The sensory and motor roles of auditory hair cells

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Abstract | Cochlear hair cells respond with phenomenal speed and sensitivity to sound vibrations that cause submicron deflections of their hair bundle. Outer hair cells are not only detectors, but also generate force to augment auditory sensitivity and frequency selectivity. Two mechanisms of force production have been proposed: contractions of the cell body or active motion of the hair bundle. Here, we describe recently identified proteins involved in the sensory and motor functions of auditory hair cells and present evidence for each force generator. Both motor mechanisms are probably needed to provide the high sensitivity and frequency discrimination of the mammalian cochlea.

Electromechanical feedback
The electrical change in an OHC caused by basilar membrane vibration generates a movement of the OHC that, in turn, feeds back to or alters basilar membrane vibration.

Reverse transduction
In normal forward transduction, hair cells convert mechanical stimuli into electrical responses. In reverse transduction, the electrical signals of hair cells evoke a mechanical output.

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Auditory sensing in mammals occurs in a subdivision of the inner ear known as the cochlea, a fluid-filled tube coiled up like a snail's shell to fit into the temporal bone at the base of the skull. Sound pressure fluctuations are transmitted to the cochlea in several steps: vibrations of each eardrum, conduction through three small middle ear bones that act in a lever-like fashion, and production of pressure waves within the cochlea to displace the basilar membrane (FIG. 1). This complex mechanical coupling ensures that sound energy is efficiently transferred from air to cochlear fluid over a wide frequency range[1,2]. Detection of the sound stimulus and its conversion to an equivalent electrical waveform, termed mechanoelectrical transduction, occurs in hair cells riding on the elongated basilar membrane. Sound-induced motion of the basilar membrane excites the hair cells by deflecting their hair bundles to activate mechanoelectrical transduction (MET) ion channels[3]. Owing to gradients in the size and stiffness of the cochlear partition (the basilar membrane and associated hair cells and supporting cells), the place of maximal vibration varies systematically with the sound frequency like resonances in a musical instrument[1]. As a consequence, each hair cell produces responses that, near the threshold of hearing, are tuned to a characteristic frequency (CF) (BOX 1).

The mammalian cochlea contains two classes of hair cell, inner and outer, with distinct functions (FIG. 2). Information about the acoustic environment — speech, music or other sounds in the outside world — is relayed primarily by the electrical signals of inner hair cells (IHCs) (BOX 2), whereas the main task of outer hair cells (OHCs) is to boost the stimulus by electromechanical feedback[4]. The OHC contribution is known as the 'cochlear amplifier', a mechanism that increases both the amplitude and frequency selectivity of basilar membrane vibration for low-level sounds. The unique features of mammalian hearing — the middle ear bones, the extended basilar membrane and the separation of function between IHCs and OHCs — have all evolved because of selective pressure to extend the upper frequency limit of hearing[5]. These changes enabled the first nocturnal mammals with small heads to spatially localize the sounds made by other animals, especially calls of their young, on the basis of interaural intensity differences.

Here, we summarize recent discoveries of hair cell mechanisms underlying mechanoelectrical transduction and frequency tuning. We describe the newly discovered proteins responsible for the precise organization of the hair bundle, many of which have been revealed by studying the genetics of hereditary deafness[6] (BOX 3). We present recent evidence about the performance and molecular identity of the MET channel. Finally, we describe the basis of reverse transduction, which enables OHCs to act as force generators and contribute to cochlear frequency selectivity. A fuller understanding of hair cell mechanisms may settle the long-standing question of how the mammalian cochlea achieves its remarkable sensitivity and frequency discrimination.

The hair bundle

Actin filaments. The site of hair cell transduction is the hair bundle, an array of modified microvilli or stereocilia arranged like a staircase in ranks of increasing height (FIG. 3). Deflections of the bundle towards its taller edge bend the stereocilia at their tapered base and open the

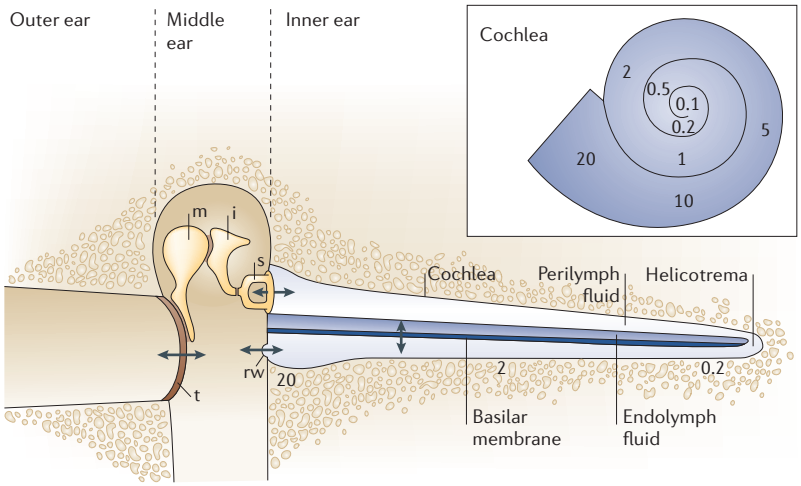


Figure 1 | **The sound conduction pathway.** A section through the temporal bone showing the sound conduction pathway in the mammalian ear. Vibrations of the eardrum or tympanum (t) are relayed via three middle ear bones, the malleus (m), incus (i) and stapes (s), and initiate pressure waves in the cochlear fluids, the pressure being relieved at the round window (rw). The pressure waves set in motion the basilar membrane, on which the organ of Corti and hair cells ride. The cochlea is divided into compartments filled with fluids of distinct ionic composition. Perilymph is similar to extracellular fluid, but endolymph, which is found in the central compartment above the tops of the hair cells, contains a high K^+ concentration and a small amount of (20 μM) Ca^{2+}. The cochlea is shown as straight to illustrate its internal structure, but it is normally coiled as in the inset. Different sound frequencies differentially excite different regions of the cochlea, the specific locations being given in kHz from 0.1 to 20 kHz in humans. Note that the frequency map is logarithmic, so that each decade occupies an equivalent distance on the basilar membrane. The components are drawn roughly to scale for the human ear, in which the cochlea is 35 mm in length.

MET channels, whereas displacements in the opposite direction close these channels[7]. Owing to this directional sensitivity, sinusoidal motion of the bundle about its resting position, as elicited by a pure tone, modulates the likelihood of channel opening to generate an electrical representation of the sound stimulus. Several of the molecular pathways that endow the hair bundle with its precise architecture revolve around filamentous actin, polymers of β- and γ-actin, both of which are present in the stereocilia[8]. Stereocilia are larger versions of the microvilli on many epithelial cells and have a core of actin filaments[9], crosslinked with espin and fimbrin[10], spanning their length from tip to base. Most of the filaments terminate in the lower shaft of the stereocilium, where they are probably anchored to the membrane by actin-binding proteins such as radixin[11]. The distribution of actin makes the stereociliary body rigid but enables it to pivot at the base by flexure of ~10% of the filaments that traverse the ankle region. These filaments are reinforced by tropomyosin and other, unidentified proteins, which appear as an osmiophilic rootlet in electron micrographs of OHCs (FIG. 3a; REFS 12,13).

