Mechanoelectrical transduction by hair cells

James O. Pickles and David P. Corey

Hair cells of the inner ear are one of nature's great success stories, appearing early in vertebrate evolution and having a similar form in all vertebrate classes. They are specialized columnar epithelial cells, with an array of modified microvilli or stereocilia on their apical surface, interconnected by a series of linkages. The mechanical stimulus causes deflection of the stereocilia, stretching linkages between them, and opening the mechanotransducer channels. On a slower timescale, hair cells adapt in order to maintain optimum sensitivity, with an adaptation motor within the stereocilia acting to keep the resting tension on channels constant.

Mechanosensitive hair cells are found in the auditory and vestibular organs of all vertebrates, as well as in the lateral line system of fishes and amphibians. While the hair cells are all variations on one basic cell type, each system is given stimulus specificity by the way that the mechanical stimulus is coupled to the hairs or stereocilia. In all these systems, deflection of the stereocilia opens mechanically sensitive ion channels to depolarize the cell.

Figures 1 and 2 show the structure of the transducing apical pole of the hair cell. This basic form is found in all mechanosensitive hair cells, whether the inner or outer hair cells of the cochlea, the hair cells of the vestibular system or the hair cells of the lateral line. The stereocilia are arranged hexagonally on the surface of the hair cell, and are composed of actin filaments packed in a dense paracrystal. This gives them a stiff, rigid structure so that they bend only at their base when deflected. The stereocilia are graded in height, being tallest on one side of the bundle and shortest on the opposite side. Adjacent to the tallest row of stereocilia is a single true cilium called the kinocilium (which in the mammalian cochlea disappears soon after birth). A line drawn from the kinocilium through the center of the bundle runs parallel to the steepest gradient in stereocilia heights, and forms an axis around which the whole bundle is bilaterally symmetrical. The stereocilia are also extensively crosslinked. Some links run parallel to the apical surface of the hair cell, joining adjacent stereocilia in all directions. These links are thought to hold the stereocilia together, so that their tips slide along one another when the bundle is deflected. There are also specialized connections known as the tip links; these emerge from the tips of the stereocilia and run almost vertically along the hair cell axis, to join the side-wall of each adjacent taller stereocilium. (Figs 1 and 2).

What does the hair cell respond to?

Micromanipulation experiments by Hudspeth and Corey have provided the most direct evidence that mechanotransduction depends on deflection of the stereocilia. In these experiments, the sensory epithelium was isolated from the sacculus of the bullfrog compared with HIV-negative controls (D. Griffin, Johns Hopkins University School of Medicine, Baltimore, MD, USA), and can lead to microgliosis (J. Merrill, UCLA, CA, USA). Nevertheless, these levels of INF-γ actually fall in HIV-infected persons with dementia compared with HIV-infected patients without neurologic disease (Griffin). In contrast, the elevated CSF concentration of quinolinate is closely associated with the degree of cognitive impairment in patients with AIDS. Thus, there does not appear to be a tight temporal correlation between INF-γ levels and quinolinate levels in the CSF of patients with HIV-associated cognitive-motor complex (of which the AIDS dementia complex is a severe form). This finding may raise questions about the possible relationship between INF-γ and quinolinate concentrations in vivo, but perhaps the requirement for such a temporal association is unwarranted.

Another cytokine that is reportedly elevated in the CSF of at least some neurologically symptomatic HIV-infected patients is tumor necrosis factor α (TNFα), which may be produced by microglia (Griffin). Moreover, glutamate can cause oscillations in $[\text{Ca}^{2+}]$ and possibly induce TNFα and interleukin 1 (IL-1) in astrocytes (Merrill). In addition, neopterin (presumably released from cells of the macrophage or microglial lineage, as mentioned by Fuchs et al.) and β₂-microglobulin are increased in the CSF of neurologically symptomatic HIV-positive patients. However, their significance, except as possible markers of disease, remains unknown.

