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Clues to the cochlear amplifier from the turtle ear

Robert Fettiplace, Anthony J. Ricci and Carole M. Hackney

Sound stimuli are detected in the cochlea by vibration of hair bundles on sensory hair cells, which activates mechanotransducer ion channels and generates an electrical signal. Remarkably, the process can also work in reverse with additional force being produced by the ion channels as they open and close, evoking active movements of the hair bundle. These movements could supplement the energy of the sound stimuli but to be effective they would need to be very fast. New measurements in the turtle ear have shown that such active bundle movements occur with delays of less than a millisecond, and are triggered by the entry of Ca^{2+} into the cell via the mechanotransducer channel. Furthermore, their speed depends on the frequency to which the hair cell is most sensitive, suggesting that such movements could be important in cochlear amplification and frequency discrimination.

In the vertebrate inner ear, sound stimuli are detected by hair cells of the cochlea via deformation of their mechanically sensitive hair bundles (Fig. 1). Although much has been learnt about this process over the past twenty years, several issues are still unresolved. One is the molecular identity of the mechanotransducer channel, a mechanosensitive ion channel responsible for converting vibrations of the sensory hair bundles into electrical signals[1–3]. Another is the precise mechanism underlying cochlear amplification, whereby sound-induced vibrations of the cochlear membranes are boosted by energy supplied by the hair cells (Fig. 1). This amplification is necessary to overcome the damping effects of the cochlear fluids, and is central to explaining not only the cochlea's high sensitivity but also its frequency selectivity, whereby each cell responds best to a narrow range of sound frequencies[4]. A variety of evidence implicates the outer hair cells as the site of force-generation in the mammalian cochlea[4], and the discovery that these cells can contract rapidly in response to changes in membrane potential has raised a possible mechanism for this process[5–7]. However, studies on lower

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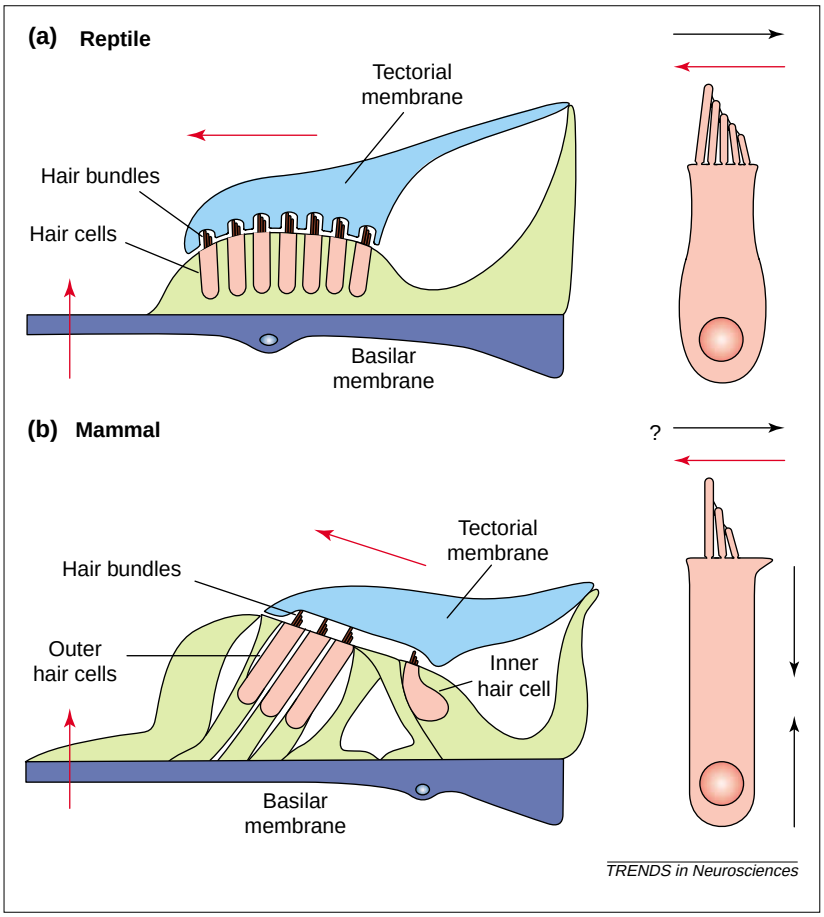

