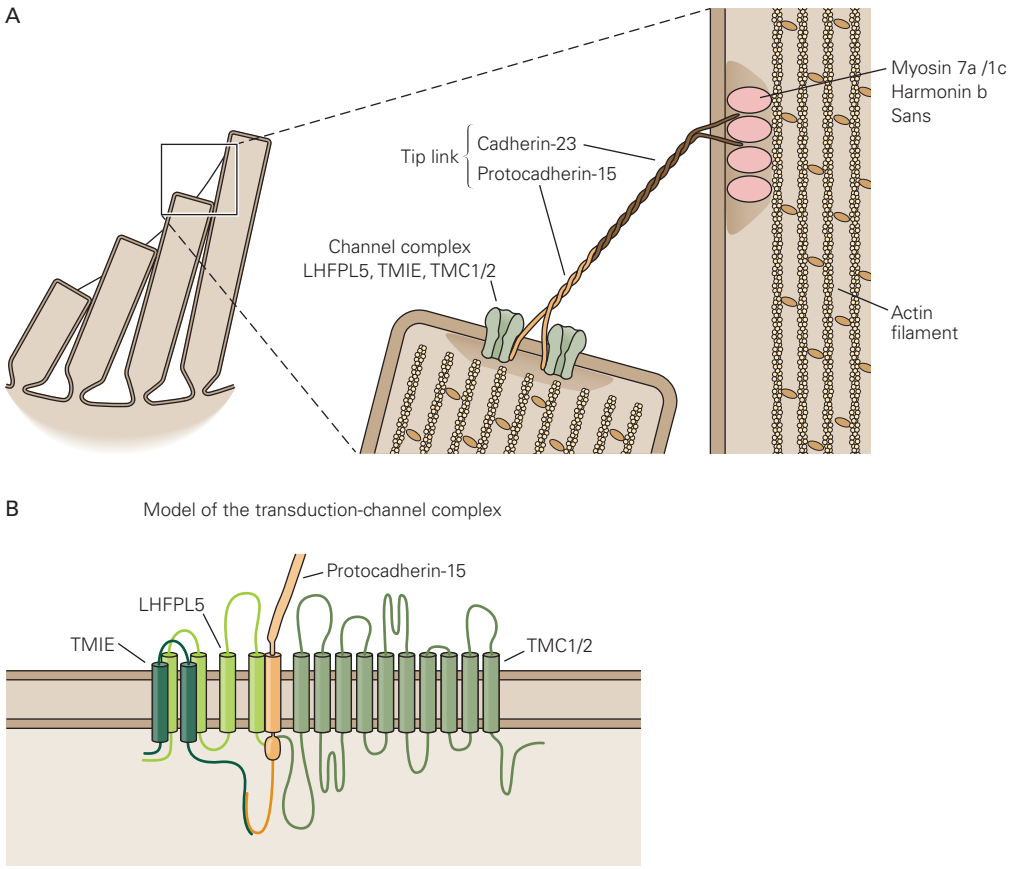

Figure 26–10 Molecular composition of the transduction machinery.

A. The tip link is composed of the heterophilic association of protocadherin 15 and cadherin 23. Two transduction channels are localized near the lower insertion point of the tip link at the tip of the shorter stereocilium. Each channel is part of a molecular complex that includes the proteins TMC1/2, LHFPL5, and TMIE. At the upper insertion point on the flank of the longer stereocilium, cadherin 23 interacts with harmonin b and the molecular motor myosin 7a, which both bind to actin and thus anchor the tip link. The protein sans serves as a scaffolding protein. In vestibular hair cells, myosin 1c may set the tip link under tension, but the presence of this motor protein is uncertain in cochlear hair cells.

B. Model of the transduction-channel complex. TMC1/2, LHFPL5, and TMIE interact with protocadherin 15 and, thus, with the lower end of the tip link. TMIE also interacts with LHFPL5. The detailed arrangement of these proteins within the transduction apparatus is still unknown. Unlike what the figure suggests, TMC1 has been proposed to assemble as a dimer, with each TMC1 molecule contributing a permeation pathway for cations. (Adapted, with permission, from Wu and Müller 2016 and Pan et al. 2018.)

2 (TMC1 and TMC2), tetraspan membrane protein in hair-cell stereocilia (TMHS; official nomenclature LHFPL5), and transmembrane inner-ear-expressed gene (TMIE; Figure 26–10). Mechanotransduction is abolished in mouse hair cells lacking TMIE, even though all other known components of the transduction machinery appear to be properly in place. However, because TMIE contains only two predicted transmembrane domains, it seems highly unlikely that this protein alone constitutes an ion channel. In the absence of LHFPL5, the conductance of the transduction channel is reduced, but significant transduction currents can still be measured, suggesting that this protein is not an essential part of the channel pore.

Multiple lines of evidence advocate for TMC1 and TMC2 as components of the transduction channel. Both proteins are localized near the lower insertion point of the tip link, where the transduction current enters the hair cell, interact with the tip-link constituent protocadherin 15, and their onset of expression coincides with that of mechanoelectrical transduction. In addition, transduction channels in a mouse carrying a single point mutation in the *Tmc1* gene show lower conductance and Ca^{2+} permeability, indicating that TMC1 is very close to the channel's pore. Individual

cysteine substitutions made at sites that are predicted to be in or near the channel pore confirm that TMC1 belongs to the main conductive pathway. Indeed, covalent modification of the cysteine residues with a positively charged reagent leads to a reduction of the single-channel conductance in several TMC1 mutants. The cysteine-modifying reagent has no effect when the reagent is applied after the hair bundle has been deflected towards its short edge (to close the transduction channels) or when access to the channel pore is prevented by a channel blocker. TMC1 is thus unlikely to constitute an accessory channel subunit that forms a vestibule to the pore-forming protein(s). Instead, the evidence is strong that TMC1 forms at least part of the pore of the transduction channel. In cochlear hair cells, TMC1 and TMC2 are coexpressed during neonatal development, but only TMC1 expression is maintained through adulthood.

