How many proteins does it take to gate hair cell mechanotransduction?

Paul Albert Fuchs¹

Department of Otolaryngology-Head and Neck Surgery, Center for Hearing and Balance and Center for Sensory Biology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Components of the Mechanotransducer Complex

Mechanosensory hair cells of the inner ear transduce sound or head motion into electrical signals through the gating of mechanically coupled ion channels. Although many cell types can respond to mechanical force (1), the disposition of mechanotransduction (MT) channels to the very tips of stereocilia (2, 3) confers onto hair cells of the inner ear their specialized sensitivity (4, 5). Differential deflection of the stereociliary tips is transmitted to the MT channels via extracellular “tip-links” that extend between taller and shorter stereocilia (6–8). The tip-links are composed of cadherin 23 and protocadherin 15 (5), the latter of which forms the lower end coupled to the MT gating complex. Positional cloning of a human deafness locus identified transmembrane channel-like protein isoforms (TMC)1 and TMC2 as potential components of the MT complex (9). TMC1 and TMC2 interact with protocadherin 15 (10) and have been nominated as subunits of the channel itself (11), perhaps combining differentially to generate the tonotopic variation in unitary conductance (larger in higher frequency hair cells) (12). The challenge for the field is to establish molecular mechanisms in a cellular organelle (the hair bundle) that couples extracellular force to channel gating through a still incompletely defined complement of interacting proteins. The problem is compounded by the fact that the MT channel and its binding partners must be transported and assembled at the stereociliary tips. In PNAS, Beurg et al. (13) shine a revealing light onto these complexities. The authors show that LHFPL5 (lipoma HMGIC fusion partner-like 5, also known as tetraspan membrane protein of hair cell stereocilia, or TMHS) is required for normal MT current, apparently by regulating the participation of TMC1 (but not TMC2). These results in themselves help to keep the story of hair-cell MT moving forward. However, the

authors’ description of “reverse-polarity” MT currents puts an intriguing twist into the plot.

Reverse-Polarity Mechanotransduction

The cornerstone of hair-cell MT is its directional specificity. Deflection toward the taller stereocilia causes MT channel opening, cationic influx, and hair-cell depolarization; the opposite motion causes channel closure (4). Incredibly, hair cells of homozygous LHFPL5 null (LHFPL5^{−/−}) mice generate

Beurg et al. propose that LHFPL5 is required for correct positioning of TMC1 in the MT complex, and in particular for the tonotopic gradient in unitary conductance of the MT channel.

reverse-polarity MT currents that can be even larger than those for the normal polarity motion. Reverse-polarity currents also have been reported for TMC1/TMC2 double mutants (14) and other MT-disrupted hair cells (15), keeping open the nomination process for MT channel proteins. Knockout of LHFPL5 produced smaller single MT channel currents and flattened the tonotopic gradient in unitary conductance (for normal polarity motion, reverse-polarity currents appear similar in low- and high-frequency hair cells, although this was not quantified). The loss of LHFPL5 is similar in effect to that for TMC1 (16) and immunolabeling showed that TMC1 was absent from stereocilia in the LHFPL5^{−/−} hair cells. Beurg et al. (13)

propose that LHFPL5 is required for correct positioning of TMC1 in the MT complex, and in particular for the tonotopic gradient in unitary conductance of the MT channel. How then to explain the large reverse-polarity currents? The authors propose that MT complexes can be assembled without the LHFPL5 required for export to the stereociliary tips. In an as yet undefined way, stereociliary deflection transmits force to these accumulated, disoriented MT channels. In addition, the authors show that TMC2 locates normally in stereociliary tips of LHFPL5^{−/−} hair cells, providing the residual normal polarity currents.

The Stereociliary Bundle Defines the Adequate Stimulus

The stereocilia muster into their oriented, tiered ranks through a complex web of intra- and intercellular commands (17, 18). Thus, it’s no surprise that the proteins making up the MT complex will include essential elements in addition to those that form the channel pore. What is more revealing is that stereociliary deflection can open MT channels that are not coupled to tip-links, indeed may not even be in the stereocilia. Future efforts to determine the exact location of reverse-polarity MT channels and their interactions with the actin cytoskeleton will provide additional definition of the MT complex.