The precise structure of the hair bundle is dynamically maintained by regulating the turnover of the actin filaments. Transfecting cultured hair cells with β-actin made visible by coupling to green fluorescent protein has shown that actin monomers are constantly being added at the tips of the stereocilia[14]. Over the course of about 2 days, the newly incorporated actin monomers make their way down each filament and dissociate at its bottom end in a process known as treadmilling (note that turnover rates were measured in developing neonatal animals and might be different in adults). Remarkably, the speed of treadmilling is proportional to the length of the stereocilium, so that in a bundle with three rows of different heights, actin filaments in all rows are recycled together[15]. The steepness of the staircase is therefore preserved by the relative rates of actin polymerization in the stereocilia of each row. The same mechanism might determine the sixfold increase in maximum stereociliary height from the base to the apex of the cochlea.

A central agent in the control of treadmilling is myosin XVa, a cousin of the myosin that powers skeletal muscle contractions. Antibody labelling in high-resolution microscopy has shown that myosin XVa is restricted to the tips of the stereocilia, the site of actin incorporation[15,16]. Furthermore, its concentration is greatest in the tallest stereociliary rank. Evidence that myosin XVa is necessary to determine stereociliary length comes from studies of shaker 2 mice, which have a mutation in this non-muscle myosin. Cochlear hair cells of shaker 2 mice lack myosin XVa expression and have abnormally short stereocilia[16]. Other molecules might assist myosin XVa in regulating actin polymerization in the stereocilia: for example, whirlin, which contains multiple PDZ domains, usually implicated in protein–protein interactions. Defects in whirlin also cause abnormally short stereocilia in whirler mice, which have hair cell pathology like that of shaker 2 mice[16,17]. The similar phenotype suggests that myosin XVa and whirlin interact functionally. Moreover, myosin XVa is required to transport whirlin to the tips of the stereocilia[16]. Whirlin might behave like a scaffolding protein and, through its PDZ domains, organize a group of proteins into a functional complex at the tip of the stereocilium to determine actin turnover and stereociliary length.

Interciliary links. Stereocilia within a hair bundle are interconnected by a series of fine extracellular filaments (FIG. 3). In mature mammalian hair bundles, adjacent stereocilia are connected along their shafts by side links, whereas tip links extend from the vertex of each stereocilium to the side of its taller neighbour[18–20]. Together, the links form an extracellular matrix coupling the stereocilia, so that when force is applied at the tip of the bundle all stereocilia rotate together, ensuring uniform excitation of the MET channels[21]. Clues to the molecular basis of the interciliary connections have been provided by studies of human Usher type I syndrome and equivalent mouse deaf mutants. These mutations result in hair bundle disorganization and splaying of the stereocilia. Three classes of protein affected in Usher's syndrome have been isolated using molecular genetics[22]. First, the cadherins (cadherin 23 and protocadherin 15), which are membrane proteins with extracellular domains that interlink, known to be important in cell adhesion. Second, intermediate coupling proteins (harmonin and SANS (Usher syndrome 1G homologue)), which, like whirlin, have PDZ domains to facilitate protein–protein crossbridging. Third, a second non-muscle myosin, myosin VIIa, which

Osmiophilic rootlet
The stem of a stereocilium that in electron micrographs is seen to be heavily stained by osmium, which probably indicates a dense concentration of protein.

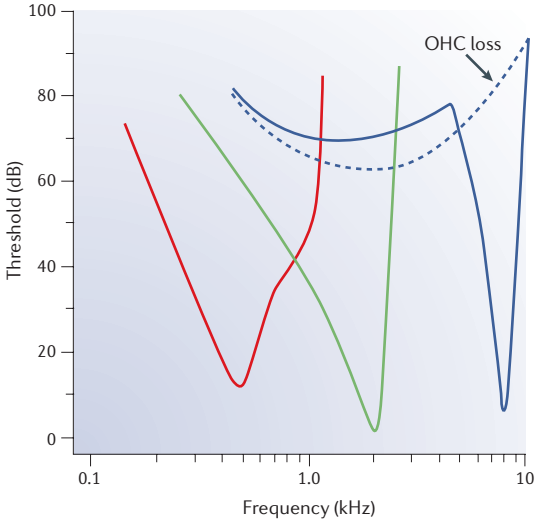
Shaker 2 mouse
A congenitally deaf mouse with a mutation in myosin XV that results in abnormally short stereocilia.

PDZ domain
A peptide-binding domain that is important for the organization of membrane proteins, particularly at cell–cell junctions. It can bind to the carboxyl termini of proteins or can form dimers with other PDZ domains. PDZ domains are named after the proteins in which these sequence motifs were originally identified (PSD-95, Discs large, zona occludens 1).

Box 1 | **Auditory frequency selectivity**

Frequency selectivity as applied to the auditory system describes its ability to distinguish different frequency components of a complex sound stimulus such as speech or music. As an example, when two strings on a guitar are plucked simultaneously, two distinct notes are heard corresponding to each of the vibrating strings. Each component reflects a sinusoidal vibration of the sound source, in this case the guitar, with a given frequency. Most sounds, even those emanating from a musical instrument, are composite, but can be represented as the sum of a set of sinusoidal components of different frequency and amplitude.

To represent a sound, the auditory system must encode the relative amplitudes of all the constituents. Frequency decomposition by the auditory system occurs largely in the cochlea, which separates the different components along its length, with high frequencies at one end and low frequencies at the other (FIG. 1). The different components then excite different hair cells, giving rise to action potentials in different auditory nerve fibres. The frequency selectivity of an auditory nerve fibre (or hair cell) can be mapped by determining its 'tuning curve' — the intensity of any sound frequency that will make just this fibre respond. This intensity is known as the threshold. The frequency selectivity of each nerve fibre is not perfect: a small range of frequencies is encoded at low sound levels, with the selectivity broadening as the sound gets louder. The tuning curves are therefore 'V' shaped, but each fibre has a particular frequency, its characteristic frequency (CF), at which the threshold reaches a minimum (at the tip of the 'V'). The cochlea contains hair cells and afferent nerve fibres with CFs that span the entire auditory range of the animal.



Examples of frequency tuning curves for three auditory nerve fibres are shown in the figure. The destruction of outer hair cells (OHCs) results in greatly elevated sound threshold and a deterioration of frequency selectivity, as shown for the neuron with the highest CF.

is thought to anchor the membrane complex to the actin cytoskeleton. Together, these three types of protein might form a functional unit for the side links, promoting cohesion of the hair bundle[22]. An additional role of the protein complex might be to keep the plasma membrane taut over the submembraneous cytoskeleton like a rubber glove stretched over the fingers of a hand. This is especially relevant for MET channel activation because it ensures that bundle deformation is rapidly transmitted to the channel without loss of mechanical energy in tissue distortion. Transduction would be impaired by loosening of the membrane, as occurs in mutations of myosin VIIa in shaker 1 mice. The hair bundles in shaker 1 mice need greater positive displacements before the MET channels start to open[23].