Dynamic Feedback Mechanisms Determine the Sensitivity of the Hair Cells

Hair cells must cope with acoustic stimuli that have a very low energy content. If the stimulus consists of a periodic signal, such as the sinusoidal pressure of a pure tone, a detection system can increase the signal-to-noise ratio by enhancing selectively the response to a relevant frequency. Hair cells respond best at a characteristic frequency of acoustic stimulation. The frequency selectivity of a given hair cell results in part from passive extrinsic filtering of its mechanical input, in particular as a result of the tonotopic arrangement of the mammalian basilar membrane. In addition, when it is appropriate that low-frequency inputs be disregarded, hair cells possess a unique mechanism of adaptation that acts as a high-pass filter. Hair cells also employ mechanical amplification that enhances and further tunes their mechanosensitivity.

Hair Cells Are Tuned to Specific Stimulus Frequencies

Every cochlear hair cell is most sensitive to stimulation at a specific frequency, termed its characteristic, natural, or best frequency. On average, the characteristic frequencies of adjacent inner hair cells differ by approximately 0.2%; adjacent piano strings, in comparison, are tuned to frequencies some 6% apart. Because the traveling wave evoked even by a pure sinusoidal stimulus spreads somewhat along the basilar membrane, the sensitivity of a cochlear hair cell extends within a limited range above and below its characteristic frequency, the more so with a greater level of stimulation. At low levels, a pure tone recruits approximately 100 hair cells. The frequency sensitivity of a hair cell may be displayed as a tuning curve. To construct a tuning curve, an experimenter stimulates the ear with pure tones at numerous frequencies below, at, and above the cell's characteristic frequency. The level of stimulation is adjusted for each frequency until the cell's response reaches a predefined criterion magnitude. The tuning curve is then a graph of sound level, presented logarithmically in decibels SPL, as a function of stimulus frequency.

The tuning curve for an inner hair cell is typically V-shaped (Figure 26–11). The curve's tip represents the cell's characteristic frequency, the frequency that produces the criterion response for the lowest level of the stimulus. Sounds of greater or lesser frequencies require higher levels to excite the cell to the criterion response. As a consequence of the traveling wave's shape, the slope of a tuning curve is far steeper on its high-frequency flank than on its low-frequency flank.

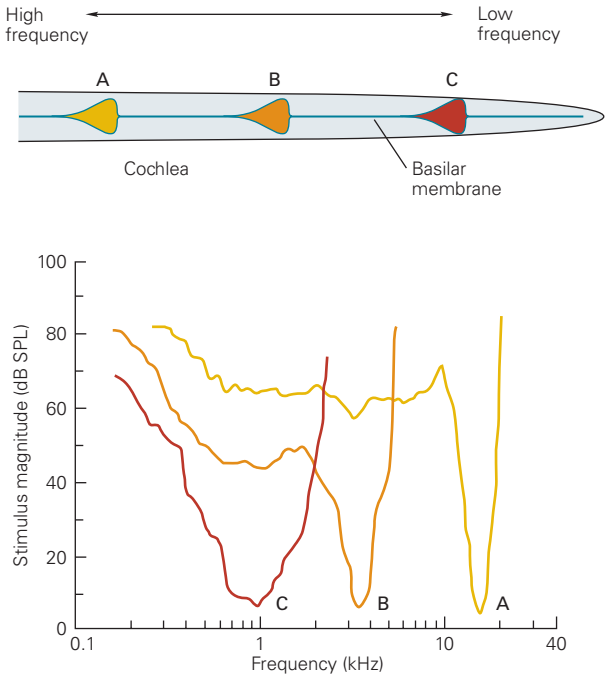

Figure 26–11 Tuning curves for cochlear hair cells. To construct a curve, the experimenter presents sound at several frequencies. At each frequency, the stimulus intensity is adjusted until the cell produces a criterion response, here 1 mV. The curve thus reflects the threshold of the cell for stimulation over a range of frequencies. Each cell is most sensitive to a specific frequency, its characteristic frequency. The threshold rises briskly—the sensitivity falls rapidly—as the stimulus frequency is raised or lowered. The characteristic frequency depends on the position of the hair cell along the longitudinal axis of the cochlea. (Reproduced, with permission, from Kiang 1980. Copyright © 1980 Acoustical Society of America.)

In the same way as a tuning fork's resonant frequency depends on the size of its tines, the heights of the hair bundles vary systematically along the tonotopic axis. Hair cells that respond to low-frequency stimuli have the tallest bundles, whereas those that respond to the highest-frequency signals possess the shortest bundles. In the human cochlea, for example, an inner hair cell with a characteristic frequency of 20 kHz bears a 4-μm hair bundle. At the opposite extreme, a cell sensitive to a 20-Hz stimulus has a bundle more than 7 μm high. A similar morphological gradient is observed with outer hair cells, supplementing the extrinsic tuning accomplished by the basilar membrane.