Thus, the picture emerging from Beurg et al.’s (13) work is that the ancient art of MT has been adapted to the architecture of the hair cell. The hair cell serves a “special sense” because it aggregates MT channels in the stereociliary bundle. This highly specialized structure directs a unique form of environmental energy to the MT channel gate.

Author contributions: P.A.F. wrote the paper.

The author declares no conflict of interest.

See companion article on page 1589.

¹Email: pfuchs1@jhmi.edu.

1 Sukharev S, Sachs F (2012) Molecular force transduction by ion channels: Diversity and unifying principles. *J Cell Sci* 125(Pt 13): 3075–3083.

2 Beurg M, Fettiplace R, Nam JH, Ricci AJ (2009) Localization of inner hair cell mechanotransducer channels using high-speed calcium imaging. *Nat Neurosci* 12(5):553–558.

3 Jaramillo F, Hudspeth AJ (1991) Localization of the hair cell’s transduction channels at the hair bundle’s top by iontophoretic application of a channel blocker. *Neuron* 7(3): 409–420.

4 Hudspeth AJ, Corey DP (1977) Sensitivity, polarity, and conductance change in the response of vertebrate hair cells to

controlled mechanical stimuli. *Proc Natl Acad Sci USA* 74(6): 2407–2411.

5 Kazmierczak P, et al. (2007) Cadherin 23 and protocadherin 15 interact to form tip-link filaments in sensory hair cells. *Nature* 449(7158):87–91.

6 Kachar B, Parakkal M, Kurc M, Zhao Y, Gillespie PG (2000) High-resolution structure of hair-cell tip links. *Proc Natl Acad Sci USA* 97(24):13336–13341.

7 Zhao Y, Yamoah EN, Gillespie PG (1996) Regeneration of broken tip links and restoration of mechanical transduction in hair cells. *Proc Natl Acad Sci USA* 93(26):15469–15474.

8 Pickles JO, Comis SD, Osborne MP (1984) Cross-links between stereocilia in the guinea pig organ of Corti, and their possible relation to sensory transduction. *Hear Res* 15(2): 103–112.

9 Kurima K, et al. (2002) Dominant and recessive deafness caused by mutations of a novel gene, TMC1, required for cochlear hair-cell function. *Nat Genet* 30(3):277–284.

10 Maeda R, et al. (2014) Tip-link protein protocadherin 15 interacts with transmembrane channel-like proteins TMC1 and TMC2. *Proc Natl Acad Sci USA* 111(35):12907–12912.

11 Pan B, et al. (2013) TMC1 and TMC2 are components of the mechanotransduction channel in hair cells of the mammalian inner ear. *Neuron* 79(3):504–515.

12 Beurg M, Evans MG, Hackney CM, Fettiplace R (2006) A large-conductance calcium-selective mechanotransducer channel in mammalian cochlear hair cells. *J Neurosci* 26(43):10992–11000.

13 Beurg M, Xiong W, Zhao B, Müller U, Fettiplace R (2015) Subunit determination of the conductance of hair-cell mechanotransducer channels. *Proc Natl Acad Sci USA* 112:1589–1594.

14 Kim KX, et al. (2013) The role of transmembrane channel-like proteins in the operation of hair cell mechanotransducer channels. *J Gen Physiol* 142(5):493–505.

15 Marcotti W, et al. (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(16):5505–5514.

16 Beurg M, Kim KX, Fettiplace R (2014) Conductance and block of hair-cell mechanotransducer channels in transmembrane channel-like protein mutants. *J Gen Physiol* 144(1):55–69.

17 Nayak GD, Ratnayaka HS, Goodyear RJ, Richardson GP (2007) Development of the hair bundle and mechanotransduction. *Int J Dev Biol* 51(6–7):597–608.

18 Deans MR (2013) A balance of form and function: planar polarity and development of the vestibular maculae. *Semin Cell Dev Biol* 24(5):490–498.