline sensation in the ASH neuron [12], we studied whether the membrane excitability of ASH is affected in the *tmc-1* mutant background. Consistent with the result obtained in the *C. elegans* egg-laying circuit, the resting membrane potential of ASH neurons in the *tmc-1* mutant is hyperpolarized and the background Na^+ -leak current is also significantly reduced [9]. Thus, TMC-1 mediates a background Na^+ -leak conductance in ASH. However, whether the Na^+ -leak conductance role of TMC-1 is involved in alkaline sensation remains unclear, as TMC-1 may be directly activated by alkaline pH. On the other hand, since both TMC-1 and TMC-2 are expressed in the body wall muscle [9, 11], it would be interesting to examine whether muscle cells are chemosensitive by responding to alkaline pH or high salt.

A major technical hurdle to study the function and regulation of TMC proteins is that it has been challenging to express them as functional ion channels in mammalian heterologous expression systems. When expressed in mammalian cell lines, most TMC proteins are trapped in the endoplasmic reticulum (ER) component. Intriguingly, ectopic expression of mammalian TMC proteins (TMC1, TMC2, and TMC3) in either neurons or muscle cells can rescue the defective egg laying and resting membrane potential observed in the *C. elegans tmc* mutants, suggesting that these TMC channels might have an evolutionarily conserved role in modulating membrane excitability.

Regulating development and sexual behavior

In the chemically defined synthetic *C. elegans* Maintenance Medium (CeMM) which is a suboptimal food source compared to the standard diet *E. coli* OP50, signaling from the pharyngeal MC neurons and body wall muscles can significantly slow larval development [11]. Interestingly, the *tmc-1* mutant exhibited an accelerated development rate in CeMM by promoting the cellular metabolism of MC neurons and body wall muscle (Fig. 2c) [11]. In addition to development, CeMM suppresses male sexual behaviors and *tmc-1* mutation attenuated the diet-induced inhibition of male sexual behaviors [11]. Thus, TMC-1 mediates the overall inhibitory effect of CeMM on larval development and male sexual behaviors. Notably, the synthetic CeMM contains no sodium. As TMC-1 promotes membrane excitability by acting as a background Na^+ -leak channel in the egg-laying circuit [9], could the low extracellular sodium concentration of CeMM trigger the inhibitory effect on development and sexual behaviors? Three lines of evidence argue against this notion. First, adding sodium salt into CeMM had no effect on development for both CeMM-fed wild type and *tmc-1* mutant worms [11]. Second, although both TMC-1 and TMC-2 channels mediate the background Na^+ -leak conductance in egg laying, the *tmc-2* mutant worm did not exhibit fast development in CeMM [11], suggesting that TMC-1 and TMC-2 must have different roles in development and male sexual behaviors. Third, TMC proteins enhance membrane excitability in the egg-laying circuit, while TMC-1 suppresses cellular respiration in the CeMM-fed worms [9, 11]. Collectively, TMC-1, but not TMC-2, appears to have Na^+ -independent functions in suppressing cellular metabolism and delaying development in the nutrient-poor CeMM.

The proper trafficking of TMC channels onto the plasma membrane requires auxiliary subunits as heterologous mammalian TMC proteins are typically retained in intracellular compartments [21]. Notably, although a large portion of *C. elegans* TMC proteins are expressed on the plasma membrane, intracellular TMCs can also be detected [9]. Since the Na^+ conductance function of TMC-1 is not required for

CeMM to delay development and suppress sexual behaviors, intracellular TMC-1 or TMC-1-specific auxiliary subunit(s) might mediate TMC-1's roles in development and male sexual behaviors. Taken together, the *C. elegans* TMCs appear to be multifunctional depending on which cells they are expressed in. Whether a unifying mechanism could explain the diverse functions of TMCs in *C. elegans* requires further investigation.