Hair Cells Adapt to Sustained Stimulation

Despite the precision with which a hair bundle grows, it cannot develop in such a way that the sensitive transduction apparatus is always perfectly poised at its position of greatest mechanosensitivity. Some mechanism must compensate for developmental irregularities, as well as for environmental changes, by adjusting the gating springs so that transduction channels are responsive to weak stimuli at the bundle's resting position. To ensure this, an adaptation process continuously resets the hair bundle's range of mechanical sensitivity. As a result of adaptation, a hair cell can maintain a high sensitivity to transient stimuli while rejecting static inputs a million times as large.

Adaptation manifests itself as a progressive decrease in the receptor potential during protracted deflection of the hair bundle (Figure 26–12). The process is not one of desensitization, for the responsiveness of the receptor persists. Instead, during a prolonged step stimulus, the sigmoidal relationship between the initial receptor potential and the bundle's position shifts in the direction of the applied stimulus. As a result, the membrane potential of the hair cell progressively returns to near its resting value. Adaptation is incomplete, however; the relation between the membrane potential and the bundle position shifts by approximately 80% of the deflected position.

How does adaptation occur? Because the mechanical force exerted by a hair bundle changes as adaptation proceeds, the process evidently involves an adjustment in the tension borne by the gating springs. It appears likely that the structure anchoring the upper end of each tip link, the *insertional plaque,* is repositioned during adaptation by an active molecular motor (Figure 26–12). The transduction channels are inherently more stable in a closed state, for they close when the tip links are disrupted. A motor is thus also required to maintain a significant fraction (10%–50%) of the transduction channels open at rest by continuously pulling on the gating springs. Several dozen myosin molecules associated with the upper end of each tip link are thought to maintain tension by ascending the actin core of the stereocilium and pulling the link's insertion with them.

When a stimulus step increases the tension in a gating spring, the associated transduction channel opens, permitting an influx of cations. As Ca^{2+} ions accumulate in the stereociliary cytoplasm, they reduce the upward force of the myosin molecules, thereby shortening the gating spring. When the spring reaches its resting tension, closure of the channel reduces the Ca^{2+} influx to its original level, restoring a balance between the upward force of myosin and the downward tension in the spring.

Hair bundles contain at least five isoforms of myosin, the motor molecule associated with motility along actin filaments (Chapter 31). In vestibular hair cells, immunohistochemical studies and site-directed mutagenesis implicate myosin 1c in adaptation. In cochlear hair cells, the role of myosin 1c in adaptation has remained elusive. Another motor protein, myosin 7a, is present near the upper insertion point of the tip link and mutations in the corresponding gene (*USH1B*) are associated with deafness. Hair-cell bundles defective for myosin 7a are disorganized, suggesting that this motor is involved at least in hair-bundle development.

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. This is the case in response to large deflections of the hair bundle, for which the time constant of adaptation is approximately 20 ms or more when endolymph bathes the hair bundle. This slow adaptation is compatible with the activity of a myosin-based motor driven by the cyclical hydrolysis of adenosine triphosphate (ATP). Yet, after being pulled open by an excitatory step stimulus of small magnitude, transduction channels reclose with typical timescales of less than 1 ms and thus short enough to be compatible with auditory frequencies. Current models posit that Ca^{2+} ions entering a hair cell through a transduction channel bind to or near the channel's pore, thereby energetically favoring channel closure. The kinetics of this fast adaptation varies systematically along the tonotopic axis of auditory organs, indicating that adaptation may help in setting the hair cell's characteristic frequency of maximal responsiveness. In addition, the reciprocal relationship between channel gating and tip-link tension means that adaptive channel rearrangements evoke internal forces that drive active hair-bundle movements. The mechanical correlate of adaptation