Fig. 1. The twin functions of cochlear hair cells. Reptiles such as the turtle (a) have one type of hair cell but mammals (b) possess two types, inner hair cells and outer hair cells with different functions. Sound stimuli vibrate the basilar membrane causing to-and-fro motion of the sensory hair bundles relative to an overlying gelatinous flap, the tectorial membrane. Red arrows show the direction of motion for excitation, which bends the hair bundle towards its taller edge and opens mechanotransducer channels. This allows entry of K^+ and Ca^{2+} ions and depolarizes the hair cell. Hair cells can also mechanically amplify the sound energy, thus greatly improving cochlear sensitivity. A ubiquitous amplification mechanism might involve the mechanotransducer channels generating force as they open and shut, which is transmitted back to the hair bundle to reinforce its vibration. An additional mechanism exists only in mammals where the outer hair cells contract in response to stimulation, thus augmenting the passive vibrations of the basilar membrane. The black arrows denote the direction of force produced in response to excitatory stimuli. The bundle amplification has not yet been seen in mammals. A single hair cell is shown on the right-hand side of the figure.

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vertebrate hair cells have shown that the hair bundles can make active movements too[8–11], but neither the mechanism nor the role of these movements is fully understood. It has been proposed that they might increase auditory sensitivity by amplifying the hair bundle vibrations to incoming sounds[12,13]. Active bundle movements might also be the origin of otoacoustic emissions[14], in which the ear spontaneously radiates sound energy. The fact that such emissions occur in all vertebrates studied, including mammals, might indicate a common mechanism of hair cell amplification across the vertebrate kingdom. The active bundle movements are intimately related to adaptation of the mechanotransducer channels[15]. These channels are permeable to most cations but have a particularly high selectivity for Ca^{2+} (Refs 1,2,16,17), which is important for regulating both adaptation and the active movements. This review focuses on recent evidence in support of force-generation by the hair bundle[15], its link to adaptation and its potential role in cochlear amplification.

Hair bundle structure and mechanics

The hair bundle is composed of between 20 and 300 modified microvilli or stereocilia arranged in rows of increasing height (Fig. 2). An extracellular matrix of fine links interconnects the stereocilia[18–22] so that when the tip of the bundle is deflected, each stereocilium pivots around its base and the ensemble moves as a whole[3,23]. One category of links, the tip links, has been suggested to transmit force to the mechanotransducer channels[18]. The exact location of these channels in relation to the links is still debatable, but most evidence places them towards the tips of the stereocilia[24,25]. An alternative suggestion is that the channels lie in a contact region just below the stereociliary tips, a region where short lateral connections can be seen between the membranes of adjacent stereocilia[26,27]. Either way, the mechanotransducer channels respond to stereociliary shearing[27,28]: deflection towards the tallest row of stereocilia results in opening of the channels and deflection towards the shortest row results in closing of the channels[1–3].

The compliance of the hair bundle, similar to the compliance of a spring, represents the amount of deformation induced by a given force. Motion of the bundle resulting from the force of the acoustic stimulus is shaped by the bundle's compliance, which has a passive component attributable to the flexibility of the stereociliary ankles[8] and the interstereociliary connections[28]. It also has an active component known as the 'gating compliance' linked to the opening and closing of the transducer channels[29–31]. Thus, molecular rearrangements of the channel during activation can exert force on the bundle via the interstereociliary connections and, in turn, influence its motion. Consequently, bundle compliance behaves in a non-linear way, increasing as the transducer channels open, reaching a maximum when the probability of opening of the channels is about 0.5 and then decreasing again at larger open probabilities[29]. An important repercussion of these findings is that factors influencing channel activation might also move the hair bundle.