TMC channels in *Drosophila*

Detecting food texture

The *Drosophila* genome encodes a single TMC protein which is required for flies to sense food texture in the tongue. *Drosophila* can discriminate distinct food based on their hardness and viscosity. Zhang et al. found that the food texture discrimination depends upon a previously unknown multidendritic (md-L) neuron which extends elaborate dendritic arbors innervating the bases of taste hairs [22]. TMC channel is expressed in md-L neurons, where it is required for sensing the hardness and viscosity of food. Specifically, loss of *tmc* greatly reduced the ability to behaviorally discriminate the preferred softness (1% agarose) and smoothness (sucrose solution only) from harder and stickier food options [22]. Furthermore, mechanical deflection of taste sensilla can induce action potentials in md-L neurons and these mechanically activated action potentials were abolished in *tmc* mutant flies [22]. Collectively, this nice study revealed the cellular and molecular components that enable external sensory bristles on the fly tongue to communicate textural features to the brain through a pre-ingestive mechanism [22]. Although TMC seems to mediate food texture-triggered mechanical responses in flies, current evidence still cannot provide definitive answer to whether the *Drosophila* TMC protein is a mechanically gated ion channel as other TMC auxiliary subunits might be the real mechanosensor.

Proprioception

Proprioception, the sense of position, orientation, and movement of body parts, provides important sensory feedback information for animals to maintain proper gestures and coordinate their body movements [23, 24]. It has been known for centuries that proprioception is mediated by mechanosensitive proprioceptors [25]. Similarly, *Drosophila* larval locomotion, which entails rhythmic body contractions, is controlled by sensory feedback from proprioceptors. However, the molecular mechanism mediating this feedback is little understood. The *Drosophila tmc* gene is expressed in larval peripheral sensory neurons as well as the larval class I and class II dendritic arborization (da) neurons and bipolar

dendrite (bd) neurons [26, 27]. Genetic disruption of *tmc* led to reduced crawling speed, increased head cast frequency, and enhanced backward locomotion. Importantly, expressing *Drosophila* TMC or mammalian TMC1 and/or TMC2 in the *tmc*-positive neurons rescued these mutant phenotypes [26], suggesting the functional conservation of TMC channels in mechanosensation. Interestingly, mechanical stimuli did not trigger any current in the *Drosophila tmc*-transfected S2 cells, while *nompC* (encoding a mechanosensitive TRPN channel)-transfected S2 cells exhibited mechanically activated currents [26]. Thus, the *Drosophila* TMC protein by itself might not be mechanosensitive and other auxiliary subunits may be required for its mechanosensitivity. An alternative explanation is that TMC proteins might not traffic to the plasma membrane in the *Drosophila* S2 expression system.

TMC channels in zebrafish

Auditory sensation

The zebrafish genome encodes three TMC channels: TMC1, TMC2a, and TMC2b of which the two TMC2 proteins are more closely related to mammalian TMC2. Similar to their mammalian counterparts, both zebrafish TMC1 and TMC2a channels can physically interact with protocadherin 15 (PCDH15), a key component of the mechanotransduction complex in auditory and vestibular hair cells [28]. In zebrafish, TMC channels seem to be restricted to hair cells of the inner ear and lateral line organ, suggesting their roles in hearing and mechanotransduction. In line with this notion, overexpression of a truncated TMC2a channel resulted in mislocalization of PCDH15 within hair bundles and disrupted mechanosensitivity of ear hair cells [28].

Several auxiliary proteins physically interact with zebrafish TMCs and regulate their proper trafficking into hair bundles. For example, TMIE is a two transmembrane domain protein expressed in the stereocilia of hair cells and mutations in TMIE cause deafness in zebrafish, mice, and humans [29–32]. In *Tmie* mutant zebrafish, TMC proteins fail to target to hair bundles, whereas TMIE overexpression promotes bundle localization of TMC proteins [33]. Mechanistically, the second transmembrane domain and adjacent regions of TMIE seem to be particularly important for targeting TMCs into hair bundles [33]. In addition, the Golgi apparatus-enriched TOMT protein interacts with TMCs within the secretory pathway, which allows TMCs to integrate into the mechanotransduction complex in zebrafish hair cells [34]. Notably, the regulation of TMCs by TOMT is also conserved in mammals as mouse TOMT and TMC1 directly interact with each other through the His183 residue of TOMT [34, 35].