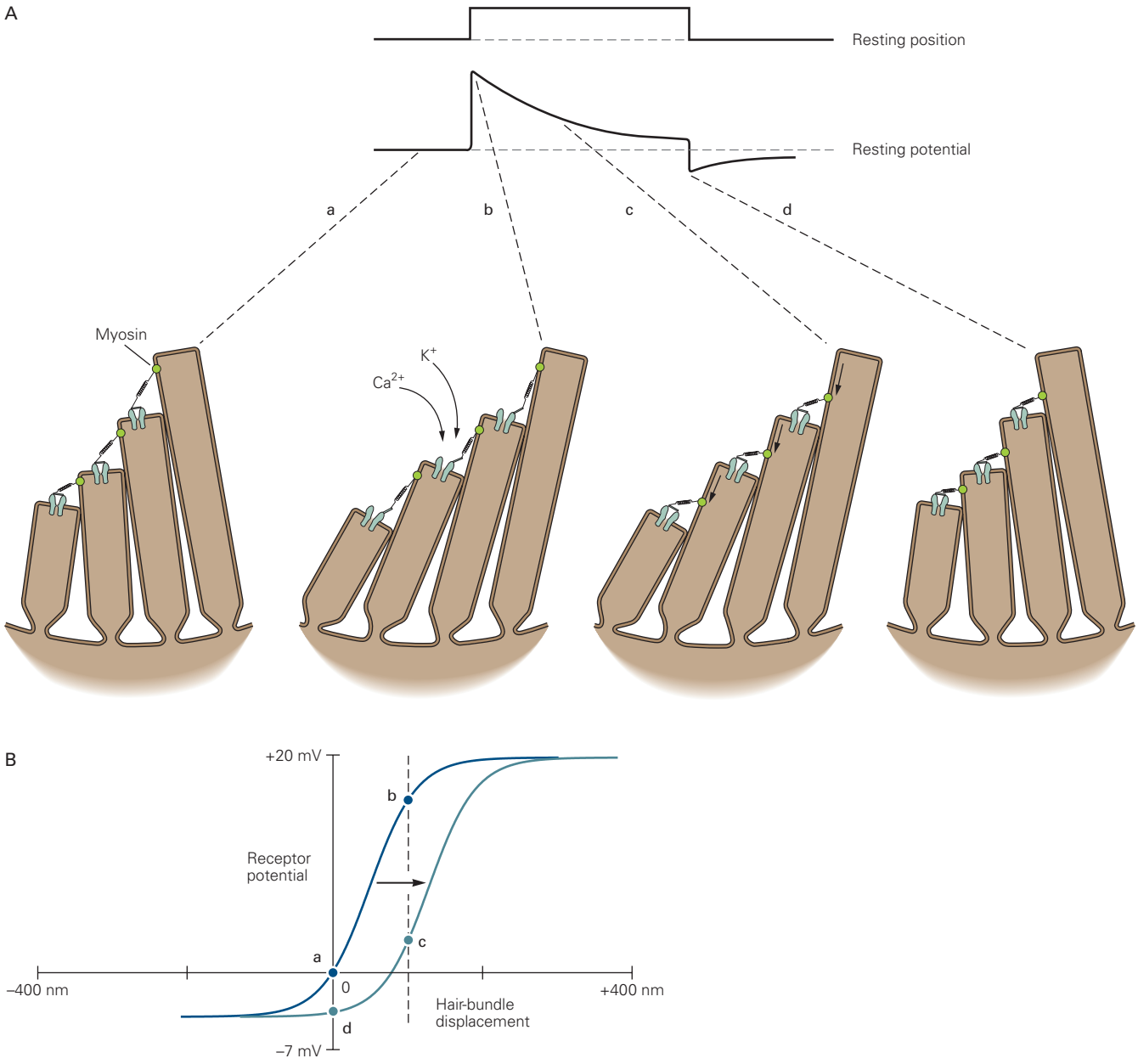

Figure 26–12 Adaptation of mechanoelectrical transduction in hair cells. **A.** Prolonged deflection of the hair bundle in the positive direction (**upper trace**) elicits an initial depolarization followed by a decline to a plateau and an undershoot at the cessation of the stimulus (**lower trace**). The four schematic hair-cell bundles illustrate the states of the transduction channels before (**a**) and during the illustrated phases of the adaptation (**b–d**). Initially, the stimulation increases tension in the tip link (second bundle), thus opening transduction channels. As stimulation continues, however, a tip link's upper attachment is thought to slide down the stereocilium, allowing each channel to close during adaptation (third bundle). Prolonged deflection of the hair bundle in the negative direction elicits a complementary response. The cell is slightly hyperpolarized at first but shows a rebound depolarization at the end of stimulation; tension is restored to the initially slack tip link as myosin molecules actively pull up the link's upper insertion. **B.** As adaptation proceeds, the sigmoidal relation between hair bundle displacement and the receptor potential of the hair cell shifts to the right along the abscissa, in the direction of the new hair-bundle position (**dashed line**), without substantial changes in the curve's shape or amplitude. The shift explains why the receptor potential decreases over time as shown in **A** between states **b** and **c**, restoring the membrane potential of the hair cell to near its value when there is no stimulus. This result implies that adaptation restores mechanical sensitivity to small rapid deflections of the hair bundle in the presence of a protracted stimulus that would otherwise saturate mechanoelectrical transduction. The four states of the transduction channels shown in **A** are marked (**a–d**). (Adapted, with permission, from Hudspeth and Gillespie 1994.)

thus provides feedback that can enhance the stimulus to the hair cell.

Sound Energy Is Mechanically Amplified in the Cochlea

The inner ear faces an important obstacle to efficient operation: A large portion of the energy in an acoustic stimulus goes into overcoming the damping effects of cochlear liquids on hair-cell and basilar-membrane motion rather than into excitation of hair cells. The sensitivity of the cochlea is too great, and auditory frequency selectivity too sharp, to result solely from the inner ear's passive mechanical properties. The cochlea must therefore possess some means of actively amplifying sound energy.

One indication that amplification occurs in the cochlea comes from measurements of the basilar membrane's movements with sensitive laser interferometers. In a preparation stimulated with low-level sound, the basilar-membrane motion is highly dependent on frequency. The movement is maximal at the appropriate frequency for the position at which the measurement is made—the characteristic frequency—but drops abruptly at higher or lower frequencies. As the sound level is increased, however, the frequency selectivity of the vibration becomes less sharp; the peak in the relationship between amplitude and frequency broadens. In addition, the membrane's sensitivity to sound, defined as the vibration amplitude per unit of sound pressure, declines precipitously. When stimulated at the characteristic frequency, the sensitivity of basilar-membrane motion to stimulation at 80 dB SPL is less than 1% of that for 10 dB SPL excitation. The basilar membrane displays a compressive nonlinearity that accommodates the millionfold variation of sound pressure that characterizes audible sounds (0–120 dB SPL) into only two to three orders of magnitude of vibration amplitude (±0.3–300 nm). The sensitivity and frequency selectivity predicted in modeling studies of a passive cochlea correspond to those observed with high-level stimuli. This result implies that the motion of the basilar membrane is augmented more than 100-fold during low-level stimulation at the characteristic frequency, but that amplification diminishes progressively as the stimulus grows in strength. Consequently, amplification lowers the threshold of hearing by more than 40 to 50 dB SPL.

