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Preview

In the Right Place at the Right Time: Is TMC1/2 the Transduction Channel for Hearing?

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Recent papers suggest that TMC1 and TMC2 constitute the ion channels mediating hearing and balance. Kurima et al. (2015) now show these proteins are expressed in the right place and at the right time, bringing us closer to definitive identification.

More than 50 years ago, Georg von Békésy, who received the Nobel Prize for elucidation of frequency tuning in the cochlea, hinted at the molecular basis of what he called "the final mechanical transformer" for converting sound into a neural signal. This is now understood to be a specific ion channel, remarkably fast (opening in microseconds) and remarkably sensitive (activated by sub-nanometer movements), located at the tips of mechanosensitive stereocilia of inner-ear hair cells. Much is known about the biophysics of this transduction channel (Fettiplace and Kim, 2014; Kawashima et al., 2015). It passes metal and organic cations up to ~1.2 nm in diameter and has a high conductance ranging from 80 to 320 pS (Pan et al., 2013; Fettiplace and Kim, 2014). A channel is opened when pulled on by filamentous tip links that run between adjacent stereocilia, and channel opening involves a large conformational change of ~4 nm (Fettiplace and Kim, 2014). But 50 years later, the channel's molecular identity remains uncertain. A new report from the Kachar, Griffith, and Holt laboratories (Kurima et al., 2015) now gets us closer to a clear identification.

Positional cloning of deafness genes in humans and mice has been exceptionally productive in revealing molecular components of the transduction machinery in hair cells. One such gene, named transmembrane channel-like 1 (*TMC1*) for its six to ten transmembrane domains, is mutated both in dominant (DFNA36) and recessive (DFNB7 and DFNB11) forms of human hereditary deafness, and in the *deafness* and *Beethoven* mouse mutants (Kurima et al., 2002). The TMC gene family comprises eight members in mammals. Despite the name, the function of the TMC1 protein is not clear; in particular, the possibility that TMC1 is a transduction channel was called into question by the persistence of the transduction current in neonatal cochlear hair cells of mutant mice lacking TMC1 (see Kawashima et al., 2015 for review). However, Kawashima et al. (2011) showed that TMC2 is also present in these cells, at least transiently, so it might compensate for loss of TMC1. By testing both single and double knockouts, they found that one or the other TMC is sufficient for hair cell function. *Tmc2* expression in cochlea then goes down, creating an absolute requirement for TMC1 at later stages.

This requirement satisfied one criterion for identification of a mechanically-gated transduction channel (Christensen and Corey, 2007), but meeting others has been more difficult. A second criterion—that the candidate protein, when heterologously expressed, should produce a mechanically-evoked current—is unlikely to be met until all components of the transduction apparatus are identified and co-expressed. A third criterion is that a heterologously expressed candidate should produce a conductance with properties similar to the native transduction channel. No group has expressed a TMC protein in a cell line and convincingly evoked a current because TMCs apparently don't reach the plasma membrane without a chaperone. However, an alternate criterion, that certain mutations of a candidate gene should change the selectivity or conductance of the transduction current in hair cells, has been met in several ways. First, mice expressing just *Tmc1* or just *Tmc2* have transduction channels with either low or high conductance and low or high Ca^{2+} permeability (Pan et al., 2013; Kim et al., 2013, Beurg et al., 2014). Second, the *Beethoven* mouse has a missense mutation (M412K) in a region predicted to be transmembrane and has transduction currents with even lower conductance and lower Ca^{2+} permeability (Pan et al., 2013). Although it is possible that the channel pore is formed by another protein and that TMCs are accessory subunits that affect conductance, the simplest hypothesis is that TMCs form the pore.

Still, other criteria must be met (Christensen and Corey, 2007), and here the new work contributes substantially. The candidate channel must be expressed in the right place at the right time. For cochlear hair cells, protein should be located where physiological experiments have shown transduction channels to be—at the tips of stereocilia. Kawashima et al. (2011) previously expressed GFP-tagged TMC1 in hair cells, and Beurg et al. (2015) used an antibody to localize TMC1. Both showed localization near stereocilia tips, but neither study was comprehensive. Kurima et al. (2015) have now created two types of BAC transgenic mouse lines, one producing TMC1 fused to mCherry under the *Tmc1* promoter and one with TMC2 fused to GFP under the *Tmc2* promoter. With spectacular resolution, the tagged TMCs were found exactly where expected. Localization is diffuse in early neonatal stereocilia but still concentrated at the stereocilia tips and is clearly restricted to tips at later ages. Ca^{2+} imaging has shown

that the tallest stereocilia, at least in mammals, do not have transduction channels (Fettiplace and Kim, 2014), and the tagged TMCs were only rarely seen in the tallest stereocilia. In a double-knockout background, the BAC transgenes rescued hearing, indicating that the fluorescently-tagged TMCs are functional and properly localized. Immunofluorescence with antibodies to TMCs independently confirmed the localization.

With BAC rescue, the time course of protein expression under the normal promoters can be followed. As expected, fluorescent TMC2 appeared first in the cochlea at postnatal day 2 (P2), when cochlear hair cells are first mechanosensitive, and TMC1 followed a few days later with full expression by P7. TMC2 then disappeared by P10, a time when cochlear function is known to require TMC1. In the vestibular system, both TMC1 and TMC2 persisted, consistent with a lack of vestibular phenotype in mice or humans with mutation of just TMC1. The exquisite resolution also allowed the authors to ask whether TMC1 associates with TMC2 in a molecular complex. They found that TMC1-mCherry fluorescent puncta appeared within 30 nm of TMC2-GFP puncta at levels higher than chance, consistent with a heteromeric channel and with the intermediate single-channel conductances often found in wild-type animals (Pan et al., 2013). Because this could result from TMC concentration at stereocilia tips, it will be important to demonstrate biochemical interaction. Finally, the localization clearly demonstrates that TMC1 and TMC2 are proteins of the plasma membrane, not restricted to the ER.

Much work remains. Localization with immunogold electron microscopy would be welcome, to localize TMCs within nanometers of the tip-link filaments that are thought to pull on transduction channels. X-ray crystal structure would elucidate the stoichiometry of TMC association and might reveal a central pore. Finally, there are additional components of the transduction apparatus, such as protocadherin-15, TMHS/LHFPL5, and TMIE. Full understanding will require both biochemical and structural assembly of this molecular jigsaw puzzle.

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