Water motion detection

Fish rely on mechanosensitive hair cells located in neuromasts of the lateral line system on the animal's surface to detect water motion. In zebrafish, TMC2b is robustly expressed in hair cells of the lateral line and *Tmc2b* null mutant displayed defective mechanotransduction [36]. Interestingly, neuromasts may contribute differentially to motor behaviors and distinct neuromasts with different orientations appear to express different levels of TMC2b, which may allow fish to detect water flow from different directions [36, 37]. For example, although M2 and MI1 neuromasts display high reliance on TMC2b, IO4 hair cells are largely independent of TMC2b [36]. Therefore, neuromasts may use different molecular mechanisms to encode water motion. Intriguingly, TOMT is highly expressed in both ear hair cells and lateral line neuromasts [34]. If TOMT plays a common role in targeting TMCs into stereocilia tips, it would be interesting to examine whether *Tomt* mutant fish are also defective in detecting water motion.

TMC channels in mammals

TMC1 and TMC2 in auditory transduction

To date, more than 40 different *Tmc1* mutations (both dominant and recessive) have been found to cause deafness in humans and mice [21]. A summary table of mutations in TMCs and their associated diseases in humans and rodents is shown in Table 1. Surprisingly, there are no known spontaneous mutations of *Tmc2* that cause a genetic disease, even though deletion mutations of *Tmc2* in mice cause deafness if *Tmc1* is absent. In rodents and humans, both *Tmc1* and *Tmc2* are expressed in inner ear hair cells, where they play an essential role in hearing transduction [38]. In addition, *Tmc1* can be detected in other organs such as the brain, eye, colon and testis [1]. In the inner ear, the temporal expression pattern differs for the two *Tmc* genes [38]. Namely, in the cochlea, *Tmc2* messenger RNA (mRNA) expression begins to rise at the base around birth and at the apex around postnatal day 2 (P2). After peak expression during the first postnatal week, *Tmc2* expression begins to decline to near zero by postnatal day 10. As the expression of *Tmc2* declines, *Tmc1* expression begins to rise and is maintained into adulthood [38]. In the vestibular organs, although *Tmc2* mRNA expression also precedes *Tmc1*, both *Tmc1* and *Tmc2* are expressed in mature vestibular hair cells. It should be noted that, in both auditory and vestibular organs, the spatiotemporal expression pattern of *Tmc2* is well correlated with the onset of mechanosensitivity of hair cells [38–40]. Interestingly, deletion of either *Tmc1* or *Tmc2* does not eliminate

mechanotransduction in early postnatal mice, while double knockouts display no conventional mechanotransduction in hair cells [38, 41, 42], indicating the potential functional redundancy between TMC1 and TMC2 channels.

Both TMC1 and TMC2 channels are located at the tips of hair cell stereocilia [43–45], where the mechanotransduction channels are located [46–48]. Very interestingly, hair cells that express *Tmc1* but not *Tmc2* exhibit smaller single-channel conductance, lower calcium permeability, and faster adaptation. By contrast, hair cells with *Tmc2* but not *Tmc1* displayed the opposite biophysical features and those with both *Tmc1* and *Tmc2* expression contain at least four distinct conductances, suggesting the formation of heteromultimers [42]. Furthermore, the Beethoven (Bth) mutation (M412K) of TMC1 altered ionic selectivity and biophysical properties [42, 49, 50], whereas two other TMC1 mutations (M412C, D569C) alter the binding affinity to the pore blocker dihydrostreptomycin [49, 51]. Collectively, these results suggest that TMC1 is a key component of hearing transduction pathway.

