The physical basis of active mechanosensitivity by the hair-cell bundle

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Purpose of review

Hearing starts with the deflection of the hair bundle that sits on top of each mechanosensory hair cell. Recent advances indicate that the hair bundle mechanically amplifies its inputs to participate in the active process that boosts the ear's technical specifications. This review integrates experimental and modeling studies to dissect the mechanisms of active mechanosensation by the hair-cell bundle.

Recent findings

The exquisite mechanosensitivity of the hair-cell bundle results from a precisely choreographed interplay between a structure of mechanically coupled stereocilia that ensures efficient transmission of sound-energy to the transduction machinery, Ca^{2+}-driven adaptation that provides fast electromechanical feedback on hair-bundle movements, and a mechanical nonlinearity inherent to the transduction process that fosters autonomous hair-bundle oscillations. In cochlear outer hair cells, cooperation between active hair-bundle motility and somatic electromotility brings the cochlear partition to the brink of an oscillatory instability, at which general physical laws ensure optimal properties for auditory detection.

Summary

The study of active hair-bundle mechanics promotes a general principle for auditory detection that is based on the generic properties of self-sustained mechanical oscillators. This principle may guide future engineering design of cochlear implants.

Keywords

adaptation, amplification, hair bundle, mechanoelectrical transduction, oscillations

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Introduction

The ear benefits from an active amplificatory process to achieve exquisite sensitivity and sharp frequency selectivity and to operate over a wide dynamical range of sound levels [1]. Hearing begins when specialized mechanosensory hair cells convert mechanical vibrations into electrical signals. Each hair cell is endowed with a stereotypical hair bundle that projects from the cell's apical surface into the surrounding endolymph. The hair bundle works as a receptor antenna. Sound-evoked deflection of the hair bundle directly modulates the open probability of mechanically-gated ion channels by affecting mechanical tension in oblique extracellular tip links to which the channels are connected near the bundle's tip. When a channel is pulled open, K^+ as well as Ca^{2+} cations flow passively into the hair cell, driven by downward gradients of electrochemical potentials.

The hair bundle is not a passive receptor of the energy derived from acoustic stimulation. The hair cell can power active hair-bundle movements, including spontaneous oscillations, that can be harnessed to amplify the cell's responsiveness to weak stimuli [2]. In lower vertebrates, active hair-bundle motility is the only candidate for the amplifier that boosts the technical specifications of hearing [3]. In mammals, active hair-bundle motility coexists with electromotility, the process by which the soma of cochlear outer hair cells change length in response to variations of their membrane potential [4]. This raises the possibility that an interplay between these two motile processes underlies cochlear amplification [5]. We review here recent experimental discoveries and physical descriptions that clarify how the hair bundle contributes to the active process that shapes sound perception at the periphery of the auditory system.

The passive mechanical substrate of active hair-bundle motility

The hair bundle is a compact array of 30–300 extended microvilli – the stereocilia – that are arranged in rows of

increasing heights. Because stereocilia apparently display a series-parallel organization, one may worry that a force applied to the bundle's top evokes splaying of the structure. Instead, the hair bundle moves as a unit and the applied force is shared equally among the stereocilia [6]. Coherent hair-bundle movements prevail because hydrodynamic interactions and horizontal top connectors keep the stereocilia in contact during acoustic stimulation [7,8•]. By preventing the relative squeezing but not the shearing mode of motion, the close apposition of stereocilia effectively immobilizes the fluid between them [8•]. As a result, hydrodynamic friction, a physical impediment to fast hair-bundle movements, is reduced to a low level that approximates friction acting on a hemiellipsoid of similar dimensions to the whole hair bundle. By coupling tip-link tension to the least dissipative mode of motion, the hair-bundle architecture ensures concerted gating of the transduction channels and thus minimizes the external force that recruits the whole set of transduction elements within the hair bundle. Mechanoelectrical transduction is so sensitive that a horizontal bundle deflection of only a few tens of nanometers can saturate the transduction process [9,10••].