Two components of transducer adaptation

The hair cell is exquisitely sensitive to mechanical stimuli, with displacements of approximately 100 nm at the tip of the bundle being sufficient to open all the transducer channels. In hair cells, as in other sensory receptors, an adaptation mechanism is required to maintain the channels within a narrow operating range and optimize their sensitivity to incoming stimuli[3,32,33]. If the bundle is pushed towards its taller edge, the channels first open, allowing an influx of K^+ and Ca^{2+} ions, and then almost

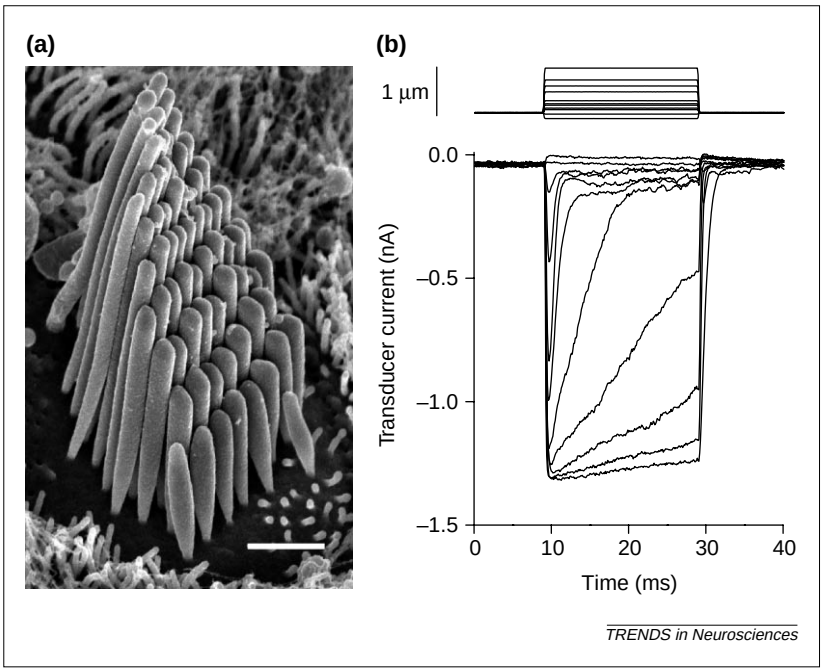

Fig. 2. Mechanosensitivity of an auditory hair cell. (a) Scanning electron micrograph of a hair bundle on a turtle hair cell, showing the increase in height of the stereocilia across the bundle, the close apposition of the stereocilia towards their tips and the narrowing at their bases. Scale bar, 1 μm. (b) Family of mechanotransducer currents recorded from a voltage-clamped turtle hair cell in response to step displacements of the hair bundle. Positive deflections towards the tallest stereociliary row evoke an inward transducer current at –80 mV holding potential. Note that for all but the largest stimuli, the current declines rapidly from its peak to a steady level. Slowing of the time course of decline in the current for large displacements might be a result of recruitment of a second adaptation mechanism.

immediately close again. This adaptation mechanism has been most extensively studied in the hair cells of lower vertebrates, such as the turtle and the frog. In turtle auditory hair cells, channel closure occurs in two stages: a fast adaptation on a sub-millisecond time scale predominates for low-level stimuli, with a slower process that extends over tens of milliseconds appearing for large stimuli[34,35] (Fig. 2b). Both components are susceptible to changes in extracellular Ca^{2+} or cytoplasmic Ca^{2+} buffer[34–36]. Moreover, the fast adaptation rate is directly proportional to the amount of Ca^{2+} entering via the transducer channels, implying that an elevation in intracellular Ca^{2+} is the trigger for channel closure[17]. Experiments on frog vestibular hair cells have largely focused on the slow component of adaptation[37,38] but these cells can also exhibit a fast component[9], suggesting that a two-stage adaptation mechanism is a general feature of hair cell mechanotransduction. The different balance between the fast and slow components in turtles and frogs might be partly as a result of the fact that the turtle hair cells are auditory and normally respond to higher frequencies of vibration compared with frog vestibular hair cells.