The tip links are thought to have a special role in transduction — one of transmitting force due to hair bundle deformation to MET channels. Because the tip links run roughly parallel to the hair bundle's axis of symmetry from the shorter to taller stereociliary rows, they are oriented to apply force during rotation of the bundle towards its taller edge, and to unload with deflection towards its shorter edge[18]. Two lines of evidence support the role of the tip links in transduction. First, exposing hair cells to submicromolar Ca^{2+}, buffered with a calcium chelator such as BAPTA, destroys the tip links[24] (probably because their structural integrity depends on association with Ca^{2+}), along with other interciliary links, and also abolishes transduction[25]. Second, experiments to localize MET channels (for example, using intracellular Ca^{2+} dyes to visualize Ca^{2+} influx through open channels) show that they are confined to the tips of the stereocilia in close proximity to the tip-link insertion[26,27]. Recent work has suggested that a Ca^{2+}-stabilized protein, cadherin 23, which is one of the proteins defective in Usher's syndrome, is also a component of the tip links based on its biochemical properties and on antibody labelling at the tips of the stereocilia[28,29]. However, there have been reports that cadherin 23 disappears in adult cochlear hair cells[30,31], so other proteins might be involved or able to substitute for cadherin 23. But the disparity might also reflect differences in expression levels or sensitivity of antibody detection. The molecular structure of the tip links and their connection to the MET channels has yet to be fully elucidated, but it is central to understanding how the channel is mechanically activated.

Whirler mouse
A mouse mutant that is unresponsive to sound and shows circling and head-tossing behaviour, indicative of both auditory and vestibular dysfunction. The mutated protein is known as whirlin and is involved in stereocilia elongation.

Usher type I syndrome
One of three subtypes (I,II and III) of hereditary disorder characterized by sensorineural deafness of cochlear origin combined with loss of vision due to retinitis pigmentosa.

The mechanoelectrical transduction channel

MET channel activation and adaptation. The hair bundle may be regarded as an accessory structure for transforming stimuli that occur during inner ear stimulation to the molecular scale suitable for gating the MET channel. Because of the bundle's geometry, there is a roughly tenfold reduction in displacement at the tip of the bundle to that at the end of the tip link. The probability of channel opening is modulated between fully closed and fully open by submicron displacements of the tip of the hair bundle (FIG. 4; REF. 3). Even with the loudest sound pressures, the hair bundles rock to and fro with amplitude no greater than the diameter of a single stereocilium, and at auditory threshold the bundles may move of the order of 1 nm — a stimulus approaching atomic dimensions.

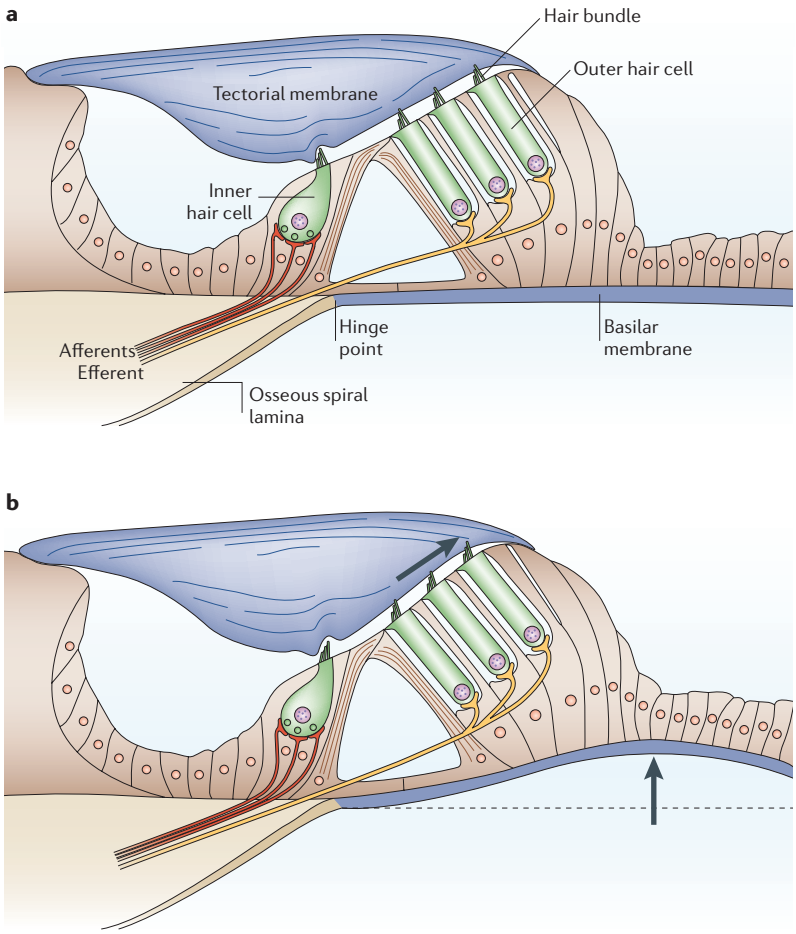


Figure 2 | **Cellular structure of the sound-detecting organ of Corti. a** | Transverse section through a middle turn of the cochlea, showing the organ of Corti, an assembly of intricately shaped supporting cells and inner and outer hair cells supported by a flexible basilar membrane. The organ of Corti is ~150 µm wide. **b** | Upward displacement of the basilar membrane stimulates the hair cells by bending their stereociliary bundles against the acellular tectorial membrane. Because of the point about which the basilar membrane hinges, the inner hair cells must be stimulated mainly by motion of the tectorial membrane. Signals from each inner hair cell are relayed to the brain via 10 to 20 afferent fibres of the VIIIth cranial nerve. Outer hair cells have both sensory and motor capabilities and possess electromotility that underlies the cochlear amplifier. They have a sparse afferent innervation (not shown) and are contacted mainly by efferent nerves, which regulate the electromotility and influence cochlear sensitivity.

The minimum auditory threshold in mammals occurs at a sound pressure of 20 µPa and, at this sound level, the displacement of the basilar membrane is ~1 nm (REF. 32). Little amplification is introduced by the geometry of the organ of Corti, and the displacement amplitude at the tips of the stereocilia is thought to be similar to that of the basilar membrane[33].

Much has been learned about the biophysical properties of the MET channel from electrical recordings of hair cells in *in vitro* preparations. It is a cation channel, most selective for Ca^{2+} but with measurable permeability to small organic cations whose size implies a wide-diameter pore[3,34–36]. This might explain its unusually large single-channel conductance of 100 pS or more[25,37,38]. It also displays ultrafast kinetics to hair bundle deformation[39,40].

