Published in final edited form as:

Trends Neurosci. 2019 March ; 42(3): 221–236. doi:10.1016/j.tins.2018.12.006.

A bundle of mechanisms: Inner-ear hair-cell mechanotransduction

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Abstract

In the inner ear, the deflection of hair bundles, the sensory organelles of hair cells, activates mechanically-gated channels (MGCs). Hair bundles monitor the head's orientation, its angular and linear acceleration, and detect sound. Force applied to MGCs is shaped by intrinsic hair-bundle properties, by the mechanical load on the bundle, and by the filter imparted by the hair bundle's environment. Channel gating and adaptation, the bundle's ability to reset its operating point, contribute to hair-bundle mechanics. Recent data from mammalian hair cells challenge longstanding hypotheses regarding adaptation mechanisms and hair-bundle coherence. Variations between hair bundles of different organelles in hair-bundle mechanics, mechanical load, channel gating, and adaptation may allow a hair bundle to selectively respond to specific sensory stimuli.

Keywords

Auditory; Vestibular; Hair Bundle; Adaptation; Mechanotransduction

Inner-ear mechanosensation

Mechanosensation is one of the oldest sensory modalities, with functions spanning single-cell volume regulation, proprioception, organismal self-localization, and hearing [1–5]. In particular, the vertebrate inner ear houses end organs specialized for sensing motion and sound. Vestibular systems primarily detect nonperiodic signals, arising from head orientation and acceleration, although their sensory hair cells also respond to low-frequency stimuli. In auditory systems, hair cells encode periodic signals from 10's of Hz to over 100,000 Hz. Moreover, fish employ hair cells in their lateral line to detect both nonperiodic and periodic inputs generated by movements and pressure gradients [6]. The hair cell, named for the bundle of mechanosensitive hairs (actin-based **stereocilia** (see **Glossary**)) protruding from its apical surface, converts mechanical input into an electrical current that drives the receptor potential. This process of mechanoelectrical transduction (MET) relies on mechanically-gated ion channels (MGCs) opening or closing in response to hair-bundle deflection. A

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similar molecular mechanism likely underlies channel activation across hair-cell based modalities. Specializations in the specific molecules present within a given hair bundle, in end-organ structures, and in hair-bundle geometry and mechanical properties determine how force is transmitted to the MGCs.

In the mammalian cochlea, hair cells detect stimuli that deflect their hair bundles by less than one nanometer near the threshold of hearing, with periods of less than one millisecond, and over a millionfold range in magnitude [7, 8]. Near threshold, all auditory and vestibular organs likely use an active process to amplify their mechanical response and extend their **dynamic range** (see below). In principle, a different type of detector is required to identify distinct input such as the onset of nonperiodic changes, the magnitude of these changes, or periodic signals. Yet, each sensory system uses the same type of organelle, the hair bundle, to detect different forms of stimulation.

In this Review, we discuss the common features shared by hair bundles across sensory systems and the specializations in different systems that allow them to detect distinct types of stimuli. Rather than focus on the molecules of mechanotransduction, which have been discussed recently in excellent reviews [9, 10], we concentrate on the biophysical mechanisms of mechanotransduction. We will summarize data revising our understanding of hair-cell adaptation, comment on the emerging role of the lipid bilayer, and describe a general physical premise that might explain how a broad set of sensory modalities are enabled by inner-ear hair cells.

Hair-bundle mechanics

The macroscopic environment

A sensory hair bundle's environment shapes the signals translated to the MGCs and determines which mechanical cue is best monitored by a particular system (Figure 1). In some hearing organs, hair bundles are free-standing; stimulated and coupled by fluid forces, which intensify with the stimulus frequency. To coordinate their motion in other settings, bundles are embedded in a cupula, tectorial structure, otolithic membrane, or sallet. The organ's mechanical structure dictates the stimulus type detected. For instance, to respond to head acceleration or orientation, hair bundles are pocketed in an otolithic membrane covered with massive calcium carbonate crystals. Bundles within a single organ may be free standing or coupled by an overlying structure. In particular, the mammalian cochlea possesses outer hair cells (OHCs) whose hair bundles are embedded in a tectorial membrane, and inner hair cells (IHCs) whose bundles are free-standing, but closely appose the membrane. Each of these configurations provides a different mechanical load on a hair bundle while also filtering the mechanical stimulus presented to the bundle. To understand the consequences of a bundle's mechanical environment, however, we must first examine the properties of the bundle itself.