There is a price to pay for the high mechanosensitivity afforded by the hair-bundle structure. Because of reciprocity between transduction channels' gating and tip-link tension, the hair bundle gets softer within the narrow range of hair-bundle deflections that elicit significant rearrangement of the channels between their open and closed states – a process termed 'gating compliance' [11–13]. Gating compliance manifests a mechanical nonlinearity inherent to the transduction apparatus and is a source of mechanical distortions [14]. The potency of this nonlinearity relies on concerted channel gating; distortions are lost in mutant mice that lack stereocilin [15], a component of the top connectors that ensure coherent hair-bundle movements [8•,16]. Gating compliance also depends on the magnitude of the change in tip-link tension associated with channel opening – the gating force. When a channel opens, the gating spring shortens by a few nanometers and tip-link tension is in turn reduced by up to 5 pN along the oblique axis of the tip link [17].

Electrophysiological measurements in rat inner hair cells indicate that there are at least two transduction channels per stereocilium [18,19]. Because force-displacement relations display a single narrow region of reduced slope, two channels of similar sensitivity would have to be connected in parallel on the same side of the tip link [20], most probably at the lower end [18]. This inference, however, does not preclude a series arrangement, with one channel at each side of the tip link, if these transduction elements respond to external forces with contrasted sensitivities [21].

Key points

- By ensuring concerted gating of mechanoelectrical transduction channels and minimizing friction, the hair-cell bundle optimizes its mechanosensitivity.
- The hair cell can power active movements of its hair bundle, even in mammals, to amplify the cell's responsiveness to sinusoidal stimuli and sharpen frequency selectivity.
- Active hair-bundle motility results from a dynamic interplay between tip-link tension, mechanical gating of the transduction channels and a fast adaptive feedback mechanism that is prompted by the calcium influx through these channels and relies on active force production by molecular motors.
- The general properties of active mechanical systems that operate on the brink of an oscillatory instability – a Hopf bifurcation – are ideally suited for hearing.
- In mammalian outer hair cells, active hair-bundle motility probably cooperates with somatic electromotility to mediate cochlear amplification.

Remarkably, gating compliance in hair cells from the bullfrog's saccule can be strong enough to yield an unstable region of negative stiffness in the bundle's force-displacement relation [17]. Thus, if all transduction channels are initially in a closed state, the opening of a few channels can trigger a cooperative avalanche of openings [22]. Again, this mechanical instability is facilitated by the parallel arrangement of the transduction elements [1,6]. Outer hair cells from the rat cochlea can also display nonmonotonic force-displacement relations, but only after fast adaptive relaxation of tip-link tension has approached steady state [23]. Nevertheless, this finding indirectly suggests that mammalian hair bundles can be endowed with negative stiffness [24].

Adaptation: an active feedback mechanism that promotes active hair-bundle movements

Although softer hair bundles respond with larger amplitudes to external forces, the nonlinear gating compliance fostered by concerted channel gating cannot, by itself, be used to extract work from the hair bundle. For any amplificatory process, an energy source is mandatory. Myosin motor molecules are involved in an active adaptation mechanism that poises the transduction apparatus to remain sensitive to small time-varying stimuli in the presence of a prolonged deflection of the hair bundle [25]. By actively pulling the upper end of the tip link toward the stereociliary tips, the motors ensure that a significant fraction of the transduction channels are open at steady state [26,27]. In the bullfrog's saccule, the resting tip-link tension has been estimated at approximately 8 pN along the oblique axis of each tip link at a resting open

probability $P_o \cong 0.15$ but may reach a maximum value of about 20 pN [28]. Functional and localization studies implicate both myosin 1c [29–31] and myosin 7a [32,33•,34] as likely candidates for the adaptation motor. If it were only to set the operating point of the transduction apparatus, adaptation could afford to operate on much slower timescales than the period of acoustic stimuli. Yet, after being pulled open by an excitatory step stimulus, transduction channels reclose with typical timescales that are compatible with auditory frequencies and that vary systematically along the tonotopic axis of auditory organs [35–37]. These observations suggest that adaptation provides high-pass filtering that may help set the hair cell's characteristic frequency of maximal responsiveness.