Adaptation
The decline in response, and therefore sensitivity, to a sustained stimulus. Adaptation is a property of many sensory receptors that enables them to adjust their sensitivity to prevailing conditions and respond only to changes in stimulus intensity.

In response to a step deflection of the hair bundle, the MET channels open in ~100 ms, but then re-close in sequential phases of adaptation (FIG. 4; REF. 41). One phase occurs rapidly in a millisecond or two, but others take tens or hundreds of milliseconds. In response to a sustained displacement of the bundle, adaptation shifts the working limits of the MET channels in the direction of the imposed stimulus without reducing the maximum current. So, at least one function of adaptation is to keep the MET channels within the most sensitive part of their operating range. The fast component of adaptation might have other roles because in auditory organs its rate varies systematically with hair cell CF[37,42]. Original conclusions about the MET channel came from work on non-mammals, but most of these have been corroborated for mammalian cochlear hair cells[38,43–46]. However, the kinetics of MET channel activation and adaptation are more than an order of magnitude faster in mammals, consistent with a need to detect higher sound frequencies than non-mammals[46].

Stereociliary Ca^{2+} is a crucial signal for MET channel performance. Ca^{2+} enters through these channels, is buffered in OHCs by millimolar concentrations of the calcium-binding protein parvalbumin-β (REF. 47), and is extruded by a high density of the PMCA2a isoform of the plasma membrane CaATPase pump[48]. Changes in stereociliary Ca^{2+} affect both fast and slow phases of adaptation, and channel activation[41,46]. So, larger Ca^{2+} influx speeds up both activation and fast adaptation. Conversely, reducing external Ca^{2+} or raising intracellular Ca^{2+} buffering both extend the time course of channel activation and fast adaptation[42,46]. Owing to the submillisecond speed of these events, the likely target for Ca^{2+} is the channel itself or some neighbouring protein. Ca^{2+} might act on the MET channel directly to re-close it[25], or induce relaxation of an adjacent element in series with the channel to counteract the stimulus[49]. The time constant of fast adaptation varies inversely with hair cell CF: cells tuned to higher frequencies have faster adaptation[42,46]. One way to achieve this variation is by larger Ca^{2+} influx in cells with higher CF. This is implemented in turtle cochlear hair cells[37] by altering the unitary conductance of the MET channels from <100 pS at low CF to >300 pS at high CF: channels with larger conductance have greater Ca^{2+} currents and faster adaptation.

Although the exact mechanism for fast adaptation is unknown, there is strong evidence that slow adaptation is mediated by another non-muscle myosin, myosin 1c (REF. 50). Myosin 1c has been proposed to control tension in the tip links, and therefore force applied to the channel, by driving the upper attachment of the link along the stereociliary wall. A possible scenario is as follows. First, a cluster of myosin 1c, connected to the upper end of the tip link, ascends the actin backbone to sustain tension in the link. Following bundle deflection, Ca^{2+} binds to myosin 1c, causing it to dissociate from actin, which allows the link's attachment point to slip and so relieve tension on the channel. On removal of the stimulus, the MET channels close, stereociliary Ca^{2+} concentration drops, and myosin 1c climbs back up the stereocilium to re-tension the links. Support for the involvement of myosin 1c has come from mutating

Box 2 | **Inner hair cells versus outer hair cells**

Inner hair cells (IHCs) convert sound-induced motion of the cochlear partition into changes in membrane potential — receptor potentials — that immediately modulate neurotransmitter release at synapses on the auditory nerve afferents (FIG. 2). IHCs lack prestin and, therefore, any associated somatic contractility, and are thought to function solely as sensory elements. Recordings from IHCs have been made to determine their receptor potentials and frequency tuning curves in intact, anaesthetized animals[90,101]. However, compared with outer hair cells (OHCs), less is known about their mechanoelectrical transduction (MET) currents in response to direct manipulation of hair bundles. Available measurements indicate that IHCs have similar sensitivity and hair bundle mechanics[102,62], although it is not known whether they show fast adaptation. The hair bundles of IHCs are of comparable size and stereociliary complement to those of OHCs, but *in vivo* they are not embedded in the tectorial membrane[99] and are probably stimulated by motion of the surrounding fluid. As a consequence, they respond primarily to basilar membrane velocity rather than displacement[90]. This velocity coupling will behave as a high-pass filter similar to adaptation of the MET channels and eliminate the direct current (DC) response. The cut-off frequency is set by the mechanical compliance and viscous damping of the hair bundle. The large afferent supply to IHCs contrasts with that of OHCs, which have a sparse afferent innervation of unknown function. However, OHCs are contacted by efferent fibres with cell bodies in the brainstem whose axons make cholinergic inhibitory synapses[103] on OHCs. They might be part of a feedback pathway to regulate cochlear sensitivity. Electrical stimulation of the efferent axons reduces IHC receptor potentials, particularly near the characteristic frequency, and blunts their frequency tuning curves[104], a finding that is consistent with the idea that OHCs are mechanical effector cells.

its ATP-binding site to confer susceptibility to inhibition by certain ADP analogues. Expression of this mutant myosin 1c rendered slow adaptation in vestibular hair cells susceptible to block by ADP analogues introduced through the recording pipette[51]. Although not seen in those experiments, more recent work, in which greater expression of the mutant myosin 1c was achieved, shows that fast adaptation can also be suppressed in the mutant[52]. This surprising observation implies a link between the two forms of adaptation, and that fast adaptation also involves myosin 1c. But it cannot require myosin 1c to progress through its ATPase cycle because this would be too slow to account for time constants of fast adaptation of <100 ms seen in mammalian OHCs[46].

The molecular identity of the MET channel. Compared with other types of ion channel, such as voltage-gated K^+ channels, much less is known about the molecular makeup of the MET channel and how the stimulus energy is coupled to channel opening. One reason for this paucity of information is the small number of channel protein molecules expected in a single cochlea. There may be no more than 200 functional channels per cell (roughly twice the number of stereocilia[26,37]) and 10,000 hair cells per cochlea. Only recently has a likely candidate for the MET channel been identified as a member of the TRP channel family. TRP channels, so named for the consequence of their mutation in *Drosophila melanogaster* photoreceptors (transient receptor potential), are distant relatives of voltage-gated K^+ channels, and many have a high Ca^{2+} permeability and large unitary conductance comparable with that of the MET channel[53]. A search of this family has revealed a TRPA1 isoform localized to mammalian hair cells[54]. TRPA1, also known as ANKTM1, was previously found in nociceptive neurons, where it is activated by cold temperatures[55]. Evidence that it is a component of the MET channel was obtained by reducing its expression in cultured mammalian hair cells. This was done by transfecting hair cell cultures with a small interfering RNA that would block translation of the endogenous message, a procedure that, after a few days, reduced the size of the MET currents. Expression of TRPA1 in HEK293 cells produced channels with a unitary conductance (50–100 pS) and a pharmacological profile similar,

Box 3 | **Hereditary hearing loss**

Hearing impairment is the most common disabling sensory defect in humans. Severe to profound hearing loss affects 1 in 1,000 newborns, and another 1 in 2,000 children before they reach adulthood, and 60% of people older than 70 years have hearing loss of at least 25 dB (REF. 105). Sensorineural hearing loss, in which there is damage to the inner ear, is the most prevalent form of hearing impairment. It has a range of causes, including noise-induced and drug-induced damage, as well as being age-related, resulting in wide variations in age of onset and in the sites and severity of the lesions. At least 60% of hearing loss may have a genetic basis, a significant proportion of which is non-syndromic (that is, no other pathology seems to be associated with the same gene), and most of these genes are inherited in an autosomal recessive mode (see Hereditary Hearing Loss Homepage).