Microscopic mechanics

A bundle's mechanical properties impact force transmission to the MGCs. **Tonotopic** gradients, such as hair-bundle heights decreasing with hair cells' increasing characteristic

frequency, exist across species [11, 12]. Differences in stereocilia thickness, the number of stereocilia, and the presence or absence of a **kinocilium** all play a role in how force applied to a hair bundle activates the MGCs. In all hair bundles, stereocilia with similar lengths form rows (Figure 2). Multiple rows form a stair-cased pattern that peaks in height at the kinocilium. The number of stereociliary rows and the step height between rows vary. A tip link comprised of protocadherin 15 and cadherin 23 dimers connects adjacent stereociliary rows, and its lower end is either directly or indirectly connected to the MGCs [13–15]. Each stereocilium acts as a stiff rod that pivots about its insertion point into the cell's apical surface [16]. An external force that deflects a hair bundle toward its tall end, tensions the tip links, which convey pulling forces to the MGCs, inducing them to open. Conversely, stimulation toward a bundle's short edge closes MGCs. In non-mammals, each hair bundle moves as a cohesive unit, such that the bundle's stereocilia move almost synchronously and in the same direction [17, 18].

Mammalian cochlear hair bundles differ substantively from non-mammalian auditory and vestibular bundles. First, inner and outer hair cells typically have three stereociliary rows, whereas hair bundles in other systems possess considerably more rows of stereocilia. How mammalian hearing benefits from the small number of rows remains unclear. Second, experimental observations have demonstrated that mammalian cochlear hair bundles do not move as a unit; they are less cohesive than other hair-bundle types [19, 20]. The lack of cohesiveness leads to **phase** differences between stereocilia, which affects the timing of channel gating. To illustrate this, we generated a simple model with three stereocilia of differing heights, each representing a stereociliary row (Figure 2). In principle, a force applied to the tallest row causes it to move, which leads the movement of row two, which in turn leads the motion of row three. To demonstrate the consequences of phase differences using the model, we fix the displacement amplitudes of each row and vary the phases between the rows (Figure 2e). In general, out-of-phase motions result in channel gating that is also out of phase, thus limiting the total MET current. The out-of-phase channel gating broadens the **activation curve** (Figure 2h), increasing the current's dynamic range but reducing its sensitivity about the bundle's resting position. Interestingly, forcing the stereocilia to be in phase does not maximize the total MET current; rather, there is an optimal phase difference between rows that maximizes the total current. These complications are compounded considering that in reality there are phase differences between stereocilia in the same row. It is possible that the mode of hair-bundle stimulation controls the phase alignment of channel gating in mammals, so that OHCs are coordinated by their attachment to the tectorial membrane and IHCs by fluid motion in the subreticular space (see Figure 1). Fluid between stereocilia may also couple them when a hair bundle is stimulated at high frequencies, ensuring the coherence of stereociliary motion [21]. Whether weak cohesion at low frequencies has functional relevance remains to be determined but the cohesiveness of bundles likely shapes the summed MET current response.

Gating-spring theory

Because many animals hear high-frequency sounds (>1 kHz), their MGCs must respond quickly to stimulation (<1 ms), implicating direct mechanical coupling between bundle

motion and channel gating. The speed of channel gating is set by the channel's properties and the mechanical components transmitting force on the bundle to force at the channel.

The opening of the MGCs is concomitant with the extension of an elastic element, known as the gating spring, in series with the channel [22]. Channel opening causes a relaxation of this gating spring, by an amount termed the gating swing (Figure 3). Gating-swing estimates are larger than predicted for a channel conformational change implying that the molecules constituting the gating element include more than the MET channel [23]. The gating element may include any component of the machinery in series with the MET channel including the lipid bilayer, the tip link, the cytoskeleton, the insertional plaque (see **upper-insertion site**), hypothesized intracellular anchors to the cytoskeleton, and any potential linker molecules or channel subunits between the tip link and the MET channel. Potential components include recently identified molecules such as TMHS/LHFPL5, TMIE, and TMC proteins [24–26]. The presence of these molecules likely varies over development and between hair-cell types, thus it is possible that the MGCs and gating elements also vary. Evidence that molecular differences exist between MET-channel complexes is accumulating. Tonotopic and cell-type differences in the single-channel conductance as well as in the distribution of proteins like the TMCs have been observed [26–29]. To better understand channel-gating mechanisms and the molecular underpinnings of the gating spring and swing, more data is needed to clarify how the identified molecules interact to form a channel and to identify how each component contributes to gating.