In addition to this circumstantial evidence, experimental observations support the idea that the cochlea contains a mechanical amplifier. When a normal human ear is stimulated with a click, that ear emits one to several measurable pulses of sound within

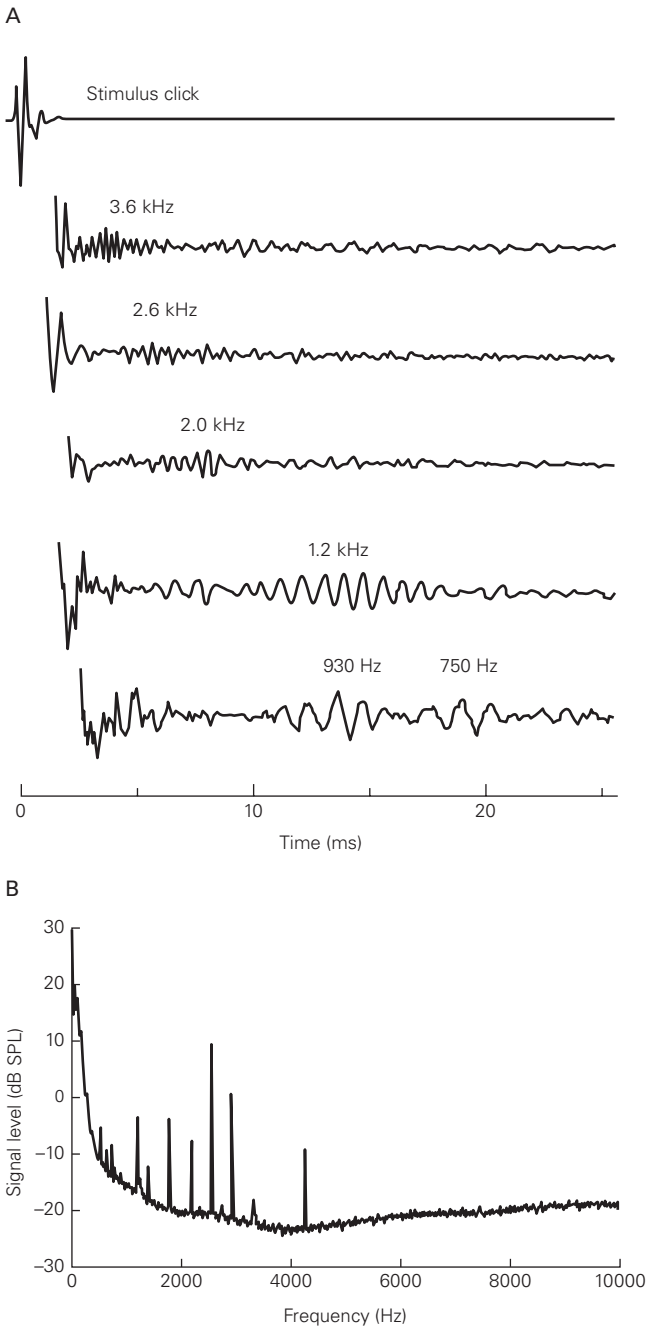

Figure 26–13 The cochlea actively emits sounds.

A. The records display evoked otoacoustic emissions from the ears of five human subjects. A brief click (**top trace**) was played into each ear through a miniature speaker. A few milliseconds later, a tiny microphone in the external auditory meatus detected one or more bursts of sound emission from the ear. (Adapted, with permission, from Wilson 1980. Copyright © 1980 Elsevier B.V.)

B. Under suitably quiet recording conditions, spontaneous otoacoustic emissions occur in most normal human ears. This spectrum displays the acoustic power of six prominent emissions and several smaller ones from one ear. (Reproduced, with permission, from Murphy et al. 1995. Copyright © 1995, Acoustical Society of America.)

milliseconds (Figure 26–13A). Because they can carry more energy than the stimulus, these so-called *evoked otoacoustic emissions* cannot simply be echoes; they represent the emission of mechanical energy by the cochlea, triggered by acoustic stimulation. In accordance with the compressive nonlinearity associated with cochlear amplification, the relative level of the emissions decreases with the stimulus level.

A still more compelling manifestation of the cochlea's active amplification is *spontaneous otoacoustic emission.* When a suitably sensitive microphone is used to measure sound pressure in the ear canals of subjects in a quiet environment, at least 70% of normal human ears continuously emit one or more pure tones (Figure 26–13B). Although these sounds are generally too faint to be directly audible by others, physicians have reported actually hearing sounds emanating from the ears of newborns!

What is the source of evoked and spontaneous otoacoustic emissions, and thus presumably of cochlear amplification as well? Several lines of evidence implicate outer hair cells as the elements that enhance cochlear sensitivity and frequency selectivity and hence act as the motors for amplification. The afferent nerve fibers that extensively innervate the inner hair cells make only minimal contacts with the outer hair cells (Figure 26–4). Instead, the outer hair cells receive an extensive efferent innervation that, when activated, decreases cochlear sensitivity and frequency discrimination. In addition, when stimulated electrically, an isolated outer hair cell displays the unique phenomenon of electromotility: The cell body shortens by up to several micrometers when depolarized and elongates when hyperpolarized (Figure 26–14). This response can occur at frequencies exceeding 80 kHz, an attractive feature for a process postulated to assist high-frequency hearing.

The energy for these movements is drawn from the experimentally imposed electrical field rather than from hydrolysis of an energy-rich substrate such as ATP. Movement occurs when changes in the electric field across the membrane reorient molecules of the protein prestin. The concerted movement of several million of these molecules, which are packed in the lateral cell membranes of outer hair cells, changes the membrane's area and thus the cell's length. When an outer hair cell transduces mechanical stimulation of its hair bundle into receptor potentials, cochlear amplification might then occur when voltage-induced movement of the cell body augments basilar-membrane motion. Consistent with this hypothesis, mutation of certain amino acid residues required for the voltage sensitivity of prestin abolishes the active process in mice.

