In addition, directly measuring oxygen changes is less sensitive to the hemodynamic factors affecting BOLD and offers a direct comparison of changes in oxygen concentration (and consequently changes in metabolism) with those in neuronal activity. The relationship of neural activity to the signals measured in human neuroimaging is complex and requires a great deal of additional work. Studies like this one are a huge step in the right direction and contribute to the necessary constraints on the interpretation of hemodynamic responses.

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Stringing the fiddle: the inner ear's two-part invention

David P Corey

The identity of the tip link, which converts mechanical force to channel opening in hair cells, has been controversial, with different groups promoting cadherin 23 or protocadherin 15. A new paper in *Nature* shows that both proteins are involved.

Inner-ear hair cells convert mechanical stimuli such as sound and head movements into neural signals. Studies of their mechanism of transduction have been blessed by an extraordinarily productive interplay between physiological and anatomical approaches. Physiology at the scale of nanometers and piconewtons correlated very well with the localization of specialized structures at the tips of the mechanosensory stereocilia mechanosensory stereocilia (**Fig. 1a**). The third leg of the experimental stool—the identification of proteins that participate in mechanotransduction—is far weaker. Particularly perplexing is the identity of the tip link, a fine filament strung between the tip of each stereocilium and the side of its tallest neighbor, which pulls on force-activated transduction channels to open them. Different laboratories have presented strong evidence supporting the involvement of two different structural proteins, cadherin 23 and protocadherin 15, in tip link formation, but also strong evidence against their participation. Now two groups, led by Bechara Kachar and Ulrich Müller, have joined hands to bridge this divide in a new paper in *Nature*[1]. Their compelling evidence suggests that the tip link is made of both cadherin 23 and protocadherin 15, joined in the middle.

Physiological experiments illuminate channel function in a dazzling array of detail. Deflection of a hair cell's stereocilia bundle (**Fig. 1a**) by ~100 nm opens ~100 'transduction channels', located at the tips of stereocilia. These ion channels are directly activated in ~10 μs by forces of ~10 piconewtons, causing the channels to move by 2–4 nm when they open. Each channel is in series with a molecular 'gating spring' of ~1 millinewton per meter stiffness that can stretch by >100 nm.

The anatomy is equally well understood. Each stereocilium contains a stiff bundle of ~300 actin filaments; when deflected, each stereocilium pivots at its base but remains in contact with its neighbors at the tip (**Fig. 1b**). Most important is the tip link. About 170 nm long, it appears as a twisted pair of strands, each ~5 nm in diameter[2] (**Fig. 1c**). Putting all of this together, the simple and elegant hypothesis for hair-cell transduction is that a deflection of the hair bundle tightens the tip links, directly pulling transduction channels open and allowing ions to flow into the cell.

To determine transduction components, researchers have tried candidate-gene approaches based on function, biochemical purification of stereocilia proteins, and oligonucleotide-array screens of hair cell mRNA. The most productive approach has been the study of genes mutated in deafness and balance disorders from humans, mice and zebrafish. Both molecules examined in the new study[1] were discovered in this way. Cadherin 23 is mutated in the human deafness and blindness syndrome Usher 1D[3], in the deaf *waltzer* mouse[4] and in the *sputnik* zebrafish[5]. Protocadherin 15 is defective in the human Usher syndrome 1F[6], in the *Ames waltzer* mouse[7] and in the *orbiter* zebrafish[8].

Understanding the functions of the two proteins was harder, however, as the inner-ear phenotypes of the *waltzer* and *Ames waltzer* mice are complex. Both are deaf, and both show the circling behavior (for which they are named) that is characteristic of vestibular defects. Within the inner ear, the most striking defect for both mice is in their hair bundles: they mature more slowly and form disorganized clumps of stereocilia[4,7]. Because the tight organization of stereocilia is so critical for hair cell function, this disorganization explained the behavioral defects, and it suggested that both cadherins were involved in the development and cohesion of the hair bundles.