Genetic studies of families with hereditary hearing loss have led to the discovery of at least 40 genes associated with non-syndromic hearing loss, and more are being found each year. Such studies are proving to be a rich source of information about which molecules are important in the inner ear[6], as well as giving hope for new therapeutic approaches. These include strategies to prevent hair cell loss by gene manipulation, through to investigations of the use of stem cell technology as a treatment for cell loss. It has also been recognized that differences in susceptibility to ototoxic agents could be a result of the variety of genetic backgrounds displayed by the human genome. A case in point is streptomycin, which probably enters hair cells through the mechanoelectrical transduction (MET) channel[106] and targets the mitochondria, culminating in cell death. Hypersensitivity to the ototoxic effects of streptomycin is associated with a point mutation in a ribosomal subunit of the mitochondrial DNA[107].

Time constant
The time required for the response of a system to decline to 37% of its initial value. For the cell membrane, this is the product of its capacitance and resistance, setting the timescale over which membrane currents change the voltage. A small time constant means that a system's response can change rapidly.

Unitary conductance
The electrical conductance of a single ion channel: the current flowing through the channel divided by the voltage applied across the cell membrane. Unitary conductance is a characteristic channel property but varies according to the ion species present.

Ototoxic agents
Procedures or drugs that damage hearing, primarily by acting on the hair cells.

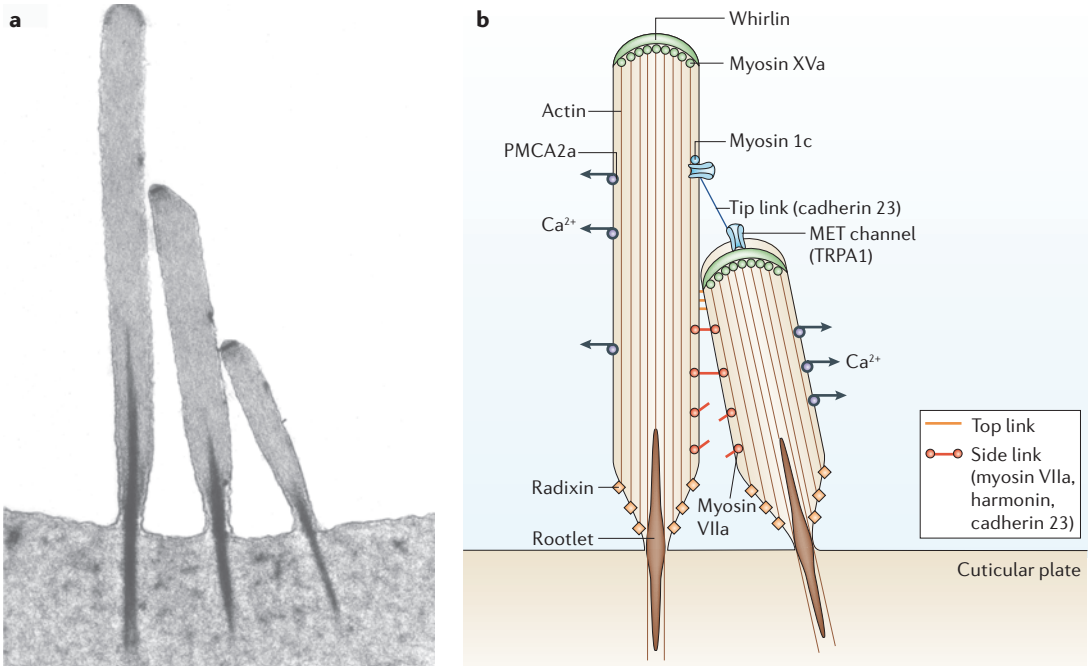


Figure 3 | **Structure and protein composition of the stereociliary bundle. a** | Transmission electron micrograph through the three rows of stereocilia on a guinea pig outer hair cell showing the osmiophilic regions at the stereociliary tips and side walls (the insertions of the tip link), and at the rootlets. The diameter of the tallest stereocilium is ~0.25 µm. The hair bundle of a mammalian outer hair cell contains 30 to 100 stereocilia and has a maximum height of between 1 and 6 µm according to its location[99]. **b** | Putative locations of some of the protein constituents of the stereocilia. Some of these (for example, myosin 1c) were first identified in vestibular hair cells and their location has not yet been confirmed in cochlear hair cells. MET, mechanoelectrical transduction; PMCA2a, plasma membrane CaATPase pump; TRPA1, transient receptor potential channel A1.

although not identical, to that of the MET channels[56]. Definitive evidence that TRPA1 is an integral part of the MET channel is still needed, and might be provided by the generation of a TRPA1 knockout.

Even if TRPA1 is the MET channel, it is unlikely to be the only isoform in mammalian hair cells, because to generate a range of MET channel properties (such as variation in unitary conductance or kinetics, as seen in the turtle) probably requires at least one other main (α) subunit or an auxiliary (β) subunit. TRP channels are tetrameric, with four α-subunits, like voltage-gated K^+ channels, and mixing two α-subunit variants could theoretically generate five species of heteromeric channel[57] with properties intermediate to channels formed from each of the parent subunits alone. Another potential α-subunit is TRPML3, which, like TRPA1, localizes to the hair cell cytoplasm and stereocilia[58]. This channel has not been fully characterized, but its mutation is responsible for early-onset hearing loss in the varitint-waddler mouse[58]. MET channel properties may also depend on the local membrane environment. The presence of the anionic lipid phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$) in the apical membrane of the stereocilia is important for transduction, and depletion of this lipid slows the rate of adaptation[59]. $PtdIns(4,5)P_2$ might act by increasing the Ca^{2+} affinity of the binding site on the MET channel or of another protein involved in the adaptation mechanism.

Varitint-waddler mouse
A mouse with deafness, vestibular and pigmentation defects due to mutation in the mucolipin 3 gene, which encodes the ion channel protein TRPML3. It is associated with progressive hair bundle disorganization and hair cell degeneration.