Because the probability of the MGCs being open is a sigmoidal function of a bundle's displacement, the MET current and force required to hold a bundle still are nonlinearly related to its displacement (Figure 3) [22]. A larger **gating force** increases the steepness of a hair cell's activation curve and decreases the slope of the displacement-force relationship, the effective stiffness of a hair bundle. The activation curve or the displacement-force relationship can be used to estimate the gating force, which is about 0.3 to 0.7 pN [30–32]. In mammals, however, the activation curve's steepness depends on stimulus modality, fluid stimuli result in narrower activation curves than stimuli using stiff probes and the activation curve's width depends on the shape of the probe, such that the inferred gating force may be underestimated [20, 33]. Channel gating reduces hair-bundle stiffness, an effect called gating compliance [22, 30, 32, 34]. Stimulation using a flexible glass fiber and a displacement clamp, reveals that hair bundles from the frog (*Rana catesbeiana*) sacculus possess **negative stiffness** [30], an extreme form of gating compliance arising from a large gating force. The observation of negative stiffness is very condition dependent, being particularly sensitive to the mode of stimulation, calcium homeostasis, and the hair-bundle load [30, 35, 36]. Although negative stiffness has not yet been seen in other types of hair bundles, it may be ubiquitous in hair bundles, as it is required for spontaneous oscillations [30], which have been observed in reptiles, amphibians, bony fish, and jawless fish [16, 37–39] and may play a role in the detection of weak sounds (see below). Overall the MET channel's current and mechanical nonlinearity may in part underlie the auditory system's nonlinear responses to sound, but the mechanism remains under debate owing to uncertainties associated with the size of the gating force [40–43].

Adaptation

Hair-bundle mechanosensitivity is also shaped by an additional dynamic process, termed adaptation, which shifts a hair bundle's **operating point** in response to a step stimulus without a loss of sensitivity at the new resting position (Figure 4)[44–46]. Adaptation can be envisioned as a change in the force sensed by MGCs that modulates their probability of opening. This process extends a hair bundle's dynamic range, provides **high-pass filtering** properties to the hair bundle, and is implicated as a component of active hair-bundle feedback underlying a hair bundle's potential role in **amplification** [46–48]. The adaptation process manifests itself in different ways depending on stimulus modality. Step displacement with a stiff probe to open MGCs results in a multiexponential current decline after the stimulus onset, in which the number of components, the time constant of each component, and the extent of adaptation depend on the stimulus rise-time [20, 49]. Conversely, a step displacement that closes MGCs produces adaptation reopening the channels during the stimulation.

Slow Adaptation

The multiexponential time course of current decay may reflect distinct adaptation mechanisms [49]. Originally, calcium entry through the MGCs was postulated to directly regulate myosin motors located at the upper tip-link insertion site [37, 50]. These motors were thought to maintain the resting tip-link tension, that is, the tension in the absence of a stimulus. This slow form of adaptation was elicited by a change in calcium controlled by channels opening/closing in response to a stimulus. Opening the channels increases calcium, weakening the interaction between the motors' heads and F-actin to reduce the total force produced by the motors, which lowers tip-link tension. Consequently, the upper-insertion point slides down (high calcium) or climbs up (low calcium) a stereocilium, in response to MGCs opening or closing. This hypothesis is supported indirectly by observations including a slow mechanical relaxation in response to bundle stimulation [37], localization of myosins at the upper-insertion site [51, 52], and adaptation's sensitivity to ATP [53] and intracellular calcium [44, 50]. In mammals, recent data questions this longstanding proposal. Localization of MGCs to the bottom of tip links and not the upper-insertion points distances the calcium influx through the channel from the motor proteins, such that in mammalian cochlear hair cells with three stereociliary rows only the MGCs in the shortest row could be modulated (Figure 2) [15]. A very slow calcium-dependent modulation of the MGC **resting open probability** was observed in mammalian hair cells. This dependence was associated more with the external calcium, however, and had a lipid-modulatory component [54, 55]. It remains under investigation whether this resting open-probability regulation involves motors, and what the relationship is between this regulation and the upper tip-link insertion site. An alternative hypothesis to the one outlined above is that motors near the upper-insertion point maintain resting tension in the gating spring, independent of adaptation [55]. Maintaining resting tension prevents slack in the gating spring, ensuring rapid channel gating in response to changes in bundle position.