Despite these and additional tantalizing cytokine findings in AIDS, other infections affecting the CNS besides HIV-1 can lead to the intrathecal production of various cytokines. For example, TNFα and IL-1 levels can increase in bacterial meningitis, and INF-γ and IL-1 can be elevated in other viral infections causing meningitis or encephalitis (K. Frei, University Hospital Zürich, Switzerland). Thus, the lack of specificity of these responses indicates that we do not yet understand their exact influence on the injury to neurons and possibly other CNS cell types that has been observed in AIDS, and highlights the need for additional work in this area.

Stuart A. Lipton
Harvard-Longwood Neurology Program, 300 Longwood Ave, Boston, MA 02115, USA

References
2 Lipton, S. A. (1992) Trends Neurosci. 15, 75-79
What is the electrophysiological mechanism of transduction?

The ion channels are relatively non-selective among the alkali cations, with Li\(^+\), Na\(^+\), K\(^+\), Rb\(^+\) and Cs\(^+\) being nearly equally permeant\(^1\).\(^2\).\(^3\). Since the apical surface of hair cells in vivo is faced with endolymphatic fluid, which contains a high [K\(^+\)], we expect that K\(^+\) will normally carry much of the transducer current. Ca\(^{2+}\) also passes through the transducer channel but with a lower efficacy. However, Ca\(^{2+}\) is a necessary cofactor in transduction, since transduction ceases if the concentration falls below about 10 \(\mu\)M\(^4\).\(^5\).\(^6\).\(^7\).\(^8\).\(^9\).\(^10\).

One remarkable finding is that there seem to be very few active transducer channels on each cell: in the order of 50–200 depending on the system, or one or two per stereocilium\(^11\). This figure, which is approximate, has been derived independently, from the analysis of mechanotransducer noise\(^12\), from the total transducer current knowing the conductance of an individual channel\(^13\), and indirectly from changes in bundle stiffness associated with transducer channel opening\(^14\) (see below). If the same numbers apply in humans, then all our auditory perception depends on a total of only \(6 \times 10^3\) mechanosensitive channels, found on the hair cells from the inner ear. This small number might be understood on energetic grounds; if there is only enough energy in an acoustic stimulus to open a certain number of channels, then having more channels available on the hair cells will give relatively little increase in sensitivity.

To permit high-frequency perception in some animals (up to 200 kHz in some bats\(^15\)), the transducer channels must be fast. Direct measurements of current responses to step-deflections in the bullfrog (\textit{Rana catesbeiana}); the otolithic membrane — which normally couples the stimulus to the stereocilia — was removed from over the stereocilia; and the stereocilia were manipulated directly while recording the intracellular potentials. Mechanoreceptor potentials were shown to depend on deflection of only the stereocilia; separate deflection of the kinocilium had no effect\(^8\). Deflection of the bundle towards the tallest stereocilia opens transduction channels, as measured by a conductance increase; this causes a depolarizing receptor potential and, ultimately, the excitation of afferent fibers that form synapses with the hair cell. Deflection in the opposite direction shuts the few channels that are open at rest, causing intracellular hyperpolarization and neuronal inhibition. Deflection at right angles to this axis has no effect\(^9\),\(^10\). The axis for maximum activation of the mechanotransducer channels therefore runs parallel to the axis of bilateral symmetry of the hair bundle, deflections in other directions having an effect depending on the vector component of the stimulus along the best axis\(^9\).
The tip-link model for transduction. (A) Mechanical stimuli act directly on ion channels in the stereocilia, by increasing tension in a 'gating spring' attached to the channel gate. (B) At any one time channels are distributed between open and closed states, but an increase in tension (dashed line) makes the open state energetically more favorable, and shifts the distribution towards the open state. (C) A simple structural model comes from the suggestion that each tip link is a gating spring. Displacing the bundle towards the tallest stereocilia (a positive stimulus) stretches the tip links to increase tension. The channels (somewhere in the distal ends of the stereocilia) are shown in this figure to be at the lower end of each tip link, but equally well could be at the upper end, or, indeed, both. (D) The sensitivity curve, relating displacement of the bundle (measured at the tips) to the probability of the channel being open, shows the very narrow operating range of hair cells. A displacement of about a third of a micrometre – approximately the diameter of one stereocilium – is a saturating stimulus. The curve also shows that some channels are open in the resting position, suggesting some resting tension in the tip links.