Adaptation seems too fast to result solely from the ATPase activity of myosin molecules [38]. By relaxing stimulus-evoked changes in tip-link tension, ATP-driven myosin movements are expected to restore the channel's open probability to near its resting value over timescales of a few tens of milliseconds or even more, whereas the measured values of the adaptation time constant can be as short as 45 μs [36]. To accelerate adaptation kinetics, the Ca^{2+}-component of the transduction current provides fast feedback on the transduction channels' state to promote channel closure. Several mechanisms have been proposed to account for the effects of Ca^{2+} on adaptation [1] but none has been accepted as definitive. Calcium ions may bind directly to the transduction channel to stabilize its closed state [39–41]. Electrophysiological measurements in mutant mice in which the staircase pattern of inner-hair-cell bundles is lost, however, challenge this hypothesis, for inner hair cells with normal activation kinetics and magnitudes of the transduction currents show no sign of fast adaptation or Ca^{2+} sensitivity [42]. Alternatively, a protein associated with the channel may change conformation [31] or reduce its stiffness [43] to relax tip-link tension and in turn allow channel reclosure. The involvement of myosin 1c in fast adaptation points to this molecular motor as a potential target for Ca^{2+} binding [31]. Moreover, this unconventional myosin isoform displays unique properties that are well suited for adaptation, including an accelerated detachment from actin at increased Ca^{2+} concentrations [44,45]. By reducing the active pulling force produced by an assembly of myosin 1c molecules on the tip link's upper insertion point into the stereocilia, Ca^{2+} could potentially mediate fast adaptation [24]. On the basis of the effects of intracellular calcium buffers on fast adaption kinetics, however, Ca^{2+} should diffuse to its target by only a few tens of nanometers [46]. This condition would be hard to meet for myosin 1c if, as Ca^{2+} imaging suggests [18], the transduction channels are located exclusively near the lower insertion points of the tip links.

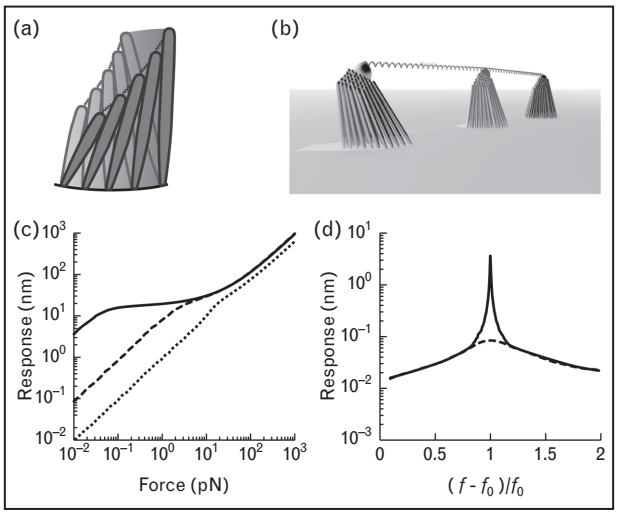
Irrespective of the detailed mechanism for Ca^{2+} feedback, the reciprocal relation between channel gating and tip-link tension imposes that adaptive channel rearrangements evoke internal forces that drive active hair-bundle movements. Accordingly, various forms of mechanical excitability have been observed with quiescent hair bundles in response to force steps [24,39,47,48], including with mammalian auditory hair cells [23], which can be understood as mechanical correlates of adaptation [2]. Strikingly, the hair bundle can also oscillate spontaneously [43,49–51]. Spontaneous hair-bundle oscillations are associated with the presence of negative stiffness in the bundle's force-displacement relation [17]. Oscillations occur because Ca^{2+}-dependent adaptation continuously attempts to set the operating point of the transduction apparatus at an unstable position of negative stiffness [24,43,52–54]. Simulations indicate that oscillations may reasonably occur at auditory frequencies, provided that Ca^{2+} binds to a low-affinity intracellular site located near the channel's pore [52,53]. In addition to the kinetic properties of the intracellular Ca^{2+} binding site, the hair-bundle stiffness and the magnitude of the calcium influx into each stereocilium [24,55] have been identified as control parameters to tune the characteristic frequency of the oscillator across the auditory range.

Spontaneous hair-bundle oscillations mediate mechanical amplification

The hair cell's ability to power oscillatory movement of its hair bundle has important functional consequences. Near the characteristic frequency of spontaneous hair-bundle oscillations, the hair cell actively resonates with sinusoidal stimuli and in turn amplifies its responsiveness [56–58]. Active oscillations offer a double benefit for auditory detection; they enlarge the range of sound intensities that can be detected by augmenting the response to the weakest stimuli and sharpen frequency selectivity by filtering the input to the hair cell (Fig. 1).