Fast Adaptation

The second component of adaptation is as brief as tens of microseconds in OHCs to milliseconds in IHCs [54], a time constant that varies tonotopically within an end organ but also across species [56, 57]. Data from non-mammalian hearing and vestibular organs suggested that fast adaptation was driven by calcium entry through the MGC interacting either directly with the channel or with an accessory protein in close approximation to the channel [58–60]. In response to a step force applied with a flexible fiber, the hair bundle rapidly follows the probe motion and then moves in the opposite direction to the stimulus, faster than the motor-attributed movement and correlating with the fast-current decline. More recent data from mammalian cochlear hair cells demonstrated that calcium entry is not required for fast adaptation [54]. The ability to resolve the calcium independence of fast adaptation in the mammalian cochlear hair bundle may result from the limited contribution of slow adaptation. Thus, it is possible that a similar mechanism exists across hair-cell types, but the calcium-dependent slow component of adaptation masks the calcium-independence of the fast component. Data from fluid-jet stimulations contrasts with stiff-probe data in arguing that fast adaptation did require calcium entry [61]. Much like data from lower-frequency end organs, the slower rise times associated with the fluid-jet stimulation tends to temporally merge adaptation components making definitive conclusions difficult [20, 49]. More work is needed to reconcile how adaptation manifests itself across stimulus modalities.

Phosphatidylinositol 4,5-bisphosphate (PIP2), a minor component of most lipid bilayers, is required for fast adaptation in both low-frequency frog saccule and mammalian cochlear hair bundles, suggesting a common molecular mechanism [62, 63]. Whether the PIP2 dependence arises from its direct interaction with the channel complex or from an indirect effect on lipid mechanics remains to be explored. Subtle effects on single-channel conductance and calcium permeation suggest a direct interaction, whereas PIP2's distribution along the stereocilia length supports a more global effect.

In general, the role of the lipid bilayer in hair-cell MET is understudied, and recent data suggest in fact that the bilayer may be more important than previously considered. GSMTX4, a compound that interferes with force transmission from the bilayer to MGCs, prevents calcium and voltage-driven changes in the MET-channel's open probability without altering fast adaptation [55, 64, 65]. Additionally, calcium-dependent open probability regulation appears to depend on an external site; perhaps altering lipid packing around the channel. Computational exploration of the lipid bilayer's mechanics further supports the feasibility of lipid modulation of MET [66–68]. A recent mathematical-modeling study suggests that the lipid bilayer can modulate cooperativity between MET channels and might explain the large gating swing [23].

It is plausible that fast adaptation is a viscoelastic relaxation of an element in series with the channel [37]. Potentially, this mechanism can be very fast and would be sensitive to the stimulus rate [49]. A viscoelastic response is not active and could be instantiated by any of the elements associated with the gating complex. Figure 5 compares fast adaptation resulting from a calcium-independent process, such as a viscoelastic relaxation, to fast adaptation arising from an active component, driven by a calcium-dependent process. For negative membrane potentials, the currents elicited by a displacement-clamp stimulus are

qualitatively similar for calcium-dependent and calcium-independent adaptation. At positive potentials, where calcium is not entering the cell, calcium-independent adaptation is possible but calcium-dependent adaptation is absent. Fast adaptation at positive potentials has thus far only been reported for mammalian cochlear hair cells [54]. It is possible that fast adaptation at positive potentials is masked in non-mammalian hair cells by robust slow adaptation creating a large change in resting open probability (>50%) which may saturate the fast-adaptation response.

Active signal detection

Signal detection by hair bundles is limited by intrinsic stochastic fluctuations setting the thresholds of all auditory and vestibular systems [69]. These fluctuations are inherently related to damping [70], which slows responses to stimuli. To encode temporal aspects of a stimulus, hair bundles must respond with sufficient speed and amplitude to elicit a neural response and distinguish external signals from intrinsic noise. Because of these fundamental limits, it is likely that both auditory and vestibular organs utilize an active mechanical feedback process to enhance their sensitivity to weak stimuli [71, 72]. In an auditory system, this process amplifies weak signals with frequencies near the natural frequencies of the system and filters out noise that differs in frequency and phase from the system's response to the stimulus [73, 74].

Under appropriate conditions, hair bundles exhibit mechanical activity that is similar to that of the auditory system. The clearest evidence for activity in hair bundles are their spontaneous oscillations [16, 37–39]. Because spontaneously oscillating bundles lose energy owing to dissipation, they require an energy source to be sustained, such as a nonequilibrium calcium gradient [37, 75]. Spontaneous oscillations are observed only if the external and internal calcium concentrations are in the right range and only in the appropriate mechanical environment [76–79] (Figure 6). Mammalian hair bundles have not yet been seen to oscillate spontaneously, but the lack of observed oscillations may be a result of inappropriate conditions rather than an inherently passive hair bundle. Spontaneous hair-cell oscillations might be ubiquitous because they are thought to produce spontaneous otoacoustic emissions, which are low-amplitude sounds produced by most vertebrate ears [80].