In one proposed mechanism for adaptation[9,37,38], the tension in the tip link is adjusted by moving its upper attachment point along the side of the stereocilium. The attachment coincides with an electron-dense patch (Fig. 3) that has been suggested to contain myosin Iβ (Refs 39,40). Ca^{2+} influx through a nearby mechanotransducer channel is postulated to detach the myosin molecules from the actin core of the stereocilium, allowing the link's attachment to slip. This would reduce the tension on the channels and close them. However, although a myosin-based mechanism might regulate bundle position on a slow time scale, it could not act quickly enough to account for the fast adaptation. Indeed, the fast adaptation observed in turtle hair cells is insensitive to inhibitors of myosin-based motors[34]. The speed of fast adaptation probably requires a direct interaction between Ca^{2+} and the mechanotransducer channel to modulate the probability of opening[41–43] (Fig. 4). The sub-millisecond time course of fast adaptation makes it improbable that this is mediated by enzymatic pathways such as phosphorylation. Because of the relationship between bundle compliance and channel-open probability (the gating compliance), channel closure following binding of Ca^{2+} might produce sufficient force to actively move the hair bundle.

Bundle movements and amplification

Active hair-bundle movements of between 1 and 100 nm have been described in several species and can occur spontaneously with amplitudes in excess of that expected for Brownian motion[8,11,13]. Such movements have also been observed as reactions to hair bundle displacements with compliant probes[8,9,15,44], and in response to changes in membrane potential, the effects of which might be secondary to alteration of Ca^{2+} influx[8,15,45]. The evoked movements fall into two categories with different time scales matching those of fast and slow adaptation. One includes fast responses that occur within a few milliseconds[8,15,44] (Fig. 5), the other embraces slower deflections extending over tens or hundreds of milliseconds. The slower type of movement might again involve the myosin-based motor proposed to regulate tip link tension and thus be related to slow adaptation[38]. However, the most conspicuous movements in turtle hair cells (Fig. 5) closely follow channel closure associated with fast adaptation, and can have a time constant as brief as 0.3 milliseconds[15]. The time course of the fast bundle movements, similar to those of adaptation, is slowed by reducing extracellular Ca^{2+}. Both processes might therefore result from the same mechanism of Ca^{2+} regulation of the mechanotransducer channels. If Ca^{2+} binds directly to the channels, the speed of adaptation and of the fast bundle movements might be limited only by the closing rate of the channels, which is ~0.1 ms in turtle hair cells[3]. Of the two kinds of active hair-bundle movements, only the fast one is fast enough to work on each cycle of the waveform at the frequencies used by the auditory system.

Channels that are opened during extrinsic bundle deflection enable Ca^{2+} ions to enter and bind to the