The proper targeting of TMC1 and TMC2 proteins to the tips of hair cell stereocilia and their functional integrity in hearing transduction depend on several accessory binding partners, including PCDH15, TMIE, LHFPL5, and CIB2 (Fig. 3) [52]. For example, the tip link protein PCDH15 may transduce tip link tension into opening of the mechanically activated hearing transduction channel by directly interacting with LHFPL5 and TMC proteins [28, 53, 54]. Meanwhile, TMIE binds to both LHFPL5 and PCDH15 (through the intracellular CD2 domain) in the hair cell mechanotransduction machinery [55]. Intriguingly, mutations of these TMC binding partners also cause deafness and defective mechanotransduction [56]. Therefore, the mechanosensitivity of hair cell transduction channels might involve multiple functional modules.

Very recently, structural studies of TMC1 suggest that TMC channels may have a similar membrane topology to the Ca²⁺-activated Cl⁻ channel TMEM16 and the mechanically activated OSCA1.2/TMEM63 channel [51, 57–60]. Namely, TMC1 channels appear to be assembled as dimers and each subunit contains ten transmembrane segments. Importantly, the transmembrane helices S4–S7 which contain the Beethoven (Bth) mutation form a lipid-facing groove that may act as a pore, raising the possibility that TMC1 in hair cells forms the ion conduction pathway for auditory transduction. In support of this view, Pan et al. performed scanning cysteine mutagenesis of TMC1 and found that at least 12 amino-acid residues within the S4–S7 segment influence the mechanically activated current in hair cells [51]. Given the large body of genetic and physiological evidence, TMC1 may function as part of the ion channel pore during hair cell auditory transduction.

Table 1 TMC mutations in diseases of humans and rodents

| Gene | Mutations | Predicted effect | Ex/in number | Phenotype | References |
|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|------------|
| Human TMC1 | c.16+1G>T, c.100C>T, c.1543T>C, c.2004T>G, c.64+2T>A, c.1330G>A, c.1333C>T, c.2030T>C, c.1696_2283del, c.195_16del, c.295delA, c.536-8T>A, c.884+1G>A, c.1534C>T, c.1960A>G, c.776+1G>A, c.767delT, c.1166G>A, c.1810C>T, c.1165C>T, c.1764G>A, c.237-6T>G, c.453+2T>C, c.628_630delATC, c.800G>A, c.1114G>A, c.1566+1G>A, c.596A>T, c.1404+1G>T, c.1788C>A, c.150delIT, c.362+18A>G, c.1714G>C, c.1714G>A, c.236+1G>C, c.458G>A, c.582G>A, c.589G>A, c.776A>G, c.797T>C, c.821C>T, c.830A>G, c.1083_1087del, c.1107C>A, c.1171C>T, c.1209G>C, c.1334G>A, c.1396_1398AAC, c.1312G>A, | Splice disruption p.R34X nonsense p.C515R missense p.S668R missense Splice disruption p.G444R missense p.R445C missense p.I677T missense 431 kb deletion 27 kb deletion p.K99KfsX4 Splice disruption Splice disruption p.R512X nonsense p.M654V missense Splice disruption p.F255FfsX14 p.R389Q missense p.R604X nonsense p.R389X nonsense p.W588X nonsense Splice disruption Splice disruption p.I210del p.G267E missense p.V372M missense Splice disruption p.N199I missense Splice disruption p.S596R missense p.N50KfsX25 p.Glu122Tyrfs*10 p.D572N missense p.D572H missense Splice disruption p.W153X nonsense p.W194X nonsense p.G197R missense p.Y259C missense p.I266T missense p.P274Lmissense p.Y277C missense p.R362PfsX6 p.N369K missense p.Q391X nonsense p.W403C missense p.R445H missense p.N466del p.A438T missense | Intron 5 Exon 7 Exon 17 Exon 21 Intron 6 Exon 16 Exon 16 Exon 21 Exon 19 Exon 5 Exon 8 Intron 10 Intron 13 Exon 17 Exon 20 Exon 7 Exon 13 Exon 15 Exon 20 Exon 15 Exon 20 Intron 7 Intron 9 Exon 11 Exon 13 Exon 15 Intron 17 Exon 11 Intron 16 Exon 20 Exon 7 Exon 8 Exon 9 Exon 9 Intron 7 Exon 10 Exon 11 Exon 11 Exon 13 Exon 13 Exon 13 Exon 13 Exon 15 Exon 15 Exon 15 Exon 15 Exon 16 Exon 16 Exon 16 | Hearing impairment | [7, 68–95] |