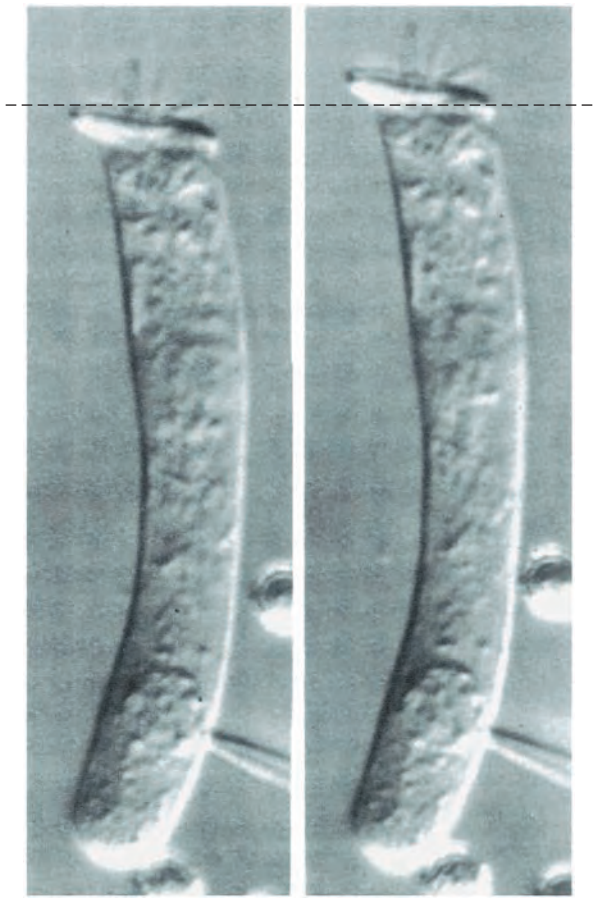

Figure 26–14 Voltage-induced motion of an outer hair cell. Depolarization of an isolated outer hair cell through the electrode at its base causes the cell body to shorten (*left*); hyperpolarization causes it to lengthen (*right*). The oscillatory motions of outer hair cells may provide the mechanical energy that amplifies basilar-membrane motion and thus enhances the sensitivity of human hearing. (Reproduced, with permission, from Holley and Ashmore 1988.)

Because sharp frequency selectivity, high sensitivity, and otoacoustic emissions are also observed in animal species that lack outer hair cells and lack high concentrations of prestin, electromotility cannot be the only form of mechanical amplification by hair cells. In addition to detecting stimuli, hair bundles are also mechanically active and contribute to amplification. Hair bundles can make spontaneous back-and-forth movements that have been shown in some nonmammals to underlie spontaneous otoacoustic emissions. Under experimental conditions, bundles can exert force against stimulus probes, performing mechanical work and thereby amplifying the input. In vitro experiments indicate that active hair-bundle motility

contributes to the cochlear active process even in the mammalian ear.

Active hair-bundle movements can be fast enough to mediate otoacoustic emissions at sound frequencies at least as high as a few kilohertz. However, it remains uncertain whether bundles can generate forces at the very high frequencies at which sharp frequency selectivity and otoacoustic emissions are observed in the mammalian cochlea. Active hair-bundle motility and somatic electromotility may function synergistically, with the former serving metaphorically as a tuner and preamplifier and the latter as a power amplifier. Alternatively, hair-bundle motility may dominate at relatively low frequencies but be superseded by electromotility at higher frequencies.

Cochlear Amplification Distorts Acoustic Inputs

When stimulated by two tones at nearby frequencies f_1 and f_2 ($f_1 < f_2$), the basilar membrane vibrates not only at these frequencies but also at additional frequencies—the distortion products—that are not present in the acoustic stimulus. As reported by the Italian violinist Giuseppe Tartini in the 18th century, distortion products can be heard as phantom tones in the auditory percept. Remarkably, the cubic difference tone $2f_1 - f_2$ is heard even at very low sound levels, and its magnitude grows in proportion to the stimulus. Correspondingly, the relative level of distortion remains practically constant over a broad range of sound levels. This phenomenon is explained by the particular form of the compressive nonlinearity associated with cochlear amplification. Clearly, the cochlea does not work as a high-fidelity sound receiver. Distorted cochlear vibrations are strong enough to be reemitted from the ear canal as *distortion-product otoacoustic emissions*. Because they are a property of healthy ears, these emissions are extensively used to screen hearing in newborns.

The Hopf Bifurcation Provides a General Principle for Sound Detection

Detailed in vivo and in vitro studies have revealed four cardinal features of auditory responsiveness. First, an active amplification process lowers the detection threshold. Second, because amplification operates only near a characteristic frequency, the input to the sensory system is actively filtered, which sharpens frequency selectivity. Third, for stimulation near the characteristic frequency, the response displays a compressive nonlinearity that represents a wide range of stimulus levels by a much narrower range of vibration amplitudes. Finally, even in the absence of a stimulus, mechanical activity can produce self-sustained oscillations that result in otoacoustic emissions.

These features have been recognized as signatures of an active dynamical system—a critical oscillator—that operates on the verge of an oscillatory instability termed the Hopf bifurcation (Box 26–1). They are generic: They do not depend on the subcellular and molecular details of the candidate mechanism that brings the system to the brink of spontaneous oscillation. The fact that active hair-bundle motility demonstrates a Hopf bifurcation in vitro provides further evidence that this mechanism contributes to cochlear amplification.

