

PNAS Commentary:

Possible titles:

Stretching out the early stages of hearing

Jonathan Ashmore

Department of Neuroscience, Physiology and Pharmacology and UCL Ear Institute, Gower St, London WC1E6BT, UK.

Author contributions: JFA wrote the paper.

The author declares no conflict of interest

[See companion article](#)

Email: j.ashmore@ucl.ac.uk

The molecular basis of mechano-transduction is one of the major problems, probably the_ unsolved problem, in hearing and balance. Sensory hair cells in both the cochlea and the vestibular systems of the inner ear have at one end a bundle of fine processes whose deflection initiates the electrical signal ultimately processed by the brain. The transduction complex, consisting of an ionic pore and the machinery to open it, lies at the top of the hair bundle and has resisted many attempts to identify its molecular components. The problem is a difficult one because there are not many copies of the complex per cell and so far no one has constructed an assay to test the proposals except in a hair cell. However during the maturation of the cell it now seems as though another set of mechanosensitive elements makes an appearance, albeit transiently [1]

The most promising candidates for the transduction channel pore itself are two proteins TMC1 and TMC2 whose genetics were first described over three decades ago in a deaf mouse [2], but only functionally identified recently [3]. How they get to the top of the bundle and connect up with a linkage, the 'tip link', necessary to make a fast response to any sound disturbance, remains unresolved. The hair bundle itself is an organelle with over 200 proteins [4] and although other components of the transduction complex are known [5] [6] their organisation remains a puzzle. To open the channel, the bundle needs to be deflected towards the tallest hairs. However, moving the bundle in the wrong or 'anomalous' direction sometimes also produces a transduction current [7], but only when the bundle is deflected with a fluid jet, not simply pushed. The current is present even when the TMC1 and TMC2 proteins are absent which suggested that either the TMCs were the wrong candidates or, more charitably, that the steps to building a functional bundle are more complex than even suspected [8].

For technical reasons, many of the reports on how hair cells work are based on early stage cochleas from rodents, typically no older than postnatal day 18 (P18). This covers the period when there is

extensive cellular remodelling of the cochlea (the comparable period in humans is gestational weeks 24-26.) Beurg et al [1] have shown now that the anomalous (or preferably, 'reverse') transduction current is associated with channels on the apical membrane surface below the bundle (Figure 1). These channels start to fall off in number normally by P2-P6 in hair cells, just as the 'normal' transduction currents reach their mature amplitudes. In the absence of TMC1 and TMC2 a reverse transduction channel can still be measured up to P8, suggesting that the reverse transduction channels may be a part of a defined programme to construct a fully working hair bundle.

What are these channels if not the transduction channel? Their pharmacology distinguishes them from the TMC candidates, but there are now many channels which have been identified as being sensitive to mechanical forces. These include members of the TRP channel family, the twin pore K channel (K2P) family, some of the ATP gated channels and more recent candidates such as the piezo channels [9]. Based on the channel's conductance and calcium permeability measured by Beurg et al, the finger points at a piezo channel. So far these channels in particular have yet to make a significant appearance in the inner ear.

Immature hair cells, like their surrounding supporting cells, have many small villi covering their apical membrane. Hard to see other than by electron microscopy, they begin to disappear on hair cells by about P4. It is quite conceivable that a fluid jet could produce a shear distortion of this irregular surface in a way that a mechanical push could not. Possibly such experimental manipulations mimic the stretching and bending of the surface as the cochlea develops. If so, 'reverse' transduction may be part of the apical specialisation controlling the formation of the hair bundle itself.

Why is this this important? Unsurprisingly, there has always been an interest in regenerating hair cells in order to restore hearing [10] [11]. Although birds appear to be able to rebuild functioning hair cells after damage, the evidence is much slimmer for any recovery in the mature mammalian inner ear. The appearance of reverse transduction as a sign that functional bundle remodelling is under way is a tempting avenue to explore. Breaking the tip-link in the transduction complex clearly leads to reverse transduction within about 5 minutes in immature hair cells [1, 7, 12] but it is not known whether the same occurs in adult hair cells. Beurg et al suggest that it does not, at least to any significant extent. Nevertheless, hair cells do show signs of membrane, and presumably protein, trafficking at their apical surface particularly around the base of the bundle and the site where the primary cilium is found. It is likely therefore that there are be multiple control signals for the regulation of this traffic for the duration of the long lifespan of the cell. The observation of transduction the wrong way round may not, as first thought, be a fatal flaw in the identification of the hair cell transducer but the elucidation of part of the programme which builds a complex mechano-transducer and indicates significant progress towards identifying what makes a hair cell special.