When a bundle's operating point is near the parameter region for which it spontaneously oscillates, activity can boost its response to periodic, step, and static stimuli. Theory predicts, and observations in the bullfrog's sacculus verify that the bundle's mechanical environment controls the bundles operating point and consequently dictates the stimulus type to which a bundle is most sensitive [36, 81–83]. For example, an accessory structure attached to the hair bundle imposes forces on the bundle. Owing to its deformation, the stiffness of the structure and its undeformed size produce a stiffness force and a constant force (Figure 6). To encode the onset and size of step inputs, vestibular bundles exhibit large mechanical twitches at the onset of step stimuli and can spike at a rate that is dependent on the size of static stimuli. These bundles and the accessory structures to which they are attached possess small stiffnesses, which places their operating points in the regions in Figure 6 labeled “Step Size Detection” and “Step Onset Detection”.

In contrast to vestibular bundles, auditory hair bundles tend to be stiffer and are often attached to stiffer structures, such that their operating points, labeled “Periodic Signal Detection” in Figure 6, are near to a parameter region for which bundles exhibit fast small spontaneous oscillations. When quiescent bundles operate under these conditions, they display resonant responses to periodic stimuli, with their sensitivity and tuning sharpness growing as the stimulus size declines. Enhanced sensitivity at resonance and sharpened frequency tuning close to a region of spontaneous oscillations are generic properties of many quiescent systems [73, 84, 85]. However, bundles operating near the border and inside the region of spontaneous oscillations are predicted to be more sensitive to sinusoidal stimuli, with frequencies around but not at the bundle’s resonant frequency, than quiescent bundles or bundles possessing larger-amplitude spontaneous oscillations [74].

For an active bundle to spontaneously oscillate and to detect stimuli as seen experimentally, it need only possess a sufficiently great gating compliance, corresponding to a large gating force, and an active feedback process. Theory shows that many different feedback processes could underlie active signal detection in the inner ear [36]. Adaptation provides mechanical feedback to a bundle, but whether fast adaptation in the mammalian cochlea is active remains a matter of current debate. The mammalian cochlea, however, possesses an additional active feedback process external to the hair bundle [86–88]. Voltage-dependent somatic length changes of outer hair cells, driven by variations in their receptor potential, could create feedback forces onto hair bundles [41]. This feedback is active owing to the receptor potential’s dependence on the endocochlear potential and the outer hair cell resting potential. Independent of the type of active feedback, however, a homeostatic mechanism is predicted to be necessary to ensure the robustness of the bundle’s performance to developmental and environmental variation [89].

In the absence of a mechanical load, a hair bundle oscillates spontaneously only if it possesses a region of negative stiffness (Figure 3). Negative stiffness is not required, however, when the bundle is loaded by the inertia of an overlying structure [36]. Raising the mass of the load increases the size of the oscillatory region and the range of parameters for which active signal detection is possible (Fig. 6d). The tectorial membrane’s mass in the mammalian cochlea might ensure that a hair bundle’s mechanical activity is not overwhelmed by the passive stiffness of the cochlear partition [90].

A hair bundle’s spontaneous oscillations, range of nonlinearity, frequency tuning, and amplification are generally suppressed by stochastic fluctuations associated with friction [91–93]. To mitigate the effects of noise, active hair bundles filter out input at frequencies differing from their resonant frequency and the coupling of multiple bundles by an overlying structure may improve their sensitivity and frequency tuning [70, 94]. Under the right conditions, however, a bundle’s response might be improved by intrinsic noise—a prediction that remains to be tested [95].

Concluding Remarks and Future Perspectives

Although it is clear that the function of auditory and vestibular organs is dictated by the environment and properties of their mechanosensory hair bundles, fundamental questions remain (see Outstanding Questions Box).

Faster and more precise techniques are needed to stimulate mammalian hair bundles [96]. Modeling predicts that a stimulus that better reproduces the natural *in vivo* input to a hair bundle will lead to faster adaptation time constants and narrower activation curves [20].

Ascertaining the cohesiveness of mammalian bundles will require better estimates of bundle properties and novel modeling strategies. Why are mammalian cochlear hair bundles so different from other end organs? Both their reduced stereociliary row number and their weak cohesion remain largely unexplained.

The lipid bilayer's properties are under-investigated yet appear to be crucial for the transmission of force to the MGCs and for adaptation dynamics. Because lipid packing is regulated by external calcium binding, which is modulated by the receptor potential, lipid dynamics might provide mechanical feedback to a bundle similar to adaptation and might allow the bundle to amplify mechanical input owing to the feedback's dependence on the non-equilibrium membrane potential.

To deduce the role of adaptation and clarify whether fast adaptation possesses an active component, one would need to suppress adaptation without blocking the mechanotransduction channel. Removing active adaptation might raise the threshold, broaden the frequency tuning, and narrow the dynamic range of hearing.