The gating spring. Following provisional identification of one subunit of the MET channel, an important step is to elucidate how the channel connects to the tip link on the external face of the membrane and is anchored to the intracellular cytoskeleton. Knowledge of the protein attachments will provide insight into how the force is exerted across the channel, an important factor in determining the speed of activation. A key premise of existing models of transduction is that the MET channels are opened by force applied by elastic elements, the 'gating springs', which are stretched when the hair bundle is deflected. One end of the spring is pulled by displacement of the hair bundle, the other end is attached to the channel's hypothetical gate. It seems unlikely that the gating springs correspond to the tip links, whose coiled double-helical structure suggests that they are inextensible and are probably rigid connections for force transmission[60]. Alternative sites for the gating spring lie in the channel itself or its coupling to the cytoskeleton.

An important implication of the gating spring model is a reciprocal relationship between channel conformational changes and force. A mechanical stimulus opens the channel, but, conversely, opening the channel can itself generate force. A consequence of the model is that the force–displacement relationship of the hair bundle is nonlinear over the range of bundle positions at which the channel is activated: it predicts that the bundle will not behave like a simple spring for which extension is

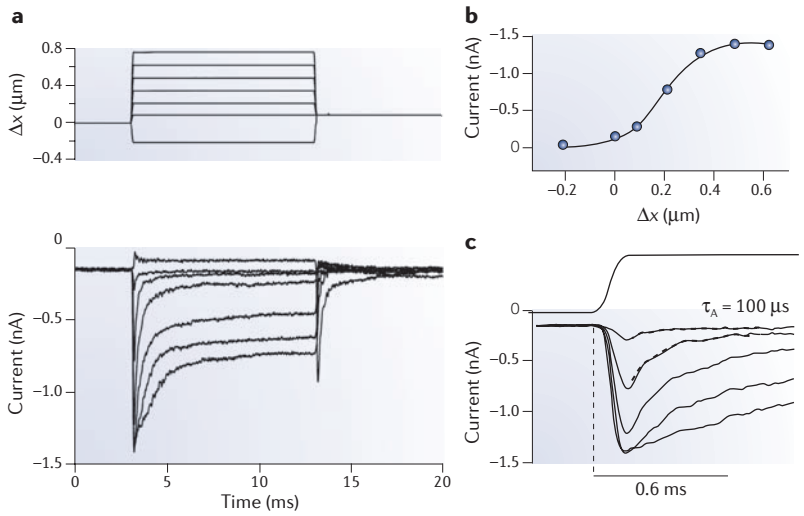


Figure 4 | **Mechanoelectrical tranduction currents in outer hair cells.** **a** | Mechanoelectrical transduction (MET) ion channel currents measured at a –84 mV holding potential from a rat outer hair cell in response to displacements of the tip of the hair bundle (Δ*x*). **b** | Sigmoidal relationship between peak current and bundle deflection. **c** | MET current onsets, showing the rapid activation of the response and the adaptation, which has a time constant (τ_A) of ~100 μs for small responses.

proportional to applied force. So, if a force (F_B) is applied to the tip of the bundle, the resulting displacement (X) is given by[61]:

$$F_B = X \cdot K_s - A \cdot p_O(X) + F_o \quad (1)$$

where K_s is the passive linear stiffness, p_O is the probability of opening of the MET channels, and F_o and A are constants. In the gating spring model, A is the product of the single-channel gating force and the total number of channels per bundle. The negative term in equation 1 signifies an active component in which channel gating generates a force in the same direction as the imposed displacement, causing hair bundle stiffness to decrease with channel opening[61] to reach a minimum when p_O is ~0.5. The existence of such a nonlinearity has been verified from measurements of bundle stiffness in various hair cell preparations[61–64]. These are performed by applying force stimuli with a flexible glass fibre, and by monitoring the resulting movements using photodiode imaging of the bundle or the attached fibre. One implication of the gating spring model is that effects of adaptation are manifested not only in channel opening, but also in bundle motion. So, fast adaptation triggered by Ca^{2+} influx is accompanied by active motion of the hair bundle[65,66]. Because the time constant of adaptation, and therefore the speed of the hair bundle's mechanical response, is matched to hair cell CF, this process might contribute to frequency tuning[66].

Photodiode imaging
The stimulator casts a shadow on a pair of photodiodes and, as it moves, the light on one photodiode increases while that on the other decreases. The difference in photocurrents can be used to measure displacements down to a few nanometres.

Cochlear frequency selectivity

A remarkable feature of auditory sensation is its frequency selectivity (BOX 1) — the ability of the cochlea to separate the acoustic frequencies along its length like an acoustic prism. As a consequence, at low sound levels each hair cell conveys information about a narrow frequency band. The origin of frequency selectivity has fascinated both biological and physical scientists for more than 100 years, but is still not fully understood. It relies partly on mechanical resonances of the basilar membrane. Sounds of different frequencies evoke localized patterns of vibration at different positions along the membrane, high frequencies near the base of the cochlea and low frequencies towards the apex[1,32] (FIG. 1). One manifestation of mechanical tuning is the correlation between auditory frequency range and cochlear size. Elephants, which have low-frequency hearing extending to just 10 kHz, have a long, 60 mm cochlea (like a tuba), whereas mice hear to 90 kHz and have a short, 7 mm cochlea (like a piccolo). Humans are in between, with a 35 mm cochlea and a 20 kHz auditory range[1]. Measurements of basilar membrane motion have been increasingly refined[67] since von Békésy's original observations of basilar membrane tuning in human cadavers, and now demonstrate a narrow mechanical tuning closely resembling that of the electrical signals of IHCs and auditory nerve fibres[32]. At least part of this tuning is passive, underpinned by longitudinal gradients in the stiffness and dimensions of the cochlear partition[1,68]. However, there is substantial evidence that the passive tuning of the basilar membrane is highly augmented by an active input from the hair cells that is contingent on their transducing ability. Both experimental and theoretical arguments have led to a consensus that the cochlear amplifier is attributable to the generation of force by OHCs during excitation to oppose the viscous damping of the cochlear partition[4,32,69,70]. The most compelling evidence for this idea is that damage to or loss of the OHCs or interference with their MET currents causes an elevation in threshold and deterioration in cochlear frequency selectivity.