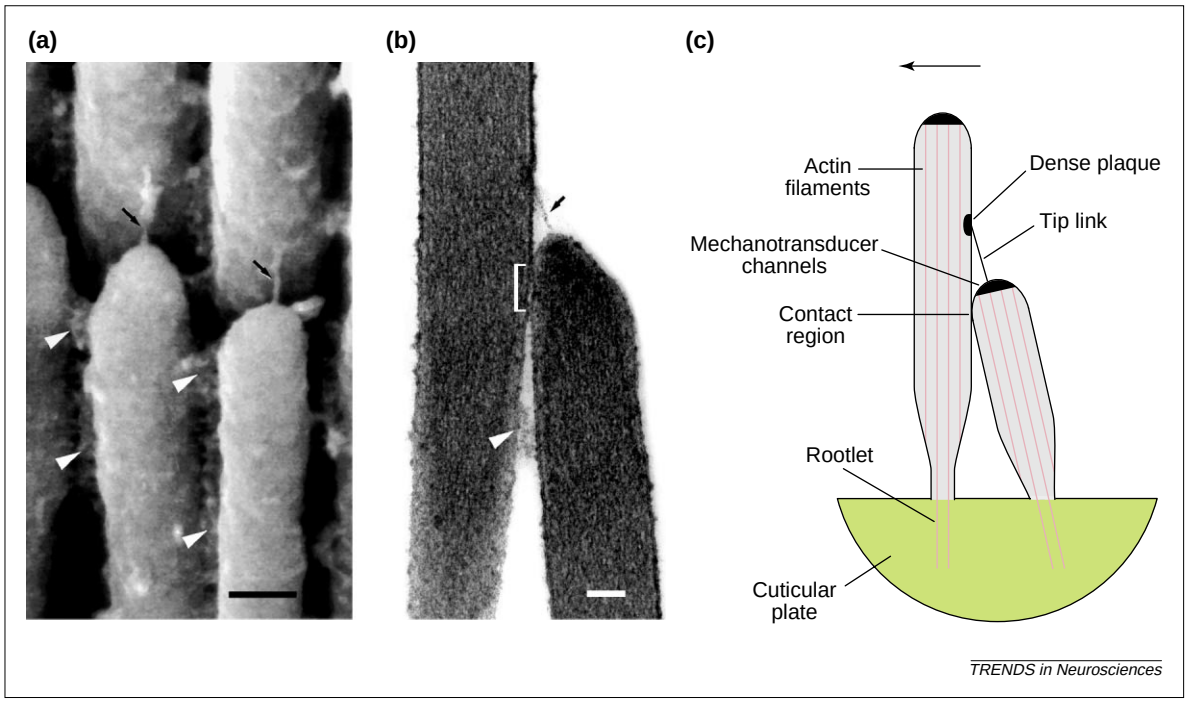

Fig. 3. Interstereociliary connections. (a) Field-emission scanning electron micrograph[21] and (b) transmission electron micrograph[27] of stereocilia from guinea pig cochlear outer hair cells. (c) Diagram of a pair of stereocilia showing the structures relevant to mechanotransduction and adaptation. Note the tip links [black arrows in (a) and (b)], lateral links [white arrowheads in (a) and (b)], the electron-dense plaque at the upper insertion of the tip link and the contact region [white square bracket, (b)] between the stereocilia. Scale bars, 0.1 μm. The stereocilia are filled with actin filaments that flex where their rootlets enter the actin meshwork of the cuticular plate. The mechanotransducer channel is thought to be located near the tip of the stereocilium[24,25], and to be opened by force transmitted by the tip link or by interstereociliary connections in the contact region. The channels are located in the general area indicated in (c). Myosin Iβ has been immunolocalized in the stereocilia and might be concentrated in the electron-dense regions at either end of the tip link[39,40]. A theory of slow adaptation[38] suggests that the plaque is a 'myosin motor' that climbs the actin filaments to tension the tip links, the activity of the motor being regulated by binding of Ca^{2+} ions that enter via mechanotransducer channels[37,38].

