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HAIR CELLS: TRANSDUCTION, TUNING, AND TRANSMISSION IN THE INNER EAR

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INTRODUCTION

Hair cells occupy the keystone position in the auditory and vestibular systems. These epithelial receptor cells carry out the crucial step of transducing mechanical stimuli into electrical responses; moreover, they help tune the neurons of the auditory system to specific frequencies of stimulation and transmit information to the brain via fibers of the eighth cranial nerve.

Investigation of the operation of hair cells has recently illuminated the

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extraordinary degree of specificity that hair cells bring to their roles. Their mechanoreceptive organelles, the hair bundles, are beautiful in large part because of the striking accuracy with which the constituent processes are sized, shaped, and arrayed. Although subcellular structure may be specified with equal exactitude in other cells, there are few if any in which this organization is so readily apparent. The precise arrangement of hair bundles is now recognized to be of great importance in mechanoelectrical transduction and frequency tuning.

So too with the degree of order in the electrical properties of the hair cell's plasma membrane. The frequency discrimination of some species ensues because each hair cell is electrically tuned to a specific frequency of stimulation. This tuning is accomplished by insertion into the membrane of a balanced complement of Ca^{2+} and K^+ channels with appropriate kinetic properties. The observed anatomical and physiological accuracy of these cells makes them unusually enticing subjects for analyses of cellular development.

STRUCTURE AND STIMULATION OF HAIR CELLS

As a derivative of the embryonic ectoderm, the hair cell is a cylindrical, epithelial cell without axons or dendrites. Each hair cell is surrounded by supporting cells, to which it is attached by a junctional complex that includes an extensive tight junction (Gulley & Reese 1976). Mechanoelectrical transduction commences when a force derived from sound, acceleration, or water motion deflects the specialized, elastic structure that gives the hair cell its name, the hair bundle (Figure 1).

The hair bundle consists of a cluster of 20–300 enlarged microvilli (the stereocilia) and a single axonemal cilium (the kinocilium) that extend from the hair cell's apical surface in a regular, hexagonal pattern. The stereocilia are not of uniform length: Every bundle has a short and a long edge with a gradation of stereociliary lengths between. A hair bundle is accordingly a bilaterally symmetrical structure whose top aspect is bevelled like the tip of a hypodermic needle. A kinocilium is initially present on every hair cell at the center of the bundle's tall edge; on mammalian cochlear hair cells, however, the kinocilium degenerates around the time of birth (Lindeman et al 1971).

Stimulation of Hair Bundles

COUPLING OF EXTERNAL STIMULI TO HAIR BUNDLES The mechanical energy of stimuli gates ion channels in hair bundles. Appreciation of this process requires some knowledge of the often intricate paths by which pressures and forces from the outside world reach the bundles.

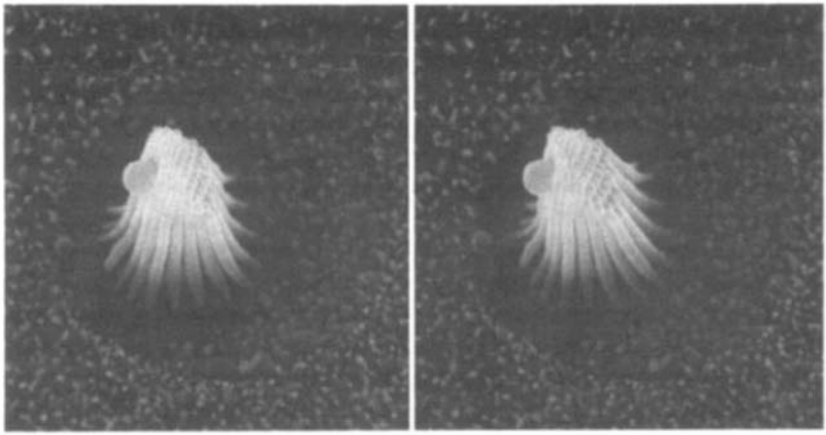

Figure 1 A hair bundle from the bullfrog's sacculus is shown in this stereoscopic pair of scanning electron micrographs. The bundle protrudes ~8 μm from the relatively smooth, circular surface of the hair cell. The bundle comprises ~ 50 stereocilia and a single kinocilium, which ends in a bulbous swelling. Supporting cells, whose surfaces are densely packed with microvilli, surround the hair cell. × 4700.