Many forms of hereditary hearing impairments arise from hair-bundle dysfunction [9, 10, 97]. Mutations can affect channel gating, adaptation, homeostasis, and hair-bundle mechanics. Mechanistic understanding, however, of how these defects lead to bundle dysfunction is limited.

Nature has composed a wondrous collection of sensors by variation on a simple theme, hair-bundle mechanotransduction. To detect a variety of signal types, acousticolateralis organs employ hair bundles in remarkably diverse configurations. Comparing these systems and the bundles within them will continue to inform us about how each system functions, and hopefully, how to treat their dysfunction.

Acknowledgements:

This work was supported by NIDCD grants DC003896 and DC014658.

Glossary

Amplification

in the context of inner-ear research, refers to a boost in the mechanical response of the living inner ear to stimulation in comparison to immediately post-mortem.

Activation curve

the MET current as a function of a hair bundle's displacement.

Bifurcation

a change in a system's qualitative behavior owing to an alteration in operating point. At a fold bifurcation, a system develops two additional steady states. At a Hopf bifurcation, a system transitions from quiescence to self-oscillation.

Dynamic range

the stimulus range over which the response changes.

Gating force

the amount of force required to open a mechanotransduction channel.

High-pass filter

a filter that allows fast stimuli to pass but attenuates slow input. In hair bundles, fast input at the start of a step stimulus produces a large MET current that declines exponentially as the stimulus slows. The time constant of current decline varies tonotopically and is inversely related to the characteristic frequency of the hair cell's location.

Kinocilium

a microtubule-based cilium that is nonmotile in hair cells. In the mammalian cochlea, the kinocilium is present during development but is absent in mature hair bundles.

Negative stiffness

the displacement-force curve relates the force required to hold a hair-bundle at rest to its displacement. The slope of this curve is the measured stiffness of a hair bundle. Owing to channel gating, the measured stiffness can be negative over a limited range of displacements.

Operating point

the set of conditions that constrain a system's state. The operating point of a hair bundle can be adjusted by controlling one or more of its parameter values and can manifest as a change in the open probability of the MGCs.

Phase

the time at which an event occurs as a fraction of a signal's period. Stereocilia exhibit a phase difference if they respond at different times relative to the period of a stimulus. If the phases of channel opening were perfectly aligned, then the channels would open at the same time relative to the stimulus period.

Resting open probability

The fraction of the maximal MET current that is present in an unstimulated static hair bundle.

Stereocilia

actin-filled structures similar to microvilli that constitute the primary components of the sensory hair bundle.

Tonotopy

the spatial arrangement of cells and tissues in auditory organs ensuring that each location responds best to a particular stimulus frequency. The mammalian cochlea detects low-frequency sound at its apex and high frequencies at its base.

Upper-insertion site

the site at which a tip link attaches to the taller of a pair of stereocilia. At this slightly invaginated location in some bundles, cadherin 23 interacts with a TEM dense region, known as the insertional plaque, composed of a variety of proteins including harmonin, whirlin, Myosin Ic and/or Myosin VIIa.

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Highlights

- Mechanically-gated channels (MGCs), located at the tops of the sensory hair bundles, translate sound as well as the head's orientation and acceleration into electrical signals.
- Mechanical loading of a hair-bundle by an accessory structure shapes the bundle's response to stimulation. Bundles can be free-standing, encased in a cupula, or embedded in a sallet, tectorial membrane, or otolithic membrane.
- Hair-bundle compliance and coordination between stereocilia control the macroscopic hair-cell response.
- Owing to the sizable **gating swing**, channel gating affects hair-bundle compliance, creating mechanical nonlinearity.
- Adaptation modulates the gating of the MGCs by means of calcium dependent and independent processes.
- Channel gating, adaptation, hair-bundle mechanics, and a hair bundle's load determine force transfer to the MGCs, specializing a bundle's response to distinct types of stimuli.