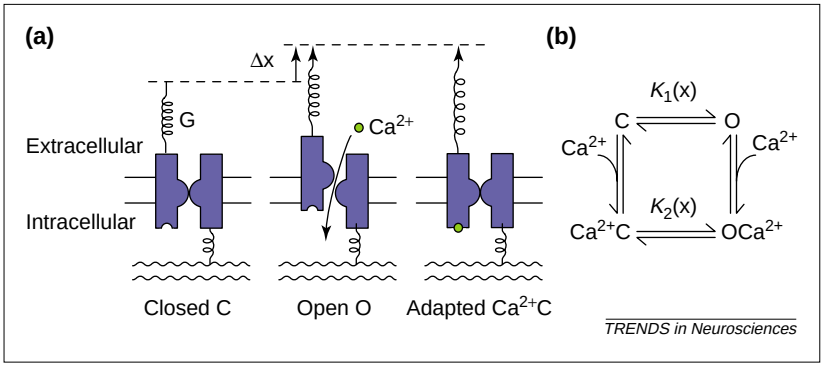

Fig. 4. A scheme for Ca^{2+} interaction with the mechanotransducer channel. (a) Deflection of the hair bundle extends the gating spring (G) causing the channel to go from closed (C) to open (O) configuration. Ca^{2+} entering the stereocilium through the open channel binds at the inner face of the channel and shuts it. Note that this generates force by increasing the tension in the gating spring. (b) A simple kinetic scheme for the mechanotransducer channel. Force applied to the channel converts it from closed (C) to open (O), allowing influx of Ca^{2+}, which binds to the channel (OCa) and then rapidly closes it (CaC). It is assumed that the equilibrium constant $K_1(x)$ is much larger than $K_2(x)$ so that the equilibrium for the Ca^{2+}-bound channel is towards the closed state. Other kinetic schemes for the mechanotransducer channel include extra closed states[3], or binding of multiple Ca^{2+} ions[47].

channels intracellularly, closing them in a manner that produces sufficient force to return the bundle back towards its original resting position. Ca^{2+} entry is then reduced, permitting the channels to open again. This is a negative feedback mechanism, with the bundle generating force to oppose its initial deflection. Feedback processes are often encountered in control of biological systems[46] and can provide both under-damped resonance and amplification, dictated by the feedback parameters. Thus, depending on the amount of Ca^{2+} entering the stereocilia and the bundle stiffness, oscillations at a specific frequency might be produced. This mechanism might underlie the damped oscillatory transducer currents[36] and oscillating hair-bundle motion[8] previously observed in turtle hair cells. Such oscillations might be important for amplifying the hair bundle vibrations set up by sound, especially near threshold. How Ca^{2+} interacts with the mechanotransducer channel is unknown, but theoretical analysis has shown that its feedback action can explain the resonance in the transducer current[34]. Furthermore, other models of hair-bundle mechanics[47,48] have exploited the capacity of feedback systems to become unstable and create spontaneous limit-cycle oscillations[46]. Under such conditions, the hair bundle would behave in a similar way to a highly tuned mechanical resonator, not only accounting for cochlear amplification but also for phenomena such as otoacoustic emissions.

Frequency selectivity

A fundamental property of the cochlea is its frequency selectivity: each hair cell responds best to a narrow range of sound frequencies[49]. To play a role in this process, feedback via the mechanotransducer channels might need to operate at different rates in