What are the structural elements involved in transduction?

Still unclear in this model are the location of the channel and what pulls on it. In order to determine the location, Hudspeth explored around a hair bundle with an extracellular electrode while deflecting the stereocilia with a probe; the extracellular potential changes were greatest around the top of the bundle, along the tips of the stereocilia. This suggests that the transducer currents were flowing into the cell near or at the tips of the stereocilia. Although challenged by Ohmori, this site has been confirmed by others. The tip link has a fine central filament (Fig. 4), presumably protein, that is surrounded by glycoalyx. This filament would be ideal for transferring the movement-induced forces onto single mechanotransducer channels in the membrane. There is also a report that, in some cases at least, the tip link is not a single strand but branches at its upper end. The kinetic model has a further implication. At a certain displacement of the stereocilia, when the channel moves from its closed to open state, the gating spring should shorten by an amount equal to the movement of the channel gate. This should add an extra compliance to the system, and should be measurable as a drop in the stiffness of the bundle at the point at which the channels are undergoing their maximum number of transitions between the open and closed states. Such changes in the stiffness of the bundle have been found by Howard and Hudspeth, and have been correlated with the probability of opening the channel. Together, these experiments indicate that the transduction channels are directly gated by mechanical tension.
stereocilia. Because the stereocilia are graded in height and the tip links are oriented nearly vertically, the links would be stretched by a deflection of the stereocilia in the direction of the tallest, that is, in the excitatory direction. Deflection in the opposite direction would be expected to take the tension off the tip links.

**A model for transduction**

The mechanical gating of channels, their localization and the function of tip links were put together in an appealingly simple model for transduction by Pickles, Osborne and Comis 5.21.26. In this model (Fig. 3) the tip link is the gating spring and pulls directly on the ion channels. It is not clear whether the transducer channels are associated with the upper or lower ends of the tip link, or both. Either end of the links would be suitable for transferring forces to membrane-bound channels.

The close correlation between the anatomical and the electrophysiological evidence supports this model; for instance, the tip links run almost exclusively along the axis of bilateral symmetry of the hair cell, that is, only along the anatomical axis along which movement of the stereocilia affects the transducer channels - since the stereocilia are hexagonally packed, other directions might be equally possible 6.32. Tip links seem to be present in all mechanotransducing hair cell systems, having been found in vestibular, cochlear and lateral line systems, and in representatives of all vertebrate classes 6,13,27,33,34. A gradation in heights of the stereocilia is required to accommodate the tip links, and such a gradation is seen in all mechanotransducing acousticolateral hair cells, although the gradation is not seen in ciliated electroreceptors, which are thought also to be of acousticolateral origin 6.30.

Recently, this model has been directly tested by cutting the tip links 2. Reducing the calcium concentration around the bundle with the fast calcium chelator BAPTA [1,2-bis(o-aminophenoxy)ethane-

$N,N',N'$-tetraacetic acid] irreversibly destroyed transduction in a few tenths of a second. A similar treatment with BAPTA for a few seconds eliminated the tip links, viewed with either scanning or transmission electron microscopy 2. When freestanding bundles were treated with BAPTA, they relaxed forward by about a tenth of a micrometre 2, in quantitative agreement with a model for tension regulation in the gating springs 36. These results indicate that tip links indeed convey tension to the transduction channels.