The external ear is a hearing horn that collects sound from a large area, often from a particular direction, and concentrates the oscillating air pressure onto the eardrum. The bones of the middle ear, which compensate in part for the acoustical-impedance mismatch that would otherwise cause sound to be reflected at this air-liquid interface, transmit the airborne vibration into an oscillatory pressure in the fluids of the inner ear. The changing pressure in turn elicits a travelling wave on the elastic basilar membrane (von Békésy 1960).

The organ of Corti lies along the basilar membrane, whose transverse vibration causes an oscillatory shear between the apical surfaces of the hair cells and the tectorial membrane (Figure 2), an overlying, collagenous shelf (Richardson et al 1987). Four rows, each containing ~4000 hair cells, run from the cochlea's base to its apex. The modiolar row, nearest the core of the helical cochlea, contains the inner hair cells. Because it is from these cells that the central nervous system receives ~95% of its auditory input (Spoendlin 1972), the inner hair cells are presumed to be the principal receptors. Inner hair cells have free-standing hair bundles (Lim 1976) that are moved by hydrodynamic forces from the surrounding fluid. Outer hair cells, which form the other three rows, have more efferent than afferent innervation and are thought to modify the motion of the basilar membrane as discussed below. Their bundles are deflected directly by the movement of the tectorial membrane, to which their longest stereocilia are attached.

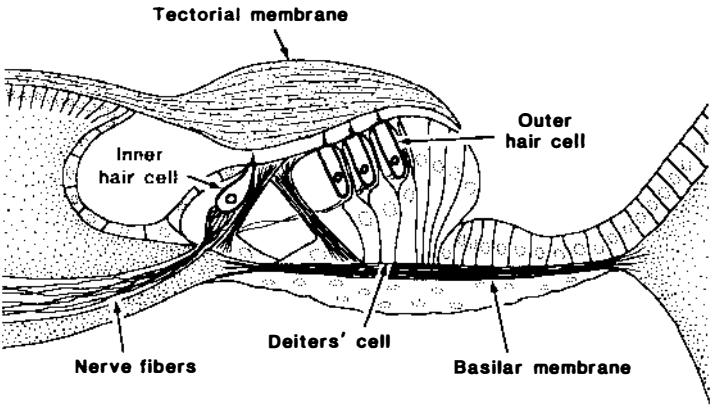

Figure 2 Schematic diagram of a cross-section through part of the human cochlea. In this view, the basilar membrane, an elastic partition stretched between two bony ridges, vibrates up-and-down. The basilar membrane supports the organ of Corti, which includes among its epithelial constituents some 16,000 hair cells in four rows. The hair bundles of inner hair cells stand free beneath the collagenous tectorial membrane; these cells make most of the synaptic contacts onto afferent nerve fibers that project into the brainstream. Outer hair cells, whose basal ends are cupped by Deiters' cells, insert their bundles firmly into the tectorial membrane.

In most other organs, the hair bundles are coupled to accessory structures through their kinocilia, which often terminate in bulbous swellings at the sites where force is applied. In these instances, stimuli are transmitted to the remainders of the bundles through bonds between the kinocilia and longest stereocilia. For example, hair cells within semicircular canals and lateral-line organs extend their kinocilia and longest stereocilia into gelatinous cupulae that are deflected by fluid flow (McLaren & Hillman 1979; van Netten & Kroese 1987). Another type of stimulation involves the inertia of the otolith, a sac of calcareous masses each a few micrometers long. The otolith rests upon an otolithic membrane to which the hair bundles attach via the tips of their kinocilia. As in a seismograph, gravity and other linear accelerations cause relative movement between the otolithic mass and the apical surface of the hair cells; this motion deflects the hair bundles of the sacculus, the utriculus, and, when present, the lagena (de Vries 1950).

DEFLECTION OF HAIR BUNDLES IN VITRO The response of a hair bundle to stimulation is most readily observed during the application of a mechanical force at the bundle's tip with a fine probe. The bundle moves as a unit, without resolvable separation of the stereocilia, whether it is pulled or pushed and regardless of the direction of stimulation (Flock et al 1977).

While remaining relatively straight along most of its length, each stereocilium evidently pivots at its base (Hudspeth 1983c). The tilting of the stereocilia within a bundle is therefore accompanied by shearing along their lengths.

Mechanical measurements from several species support the view of hair-bundle motion suggested by light-microscopic observations. When force is applied at the tip of a bundle 5–10 μm high, the bundle's stiffness measures ~ 1 mN $\cdot$ m^{-1} (Flock & Strelioff 1984; Crawford & Fettiplace 1985; Howard & Ashmore 1986; Howard & Hudspeth 1987a, b). The stiffness varies inversely with the square of the height at which the force is applied, a result consistent with each stereocilium's pivoting about its point of insertion rather than bowing along its entire length (Crawford & Fettiplace 1985; Howard & Ashmore 1986). The stiffness increases in direct proportion to the number of constituent stereocilia, as expected for relative sliding of the stereocilia rather than for compression of some and extension of others.