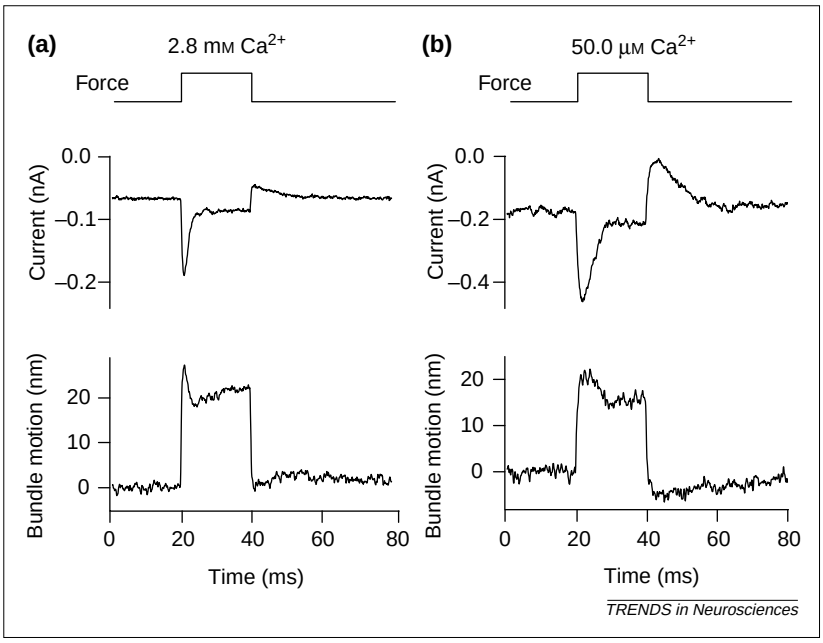

Fig. 5. Adaptation and hair bundle movements in turtle hair cells. (a) The hair bundle was stimulated with a fine glass fiber producing an adapting mechanotransducer current (middle) and displacement of the bundle (bottom). The stiffness of the fibre was less than that of the bundle so the stimulus (top) approximated a force step of 28 pN. The displacement of the bundle was measured by projecting its image onto a pair of photodiodes and determining the movement from the change in the photocurrent[8,15]. The bundle initially moved in the positive direction, but then recoiled with a time course identical to that of adaptation of the current. This rapid transient in the displacement record indicates the active mechanical response. (b) Reducing Ca^{2+} concentration from 2.8 mM to 50.0 μM slowed both the current adaptation and the bundle recoil. At the end of the force step, overshoots in the current and movement were caused by resetting of the adaptation on return to the resting position. The results show how active bundle motion is linked to the opening and closing of the mechanotransducer channels.

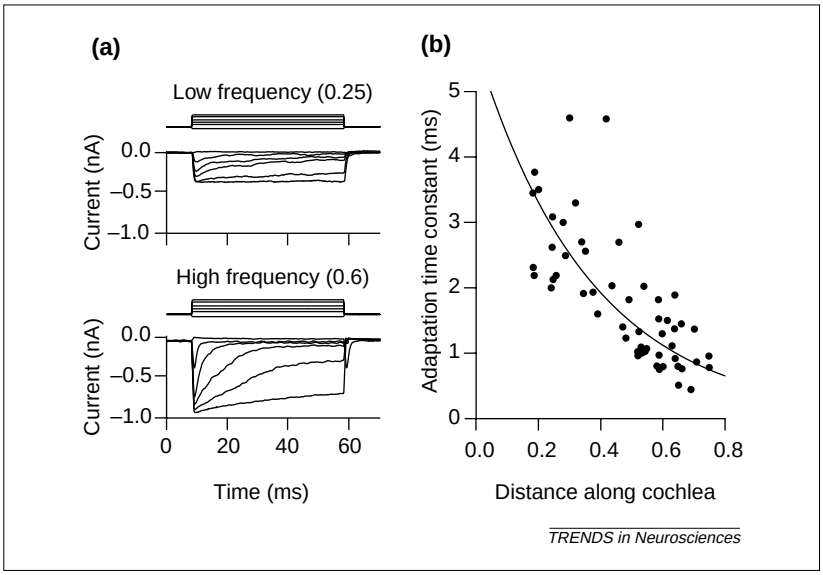

Fig. 6. Tonotopic variations of adaptation rate in auditory hair cells. (a) Mechanotransducer currents from hair cells near the low-frequency end and the high-frequency end of the turtle cochlea. The currents in the cell tuned to the higher frequency are larger (implying a greater number of mechanotransducer channels) and adapt more rapidly. The numbers in parentheseis give the positions of the hair cells, expressed as the functional distance along the cochlea for the low frequency end. In the turtle, and in other vertebrates, the position of the cell in the cochlea specifies the sound frequency to which it is most sensitive. (b) The time constant of adaptation for small bundle deflections in different hair cells is plotted against the fractional distance of each cell from the low frequency apex end of the cochlea. Note that adaptation is faster in cells tuned to higher frequencies. In other measurements of bundle motion[15] the time course of its recoil during a force step (Fig. 5) also varied with the frequency to which the hair cell was tuned. Modified, with permission, from Ref. 49.

