membrane in a continuous array (Figure 26–3F). In the 19th century, the German physiologist Hermann von Helmholtz was the first to appreciate that the basilar membrane's operation is essentially the inverse of a piano's. The piano synthesizes a complex sound by combining the pure tones produced by numerous vibrating strings; the cochlea, by contrast, deconstructs a complex sound by isolating the component tones at the appropriate segments of the basilar membrane.

For any frequency within the auditory range, there is a characteristic place along the basilar membrane at which the magnitude of vibration is maximal. Although the morphological gradients of the basilar membrane are key to the process, the actual dispersion of a sound's frequency components along the cochlea's longitudinal axis depends on the mechanical properties of the cochlear partition as a whole. In particular, as we shall detail later, the hair cells within the organ of Corti provide active mechanical feedback that sharpens mechanical tuning of the basilar membrane and enhances its sensitivity to sound. The arrangement of vibration frequencies along the basilar membrane is an example of a *tonotopic map.* The relationship between frequency and position along the basilar membrane varies monotonically, but is not linear; the logarithm of the frequency decreases roughly in proportion to the distance from the cochlea's base. The frequencies from 20 kHz to 2 kHz, those between 2 kHz and 200 Hz, and those spanning 200 Hz to 20 Hz are each represented by approximately one-third of the basilar membrane's extent.

Analysis of the response to a complex sound illustrates how the basilar membrane operates in daily life. A vowel sound in human speech, for example, ordinarily comprises three dominant frequency components termed formants. Each frequency component of the stimulus establishes a traveling wave that, to a first approximation, is independent of the waves evoked by the others (Figure 26–3G) and reaches its peak excursion at a point on the basilar membrane appropriate for that frequency component. The basilar membrane thus acts as a mechanical frequency analyzer by distributing the energies associated with the different frequency components of the stimulus to hair cells arrayed along its length. In doing so, the basilar membrane begins the encoding of the frequencies in a sound.

The Organ of Corti Is the Site of Mechanoelectrical Transduction in the Cochlea

The organ of Corti, a ridge of epithelium extending along the basilar membrane, is the receptor organ of the inner ear. Each organ of Corti contains approximately 16,000 hair cells that are innervated by approximately 30,000 *afferent* nerve fibers; these are fibers that carry information into the brain along the eighth cranial nerve. Like the basilar membrane itself, each hair cell is most sensitive to a particular frequency, and these frequencies are logarithmically mapped in descending order from the cochlea's base to its apex. Thus, the information transmitted by these sensory cells to their innervating nerve fibers is also tonotopically organized.

The organ of Corti includes a variety of cells, some of unknown function, but four types have obvious importance. First, there are two types of hair cells. The *inner hair cells* form a single row of approximately 3,500 cells, whereas approximately 12,000 *outer hair cells* lie in three rows farther from the central axis of the cochlear spiral (Figure 26–4). The space between the inner and outer hair cells is delimited and mechanically supported by pillar cells. The outer hair cells are supported at their bases by Deiters's (phalangeal) cells.

A second epithelial ridge adjacent to the organ of Corti, but nearer the cochlea's central axis, gives rise to the tectorial membrane, a gelatinous shelf that covers the organ of Corti (Figure 26–4). The tectorial membrane is anchored at its base, and its tapered distal edge forms a fragile connection with the organ of Corti.

Hair cells are not neurons; they lack both dendrites and axons (Figure 26–5A). A special saline solution, the endolymph that fills scala media, bathes the cell's apical aspect. Tight junctions between hair cells and supporting cells separate this liquid from the standard extracellular fluid, or perilymph, that contacts the basolateral surface of the cell. Immediately below the tight junctions, a desmosomal junction provides a strong mechanical attachment for the hair cell to its neighbors.

The hair bundle, which serves as a receptive antenna for mechanical stimuli, projects from the flattened apical surface of the hair cell. Each bundle comprises a few tens to a few hundred cylindrical processes, the *stereocilia*, arranged in 2 to 10 parallel rows and extending several micrometers from the cell surface. Successive stereocilia across a cell's surface vary monotonically in height; a hair bundle is beveled like the tip of a hypodermic needle (Figure 26–5B). The inner hair-cell bundles of the mammalian cochlea, when viewed from above, have a roughly linear form. Outer hair-cell bundles, in contrast, have a V or W shape (Figure 26–6).

Each stereocilium is a rigid cylinder whose core consists of a fascicle of actin filaments that are heavily cross-linked by the proteins plastin (fimbrin), fascin, and epsin. Cross-linking renders a stereocilium