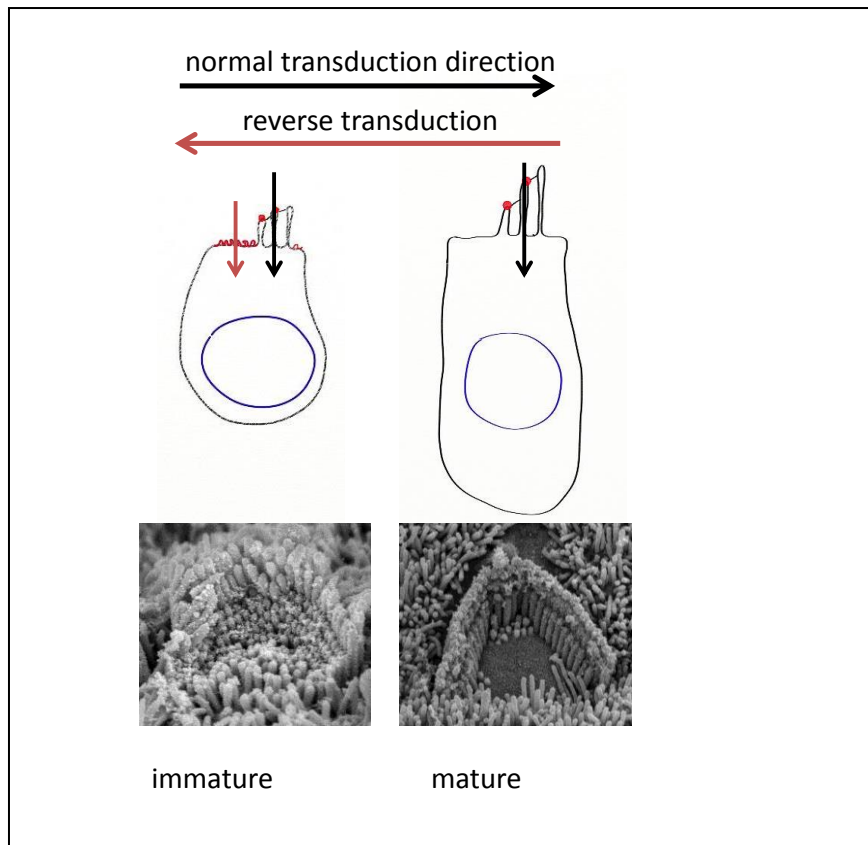


Figure 1 Legend:

Deflection of the hair bundle towards the tallest stereocilium on a hair cell opens channels and allows current to flow into the cell. In immature cells, forces in the opposite direction opens cation channels on the apical surface [1]. Such cells have an apical membrane covered in small microvilli, approximately 100 nm in diameter, possibly associated with the ‘reverse transduction’ mechanosensitive channels. Below, scanning electron micrographs of an immature (P3) and mature (P6) hair bundle from an apical turn cochlear outer hair cell showing microvilli covering the surface membrane.

Acknowledgements:

The author’s research is supported in part by a project grant from the Biotechnology and Biological Sciences Research Council (BBSRC) to A.Forge who kindly provided the electron micrographs for Figure 1.

References

1. Beurg, M., et al., *Development and localization of ‘reverse-polarity’ mechanotransducer channels in cochlear hair cells*. PNAS, 2016.
2. Steel, K.P. and G.R. Bock, *The nature of inherited deafness in deafness mice*. Nature, 1980. **288**(5787): p. 159-61.
3. Pan, B., et al., *TMC1 and TMC2 are components of the mechanotransduction channel in hair cells of the mammalian inner ear*. Neuron, 2013. **79**(3): p. 504-15.
4. Shin, J.B., et al., *Molecular architecture of the chick vestibular hair bundle*. Nat Neurosci, 2013. **16**(3): p. 365-74.
5. Zhao, B., et al., *TMIE is an essential component of the mechanotransduction machinery of cochlear hair cells*. Neuron, 2014. **84**(5): p. 954-67.

6. Maeda, R., et al., *Tip-link protein protocadherin 15 interacts with transmembrane channel-like proteins TMC1 and TMC2*. Proc Natl Acad Sci U S A, 2014. **111**(35): p. 12907-12.
7. Kim, K.X., et al., *The role of transmembrane channel-like proteins in the operation of hair cell mechanotransducer channels*. J Gen Physiol, 2013. **142**(5): p. 493-505.
8. Nayak, G.D., et al., *Development of the hair bundle and mechanotransduction*. Int J Dev Biol, 2007. **51**(6-7): p. 597-608.
9. Woo, S.H., et al., *Piezo2 is required for Merkel-cell mechanotransduction*. Nature, 2014. **509**(7502): p. 622-6.
10. Chen, W., et al., *Restoration of auditory evoked responses by human ES-cell-derived otic progenitors*. Nature, 2012. **490**(7419): p. 278-82.
11. Izumikawa, M., et al., *Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals*. Nat Med, 2005. **11**(3): p. 271-6.
12. Marcotti, W., et al., *Transduction without tip links in cochlear hair cells is mediated by ion channels with permeation properties distinct from those of the mechano-electrical transducer channel*. J Neurosci, 2014. **34**(16): p. 5505-14.