Although active hair-bundle motility provides a plausible component of the active process *in vivo*, amplification at the scale of a single hair bundle [57] is much less effective than the active process in an intact organ [59]. The gain of a single oscillatory hair bundle is in fact seriously limited by intrinsic noise, which destroys the phase coherence of active force production [53,60]. Hair bundles, however, do not work in isolation but are usually mechanically coupled by overlying membranous structures. By effectively reducing noise, the elastic coupling of oscillatory hair bundles with similar characteristics enhances the performances of each oscillator as a frequency-selective amplifier [61,62•] (Fig. 1). Simulations indicate that an oscillatory module comprising only a few tens of coupled hair bundles would be sufficient to account for the gain of

Figure 1 Nonlinear, frequency-selective amplification from active hair-bundle oscillations



(a) Spontaneous hair-bundle oscillations. (b) Elastic links that connect the top of neighboring hair bundles mimic mechanical coupling by accessory structures. (c) With a passive hair bundle or when an oscillatory hair bundle is stimulated at a much higher frequency than its characteristic frequency, the mechanical response of a single hair bundle is almost linearly related to the applied sinusoidal force (dotted line). When a single oscillatory hair bundle is stimulated near its characteristic frequency, the stimulus intensity that elicits a threshold response of 0.3 nm is reduced by ten-fold (dashed line). The elastic coupling of 81 similar hair bundles further reduces the stimulus amplitude required to cross threshold; amplification extends the range of responsiveness by 50 dB (continuous line). (d) At low forcing amplitude (0.01 pN), the response of a single hair-bundle displays an active resonance when the stimulus frequency f matches the frequency of spontaneous oscillation f_0 (dashed line). Upon coupling, frequency tuning and hair-bundle response are enhanced (continuous line).

the cochlear amplifier that is measured *in vivo* in mammalian species. In lower vertebrates, autonomous electrical oscillations of the membrane potential afford another means to enhance the performances of the hair-bundle amplifier, through a bidirectional coupling between electrical and mechanical oscillators within the same hair cell [63].

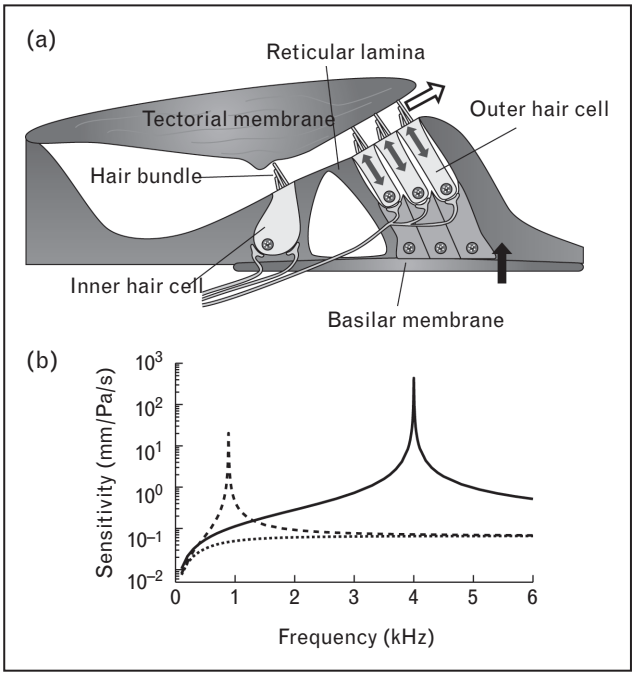
The Hopf bifurcation: a general principle for auditory amplification

Qualitatively, an oscillatory hair bundle recapitulates the four salient features of the cochlear amplifier that enhances mammalian hearing *in vivo* [1] (Fig. 1). First, amplification lowers the stimulus intensity that elicits a threshold response. Second, frequency selectivity is sharpened, for amplification is efficient only near a characteristic frequency. Third, near this characteristic frequency, a wide range of stimulus intensities is compressed into a much narrower range of vibration amplitudes. Finally, the active process can power mechanical vibrations even in the absence of stimulation, a phenomenon that may underlie spontaneous otoacoustic emissions. These four properties have been recognized by physicists as signatures of an active dynamical system that operates close to an oscillatory instability called 'Hopf bifurcation' [52,64–66]. Any system whose workings are described by a Hopf bifurcation must display the same generic properties, which do not depend on the detailed mechanism of active force production and are ideally suited for auditory detection [67,68].