Structure of Hair Bundles

STEREOCILIARY CYTOSKELETONS The dominant structural feature of the stereocilium is its rigid, microfilamentous cytoskeleton (Figure 3). Stereo-

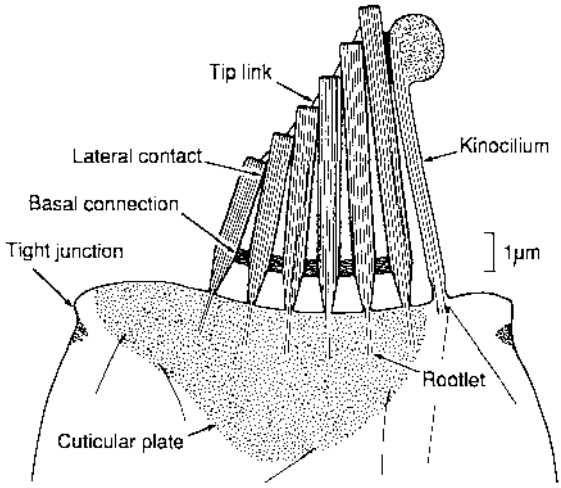

Figure 3 Schematic drawing of the constituents of a representative hair bundle. Each stereocilium has at its core a fascicle of cross-linked actin filaments, a few of which extend as a rootlet into the fibrous cuticular plate. Three types of contacts interconnect the stereocilia; the longest stereocilia are also attached to the axonemal kinocilium at the bundle's tall edge. The hair cell is bound to the supporting cells by a junctional complex with a prominent tight junction.

cilia range in diameter from ~100 nm to >800 nm; because the center-to-center spacing of the microfilaments is ~ 10 nm (DeRosier et al 1980), there are from ~50 to >3000 filaments per stereocilium. Although isodiametric along most of their 1–50-μm lengths, stereocilia taper over a distance of ~1 μm above their insertions into the cellular apex. In this region, the number of microfilaments decreases dramatically as most of the filaments terminate on the plasma membrane. A few dozen central filaments, which become embedded in osmiophilic material in the tapered region, extend as a rootlet a few micrometers into the elaborate, filamentous specialization at the hair cell's apical cytoplasm, the cuticular plate.

Like other 6-nm filaments, the microfilaments of stereocilia consist of actin. This has been confirmed both by decoration of the filaments with the S1 fragment of myosin (Flock & Cheung 1977; Flock et al 1981; Slepecky & Chamberlain 1982) and by immunohistochemistry of intact hair bundles and of isolated stereocilia (Flock et al 1981; 1982; Drenckhahn et al 1982). As in microvilli, the actin filaments of stereocilia are oriented with their "barbed," growing ends directed away from the cell body.

For stereocilia to behave as rigid rods, the actin filaments at their cores are extensively cross-linked in a manner unknown in other cells. A filament consists of a double strand of actin monomers in a helix of constant pitch. Adjacent stereociliary actin filaments are parallel and nearly in phase with one another: At each cross-sectional level of a stereocilium, every actin filament is at approximately the same position on its helical course (DeRosier et al 1980). The alignment of the filaments is brought about by cross-links that extend between adjacent actin filaments at regular intervals of ~12.5 nm (Tilney et al 1980; DeRosier et al 1980; Tilney et al 1983). Because the periodicity of actin monomers is not integrally related to the spacing between successive cross-links, bonding between the filaments requires a modicum of radial rotational flexibility at the sites of attachment of cross-links; the observed bonding necessitates a flexibility of ~13° (DeRosier et al 1980). Although the material that cross-links stereociliary actin filaments has not been identified, immunofluorescence studies indicate that stereocilia contain fimbrin (Flock et al 1982; Slepecky & Chamberlain 1985), a protein that cross-links actin in microvilli (Bretscher & Weber 1980).

Because some hair bundles are capable of active movement, investigators have sought to demonstrate myosin in stereocilia. Although one immunofluorescence study provided evidence for myosin (Macartney et al 1980), there is also a negative report based upon similar techniques (Drenckhahn et al 1982). It is possible that stereocilia contain a form of myosin that does not cross-react with antibodies raised to myosin from the usual

sources. This issue will likely be clarified only after a variety of antibodies to a broad range of myosin isoforms have been tested.