Other evidence pointed toward a more specialized role for cadherin 23 as the tip link itself. This cadherin is particularly long: with each of its 27 ectodomains estimated at 4.5 nm in length (**Fig. 1d**), it could span at least 120 nm, close to the 150–170 nm needed for a tip link. Also, the tip link can be cut by removing extracellular Ca^{2+} with the chelator BAPTA, a property reminiscent of cadherin Ca^{2+} sensitivity. Antibodies to cadherin 23 label the tips of stereocilia, but this immunoreactivity disappears if the tip links are disrupted with BAPTA, suggesting that the tip links include cadherin 23 (ref. 9). In addition, *sputnik* zebrafish carry mutations in cadherin 23, antibodies to this cadherin label the tips of zebrafish stereocilia, and a mild allele of *sputnik* with otherwise normal hair bundle morphology lacks tip links[5].

This might have been the satisfying end of the story, but there were problems. First, antibodies to cadherin 23 brightly label most of the developing hair bundle, but immunoreactivity disappears from the tips of stereocilia in older animals[10]. As older animals can still hear, of course, this pattern was inconsistent with a role for cadherin 23 in tip links. Also, tip links are sensitive to BAPTA but not to the protease subtilisin, whereas the broad cadherin 23 immunoreactivity has the opposite sensitivity[10].

David Corey is in the Howard Hughes Medical Institute and Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115, USA.
e-mail: dcorey@hms.harvard.edu

Masking of antibody-binding sites during the maturation of the transduction complex could explain the discrepancy, but it did not look good for cadherin 23 as the tip link protein.

A mystery 'tip link antigen' raised further problems. A monoclonal antibody exquisitely and specifically labeled tip links in electron microscopic images[11]. However, the target of the antibody—although unidentified—has a molecular mass of less than two-thirds the mass of cadherin 23.

Meanwhile, work on protocadherin 15 became equally confusing. Some antibodies to protocadherin 15 label the entire hair bundle[12], whereas others show specific labeling at the tips of all but the tallest stereocilia[12,13]. Protocadherin 15 binds two other proteins that cause Usher syndrome when mutated: harmonin (Usher1C)[14] and myosin 7a (Usher1B)[13]. Mutations in all five known Usher type 1–associated genes cause disorganized hair bundles, suggesting that these proteins function together to influence bundle development and cohesion. Still, the specific labeling of stereocilium tips, observed at least sometimes, hinted at something more specialized.

Sometimes complexity begets simplicity. Protocadherin 15 turns out to be expressed in at least 24 alternatively spliced variants, organized into four different groups on the basis of differing C termini. Antibodies to some splice forms label the whole hair bundle, whereas antibodies specific to another form label only stereocilium tips[12]. Thus, different splice forms may have alternative functions at different times in development. Moreover, enough of the mysterious tip link antigen was eventually purified to identify it using mass spectroscopy, and it was none other than protocadherin 15 (ref. 12). But where did that leave cadherin 23?

The newest work[1] puts it all together in an elegant series of experiments. Beautiful antibody labeling showed both cadherin 23 and protocadherin 15 at the location of the tip links. The length of a tip link is almost exactly the combined length of their extracellular domains. Antibodies to specific epitopes along these extracellular domains (arrowheads, **Fig. 1d**) labeled the tip link in corresponding locations, and showed that cadherin 23 was at the upper end and protocadherin 15 at the lower end (**Fig. 1f**). Consistent with the model, cutting the tip links to let the two ends spring apart revealed an upward movement for cadherin 23, but not protocadherin 15. What about the helical dimer structure[2] of tip links? Purified and negatively stained cadherin 23 appears as a twisted parallel dimer of about two-thirds the tip-link length, as observed with electron microscopy. Under the same conditions, protocadherin 15 manifests as a parallel dimer of about one-third the length. Mixtures of the two are seen bound end to end, looking very much like a tip link. Finally, a mutation in protocadherin 15 that causes deafness disrupts the binding of these two cadherins. And the discrepancies? Many might be attributed to other splice forms of the cadherins carrying out other functions in the hair bundle.