Within this framework, the characteristic frequency is set by that of the critical oscillator. The cochlear partition may be viewed as a set of active oscillatory modules that are hydrodynamically coupled by the cochlear fluids and with characteristic frequencies tonotopically distributed along the longitudinal axis of the cochlea. The hypothesis of critical oscillation facilitates modeling of the traveling wave and cochlear amplification. This is because the generic behaviors of a critical oscillator can be described by a single equation termed the "normal form" (Box 26–1). A critical oscillator is ideally suited for auditory detection, even if its inherent nonlinearity yields pronounced distortions in response to complex sound stimuli. Nonlinear interference between the frequency components of complex stimuli in fact appears as a necessary price to pay for the exquisite sensitivity, sharp frequency selectivity, and wide dynamic range of auditory detection afforded by a critical oscillator. The Hopf bifurcation provides generic properties that account for numerous disparate experimental observations at the level of a single hair bundle, of the basilar membrane, and even in psychoacoustics. This physical principle of auditory detection thus greatly simplifies our understanding of hearing. Although this chapter focusses primarily on mammalian hearing, the common necessity to hear with high sensitivity and sharp frequency selectivity poses similar physical constraints to the ears of all land vertebrates. These constraints have led to the independent evolution of ears that share similar structural features and whose operation is based on similar physical principles, including the use of critical oscillators to amplify sound (Box 26–2).

Hair Cells Use Specialized Ribbon Synapses

Being sensory receptors, hair cells form synapses with sensory neurons. The basolateral membrane of each cell contains several presynaptic active zones at which

Box 26–1 Generic Properties Near a Hopf Bifurcation

A dynamical system displays a Hopf bifurcation when it abruptly transits from quiescence to a state of spontaneous oscillation while subject to continuous variation of a control parameter C. If the system is poised in the vicinity of the critical point, at which $C = C_C$, its steady-state response to sinusoidal forces can be described by a single equation—the "normal form"—of a complex variable Z:

$$\Lambda \frac{dZ}{dt} \cong -\Lambda(C_c - C - 2i\pi f_c)Z - B|Z|^2 Z + F \qquad \textbf{(26–1)}$$

Here, the real part of Z may represent the position of the basilar membrane or of the hair bundle, Λ is a friction coefficient, and F is the external force provided by a sound stimulus. In the absence of an external force ($F = 0$), spontaneous oscillations emerge when the control parameter becomes larger than the critical value C_C (Figure 26-15A); the parameter f_C corresponds to the frequency of spontaneous oscillation at the critical point. A Hopf bifurcation must be driven by an active process, whereby the system mobilizes internal resources of energy to power spontaneous movements. A system operating precisely at the critical point is called a critical oscillator. The response of a critical oscillator to sinusoidal stimuli is endowed with generic properties (Figure 26–15B,C) that are characteristic of sound detection in the ear.

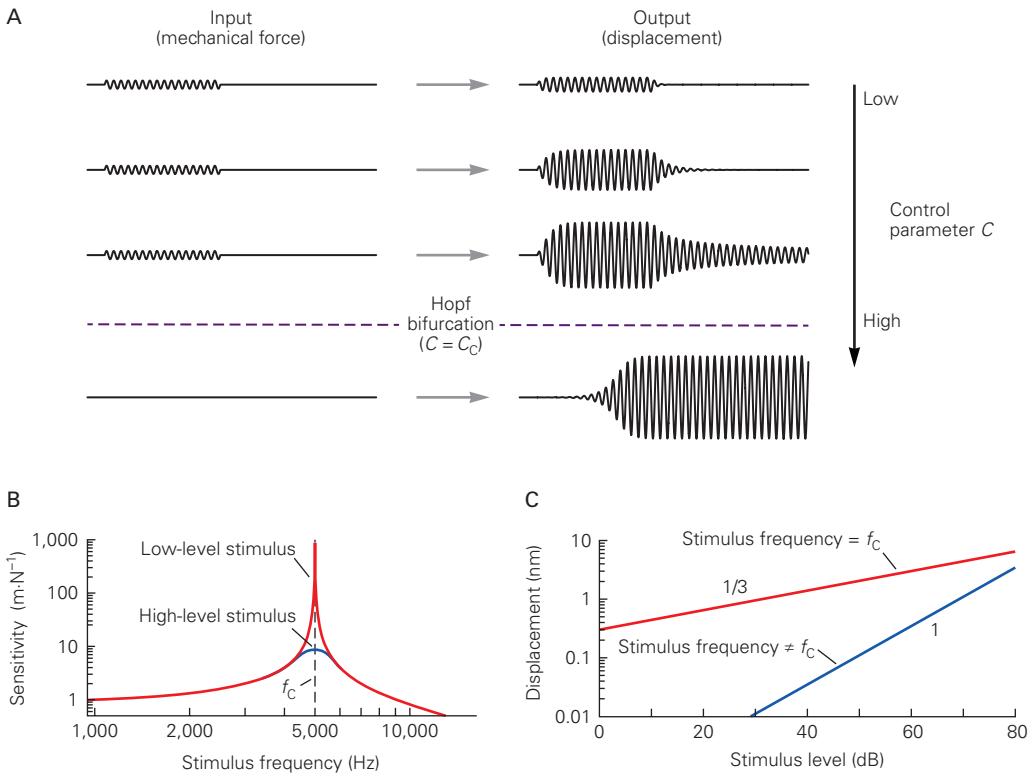

Figure 26–15 Frequency-selective amplification near a Hopf bifurcation.