Deep-etch, freeze-fracture electron microscopy reveals links extending from the core of actin filaments to the surface membranes of stereocilia (Hirokawa & Tilney 1982); by analogy with the comparable structures in microvilli, these links may consist of 110-kDa protein (Matsudaira & Burgess 1979; Collins & Borysenko 1984). It is conceivable that the positive report of myosinlike immunoreactivity reflects the interaction of a particular antimyosin with an epitope shared by myosin and the 110-kDa protein, which is a related, K^+- or Ca^{2+}-activated, actin-binding MgATPase (Coluccio & Bretscher 1987; Conzelman & Mooseker 1987).

LINKAGES WITHIN HAIR BUNDLES For a hair bundle to move as a unit, there must be linkages that transmit forces throughout the bundle. Four types of filamentous linkages interconnect the stereocilia and kinocilium of a bundle (Figure 3). When present, the kinocilium is ligated to the adjacent stereocilia by strands of osmiophilic material (Hillman 1969). Because in many organs the stimulus is applied to the hair bundle solely at the kinocilium, these strands must transmit force to the stereocilia, where mechanoelectrical transduction transpires (Hudspeth & Jacobs 1979). The chemical nature of these strands and of the other linkages within the hair bundle is unknown.

Each stereocilium is attached to its six neighbors by a web of basal connections that run parallel to and ~ 1 μm above the cell's apical surface (Bagger-Sjöbäck & Wersäll 1973; Csukas et al 1987). Nebulous lateral contacts occur along the upper reaches of the stereocilia (Flock et al 1977); it is not certain whether these are specific interstereociliary junctions or simply represent intermeshing of the dense glycocalices of adjacent stereocilia. The final type of interciliary contact is better defined, but more difficult to demonstrate ultrastructurally. In appropriately fixed material, a thin strand connects the tip of each stereocilium to its tallest neighbor (Pickles et al 1984; Furness & Hackney 1985). Unlike the other two types of contacts between stereocilia, these tip links occur only along axes parallel with the bundle's plane of bilateral symmetry.

CUTICULAR PLATES As the stereociliary rootlets of the hair bundle penetrate into the apical cytoplasm of the hair cell, they are associated with a dense, filamentous feltwork. This structure, the cuticular plate, is an elaboration of the terminal web of other epithelial cells. The plate's association with the stereociliary rootlets and, in many cells, with the intermediate junctions around the cell's apical periphery suggests that the cuticular plate provides a stable platform upon which the stereocilia can tilt.

The cuticular plate consists of several proteins that make or associate with filaments: actin, α-actinin, fimbrin, tropomyosin, and myosin (Flock et al 1981; Drenckhahn et al 1982; Slepecky & Chamberlain 1985). In addition, the basal surfaces of many cuticular plates are associated with microtubules or with striated, actin-containing organelles (Friedmann bodies; Slepecky & Chamberlain 1982). Its complex composition hints that the cuticular plate is more than a passive anchor for the stereocilia. Alterations of the plate's shape, which would presumably change the relative orientations of the stereocilia, could contribute to adaptation of mechanoelectrical transduction (Hudspeth 1983b) or to active motility of the hair bundle, two phenomena described below. To date, however, there has been no demonstration of motility by the cuticular plate.

Development and Repair of Hair Bundles

The formation of the hair bundle is a complex process that has been investigated extensively only in the chick's basilar papilla (Tilney & DeRosier 1986; Tilney et al 1986; Cotanche 1987a). Stereocilia commence their growth from the apical surface of a hair cell as microvilluslike processes arrayed with the regular, hexagonal packing that characterizes the mature bundle. In a second stage of development, the miniature stereocilia cease to lengthen; instead, they increase in girth while extending their rootlets into the cuticular plate. The stereocilia then elongate to their final lengths. The bevelled top aspect of the bundle is established by differential arrest of the growing stereocilia: The processes in the shortest ranks cease elongation first, with successively longer stereocilia stopping growth progressively.