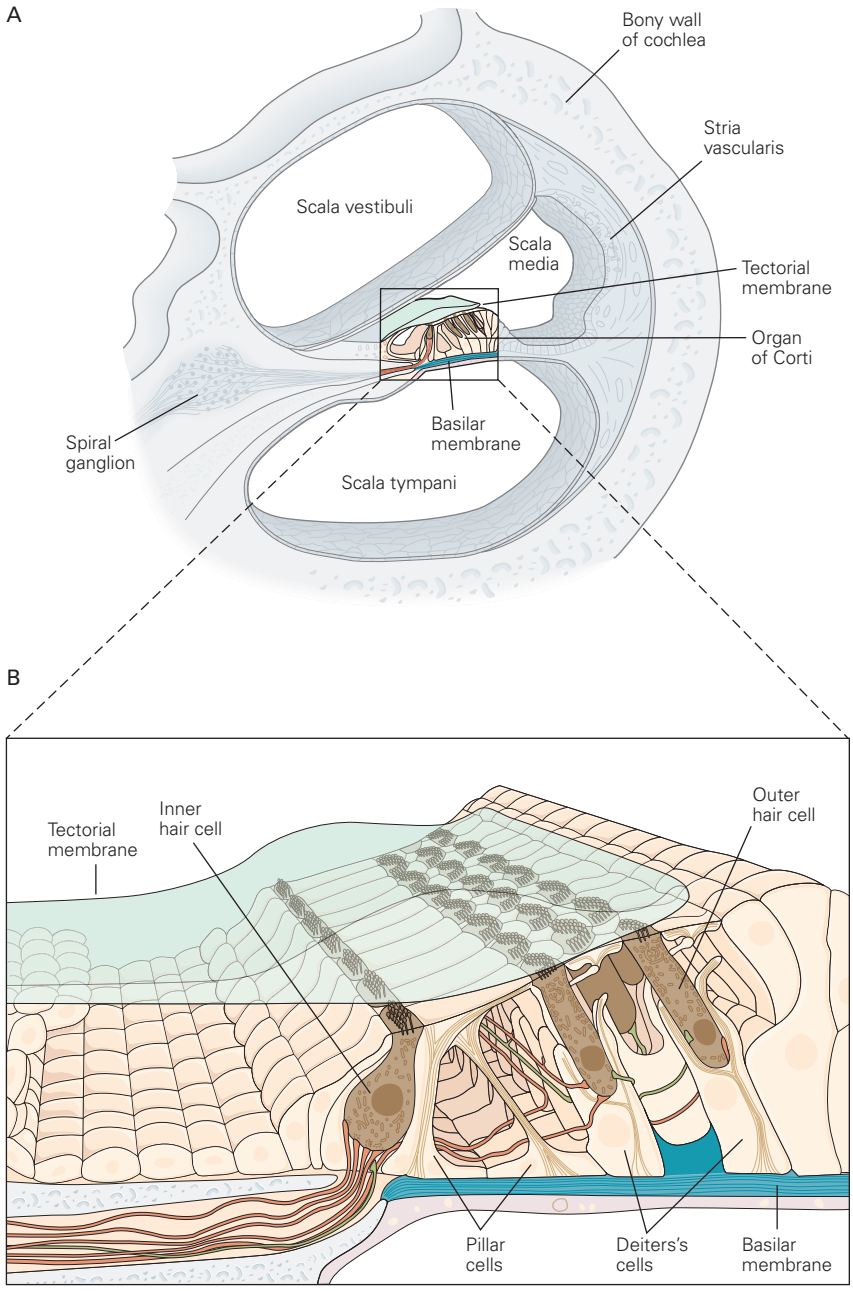


Figure 26–4 Cellular architecture of the human organ of Corti. Although there are differences among species, the basic plan is similar for all mammals.

A. The organ of Corti, the inner ear's receptor organ, is an epithelial strip that surmounts the elastic basilar membrane. The organ contains some 16,000 hair cells arrayed in four rows: a single row of inner hair cells and three rows of outer hair cells. The mechanically sensitive hair bundles of these receptor cells protrude into endolymph, the liquid within the scala media. Reissner's membrane, which provides the upper boundary of the scala media, separates the endolymph from the perilymph in the scala vestibuli. The hair bundles of outer hair cells are attached at their tops to the lower surface of the tectorial membrane, a gelatinous shelf that extends the full length of the basilar membrane.

B. The hair cells are separated and supported by pillar cells and Deiters's cells. One hair cell has been removed from the middle row of outer hair cells to reveal the three-dimensional relationship between supporting cells and hair cells. Afferent and efferent nerve endings are colored in **red** and **green**, respectively.

much more rigid than would be expected for a bundle of unconnected actin filaments. The actin core of the stereocilium is covered by a tubular sheath of plasma membrane. Although a stereocilium is of constant diameter along most of its length, it tapers just above its basal insertion (see Figure 25–5B). Correspondingly, the number of actin filaments diminishes from several hundred to only a few dozen. This thin cluster of microfilaments anchors the stereocilium in the cuticular plate, a thick mesh of interlinked actin filaments beneath the apical cell membrane. Because of this tapered structure, a mechanical force applied at the tip causes the stereocilium to pivot around its basal insertion. Horizontal top connectors interconnect adjacent stereocilia near their tips. These extracellular filaments restrict the bundle to move as a unit during stimulation at low frequencies. At high frequencies, the viscosity of the liquid between the stereocilia also opposes their separation and thus ensures the unitary motion of the hair bundle.

During its early development, every hair bundle includes at its tall edge a single true cilium, the *kinocilium* (Figure 26–5). Like other cilia, this structure possesses at its core an axoneme, or array of nine paired microtubules, and often an additional central pair of microtubules. The kinocilium is not essential for mechanoelectrical transduction, for in mammalian cochlear hair cells, it degenerates around the time of birth.