Table 1 (continued)

| Gene | Mutations | Predicted effect | Ex/in number | Phenotype | References |
|----------------|---------------------------------------------|--------------------|--------------|---------------------------------------|---------------------|
| | c.1253T>A, | p.M418K missense | Exon 16 | | |
| | c.1249G>A, | p.G417R missense | Exon 16 | | |
| | c.1247T>G, | p.L416R missense | Exon 16 | | |
| | c.1589_1590delCT, | p.S530X frameshift | Exon 18 | | |
| | c.1718T>A, | p.I573N missense | Exon 19 | | |
| | c.1714G>A, | p.D572N missense | Exon 19 | | |
| | c.1714G>C, | p.D572H missense | Exon 19 | | |
| | c.1763+3A>G, | p.W588WfsX81 | Intron 19 | | |
| | c.IVS19+5G>A, | splice disruption | Intron 19 | | |
| | c.1939T>C, | p.S647P missense | Exon 20 | | |
| | c.1959C>G, | p.Y653X missense | Exon 20 | | |
| | c.1979C>T, | p.P660L missense | Exon 20 | | |
| | c.2035G>A, | p.E679K missense | Exon 21 | | |
| | c.2050G>A, | p.D684N missense | Exon 21 | | |
| | c.2130_1delG, | splice disruption | Exon 22 | | |
| | c.2210_2211insCT, | p.E737HfsX2 | Exon 23 | | |
| | c.2260+2T>A | splice disruption | Intron 23 | | |
| TMC2 | Unknown | Unknown | Unknown | Unknown | |
| TMC3 | Unknown | Unknown | Unknown | Unknown | |
| TMC4 | Unknown | Unknown | Unknown | Unknown | |
| TMC5 | Up-regulated | Unknown | Unknown | Intrahepatic cholangiocarcinoma (ICC) | [96] |
| TMC6 (EVER1) | c.280C→T, | p.R94X nonsense | Exon 5 | Epidermodysplasia verruciformis (EV) | [97, 98] |
| | c.1726G→T | p.G576X nonsense | Exon 14 | | |
| TMC7 | Unknown | Unknown | Unknown | Unknown | |
| TMC8 (EVER2) | c.754/755delT, | p.F252fs X283 | Exon 7 | Epidermodysplasia verruciformis (EV) | [97, 98] |
| | c.1084G→T | p.E362X nonsense | Exon 9 | | |
| Rodents | | | | | |
| TMC1 | c.1235T>A (<i>Beethoven</i> mutation), | p.M412K missense | Exon 13 | Deafness | [7, 8, 38, 99, 100] |
| | c.1387_1557del (<i>deafness</i> mutation), | p.I463_Q519del | Exon 14 | | |
| | c.545A>G (<i>baringo</i> mutation), | p.Y182C missense | Exon 8 | | |
| | c.1345T>C (<i>nice</i> mutation), | p.Y449H missense | Exon 13 | | |
| | c.1661G>T (<i>stitch</i> mutation), | p.W554L missense | Exon 15 | | |
| | Tmc1Δ/Δ | Targeted deletion | | | |
| TMC2 | Unknown | Unknown | Unknown | Unknown | |
| TMC3 | Unknown | Unknown | Unknown | Unknown | |
| TMC4 | Unknown | Unknown | Unknown | Unknown | |
| TMC5 | Unknown | Unknown | Unknown | Unknown | |
| TMC6 | Unknown | Unknown | Unknown | Unknown | |
| TMC7 | Unknown | Unknown | Unknown | Unknown | |
| TMC8 | Unknown | Unknown | Unknown | Unknown | |