In order to be as sensitive as possible to sound, the ear efficiently focuses acoustical energy upon small targets, the hair bundles. As a consequence, very loud sounds can overstimulate the bundles. Modest degrees of acoustical trauma that result in a temporary diminution of the ear's sensitivity cause only a slight reduction in the extent to which actin filaments are associated in the stereociliary tapers (Liberman & Dodds 1987). More violent acoustical trauma results in a permanent hearing loss that is most extreme at the frequencies represented in the sound; the rootlet of each affected stereocilium is ruptured, generally with disruption of the stereocilium's normal taper (Tilney et al 1982; Engström et al 1983; Liberman 1987). The actin microfilaments within stereocilia may also be disheveled and lack their normal cross-links (Tilney et al 1982); finally, the tip links between stereocilia may be broken (Pickles et al 1987). Although decreased hair-bundle stiffness (Hunter-Duvar 1977; Canlon et al 1987) and diminished mechanosensitivity are associated with these morphological lesions, it is not yet apparent which aspects of the stereociliary damage contribute to these functional changes.

Recovery from acoustical trauma occurs both by repair of damaged cells and, in some species, by replacement of destroyed ones. Following moderate noise trauma to lizards, the stereocilia's disarrayed actin filaments regain their regular alignment within days; the temporary threshold shift abates in the same period (Tilney et al 1982). Permanent threshold shifts are associated with loss of stereocilia or fusion of their membranes (Engström et al 1983; Liberman 1987). In mammalian cochleas, severely damaged hair cells die, leaving epithelial scars (Bredberg 1968); few if any cells are replaced. In the basilar papilla of the chick, however, hair cells destroyed by noise are replaced through the differentiation of unknown progenitors (Cotanche 1987b).

We know nothing about the turnover of the hair cell's crucial components, its hair bundle and the associated cuticular plate. Vertebrate photoreceptors constantly renew their photoreceptive outer segments through a complex process involving phagocytotic and biosynthetic steps (Young 1976). Although it is probable that the components of hair bundles are similarly in a state of flux, the labelling experiments required to demonstrate this point have yet to be performed.

MECHANOELECTRICAL TRANSDUCTION

Response to Hair-bundle Displacement

Deflection of the hair bundle in the positive direction, toward the longest stereocilia, is excitatory; the cell's membrane is depolarized with respect to the resting potential of about -60 mV. Displacement in the negative direction is inhibitory and deflection in the orthogonal direction produces no electrical response (Hudspeth & Corey 1977; Shotwell et al 1981).

RECEPTOR CURRENTS AND RECEPTOR POTENTIALS The cell's voltage response, or receptor potential, is due to a mechanically activated current, the receptor current (Corey & Hudspeth 1979b). As the bundle is moved from a large negative to a large positive deflection, the receptor current rises sigmoidally from zero to a maximum of a few hundred picoamperes (Corey & Hudspeth 1983a; Ohmori 1985, 1987; Holton & Hudspeth 1986a). The resulting receptor potential is shaped by the membrane's properties: its capacitance and its ion- and voltage-sensitive ion channels (Crawford & Fettiplace 1981; Lewis & Hudspeth 1983a; Ohmori 1985; Art & Fettiplace 1987; Hudspeth & Lewis 1988a, b). The maximal receptor potential is on the order of tens of millivolts (Hudspeth & Corey 1977; Crawford & Fettiplace 1980; Russell & Sellick 1978).

The range of displacements over which the transducer is sensitive is ~ 100 nm ($\sim 1°$ of angular deflection) for a hair bundle several micrometers

high (Corey & Hudspeth 1983a; Holton & Hudspeth 1986a; Ohmori 1987; Russell et al 1986; Howard & Hudspeth 1988b). The maximal sensitivity to small displacements is about -2 mA·m^{-1} or 400 kV·m^{-1}. It is estimated that, at the perceptual threshold of hearing, the bundles of inner hair cells are deflected 0.1–1 nm (Rhode & Geisler 1967; Rhode 1984); this stimulus corresponds to a response in the hair cell of $\sim$1 pA or $\sim$100 μV.

When the hair bundle is in its undisturbed position, the inward receptor current is 10–20% of its maximal level (Corey & Hudspeth 1983a; Ohmori 1987). This resting activation is important for two reasons. First, because a slight movement of the bundle in the positive or the negative direction respectively increases or decreases the current, the hair cell's transducer has no threshold. Second, resting activation situates the transducer near the point of maximum curvature on the sigmoidal relation between current and displacement, where the responses to positive and negative deflections differ most in magnitude. Symmetrical oscillation of the hair bundle about its resting position thus produces an asymmetrical, rectified response: The receptor current increases more during the positive phase of the displacement than it decreases during the negative phase. The resultant net increase in receptor current causes a depolarization of the hair cell even if the stimulus frequency is so high that the membrane potential cannot follow the receptor current on a cycle-by-cycle basis (Russell & Sellick 1978). Hair cells can accordingly respond to oscillatory stimuli of frequencies up to 20 kHz in man and over 100 kHz in bats and whales.