**An adaptation mechanism regulates tension in the tip links**

The extraordinary sensitivity of the transduction mechanism poses a problem for the hair cell, in that larger stimuli easily saturate the transduction. This is a problem for hair cells in the vestibular system that sense acceleration, because the constant acceleration of gravity imposes a stimulus a thousandfold larger than their threshold sensitivity. On a different timescale, this high sensitivity creates a developmental problem for all hair cells, because tip links and channels must be positioned within the hair bundle with an accuracy of 10–20 nm.

As a consequence, hair cells have developed an adaptation mechanism that continuously adjusts the tension in the tip links. *In vitro* experiments initially showed that steady displacements of a hair bundle caused the sensitivity curve to move along the displacement axis to match the new position of the bundle without appreciably changing shape (Fig. 5) 16. For saturating displacement stimuli, this both reduces the response and repositions the maximum sensitivity at the steady bundle position. The mechanism is apparently active *in vivo*, at least in the bullfrog saccule where it is thought to underlie an adaptation of the saccular microphonic potential 37.

This shift of the sensitivity curve can be reconciled with the tip-links model for transduction by supposing that adaptation involves an adjustment of tension in the tip link, specifically by sliding the upper attachment point of the tip link along the side of the stereocilium (Fig. 5) 36. More recently, a quantitative model for adaptation has been developed, based on an active motor element 36. This model can account for a variety of phenomena associated with adaptation, and also predicts active movements of the hair bundle under certain conditions. Such movements of the whole bundle have been observed, which tends to confirm the mechanical basis of adaptation 36.

Interestingly, the rate of tension adjustment depends on the concentration of Ca$^{2+}$ inside the tips of stereocilia.
the stereocilia, suggesting that the adjustment motor is in the tips very near the transduction machinery. Indeed, displacement of the upper tip-link insertion site has been observed in hair cells fixed in various adaptation states, which is in agreement with the model and confirms the involvement of the tip links.39

Alternative models for transduction

No alternative models for hair-cell mechanotransduction, beyond that presented here, have been put forward in the past decade. Earlier, it was suggested that transduction might depend on distortion of the whole hair-cell body40 (which has been ruled out by experiments in which mechanotransduction was associated with micromanipulation of the stereocilia1,5), or of the kinocilium alone41 (which has been ruled out by experiments in which the stereocilia were manipulated separately from the kinocilium, showing that transduction depends only on the former5). Suggestions that mechanotransduction depended on membrane distortion at the sites at which the stereocilia flexed in response to deflection, that is, at their bases where they enter the cell body42, were countered by the experiments described above suggesting that the channels were instead located near the tips of the stereocilia.22,24,25

It was found recently that antibodies to an amiloride-sensitive ion channel from renal epithelium bind near the tips of the stereocilia.43 Since amiloride also blocks transduction channels44, although at a higher concentration than that required to block the renal channel, the antibody might recognize the hair-cell transduction channels. Antibody binding occurred mainly at the points of contact between stereocilia and not at either end of the tip links. Although this finding is obviously inconsistent with the simple tip-links hypothesis, it may also be that the antibody recognizes latent channels and not those mechanically connected to tip links, or that the epitope recognized might not be the transduction channel at all.

Towards a molecular basis

The next level of understanding will entail a molecular description of the elements in the transduction chain. The excitement of tracing the basis of hearing to a few molecules will be tempered by the great difficulty in purifying the relevant proteins, particularly since we expect them to be present in amounts of the order of an attomole per animal45,46. The channel, the tip link and the motor remain biochemically unidentified at present. Of these, the motor molecule responsible for adaptation may be the most tractable, since it resembles the family of myosins in its properties. The actin core of stereocilia provides the first clue, since the only molecules known to move on actin are of the myosin family. The maximum rate of adaptation that tightens the tip links is in the range of 1–2 μm/s, the same rate at which myosin moves on actin, and myosin-coated glass beads have been found to climb towards the tips of stereocilia cores at a similar velocity.47 However, immunological and biochemical approaches to identifying the motor are still inconclusive. Perhaps the best hope for identifying the channel is by molecular probing for similarity to other ion channels48.