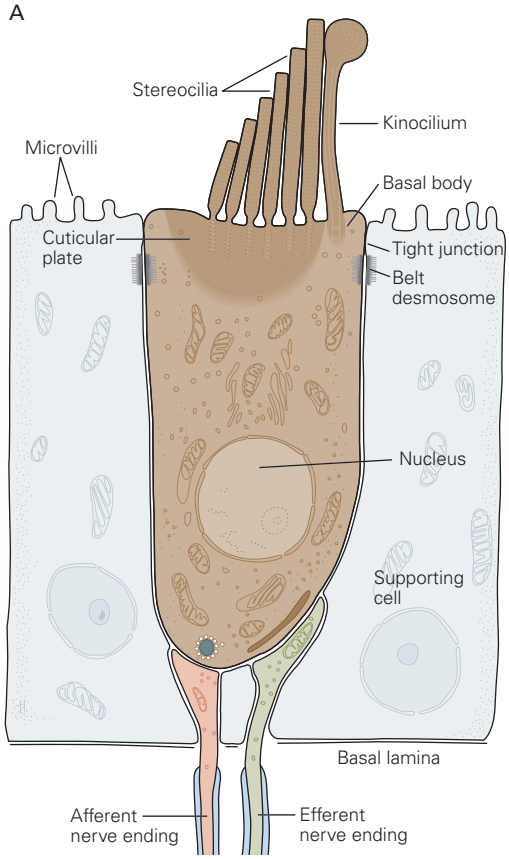
Hair Cells Transform Mechanical Energy Into Neural Signals

Deflection of the Hair Bundle Initiates Mechanoelectrical Transduction

Just as in vestibular organs (Chapter 27), mechanical deflection of the hair bundle is the stimulus that excites hair cells of the cochlea. Stimuli elicit an electrical response, the receptor potential, by opening or closing—a process termed "gating"—mechanically sensitive ion channels. The hair cell's response depends on the direction and magnitude of the stimulus.

In an unstimulated cell, 10% to 50% of the channels involved in stimulus transduction are open. As a result, the cell's resting potential, which lies within a range of approximately –70 to –30 mV, is determined in part by the influx of cations through these channels. A stimulus that displaces the bundle toward its tall edge opens additional channels, thereby depolarizing the cell (Figure 26–7). In contrast, a stimulus that displaces the bundle toward its short edge shuts transduction channels that are open at rest, thus hyperpolarizing

A



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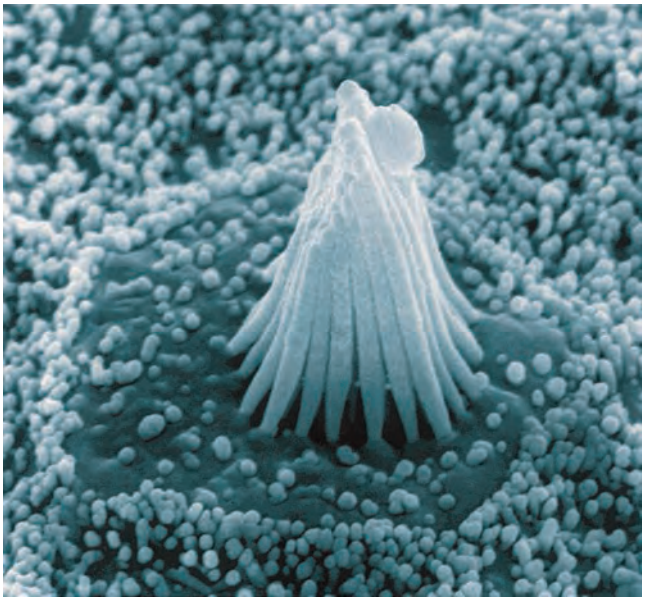


Figure 26–5 (Left) Structure of a vertebrate hair cell.

A. The epithelial character of the hair cell is evident in this drawing of the sensory epithelium from a frog's internal ear. The cylindrical hair cell is joined to adjacent supporting cells by a junctional complex around its apex. The hair bundle, a mechanically sensitive organelle, extends from the cell's apical surface. The bundle comprises some 60 stereocilia arranged in stepped rows of varying length. At the bundle's tall edge stands the single kinocilium, an axonemal structure with a bulbous swelling at its tip; in the mammalian cochlea, this organelle degenerates around the time of birth. Deflection of the hair bundle's top to the right depolarizes the hair cell; movement in the opposite direction elicits hyperpolarization. The hair cell is surrounded by supporting cells, whose apical surfaces bear a stubble of microvilli. Afferent and efferent synapses contact the basolateral surface of the plasma membrane.

B. This scanning electron micrograph of a hair cell's apical surface reveals the hair bundle protruding approximately 8 μm into the endolymph. (Image reproduced, with permission, from A.J. Hudspeth.)