TRANSDUCTION CHANNELS The flow of receptor current is associated with an increase in the conductance of the hair cell's membrane (Hudspeth & Corey 1977). The current is presumed to flow through ion channels with hydrated pores. When open, these transduction channels are equally permeable to small, monovalent cations such as Li^+, Na^+, K^+, Cs^+ and can even pass, although to a lesser extent, organic cations such as choline and tetraethylammonium with diameters up to 700 pm (Corey & Hudspeth 1979b; Ohmori 1985). The bore of the transduction channel's pore thus resembles that of the acetylcholine-receptor channel. Divalent cations such as Mg^{2+}, Ca^{2+}, and Sr^{2+} have an even higher affinity for the channel than do monovalent cations, but have a lower flux through it (Corey & Hudspeth 1979b; Ohmori 1985). The permeability data are consistent with the existence of a negatively charged site within the hydrated pore that excludes anions and determines the selectivity for cations (Howard et al 1988). The channel is blocked by the polycationic aminoglycoside antibiotics, such as streptomycin and gentamicin, at concentrations of 1–1000 μM. Although transduction channels are not intrin-

sically voltage sensitive (Holton & Hudspeth 1986b; Ohmori 1987), their blockage by antibiotics is. The blockage is consistent with the antibiotics' binding to the negatively charged selectivity site located within the pore, partway across the membrane's electric field (Hudspeth & Kroese 1983; Ohmori 1985).

The receptor current in vivo results chiefly from the flow of K^+ into the cell; the endolymph that bathes hair bundles contains about 150 mM K^+, but only 1 mM Na^+, 10 μM Mg^{2+}, and 10–300 μM free Ca^{2+} (Bosher & Warren 1978). The transduction channel's permeability to Ca^{2+} is sufficiently high that entry of this ion into the cytoplasm could mediate physiological functions (Corey & Hudspeth 1979b; Ohmori 1985; Assad & Corey 1988).

The conductance and number of transduction channels have been estimated from tight-seal, whole-cell recordings. Vestibular hair cells of the chick manifest discrete current steps during bundle deflection (Ohmori 1984, 1985); because the maximal conductance change for large stimuli is $\sim$2 nS (nanosiemens) there are evidently $\sim$40 channels per bundle (Ohmori 1987). The single-channel current in hair cells of the bullfrog is estimated to be 20 pS from analysis of the cell's current noise; the number of channels is 50–250 (Holton & Hudspeth 1986a). If evenly distributed throughout the bundle, the transduction channels thus number 1–5 per stereocilium. This fact importantly constrains models for transduction. The small number of channels per cell and the modest number ($<10^5$) of hair cells in most ears conspire to make the biochemical characterization of the transduction channel a formidable technical challenge (Hudspeth 1983a).

Mechanics of Gating

Transduction channels appear to be gated directly when forces applied at the bundle's tip act upon the channels (Corey & Hudspeth 1983b; Howard et al 1988). Less direct mechanisms that require enzymes or second messengers are excluded by the remarkably short delay, <25 μs at 25°C, between bundle deflection and channel opening (Corey & Hudspeth 1979a, 1983b).

We suppose that positive deflection of the hair bundle pulls upon a linkage that is attached to the molecular gate of the transduction channel (Figure 4; Howard et al 1988). The gate can exist in either of two configurations, open or closed. Because transduction channels can rattle between these configurations while the hair bundle is held fixed (Holton & Hudspeth 1986a), the mechanical linkage must be elastic. The length of this elastic element, the gating spring, is different in the two configurations; opening of the channel shortens the gating spring by a distance that is

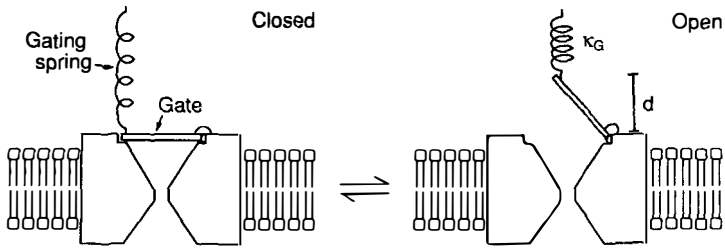

Figure 4 A model for mechanical gating of the hair cell's transduction channel. Each channel has a molecular gate attached to an elastic element, the gating spring, of stiffness κ_G. Opening of the channel (*right*) shortens the gating spring by a distance *d*. When the hair bundle is deflected in the excitatory direction, the increased tension in the gating spring increases the channel's probability of being open.

envisaged to be of molecular dimensions. The distance may be that traversed by a mobile protein domain that obstructs the channel's pore only in the closed state. The larger the positive displacement of the hair bundle, the greater the tension in the gating spring, and the lower the energy of the open state with respect to the closed; according to thermodynamic principles, the channel is then biased to spend more time open than closed. A transduction element consisting of a gating spring and channel thus functions as a displacement sensor.