In 1962 Georg von Bekesy concluded several decades of work on the cochlea by suggesting that 'it does not seem impossible that the final mechanical transformer is of molecular dimensions'. Now, thirty years later, we think we know where that mechanical transformer is, and how it works. Hair cell research is presently at a turning point, as efforts are directed towards identifying and understanding the 'transformer' in molecular terms.

Selected references

Changes in $\text{Ca}^{2+}$-binding proteins in human neurodegenerative disorders

Claus W. Heizmann and Katharina Braun

The cellular distribution of $\text{Ca}^{2+}$-binding proteins has been extensively studied during the past decade. These proteins have proved to be useful neuronal markers for a variety of functional brain systems and their circuitries. Their major roles are assumed to be $\text{Ca}^{2+}$ buffering and transport, and regulation of various enzyme systems. Since cellular degeneration is accompanied by impaired $\text{Ca}^{2+}$ homeostasis, a protective role for $\text{Ca}^{2+}$-binding proteins in certain neuron populations has been postulated. As massive neuronal degeneration takes place in several brain diseases of humans, such as Alzheimer's disease, Parkinson's disease and epilepsy, changes in the expression of $\text{Ca}^{2+}$-binding proteins have therefore been studied during the course of these diseases. Although the data from these studies are inconsistent, the detection and quantification of $\text{Ca}^{2+}$-binding proteins and the neuron populations in which they occur may nevertheless be useful to estimate, for example, the location and extent of brain damage in the various neurological disorders. If future studies advance our knowledge about the physiological functions of these proteins, the neuronal systems in which they are expressed may become important therapeutical targets for preventing neuronal death in an array of neurodegenerative diseases.

More than 7000 articles on calcium were published in 1991 (a figure found using the Medline database, with 'calcium' as the search word). This emphasizes the tremendous interest and progress in $\text{Ca}^{2+}$-related research. In nerve cells, $\text{Ca}^{2+}$ ions activate and regulate a number of key processes, including fast axonal transport of substrates, synthesis and release of some neurotransmitters, and membrane excitability. It is also thought that in the CA1 hippocampal region of the brain, $\text{Ca}^{2+}$ might induce long-term potentiation and memory storage mechanisms.

The $\text{Ca}^{2+}$ message is converted into an intracellular response — in many cases by $\text{Ca}^{2+}$-binding proteins that are involved in a wide variety of activities, such as cytoskeletal organization, cell motility and differentiation, cell-cycle regulation, and $\text{Ca}^{2+}$ buffering and transport. It might therefore be possible that altered levels of some $\text{Ca}^{2+}$-binding proteins (e.g. due to deletion or mutation of the corresponding genes) could lead to an impaired $\text{Ca}^{2+}$ homeostasis in nerve cells and to pathological conditions. Using mostly immunohistochemical techniques, several research groups have now started to search for altered expression of the $\text{Ca}^{2+}$-binding proteins parvalbumin, calbindin-D28K and S100 in affected brain regions of patients suffering from acute insults, such as stroke and epileptic seizures, and from chronic neurodegenerative disorders, such as Alzheimer's, Huntington's, Parkinson's and Pick's diseases. In addition, altered $\text{Ca}^{2+}$ levels have been found in platelets of patients with bipolar affective disorders, and $\text{Ca}^{2+}$ antagonists have been suggested for treatment of psychotic depression.

The few proteins that have been investigated in these pathological states belong to a large family of more than 200 members. These proteins are characterized by a common structural motif, the EF-hand, because of the arrangement of the E and F helices of this motif has aided the identification of many new proteins with known functions, such as calmodulin, troponin C, myosin light chains, calpain, calcineurin and recoverin, are far outnumbered by those with unidentified roles. Most $\text{Ca}^{2+}$-