Outstanding Questions Box

- How does the hair bundle's structure and geometry determine its function? In particular, why do mammalian cochlear hair bundles differ in shape and cohesion from bundles found in other systems?
- What is the functional role of hair-bundle cohesion in the mammalian cochlea?
- What are the functional consequences of outer-hair-cell hair bundles being embedded in a tectorial membrane? How do tectorial-membrane properties shape hair-bundle motion?
- What are the functional consequences of inner-hair-cell bundles being free-standing but in a restricted environment? Does the fluid's boundary layer control hair-bundle motion?
- What is the role of the lipid bilayer in modulating force transfer to MGCs? Does PIP2 directly interact with channel proteins? Are lipid mechanics regulated to aid in force transmission to MGCs? Is this regulation due to membrane cytoskeletal connections and/or specific lipid components along the stereocilia?
- What are the molecular components contributing to the gating-spring? How do these components vary across species and within an organ? What are the functional consequences of these variations?
- What are the mechanisms underlying fast and slow adaptation? Is fast adaptation active? What is responsible for tonotopic variations in fast-adaptation rates?
- What role does adaptation play in frequency selectivity in the mammalian cochlea?
- How do hair bundles detect weak stimuli despite the presence of substantial intrinsic noise?
- How are hair-bundle mechanics and outer-hair-cell motility integrated to generate nonlinear cochlear amplification?
- What are the molecular underpinnings of hair-cell mechanotransduction? What are the pore forming domains of the MGCs? How are the molecules coupled and what is the specific function of each?

Figure 1:

Hair bundles of various sizes are attached to a variety of accessory structures in different sensory organs. Hair bundles consist of stereocilia (blue) and often a kinocilium (red). (a) The basilar papilla of the brown anole *Anolis sagrei* possesses free standing hair bundles of differing heights [98]. (b) A zebrafish *Danio rerio* neuromast comprises pairs of similarly-oriented bundles with long kinocilia protruding into an overlying cupula [99]. (c) Rotations of the head are detected by hair bundles embedded in the cupula of a semicircular canal. (d) Translational acceleration and orientation of the head displace hair bundles with kinocilia attached to the otolithic membrane of the utricle or saccule. (e) Two small groups of

oppositely-oriented hair bundles are coupled by massive sallets in the basilar papilla of the Tokay gecko *Gekko gecko* [100]. (f) Larger groups of hair bundles in the Tokay gecko can also be coupled together by a tectorial curtain. (g) In the mammalian cochlea, the hair bundles of outer hair cells are embedded in the overlying tectorial membrane (three rightmost rows), whereas the bundles of inner hair cells (left) closely appose but are not attached to the membrane.

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Figure 2:

Cochlear hair bundles in mammals differ from those in non-mammals and vestibular organs. Rows of stereocilia with similar heights are connected by tip links (black) to shorter rows. MGCs at the bottom of each tip link are opened by an increase in tip-link tension. Because blue stereocilia do not have mechanotransduction channels through which calcium might flow, slow adaptation does not change tension in the tip-links connected to the pink stereocilia, whose channels are therefore unregulated by slow adaptation. (a) Vestibular and non-mammalian auditory bundles comprise several rows of stereocilia arranged in a cluster,

with the tallest row connected to a kinocilium (red). (b) Three rows of stereocilia are arranged like a fan in the hair bundles of inner hair cells. (c) Outer hair-cells bundles are V-shaped and possess three rows of stereocilia. (d) A force on the tallest row (row 1) stretches gating springs between rows. (e) In response to a sinusoidal stimulus, the displacement of row 1 ($X_1=A_1\sin(2\pi ft)$) leads that of row 2 ($X_2=A_2\sin(2\pi ft-\phi_2)$), which in turn leads that of row 3 ($X_3=A_3\sin(2\pi ft-\phi_3)$). (f) Mechanotransduction currents flow through the channels associated with each gating spring ($PO(G(X_1, X_2))/2$, blue; $PO(G(X_2, X_3))/2$, red) yielding the total macroscopic current ($PO(G(X_1, X_2))/2+PO(G(X_2, X_3))/2$, green), in which the $G(X_n, X_{n+1})$ is the gating-spring length corresponding to rows n and $n+1$. P_o and G are determined by the bundle's properties, but the results shown are not qualitatively dependent on parameter values. (g) Macroscopic currents are shown for adjacent stereocilia moving in phase (gray), in anti-phase (brown), and corresponding to panel f (green). (h) The activation curve measured when channels are greatly out of phase is wider (dark purple) than the activation curve for more synchronous channel gating (light purple). Currents are normalized to the amplitude of the macroscopic current for saturating displacements and synchronous channel opening. (a-c) Modified from [101].