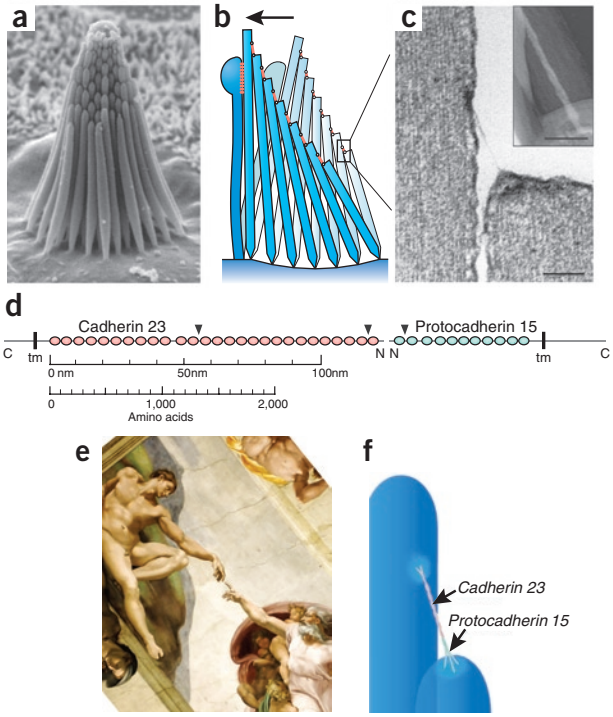

Figure 1 Structure of hair bundle stereocilia and tip links. (**a**) Hair bundle from a frog vestibular organ, composed of ~60 stereocilia. (**b**) An excitatory deflection of the hair bundle (arrow) causes stereocilia to shear past one another, separating the tips. The single kinocilium of a bundle is attached to neighboring stereocilia. (**c**) Tip link extending from the tip of a shorter stereocilium to the side of the next taller, along the excitatory axis. Inset, quick-freeze deep-etch image showing the double helical structure of the tip link. Inset reprinted by permission from ref. 2, copyright 2000 National Academy of Sciences USA.) Scale bars: 100 nm, 50 nm in inset. (**d**) Domain structure and approximate length of cadherin 23 and protocadherin 15. N and C termini transmembrane domains are indicated as tm. Ovals represent cadherin ectodomains. Epitopes used for immunogold electron microscopy are indicated by arrowheads, and the antibody-binding positions on the tip links matched the spacing of epitopes on the cadherins. (**e**) The problem of connection: finding and binding the correct target (Michelangelo Buonarroti, 1511; photograph by Sam Bloomberg-Rissman, reproduced with permission from David Sanger Photography). (**f**) Proposed molecular composition of a tip link. A parallel homodimer of cadherin 23 joins a parallel homodimer of protocadherin 15, binding at their N termini (reproduced from ref. 1 by permission of Macmillan Publishers Ltd., copyright 2007).

With the identity of the tip link in hand, researchers can begin to answer many other questions. The biophysicists will want to know whether these cadherins are elastic enough to be the gating spring that stretches with hair bundle deflection, or whether they should look elsewhere in the bundle for an elastic protein. A previous Ca^{2+} imaging study suggesting that a transduction channel exists at each end of the tip link is less consistent with the asymmetric molecular structure and might need to be revisited. A perplexing question remains: how do the two cadherins find each other during hair bundle development (**Fig. 1e**)? Each must be independently inserted into the bases of neighboring stereocilia, but the bases are too far apart for an intact tip link to span. Instead, they might find each other during their ascent to the tips. A similar problem of molecular connection would arise during the repair of tip links, which may occur on a daily basis. Structural biologists will want to know precisely how the N termini of the different cadherins grasp each other so tightly, as the conserved tryptophan residues that interlock other cadherins[15] are not present in cadherin 23. Finally, the great unknown at center stage of the mechanism is the mechanically gated transduction channel. Could these cadherins give us a cord with which to unmask the channel?

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Erratum: Stringing the fiddle: the inner ear's two-part invention

David P Corey

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In the version of this article initially published, the legends for Figure 1d and f were switched, and citations to these panels in the text were incorrect. In addition, references to specific animal models in ref. 10 were inaccurate. The errors have been corrected in the HTML and PDF versions of the article.