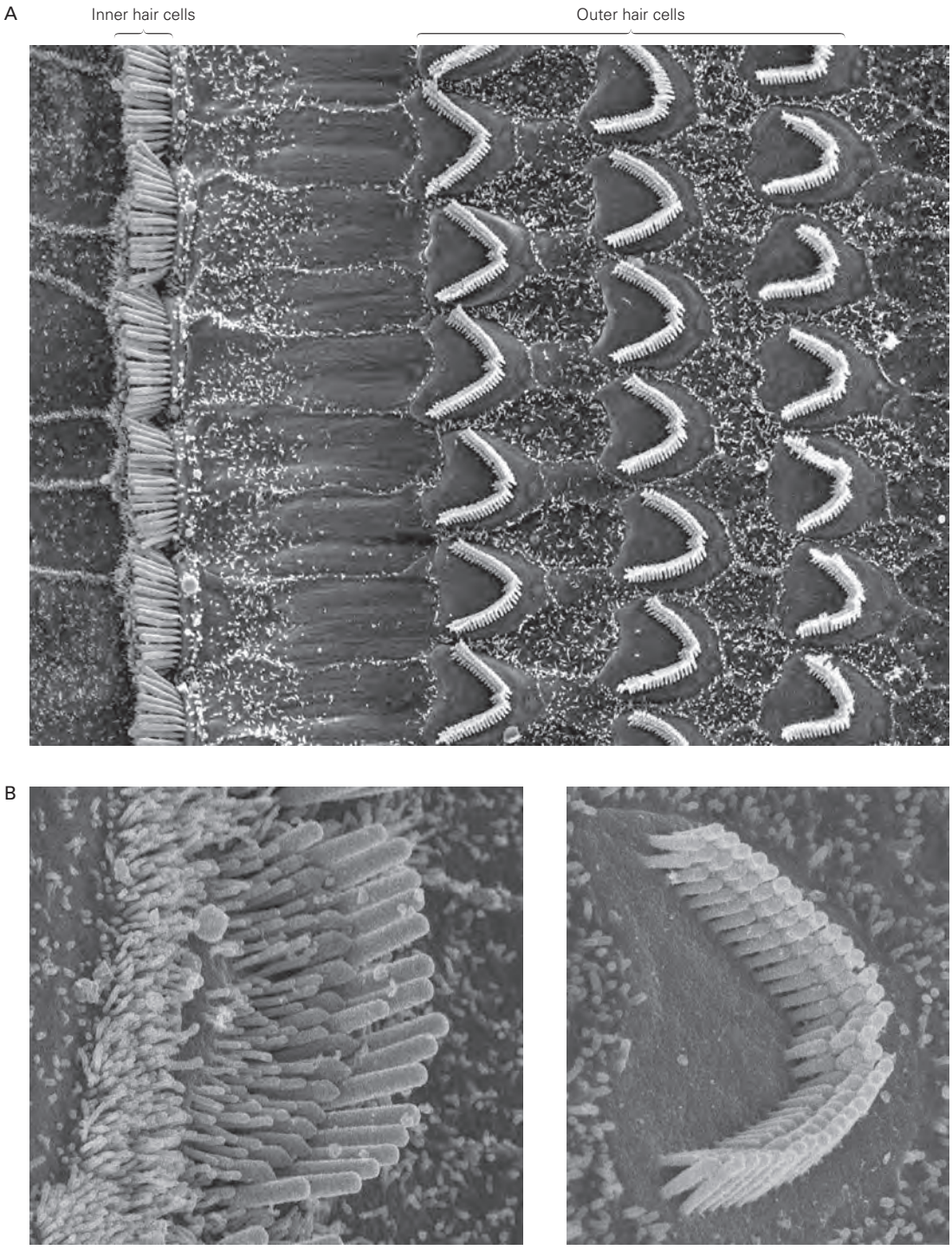
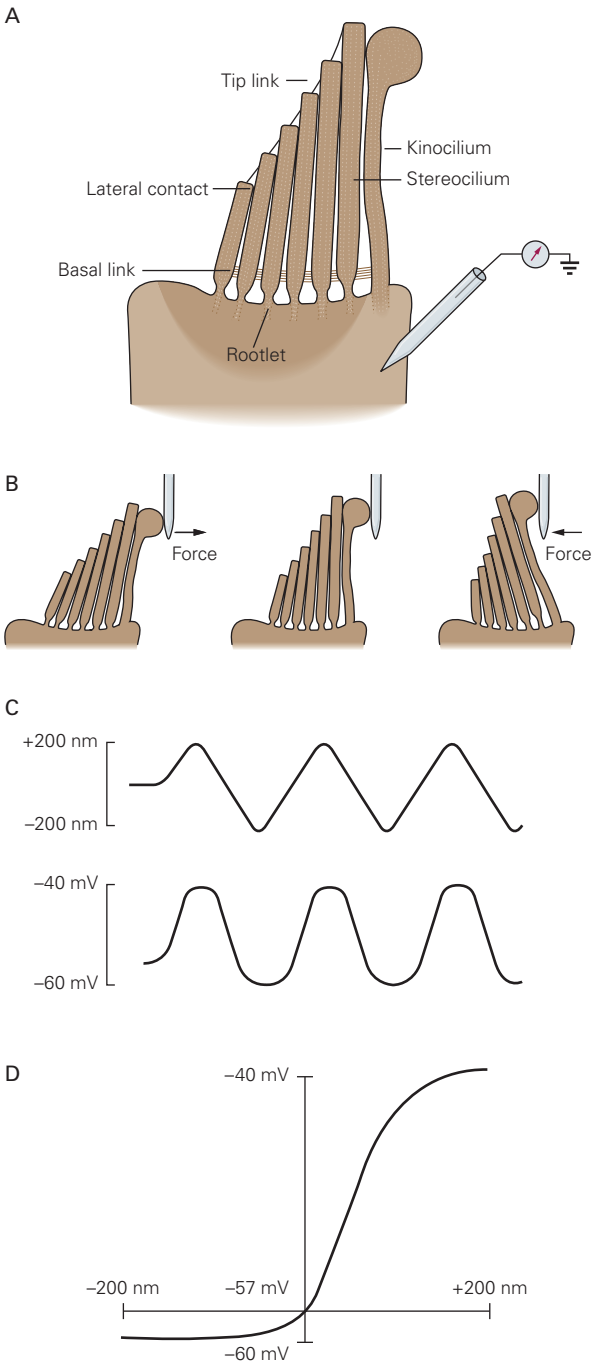


Figure 26–6 Arrangement of the hair cells in the organ of Corti. (Images reproduced, with permission, from D. Furness, Keele University, United Kingdom.)