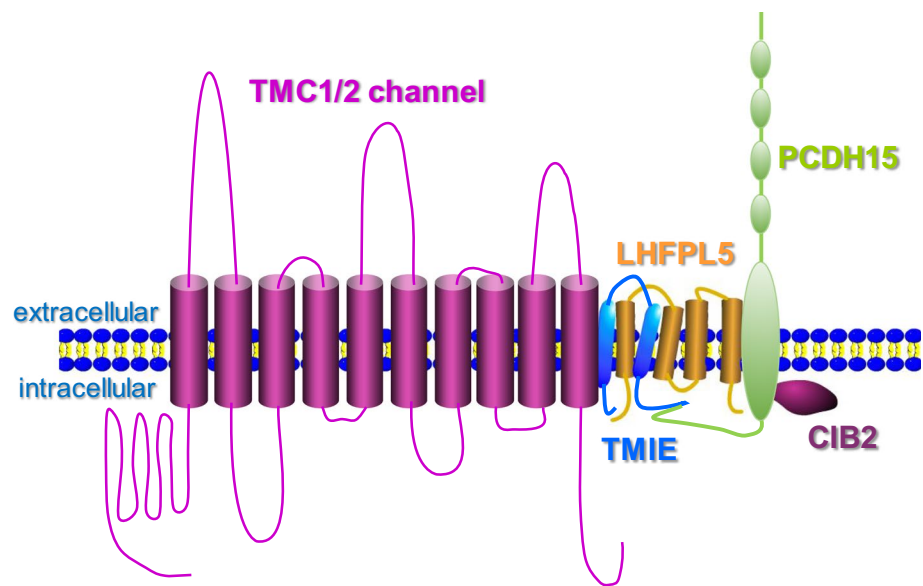
All annotations are based on cDNAs (c.) of TMC proteins

p protein, *del* deletion, *fs* frame shift, *ex* exon, *in* intron

Notably, despite the strong structural and physiological evidence on TMC1 channel in auditory transduction, it remains unclear whether the transduction pore is completely

bounded by S4–S7 helices or bounded in part by other accessory proteins like TMIE. Alternatively, the pore could be exposed to the lipid membrane. Further studies from

Fig. 3 Potential arrangement of proteins within the vertebrate auditory transduction complex. By interacting with multiple auxiliary proteins (TMIE, LHFPL5, PCDH15, and CIB2), TMC1 channel may form the pore of auditory transduction complex (modified from Corey et al. [67]). For simplicity, a TMC monomer is depicted in the model figure (according to recent structural studies, TMC channels form dimers)



cryo-EM or X-ray crystallography may help clarify additional structural details of TMC1 channel, including the pore region, location of the gate, and force-dependent transformation that occurs as TMC1 channels' transition from closed to open states. In addition, to firmly establish TMC1 as a mechanosensitive hearing transduction ion channel, an important experiment remains unfulfilled: reconstitution of TMC1 channels and mechanosensitivity in a heterologous expression system. As TMC1 does not traffic to the plasma membrane in heterologous cells, if reconstitution of hair cell-like mechanosensitivity in a heterologous system eventually proves successful, it will likely require expression of TMC1 in the correct lipid environment, co-expression with correct binding partners, scaffold proteins, and chaperones. On the other hand, since mammalian TMC1 and TMC2 channels have been ectopically expressed as functional proteins in *C. elegans* and *Drosophila* [9, 12, 26], it would be very interesting to examine whether the ectopically expressed mammalian TMC1 channel exhibits mechanosensitivity in worms and flies. If not, could the co-expression of TMC1 with its binding partners (i.e., PCDH15, TMIE, LHFPL5, and CIB2) reconstitute the mechanosensitivity? In this regard, *C. elegans* and *Drosophila* might provide useful expression systems to conduct structure–function studies of mammalian TMC channels.