The somatic motor. The prevailing view is that OHCs produce force by longitudinal contractions of their cell body, the somatic motor, driven by changes in membrane potential (FIG. 5). Individual OHCs, dissociated from the cochlea and stimulated electrically, shorten in response to depolarization and extend for hyperpolarization[71,72]. Because of the speed of the mechanical response, these cells can theoretically change their shape in synchrony with the sound stimulus for frequencies spanning the entire auditory range. Although behaving like diminutive muscle fibres, OHCs use a quite different contractile mechanism from the ATP-driven actomyosin reaction in muscle. This involves a membrane protein, prestin[73], which belongs to a solute carrier (SLC26) family of anion transporters and is densely packed in the lateral membrane of OHCs[74]. Whether prestin transports anions is unclear[75], but the presence of an anion-binding site is believed to bestow voltage-sensitivity on the protein[76]. So, hyperpolarization promotes binding of intracellular Cl^- ions, inducing a conformational switch in prestin that expands its surface area and causes cell elongation. In support of this mechanism, it has been shown that substituting other anions for intracellular Cl^- changes the voltage dependence of the electromotile process. The

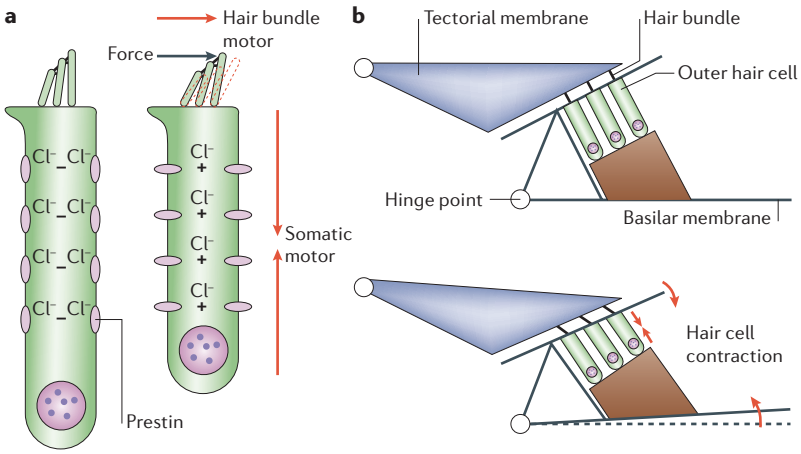


Figure 5 | **The putative motors of outer hair cells.** Outer hair cells can generate force, mechanically boosting sound-induced vibrations of the hair bundle and augmenting frequency tuning. Two mechanisms have been advanced to explain this cochlear amplifier: the somatic motor and the hair bundle motor. **a** | In the resting state (–), Cl^- ions are bound to prestin molecules in the lateral membrane of the hair cell. When force is applied to the hair bundle, the cell is depolarized (+), the Cl^- ions dissociate and the prestin changes conformation, reducing its area in the plane of the membrane and shortening the hair cell body (the somatic motor). Adaptation of mechanoelectrical transduction (MET) channels, which are activated by bending of the stereocilia at their tapered base, also causes the hair bundle to produce extra force in the direction of the stimulus (the hair bundle motor). The amplitudes of the hair bundle movements have been exaggerated to illustrate the concept. **b** | Schematic diagram showing the effects of the somatic motor (red arrows) on the organ of Corti mechanics[100], which leads to downward motion of the reticular lamina (the upper surface of the organ of Corti) and a negative deflection of the hair bundle. This is a negative feedback pathway, as a positive deflection of the hair bundle causes outer hair cell depolarization, cell contraction and opposing motion of the bundle.

importance of prestin is underscored by construction of mutations that reduce its expression. Targeted deletion of prestin in mice causes loss of OHC electromotility and a 100-fold or more reduction in cochlear sensitivity[77] without affecting forward mechanoelectrical transduction[78].

There is a major unresolved problem with the somatic motor as the site of the cochlear amplifier. Because it is voltage-dependent, cell length is controlled by receptor potentials that result from hair bundle motion. At high frequencies, the receptor potential is low-pass filtered by the membrane time constant, the product of membrane resistance and capacitance. Minimum time constants of a few tenths of a millisecond have been reported[79,80], which implies that the periodic component of the receptor potential will be attenuated in proportion to frequency above ~1 kHz. So, although somatic motor transitions are potentially very fast[81] and extend up to 80 kHz, their recruitment must be limited by the size of the driving voltage. The roll-off with increasing frequency might be partially offset by a larger size of MET current[45,46] as in turtles, and a greater density of prestin[82] in OHCs with high CF. However, there is a more stringent demand on the active process, because, in order to cancel a frictional force proportional to basilar membrane velocity, force production by the OHCs might need to increase with frequency rather than decline. A way of circumventing the time constant limitation has been proposed, by activating the prestin with extracellular potentials instead of the usual excursions in intracellular potential[83]. Extracellular potentials are generated by flow of transduction currents summed over a group of hair cells and are not attenuated by the OHC membrane time constant. However, measurements of these voltages adjacent to the OHC indicate that they are at most a few millivolts even at high sound levels[84], which might be insufficient to affect prestin. Although a small periodic component of the receptor potential will remain at high frequencies, unless a new mode of prestin gating is invoked[75], the simplest hypothesis is that the somatic motor is restricted to operating on a cycle-by-cycle basis at frequencies less than a few kilohertz. This is not to say that somatic motility is useful only at low frequencies. At frequencies above that imposed by the membrane time constant, prestin would be controlled by sustained depolarizing receptor potentials, acting as another form of adaptation to keep the hair bundle in its most sensitive working range. Such negative feedback by prestin activation (FIG. 5b) would decrease the proportion of MET conductance at rest and reduce the direct current (DC) component of the OHC receptor potential. In line with this suggestion is the finding that changes in OHC membrane potential produced by current injection can alter the operating range of the MET channels: depolarization, which would cause OHC contraction, decreased the fraction of channels open at rest, equivalent to adaptation[85].

The hair bundle motor. An alternative mode of amplification arises from force generated by the hair bundle[86,87]. Active hair bundle movements have been well documented in non-mammalian hair cells, where they have been linked to the two phases of MET channel adaptation. The most prominent movement in turtle auditory hair cells closely parallels fast adaptation[66]. A positive deflection of the bundle opens MET channels and increases stereociliary Ca^{2+}, which re-closes the channels and causes the bundle to spring back, opposing the stimulus. This has been referred to as a 'twitch' or recoil[65,66], and it has sufficient speed to operate at frequencies in the auditory range. The time constant of the mechanical response, like that of fast adaptation, varies with hair cell CF and is smaller in cells tuned to higher frequencies. The mechanism follows from the gating spring model of transduction, in which channel opening increases hair bundle compliance: Ca^{2+}-evoked re-closure of the channel decreases compliance, causing the bundle to move back towards its original resting position. The hair bundle motor is probably controlled by conformational changes in the MET channel protein, and so, unlike the somatic motor, is not limited by the membrane time constant, only by the kinetics of the channel activation and adaptation. However, compared with the somatic motor, the force developed may be small. According to the gating spring model (equation 1) the maximum force is *A*, the product of the single-channel gating force (~0.5 pN) and the total

Low-pass filter
A filter that suppresses all frequencies above a certain point known as the cut-off frequency.

Periodic component
A repetitive waveform: for example, a sine wave.

Compliance
The inverse of stiffness; the displacement of an elastic element produced by a known force.