A. Inner hair cells form a single row, and the stereocilia of each cell are arranged linearly. In contrast, outer hair cells are distributed in three rows, and the stereocilia of each cell are arranged in a V configuration. The apical surfaces of several other cells are visible: from left to right, inner spiral sulcus cells, pillar cells, Deiters's cells, and Hensen's cells (see Figure 26–4).

B. Higher magnification shows the linear configuration of the hair bundle atop an inner hair cell (*left*) and the V configuration of an outer hair-cell bundle (*right*), as well as the arrangement of the stereocilia in rows of increasing heights.

the cell. Hair cells respond most to stimuli parallel to the hair bundle's axis of morphological mirror symmetry: Stimuli at right angles to the axis produce little change from the resting potential. An oblique stimulus elicits a response proportional to its vectorial projection along the axis of sensitivity.



A hair cell's receptor potential is graded. As the stimulus amplitude increases, the receptor potential grows progressively larger up to the point of saturation. The receptor potential of an inner hair cell can be as great as 25 mV in peak-to-peak magnitude. The relation between a bundle's deflection and the resulting electrical response is sigmoidal (Figure 26–7D). A displacement of only ±100 nm represents approximately 90% of the response range. During normal stimulation, a hair bundle moves through an angle of ±1° or so, that is, by much less than the diameter of one stereocilium.

When observed in vitro, a hair bundle exhibits Brownian motion of approximately ±3 nm, whereas the threshold of hearing corresponds to basilar-membrane movements of as little as ±0.3 nm. There are at least three mechanisms that explain how the hair bundle may respond to motion smaller than its own noise. First, because the cochlear partition does not move as a rigid body, the movement of the hair bundle is larger than that of the basilar membrane. Second, frequency-selective amplification of low stimuli actively pulls the signal out of the noise. Finally, mechanical coupling to a group of neighbors results in synchronization that effectively reduces noise. At hearing threshold, a stimulus evokes a receptor potential near 100 μV in amplitude.

The ion channels in hair cells that mediate mechanoelectrical transduction are relatively nonselective, cation-passing pores with a conductance near 100 pS. From the known size of small organic cations and fluorescent molecules that can traverse the channel, the transduction channel's pore must be about 1.3 nm in diameter. Most of the transduction current is carried by K^+, the cation with the highest concentration in the endolymph bathing the hair bundle. Although

Figure 26–7 (Left) Mechanical sensitivity of a hair cell.

A. A recording electrode is inserted into a frog hair cell.

B. A probe attached to the bulbous tip of the stereocilium is moved by a piezoelectric stimulator, deflecting the elastic hair bundle from its resting position. The actual deflections are generally only one-tenth as large as those portrayed.

C. When the top of a hair bundle is displaced back and forth (**upper trace**), the opening and closing of mechanically sensitive ion channels produce an oscillatory receptor potential (**lower trace**) that—as here—may saturate in both the depolarizing and the hyperpolarizing directions.

D. The relation between hair-bundle deflection (abscissa) and receptor potential (ordinate) is sigmoidal. The entire operating range is only approximately 100 nm, less than the diameter of an individual stereocilium. At rest, the hair bundle operates within the steep region of the sigmoid, which ensures significant receptor potentials in response to weak stimuli.

endolymph is relatively poor in Ca^{2+}, a small fraction of the transduction current is carried by this ion. Fluorescent indicators indicate that Ca^{2+} entry, and thus mechanoelectrical transduction, occurs precisely at the stereociliary tips of a deflected hair bundle. Single-channel recordings, together with the observation that the magnitude of the transduction current is roughly proportional to the number of functional stereocilia remaining in a microdissected bundle, indicate that there are probably only two active transduction channels per stereocilium.

The large diameter and poor selectivity of the pore permit transduction channels to be blocked by aminoglycoside antibiotics such as streptomycin, gentamicin, and tobramycin. When used in large doses to counter bacterial infections, these drugs have a toxic effect on hair cells; the antibiotics damage hair bundles and eventually kill hair cells. These drugs pass through transduction channels at a low rate and thus cause long-term toxic effects by interfering with protein synthesis on the mitochondrial ribosomes, which resemble bacterial ribosomes. Consistent with this hypothesis, human sensitivity to aminoglycosides is maternally inherited, as are the mitochondria, and in many instances reflects a single base change in the 12S ribosomal RNA gene of the mitochondrion.

Mechanical Force Directly Opens Transduction Channels

The mechanism for gating of transduction channels in hair cells differs fundamentally from the mechanisms used for electrical signals in neurons such as the action potential or postsynaptic potential. Many ion channels respond to changes in membrane potential or to specific ligands (Chapters 8, 10, and 12–14). In contrast, two lines of evidence suggest that the mechanoelectrical transduction channels in the hair cell are activated by mechanical strain.

First, a bundle is stiffer along its axis of mechanical sensitivity than at a right angle. This observation suggests that a portion of the work done in deflecting a bundle goes into elastic elements, termed *gating springs,* which pull on the molecular gates of the transduction channels. Because the gating springs contribute over half of a hair bundle's stiffness, the transduction channels efficiently capture the energy supplied when a bundle is deflected. In addition, the mechanical properties of a hair bundle vary during channel gating: When channels open or close, stiffness decreases and friction increases. Both phenomena are expected if the channels are gated directly through a mechanical linkage to the hair bundle.