Other mammalian TMC channels

In addition to TMC1 and TMC2, six other TMC channels are present in mammals. However, very little is known about the function and regulation of these TMC proteins. *Tmc3* mRNA can be detected in most neuronal organs as well as some non-neuronal organs. The mRNAs of *Tmc4*, *Tmc5*, *Tmc6*, and *Tmc7* are expressed in most murine organs tested,

while *Tmc8* mRNA is detectable in thymus, lung, and spleen [1]. Mutations in *Tmc6* (*EVER1*) and *Tmc8* (*EVER2*) are implicated in epidermodysplasia verruciformis, a recessive disorder comprising susceptibility to cutaneous human papilloma virus infections and associated nonmelanoma skin cancers [2]. Mechanistically, mutations in *Tmc8* appears to be linked to up-regulated Zn^{2+}/Ca^{2+} signaling and anocytamin 1 (TMEM16A) activation [61, 62]. More investigations are clearly required to reveal the physiological roles of these understudied TMC channels.

Discussion

Among eight TMC channels in mammals, TMC1 and TMC2 are best studied because of their essential role in hearing transduction. Similarly, homologues of TMC1 and TMC2 have been implicated in mechanotransduction of *Drosophila* and zebrafish. This raises an intriguing question: are TMC1 and/or TMC2 channels gated by mechanic force? Mechanosensitive ion channels are ubiquitously present in nearly all organisms [63]. In general, mechanosensitive channels can be divided into two categories depending on whether they require other auxiliary components for the mechanosensitivity [64]. The bacterial Msc channels and mammalian PIEZO channels are inherently mechanosensitive and they can be functionally reconstituted in lipid bilayers [65, 66]. By contrast, TMC channels require multiple auxiliary factors for proper membrane trafficking and mechanosensitivity. In fact, at this stage, we still do not know whether TMC channels are intrinsically mechanosensitive, because many essential binding partners of TMC channels could act as the real mechanosensor. Functional reconstitution of TMC1/2 channels in a heterologous system may help bridge this knowledge gap.

In addition, the recent development of cryo-EM microscopy may provide more structural insights into the gating mechanism of TMC channels.

C. elegans TMC channels display the most similarity to *Drosophila* TMC and vertebrate TMCs1-3 proteins (Fig. 1a) [9]. However, defective mechanosensation has not been reported for the *tmc* mutant worms. Very interestingly, ectopic expression of mammalian TMC1, TMC2, and TMC3 in the egg-laying circuit of *C. elegans* can functionally rescue the egg-laying defect and membrane excitability of *tmc* mutants. This finding has two implications: first, mammalian TMC1–3 channels might also modulate membrane excitability by acting as background Na⁺-leak channels, and second, we may use *C. elegans* as a heterologous expression system to study the biophysical and pharmacological features of mammalian TMC channels. Notably, unlike their mammalian counterparts, little is known about the binding partners of TMC channels in *C. elegans*. As *tmc* mutant worms display various defects in chemosensation, development, and egg laying, genetic screens using these defective behaviors might reveal novel binding proteins of TMC channels.

In summary, pioneering genetic and physiological studies of TMC proteins have revealed a novel family of ion channels involved in many important cellular processes. Although mammalian TMC1 and TMC2 channels clearly have an essential role in hearing, more studies are needed to define their precise action during auditory transduction. Meanwhile, further investigations are required to understand the physiology of other understudied TMC channels (TMC3–8).

Funding The work in the Xiao lab is supported by the American Cancer Society Research Scholar Grant (RSG-17-171-01-DMC), UF Center for Smell and Taste Pilot Grant, and the American Federation for Aging Research (AFAR) Research Grant (17146). The work in the Kang lab is supported by the National Foundation of Natural Science of China (31771113, 31900736, 31800878), the Fundamental Research Funds for the Central Universities (2018FZA7004), China Postdoctoral Science Foundation (2018M640551, 2018M642412, 2019T120503, 2019T120505), the 111 project, and the Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (2018PT31041).

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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