cells tuned to different frequencies. Several factors, morphological and molecular, could affect the rate of the feedback. For example, both the maximum height of the hair bundle and numbers of stereocilia vary with optimal frequency[50–53] and can influence the bundle's passive mechanical properties[54,55]. In the turtle cochlea, there is also evidence that adaptation and active bundle movements are faster in hair cells tuned to higher frequencies (Fig. 6), which can be partly explained by an increase in the number of mechanotransducer channels per stereocilium[35]. But the properties of individual channels, such as their Ca^{2+} permeability or the speed with which they respond to changes in internal Ca^{2+}, might also vary[36]. As yet, the molecular identity of the mechanotransducer channels is unknown. However, recent characterization of Ca^{2+}-activated K^+ (BK) channels, which underlie electrical tuning in lower vertebrate hair cells, indicates a variation in channel structure with resonant frequency[56–58]. Such variation results in differences in both Ca^{2+} sensitivity and kinetics among channel isoforms[59,60]. Therefore, it would not be surprising to find multiple variants of the mechanotransducer channels that are differentially distributed along the cochlea.

Amplification in the mammalian cochlea

The mammalian cochlea encodes a much wider frequency range than the auditory organs of lower vertebrates. It also contains inner and outer hair cells with distinct roles. Both types of hair cell convert bundle deflections into receptor potentials but outer hair cells also have a motor function and can undergo rapid length changes[5–7] that might amplify the vibrations of the basilar membrane[61]. This motility is thought to involve a novel motor protein, recently identified as prestin[62]. It has been suggested that voltage-dependent changes in the shape of the motor protein, which is present at high density in the lateral membrane of the hair cell, alter the surface area and thus the length of the cell. Discovery of the specialized form of force production by outer hair cells has meant that less attention has been paid to the stereociliary bundle as a possible source of mechanical amplification in mammals. Furthermore, it has proved difficult to study mechanotransduction in the adult mammalian cochlea, and there is no evidence so far for active bundle movements in outer hair cells. However, recordings in neonatal preparations have shown that the mechanotransducer currents in neonatal mammalian hair cells possess properties similar to those in lower vertebrates, and exhibit both adaptation[63,64] and gating compliance[30,65]. If the technical difficulties of measuring transduction in adult mammalian preparations can be overcome, it is probable that active bundle movements will be found there too.

Why have mammals added the extra process of somatic motility? It might be that the bundle-based

mechanism found in lower vertebrates cannot deliver sufficient force to overcome the viscous damping at the higher frequencies used by mammals, and needs to be boosted by larger movements of the outer hair cells themselves. Some insight into this might be gained by calculating the forces generated by the two processes. The peak force produced by electrically stimulating isolated outer hair cells has been estimated as up to 100 pN per millivolt change in membrane potential[66,67]. Maximum receptor potentials of 5–10 mV have been recorded from outer hair cells[68,69], indicating a force caused by somatic shortening of 1000 pN (1 pN is 10^{-12} Newtons, which is about half the force generated by a single muscle myosin II head[70]). To calculate the force contributed by the mechanotransducer channels requires knowledge of the gating force per channel and the number of channels. Assuming a gating force[65,71] of the order of 0.5 pN, and 200 channels per hair cell[35], this sets an upper limit to the hair bundle force of 100 pN. Thus, the force generated by somatic contractility could be ten times that caused by active hair-bundle motion. However, these calculations rely upon extrapolations from lower vertebrate measurements for the number and properties of mechanotransducer channels. They also give no clue as to how efficiently either force generator influences cochlear micromechanics[4].

In order to determine the contribution of active bundle movements to cochlear amplification, several key issues need to be resolved. One is the molecular nature of the mechanotransducer channel, its interactions with Ca^{2+} and whether its characteristics or numbers vary with position along the cochlea. The recent cloning of a *Drosophila* mechanosensitive channel[72] will probably lead us closer to the identification of a vertebrate homologue in hair cells. It also remains to be determined whether the active movements observed in the hair bundles of lower vertebrates can be detected in mammalian hair cells, especially in the adult cochlea. Nevertheless, it is clear from work on the former that the evolutionary substrate exists for fast mechanical amplification by active bundle motion, raising the possibility that it might be an important component of the cochlear amplifier.

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