A second indication that transduction channels are directly controlled by gating springs is the rapidity with which hair cells respond. The response latency is so brief, only a few microseconds, that gating is more likely to be direct than to involve a second messenger (Chapter 14). Moreover, the electrical responses of hair cells to a series of step stimuli of increasing magnitude become both larger and faster. This behavior favors a kinetic scheme in which mechanical force controls the rate constant for channel gating.

The *tip link* is a probable component of the gating spring. A tip link is a fine molecular braid joining the distal end of one stereocilium to the side of the longest adjacent process (Figure 26–8A). Deflection of a hair bundle toward its tall edge tenses the tip link and promotes channel opening; movement in the opposite direction slackens the link and allows the associated channels to close (Figure 26–8B).

Three experimental results suggest that the tip links are components of the gating springs. First, tip links are universal features of hair bundles and are situated at the site of transduction. The transduction channels are indeed located at the stereociliary tips, thus near the lower insertion point of the tip link. Second, the orientation of the links is consistent with the vectorial sensitivity of transduction. The links invariably interconnect stereocilia in a direction parallel with the hair bundle's axis of mechanosensitivity. Finally, when tip links are disrupted by exposing hair cells to Ca^{2+} chelators, transduction vanishes. As the tip links regenerate over the course of approximately 12 hours, a hair cell regains mechanosensitivity. It remains unclear whether the elasticity of gating springs resides primarily in the tip links or in the structures at their two insertions.

In the mammalian cochlea, hair bundles are deflected through their linkage to the tectorial membrane. When the basilar membrane oscillates up and down in response to a sound, the organ of Corti and the overlying tectorial membrane move with it. Because the basilar and tectorial membranes pivot about different lines of insertion, however, their up-and-down motion is accompanied by a back-and-forth shearing motion between the upper surface of the organ of Corti and the lower surface of the tectorial membrane. This is the motion that is detected by hair cells (Figure 26–9).

The hair bundles of outer hair cells, whose tips are firmly attached to the tectorial membrane, are directly deflected by this movement. The hair bundles of inner hair cells, which do not contact the tectorial membrane, are deflected by movement of the liquid beneath the membrane. This mode of stimulation affords some mechanical magnification of the signals reaching hair

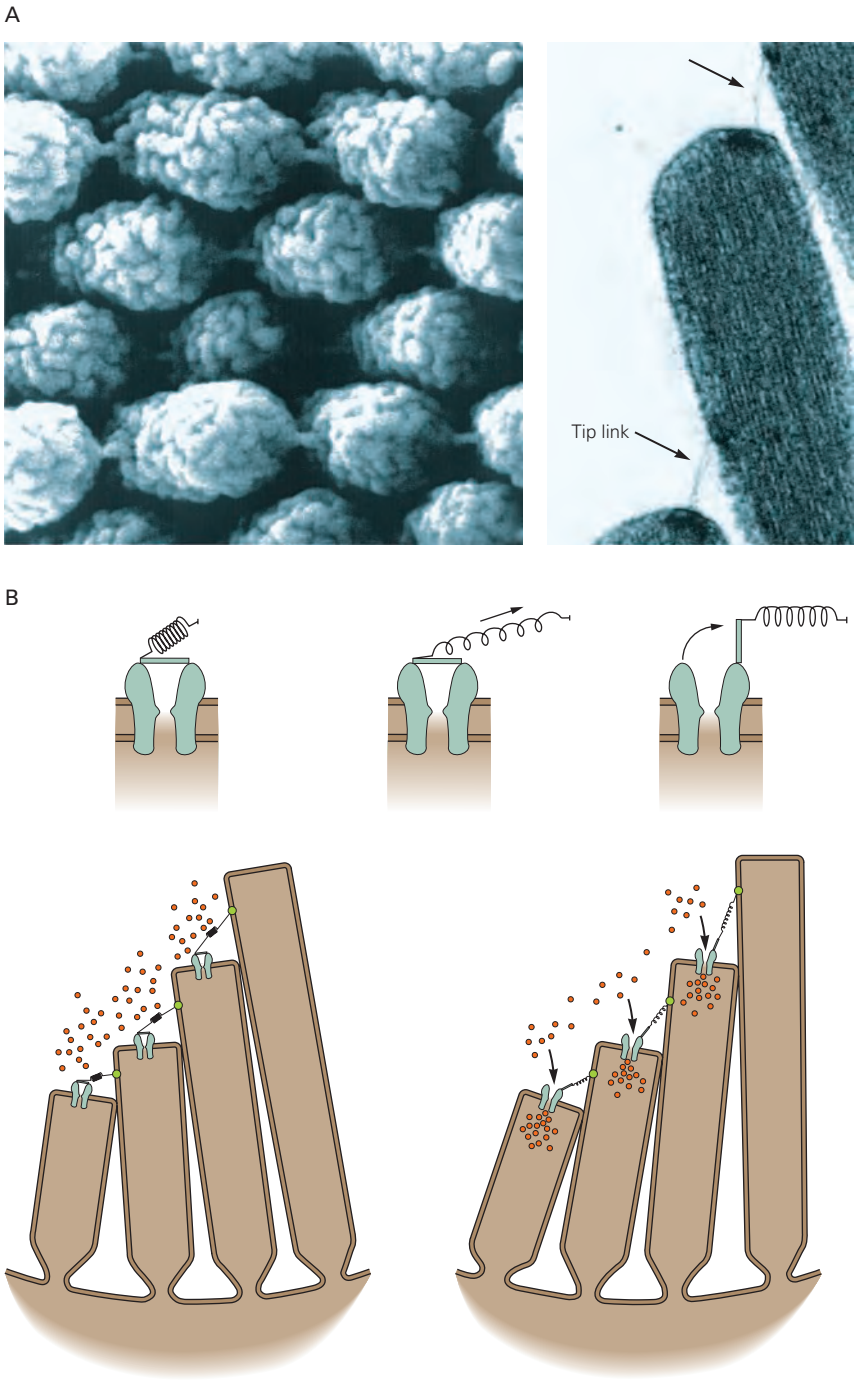


Figure 26–8 Mechanoelectrical transduction by hair cells.