The role of active hair-bundle motility in the mammalian cochlea

In the mammalian cochlea, the active process that augments hearing manifests itself in the amplified vibrations of the basilar membrane that supports the organ of Corti [59] (Fig. 2A) [69••]. Modeling studies suggest that hair

Figure 2 A possible interplay between active hair-bundle motility and somatic electromotility in outer hair cells of the mammalian cochlea



(a) In cross-section, the cochlear partition contains one inner hair cell and three outer hair cells. Sound-evoked vibrations of the basilar membrane (black vertical arrow) elicit shearing between the reticular lamina and the tectorial membrane, which deflects the hair bundle of outer hair cells (white arrow). Hair-bundle movements are enhanced by active hair-bundle motility and produce receptor potentials that drive electromotile movements of the hair-cell soma at the right phase to reduce friction acting on the hair bundle (double-headed grey arrow). (b) Active hair-bundle motility alone can enhance the sensitivity of the cochlear partition to sinusoidal stimuli but is too weak to drive movements at a frequency high enough to match the characteristic frequency of the cochlear partition at this location, here 4 kHz, and to provide sufficient sensitivity (dashed line). The synergic interplay between active hair-bundle motility and electromotility shifts the frequency of active resonance to higher frequencies and enhances sensitivity (continuous line). When active hair-bundle motility is turned off so that hair bundles become passive, the sensitivity is low and displays no frequency selectivity (dotted line). Adapted from [69••].

bundles are too weak to impinge on the responsiveness to sound of this macroscopic structure, at least at high frequencies [70,71]. When assessed in an in-vitro preparation of the gerbil's cochlea, hair bundles nevertheless contribute a significant fraction to the stiffness of the cochlear partition [72]. Moreover, these hair bundles display a compressive nonlinearity that may reflect active hair-bundle movements driven by the Ca^{2+}-component of transduction currents [73]. Together with the finding that hair bundles can produce force on a timescale compatible with auditory frequencies [23], it is reasonable to assume that active hair-bundle motility participates in cochlear amplification. If so, hair-bundle motility probably cooperates in cochlear outer hair cells with somatic electromotility, a biological form of piezoelectricity that has been shown to be necessary to the active process [74,75] but is linear over the physiological range of receptor potentials and displays no frequency selectivity [4].

A theoretical description of the interplay between these two forms of motility indicates that the cochlear partition can be brought to the proximity of a Hopf bifurcation and in turn evince all the hallmarks of the cochlear amplifier [69••]. In the most likely scenario, the electromotile feedback provides negative friction and positive stiffness to the hair bundle. This coupling results in faster oscillations of the basilar membrane than hair bundles would be able to provide alone as well as higher sensitivity and sharper frequency selectivity (Fig. 2). The impact of electromotility could be greater still, for the receptor potentials that drive this motile process are probably much larger than previously anticipated [10••]. Notably, in contrast to previous modeling assumptions [76], it is not necessary to take inertia into account to get frequency tuning. The characteristic frequency of the amplificatory process is set instead by the local properties of the active process and may thus vary over a broader range than passive mechanical properties of the cochlear partition would allow [77]. Alternatively, a provocative description of the synergic cooperation between active hair-bundle motility and electromotility suggests that the two processes could operate together to extend the frequency range of hearing towards low frequencies by preventing feedback of active bundle forces on basilar-membrane vibrations [78]. Clearly, the relative implication and interaction of these two forms of motility in cochlear amplification remains a central question of auditory physiology, and the subject of an intense debate [5,79].

Conclusion

The hair bundle is not a passive receptor of mechanical vibrations – a mechanical antenna – whose mechanosensitivity would result solely from the coupling of ion channels to elastic gating springs. This organelle is an active mechanical structure, which can convert energy of biochemical origin to power active movements that amplify its responsiveness to sound. Active hair-bundle motility results from the reciprocal relationship between the direct mechanical gating of transduction channels and an adaptation mechanism that is prompted by the calcium influx through these channels. This active process is fast enough to operate at auditory frequencies, though it remains to be demonstrated experimentally that it can work autonomously on a cycle-to-cycle basis at high frequencies. In mammals, the interplay between active hair-bundle movements and electromotility may position the cochlear partition on the brink of an oscillatory instability. This condition is ideal for hearing, for it automatically ensures exquisite sensitivity, sharp frequency selectivity and a wide dynamic range of responsiveness.

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Conflicts of interest

There are no conflicts of interest.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 411–412).

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