A. As the control parameter C increases to approach the critical value C_C, the response to a sinusoidal stimulus of constant amplitude increases: The system gets more sensitive. For $C > C_C$, the system oscillates spontaneously at a constant amplitude and frequency, even if no stimulus is applied. The Hopf bifurcation corresponds to $C = C_C$. (Reproduced, with permission, from Hudspeth 2014.)

B. When the system is poised at the bifurcation ($C = C_C$), the sensitivity to a low-level stimulus is greatly enhanced near the characteristic frequency f_C (here 5000 Hz; **dashed line**), but drops rapidly if the stimulus is detuned from this frequency (**red line**). For a stimulus 60 dB more intense, the maximal sensitivity is 100-fold lower, and the peak in sensitivity is 100-fold broader (**blue line**): A critical oscillator enhances the weakest stimuli much more than the strongest and with much sharper frequency selectivity. (Adapted, with permission, from Hudspeth, Jülicher, and Martin 2010.)

C. At the characteristic frequency f_C, the response—here displacement—displays a compressive growth that corresponds to a line of slope 1/3 in this doubly logarithmic plot (**red line**): A large range of stimulus levels is represented by a much narrower range of response amplitudes. In contrast, when the frequency of the stimulus departs significantly from the characteristic frequency (**blue line**), the response is proportional to the input, corresponding to a line of slope unity. By amplifying weak inputs near its characteristic frequency, the critical oscillator lowers the stimulus level required to elicit a threshold vibration, here by 60 dB for a threshold of 0.3 nm. (Adapted, with permission, from Hudspeth, Jülicher, and Martin 2010.)

Box 26–2 The Evolutionary History of Hearing Resulted in Similarities Between Groups

Mammals are not alone in possessing sensitive and frequency-selective hearing. Amphibians and reptiles, including birds, also do. It is a remarkable fact that these various groups of land vertebrates actually acquired their good hearing systems largely independently. The small, dedicated auditory receptor organ was present in the inner ear of their common ancestor. Much later, the ancestors of modern lizards, birds, and mammals each independently evolved middle-ear systems with eardrums collecting sound from the outside world. Some species, such as birds and their relatives, even evolved two groups of sensory hair cells that have a division of labor similar to that of mammalian inner and outer hair cells. Comparison of middle-ear and inner-ear structures and functions across all living vertebrate groups has revealed that they share many common features and that hearing performance is largely comparable between them. Sound amplification associated with active hair-bundle motility was already present in the very first hair cells that evolved, even before the first fishes. This amplifying system was inherited by all groups and, as described earlier, plays a critical role in improving hearing sensitivity and sharpening frequency selectivity. The greatest difference between mammals and the other groups is that the upper frequency limit of hearing is generally higher in mammals. Nonmammalian ears are limited in response to frequencies lower than about 12 to 14 kHz, whereas some mammals can hear beyond 100 kHz.

In addition to active hair-bundle motility, the second mechanism that tunes individual hair cells to specific frequencies in many nonmammalian ears is electrical in nature. In many fishes, amphibians, and birds, the membrane potential of each hair cell resonates at a particular frequency. Several factors, including alternative splicing of the mRNA encoding cochlear K^+ channels and expression of these channels' auxiliary β subunit, tune the characteristic frequency of the resonance along the tonotopic axis of the auditory organ. Whether electrical resonance contributes to frequency tuning in the ears of mammals, including humans, remains uncertain. It is plausible that mammalian hair cells use instead an interplay between somatic electromotility, which seems absent in nonmammalian species, and the micromechanical environment, including hair-bundle motility, to actively amplify and filter their inputs.

The key signatures of a Hopf bifurcation have been recognized in spontaneous mechanical oscillations of the hair bundle, in electrical oscillations of the membrane potential, and in sound-evoked vibration of the basilar membrane. It is likely that the parallel evolution of hearing organs in different groups of vertebrates resulted in several ways of benefitting from the generic properties of critical oscillation.

chemical neurotransmitter is released. An active zone is characterized by four prominent morphological features (Figure 26–16).

A presynaptic dense body or synaptic ribbon lies in the cytoplasm adjacent to the release site. This fibrillar structure may be spherical, ovoidal, or flattened, and usually measures a few hundred nanometers across. The dense body resembles the synaptic ribbon of a photoreceptor cell and represents a specialized elaboration of the smaller presynaptic densities found at many other synapses. In addition to molecular components shared with conventional synapses, ribbon synapses contain large amounts of the protein ribeye.

The presynaptic ribbon is surrounded by clear synaptic vesicles, each 35 to 40 nm in diameter, which are attached to the dense body by tenuous filaments. Between the dense body and the presynaptic cell membrane lies a striking presynaptic density that comprises several short rows of fuzzy-looking material. Within the cell membrane, rows of large particles are aligned with the strips of presynaptic density. These particles include the Ca^{2+} channels involved in the release of transmitter as well as the K^+ channels that participate in electrical resonance in nonmammalian vertebrates.

Studies of nonmammalian experimental models show that, as with most other synapses (Chapter 15), the release of transmitter by hair cells is evoked by presynaptic depolarization and requires influx of Ca^{2+} from the extracellular medium. Hair cells lack synaptotagmins 1 and 2, however, and the role of those proteins as rapid Ca^{2+} sensors has probably been assumed by the protein otoferlin, which also promotes the replenishment of synaptic vesicles. Although glutamate is the principal afferent neurotransmitter, other substances are released as well.

The presynaptic apparatus of hair cells has several unusual features that underlie the signaling abilities of