A. A tip link connects each stereocilium to the side of the longest adjacent stereocilium, as seen in a scanning electron micrograph (*left*) and a transmission electron micrograph (*right*) of a hair bundle's top surface. Each tip link is only 3 nm in diameter and 150 to 200 nm in length. The links appear stouter in the illustration on the left because of metallic coating during specimen preparation. (Reproduced, with permission, from Assad, Shepherd, and Corey 1991; reproduced, with permission, from Hudspeth and Gillespie 1994.)

B. Top: Ion flux through the channel that underlies mechanoelectrical transduction in hair cells is regulated by a molecular gate. The opening and closing of the gate are controlled by the tension in an elastic element, the gating spring, which senses hair-bundle displacement. (Adapted, with permission, from Howard and Hudspeth 1988.)

Bottom: When the hair bundle is at rest, each transduction channel fluctuates between closed and open states, spending most of its time shut. Displacement of the bundle in the positive direction increases the tension in the gating spring, here assumed to be in part a tip link, attached to each channel's molecular gate. The enhanced tension promotes channel opening and the influx of cations, thereby producing a depolarizing receptor potential. (Adapted, with permission, from Hudspeth 1989.)

bundles. At least for high-frequency stimuli, the movements of hair bundles are thought to be severalfold greater than that of the basilar membrane.

Direct Mechanoelectrical Transduction Is Rapid

Hair cells operate much more quickly than do other sensory receptor cells of the vertebrate nervous system and, indeed, more quickly than neurons themselves. To deal with the frequencies of biologically relevant sounds, transduction by hair cells must be rapid. Given the behavior of sound in air and the dimensions of sound-emitting and sound-absorbing organs such as vocal cords and eardrums, optimal auditory communication occurs in the frequency range of 10 Hz to 100 kHz. Much higher frequencies propagate

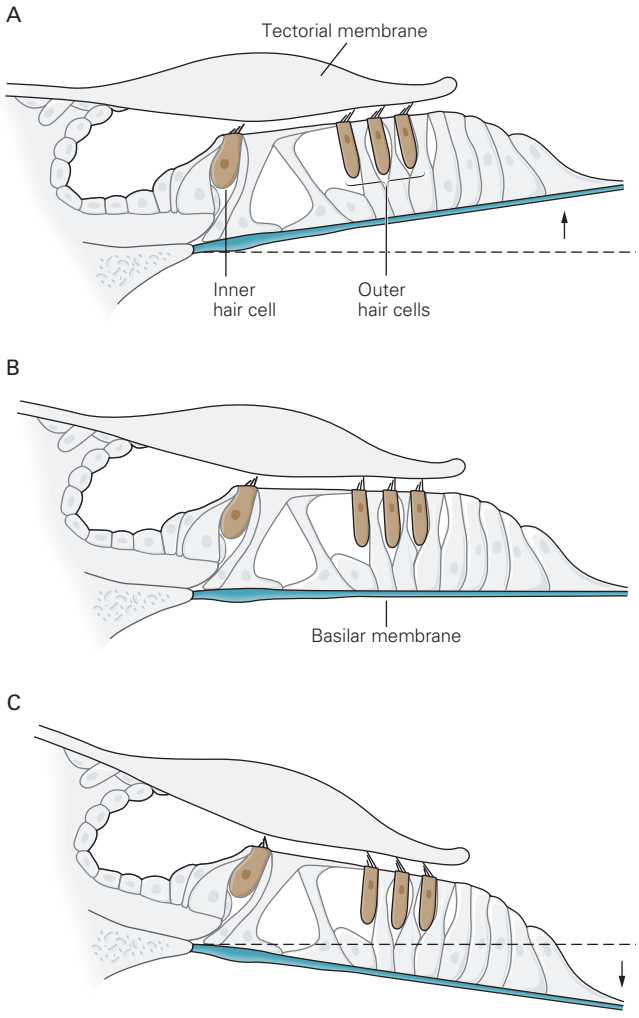


Figure 26–9 Forces acting on cochlear hair cells. Hair cells in the cochlea are stimulated when the basilar membrane is driven up and down by differences in the pressure between the scala vestibuli and scala tympani. This motion is accompanied by shearing movements between the tectorial membrane and organ of Corti. These motions deflect the hair bundles of outer hair cells, which are attached to the lower surface of the tectorial membrane. The hair bundles of inner hair cells, which are not attached to the tectorial membrane, are deflected by the movement of liquid in the space beneath that structure. In both instances, the deflection initiates mechanoelectrical transduction of the stimulus.

A. When the basilar membrane is driven upward, shear between the hair cells and the tectorial membrane deflects hair bundles in the excitatory direction, toward their tall edge.