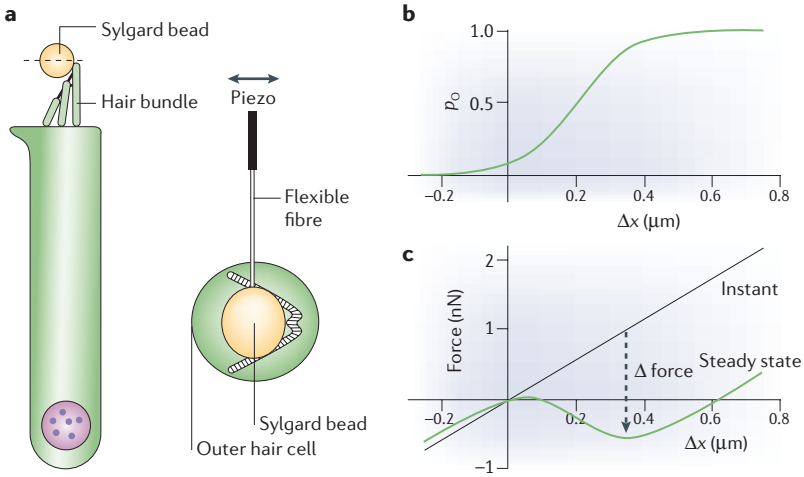


Figure 6 | **Mechanical properties of an outer hair cell bundle. a** | A method of measuring hair bundle mechanics. The outer hair cell bundle is deflected with a Sylgard bead on the tip of a flexible glass fibre whose fixed end is deflected by a piezoelectric actuator (piezo). Hair bundle motion is inferred from the change in photocurrent in a photodiode array (not shown) on which the image of the bead is projected. **b** | Mechanoelectrical transduction (MET) channels increase their probability of opening (p_O) with positive hair bundle displacement (Δx). **c** | Relationship between applied force and bundle displacement. At early times (<0.1 ms; instant) the bundle behaves like a simple spring in which displacement is approximately proportional to force. At later times (1 ms; steady state), with the adaptation of the MET channels, the force–displacement relationship becomes nonlinear. Both plots have been calculated from equation 1 (main text) using the p_O–Δx relationship shown in **b**, a passive stiffness, *K*, equal to 3 mN m^{-1} and values for the constant *A* of 100 pN (instant) and 2,000 pN (steady state). Note that in this description, *A* is a function of time, whereas in the original derivation of equation 1, *A* was a constant. The deviation between the instantaneous and steady-state plots represents the force developed by the bundle. The time course of force production mirrors that of fast adaptation.

number of channels (200)[61,63]. This sets an upper limit of 100 pN, less than a tenth of that generated by the somatic motor. The peak force produced by electrically stimulating isolated OHCs has been estimated as ~100 pN per millivolt change in membrane potential[88,89]. As maximum receptor potentials above 20 mV have been recorded in OHCs[45,90], the force produced by the somatic motor is more than 2,000 pN. The active bundle movements characterized in lower vertebrates might be the primary source of mechanical amplification in the avian cochlea[5], where force generation is summed from up to 12 rows of short hair cells that are equivalent to mammalian OHCs but lack prestin or any sign of somatic motility[91,92].

Although OHCs in mammals, similar to those in frogs and turtles, show fast adaptation, until recently there has been no evidence that they could generate forces comparable to those seen in non-mammals. However, new experiments on rat OHCs have revealed the presence of a strong force generator[93]. The force–displacement relationship of the hair bundle was found to be nonlinear, the bundle becoming increasingly compliant with MET channel gating (FIG. 6). Furthermore, the nonlinearity developed with a time course similar to that of fast adaptation of the MET current. This phenomenon differs from the process linked to adaptation in non-mammalian hair cells in two respects: it has opposite polarity (equivalent to positive feedback) and it generates much larger forces. Maximum force generation, estimated as the difference between steady state and instantaneous force–displacement plots, is >500 pN. The time course of force production is similar to that of adaptation, and, like adaptation, is slowed by lowering the extracellular Ca^{2+} concentration. The temporal limits of this mechanical process are unknown because of the limited speed with which forces can be delivered to the hair bundle using the flexible-fibre technique. However, fast adaptation time constants of <50 μs have been measured in rat OHCs, and when corrected for *in vivo* conditions they might be substantially faster[46]. So, even in the relatively low Ca^{2+} concentration of 20 μM in cochlear endolymph, prominent fast adaptation is present with a submillisecond time constant that varies with CF[46]. When these time constants are extrapolated to the conditions prevailing *in vivo* (for example, temperature and endolymphatic potential), cut-off frequencies similar to the CF are predicted[46].

At present, the mechanism of force generation is unknown, but its speed and relationship to adaptation implicate the mechanotransduction apparatus as the source. The OHC hair bundle motor might be sufficiently powerful to complement forces generated by the somatic motor, especially at high frequencies at which the intracellular receptor potential is attenuated. This conclusion is bolstered by measurements of IHC bundle motion in an *in vitro* cochlear preparation, which show a nonlinear mechanical amplification dependent on the Ca^{2+} influx through MET channels[94]. So, vibrations of the organ of Corti evoked by acoustic stimulation were augmented by a mechanism requiring only Ca^{2+} current to flow through the MET channels, which would generate meagre receptor potentials unable to activate prestin.

Conclusions

During the past decade, important advances have been made in uncovering the molecular basis of auditory sensation, but crucial steps in the transduction chain still need to be determined. One is the complete characterization of the MET channel and its attachments to the cytoskeleton and the tip link. This may clarify how force is delivered to the channel to explain its sensitivity and speed, enabling the hair cell to detect bundle vibrations with subnanometre amplitude and microsecond delay. How many different channel subunits are needed to produce a variation in channel conductance with CF? A second area is apportioning the relative contributions of the OHC bundle motor and the somatic motor in cochlear amplification. The simplest hypothesis is that both mechanisms are important[95], but perhaps their relative contributions vary with frequency. Such division of labour might accord with the change in form of the neural tuning curves with increasing frequency[96]. How much does hair bundle[97] or somatic stiffness[87,98] contribute to the mechanical properties of the cochlear partition and how does the organ of Corti deform during

Sylgard bead
A drop of silicone rubber, a mixture of a base and a curing agent, that is deposited on the tip of a glass fibre while liquid, then polymerized and hardened by heating.

Piezoelectric actuator
A ceramic crystal that deforms when a potential difference is applied across it. Piezoelectric devices require large voltages to induce movements of a micron. Such devices are reversible and also generate a voltage in response to mechanical stress.

sound stimulation? Resolving these issues will require several lines of inquiry: defining the molecular basis of force generation by the hair bundle; exploring the micromechanics of the cochlear partition; and devising models that realistically incorporate OHC properties. It will also be important to distinguish the contributions of proteins such as myosin VIIa to hair bundle development from their role in the adult cochlea. Identifying the molecular constituents alone will not solve the problem, which will require a broader approach that incorporates both physiological measurements and modelling to dissect the cochlear machine.

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Competing interests statement
The authors declare no competing financial interests.

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