The sigmoidal dependence of the receptor current on displacement, measured by whole-cell, tight-cell recordings from hair cells of the bullfrog's sacculus and the chick's labyrinth (Holton & Hudspeth 1986a; Ohmori 1987), is consistent with this gating-spring model. The model also predicts the observed increase in the rate constant of channel opening with increasingly positive stimulation (Corey & Hudspeth 1983b).

A passive gating mechanism, such as in Figure 4, is inherently reciprocal. Not only should the application of force to the hair bundle affect the probability of a channel's being open, but the opening of a transduction channel should also exert a force upon the bundle. High-resolution mechanical measurements on hair bundles from the bullfrog's sacculus demonstrate that, within the range of bundle positions in which transduction channels switch between their open and closed states, the bundle's compliance nearly doubles (Howard & Hudspeth 1988a, b). This gating compliance is comparable to the gating current contributed by the movement of the charged groups that constitute the voltage sensors of voltage-sensitive ion channels (Armstrong & Bezanilla 1974). A force of $\sim$300 fN at the bundle's tip is required to open a single transduction channel. This force is about one tenth that produced by myosin as it splits a single ATP molecule during its interaction with actin (Huxley & Simmons 1971).

The mechanical recordings are consistent with one to three transduction channels per stereocilium, a value similar to that deduced from the receptor current.

The mechanical and electrical data provide strong support for the gating-spring model of Figure 4. According to this model, a small amount of energy, of the order of the thermal energy kT, suffices to gate a transduction channel, through which many thousands of ions can then flow down their electrochemical gradients. In this way the human ear, for example, can detect sounds so faint that they are almost drowned out by the random collision of air molecules against the eardrum.

Anatomical Substrate for Gating

The physical nature and location of the gating springs and transduction channels remains an important open question. Analysis of the current flow into hair cells indicates that the channels are located in the membrane at the apex of the cell, above the junctional complex (Tasaki et al 1954; Corey & Hudspeth 1979b, 1983a). Neither the channels nor the gating springs are within the kinocilium: Certain hair cells, such as those of the mammalian cochlea, lose their kinocilia during development; kinocilia can be amputated from other hair cells without altering their electrical response to bundle displacement (Hudspeth & Jacobs 1979). Measurements of extracellular current density localize the channels at the top of the hair bundle, near the tips of the stereocilia (Hudspeth 1982). Mechanoelectrical transduction by vertebrate hair cells is thus fundamentally different from that by arthropod auditory sensilla and protists, which lack stereocilia and whose mechanosensitivity is associated with axonemal cilia.

The filamentous link that runs from the tip of each stereocilium to its tallest neighbor is presently the best candidate for the gating spring (Pickles et al 1984; Furness & Hackney 1985). As well as according with the location and number of channels, these links, which are ideally situated to detect shear between neighboring stereocilia, would confer the appropriate directionality to the cell's response: Positive deflection is expected to tense the links, negative displacement to slacken them, and orthogonal deflections to affect them little if at all. The tip links confer upon the bundle an orientation that the radially symmetrical stereocilia cannot.

If the links are stretched as expected during bundle deflection, they must be quite resilient: The bundle's tip can be moved up to 1 μm without damage to the transducer. Such movement would elongate the 100-nm-long filaments by up to 100%. Assuming that the 5-nm-diameter threads consist of a material such as rubber or elastin, the tip links would contribute a stiffness to the bundle that accords with the mechanical properties of the gating springs (Howard & Hudspeth 1987a, 1988b). If the tip links are the

gating springs, then the mechanical recordings imply that the swing of a gate, the distance by which the gating spring shortens as the channel opens, is ~4 nm (Howard & Hudspeth 1988b). This conformational change is sufficiently large to effect the gating of a pore and is comparable to the inferred displacement of the myosin molecule during its power stroke (Huxley & Simmons 1971).