B. At the midpoint of an oscillation, the hair bundles resume their resting position.

C. When the basilar membrane moves downward, the hair bundles are driven in the inhibitory direction.

poorly through air; much lower frequencies are inefficiently produced and poorly captured by animals of moderate size. Even in animals sensitive to relatively low frequencies, such as frogs, the in vitro transduction current in response to a step stimulus of moderate intensity rises with a time constant of only 80 µs at room temperature. For mammals to be able to respond to frequencies greater than 100 kHz, the hair cells evidently display gating times that are an order of magnitude smaller. Locating sound sources, one of the most important functions of hearing, sets even more stringent limits on the speed of transduction (Chapter 28). A sound from a source directly to one side of a person reaches the nearer ear somewhat sooner than the farther, by at most 700 µs in humans. An observer can locate sound sources on the basis of much smaller delays, about 10 µs. For this to occur, hair cells must be capable of transducing acoustic waveforms with microsecond-level resolution.

Deafness Genes Provide Components of the Mechanotransduction Machinery

Genetic studies of deafness in both humans and mouse models have provided entry points into the molecular composition of the mechanotransduction machinery of the hair cell. In particular, the upper two-thirds of the tip link consist of two parallel molecules of cadherin-23, whereas the lower third comprises two parallel molecules of protocadherin-15 (Figure 26–10). The two components are joined at their tips in a Ca^{2+}-sensitive manner; lowering the extracellular Ca^{2+} concentration below approximately 1 µM disrupts their association. In humans, mutations in the genes coding for cadherin 23 (*USH1D*) and protocadherin 15 (*USH1F*) lead to the most severe form of the Usher syndrome, an autosomal recessive disorder that associates severe-to-profound congenital deafness, constant vestibular dysfunction, and retinitis pigmentosa with a prepubertal onset. The study of other genes involved in this type of Usher syndrome has revealed that the upper end of the tip link is anchored to the actin core of a stereocilium by a protein complex that includes the scaffolding proteins sans (*USH1G*) and harmonin (*USH1C*), as well as the molecular motor myosin 7a (*USH1B*).

The small number of channels in a hair cell, along with the lack of high-affinity ligands with which to label them, explains why the biochemical identity of the transduction channels has long remained uncertain. However, recent genetic, biochemical, and biophysical experiments indicate that four integral transmembrane proteins are intimately related to the transduction channel: transmembrane channel-like proteins 1 and

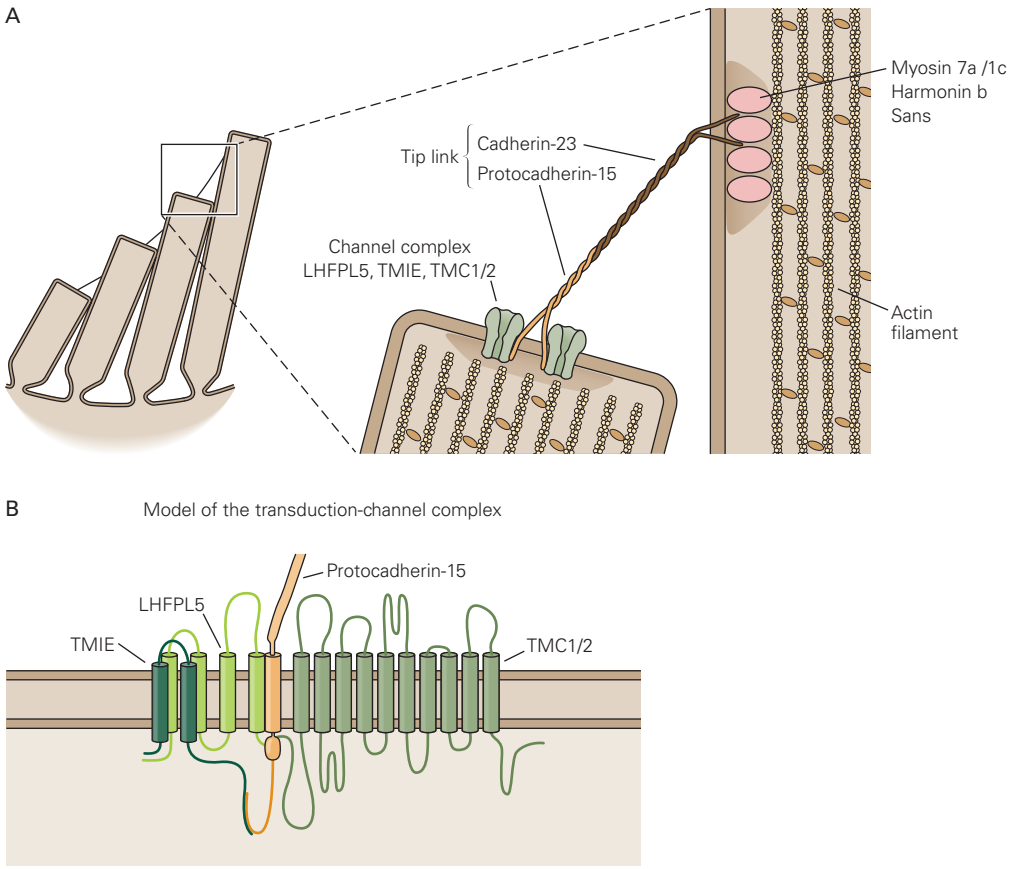


Figure 26–10 Molecular composition of the transduction machinery.

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