Figure 3:

The gating-spring mechanism. (a) A stereocilium is connected to its smaller neighbor by a tip link. Two mechanically sensitive ion channels are attached to the lower end of the tip link either directly or indirectly through accessory proteins or the lipid bilayer. The tip link's upper end is connected to the actin cytoskeleton by the insertional plaque, whereas the channels may be coupled to the cytoskeleton by anchor proteins. (b) Because a channel's state is described by a Boltzmann distribution, its open probability (upper) and the force required to hold the bundle at a fixed displacement (lower) are nonlinear functions of the displacement. (Upper) A channel with a large gating force possesses a narrower activation curve (cyan) than a channel with a smaller gating force (green). (Lower) For a sufficiently large gating force, the displacement-force relation can exhibit a region of negative slope. (c) (Upper) The gating-spring complex comprises all elastic elements in series with the channel complex and may include the cytoskeleton, anchor proteins, the tip link, the lipid bilayer, accessory proteins, channel proteins, and the insertional plaque. The channel complex may include two channels, the lipid bilayer, and accessory proteins. Viscoelastic elements in parallel with the channel complex include the cytoskeleton and the lipid bilayer. (Lower) An

external force displaces a hair bundle and extends the gating-spring complex and the parallel springs. Owing to increased tension in the gating-spring complex, the channel opens. Opening of the channel decreases the gating-spring's extension by a distance known as the gating-swing.

Figure 4:

Measurements and mechanisms of adaptation. (a) Resting tension in the gating-spring complex is maintained by myosin motors at the tip link's upper end and requires that the MET channels are anchored to the cytoskeleton at the tip link's lower end. Calcium entry through the channels can reduce tension in the gating-spring, and thus close the channels, by inhibiting the myosin motors or by inducing a conformational change in the channels or the gating-spring complex. Because calcium must diffuse the large distance between the channels and the upper end of the tip link, adaptation at the upper end of the tip link is slow. Conversely, adaptation owing to calcium binding near the channel is fast. (b) A step displacement applied to a hair bundle (upper) increases the current into the bundle (middle). The current adapts with at least two timescales. To estimate the activation curve (lower), the peak currents for a set of displacement pulses (upper) are normalized to the maximum current. (c) In response to a step displacement, adaptation is schematized as a fast, viscoelastic adaptation element and an element producing a slow adaptation force. (1) Before stimulation, the adaptation processes help maintain resting tension in the gating-spring complex. (2) After stimulation, but before significant adaptation, the channel opens,

and the gating spring extends. (3) Calcium enters the hair cell and binds to the fast adaptation element inducing it to extend. In principle, the fast, viscoelastic element could extend independently of calcium. (4) Calcium reduces the force produced by the slow element.

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Figure 5:

Adaptation might arise from a calcium-independent relaxation. (a) A stiff probe is used to apply step displacements to a hair bundle. Example displacements pulses are shown, which are applied before and after the primary displacements to measure the adapted activation curves. (b) (Top) If adaptation required calcium influx, then preventing calcium entry by applying a positive membrane potential would eliminate adaptation. In experiments, however, the current adapts for both positive (middle) and negative (bottom) holding potentials. Consequently, a component of adaptation is independent of calcium entry.

Current responses to displacement pulses are also illustrated. (c) The activation curves' adaptive shift is slightly greater at a positive holding potential in comparison to a negative holding potential. Wider activation curves at positive potentials may indicate a calcium-dependent gating element. (d) For calcium-dependent adaptation, spontaneous hair-bundle oscillations are possible over a limited range of calcium concentrations. There is no evidence supporting a calcium-independent mechanism for spontaneous oscillations, which implies that there are no spontaneous oscillations at any calcium concentration.

Figure 6:

(a) By changing its operating point, a vestibular hair bundle, which detects steps, can be induced to detect periodic signals, an auditory function. A bundle's behavior and response to time-dependent forcing are shown as functions of its stiffness and constant-force load (see panel b). Orange lines of supercritical (thick) and subcritical (thin) Hopf **bifurcations** and lines of fold bifurcations (wine) are shown. (Bistability) Displacements (Disp.) of a bundle are illustrated as functions of time for different initial conditions, yielding two distinct steady states. (Spon. Osc.) As the load stiffness grows, the amplitude of spontaneous oscillations declines but their frequency rises. (Periodic Signal Detection) A bundle's

response amplitude (Ampl.) at the stimulus frequency (Freq.) is larger and more sharply tuned the closer the bundle operates to the region of spontaneous oscillations. (Step Onset Detection) A bundle best detects the onset of a step stimulus when it operates near the zone of spontaneous oscillations. (Step Size Detection) Starting from the same operating point, the frequency of noise induced spikes is correlated with the magnitude of a step stimulus. The trajectories illustrated in distinct regions have different time, displacement, and force scales. (b) An accessory structure applies a constant force on a bundle and forces that depend on the structure's stiffness, damping coefficient, and mass. The adaptation process and an external stimulus produce additional forces. (c) The sector of spontaneous oscillations shrinks and eventually vanishes as the damping coefficient's value grows. (d) The area of spontaneous oscillations expands as the accessory structure's mass rises.