Other possible anatomical substrates for the gating springs cannot be excluded. The gating spring may, for example, be the elasticity of the membrane in which each channel sits (Sachs 1986); gating would then be analogous to that of stretch-activated channels found in the membranes of several types of cell (Guharay & Sachs 1984; Martinac et al 1987; Lansman et al 1987). Deflection of the hair bundle may alternatively stress a cytoskeletal element that is connected to the channels from the cytoplasmic side. Gating of the channel must in any case involve a conformational change that relieves the tension in the gating spring, whatever this may be (Howard et al 1988).

Adaptation

In response to sustained deflection, the operating range of the transducer in the bullfrog's saccular hair cell adapts within milliseconds to accord with the new tonic position (Eatock et al 1987). The bundle's position of least stiffness moves as well, indicating that the range of displacements in which the channels are gated also shifts (Howard & Hudspeth 1988a, b). Although nearly complete for displacements up to a few hundred nanometers, adaptation is less extensive beyond 800 nm (Eatock et al 1987). The function of adaptation in the frog's sacculus is clear; adaptation nulls the large, tonic, gravitational input to the hair bundle (Howard & Hudspeth 1987c) and leaves the hair cell highly sensitive to much smaller vibrations of the substrate (Koyama et al 1982). Adaptation may serve every hair cell in the fine adjustment of the transducer's operating range to its point of maximum sensitivity or greatest rectification (Hudspeth 1983a).

Because the hair bundle relaxes mechanically as the receptor current adapts, adaptation to positive stimuli probably reflects a gradual reduction of the tension in the gating springs (Howard & Hudspeth 1987a). The rate of adaptation depends on the extracellular Ca^{2+} concentration. The extent of adaptation is reduced by pharmacological blockers of calmodulin (Corey et al 1987). The voltage sensitivity of the rate of adaptation is consistent with the idea that adaptation is mediated by the entry of Ca^{2+} through transduction channels (Assad & Corey 1988). Adaptation may thus be caused by a Ca^{2+}-dependent change in the cytoplasmic anchoring of the gating springs, which in turn would modify their tension (Howard

& Hudspeth 1987a), in the helical twist of the actin filaments, which would alter the lengths of the stereocilia, or in the shape of the cuticular plate, which in turn would alter the geometrical relations among the stereocilia (Hudspeth 1983b).

FREQUENCY SELECTIVITY OF HAIR CELLS

The auditory centers of the brain are generally organized tonotopically: The spectrum of sound frequencies is mapped onto each nucleus or tract so that neighboring cells are most effectively stimulated by nearby frequencies. This tonotopy is established within the sensory epithelium of the inner ear. Recordings from mammalian cochleas have shown that each hair cell is maximally sensitive to a particular frequency of sound. From the cochlea's apex to its base, successive hair cells respond best to increasingly high frequencies. The basilar papillas of birds (von Békésy 1960) and reptiles (Crawford & Fettiplace 1980; Holton & Weiss 1983a, b), and the amphibian papillas of amphibians (Lewis et al 1982; Pitchford & Ashmore 1987) are also organized tonotopically.

By acting as mechanical filters, the structures that transmit stimuli to hair cells clearly play an important role in establishing the frequency selectivity of some receptor organs. In the mammalian cochlea, for example, oscillatory motions of the fluid drive a travelling wave that propagates along the basilar membrane (von Békésy 1960). Because the basilar membrane becomes progressively thinner and broader, and therefore more compliant, as it extends the tens of millimeters from the base to the apex of the cochlea, each position along the membrane is most effectively set into vibration by a particular frequency of sound. Tonotopy could, in principle, therefore be established using equivalent hair cells that differ only in their positions and hence mechanical inputs.

The last two decades have brought an appreciation that passive mechanical filtering of the input to hair bundles is insufficient to account for the sharpness of frequency selectivity by many hair cells. In the mammalian cochlea, the sharp tuning of the basilar membrane (Rhode 1971, 1984; Khanna & Leonard 1982; Sellick et al 1982) cannot readily be explained by the membrane's mass, stiffness, and damping (Neely & Kim, 1983; but see Zwislocki & Kletsky 1980). Furthermore, both the heights and the mechanical properties of hair bundles vary continuously from one end of the cochlea to the other, the high-frequency-responsive cells having the shortest (Lim 1980) and stiffest bundles (Flock & Strelioff 1984). Each hair cell, with its structurally distinct hair bundle (Tilney & Saunders 1983), may therefore contribute uniquely to tuning.

In nonmammalian auditory organs there is even greater reason to believe

that hair cells have intrinsic tuning mechanisms. Although the basilar membranes of reptiles are only coarsely frequency selective, hair cells in the auditory organs of these animals are tuned within an octave (Weiss et al 1978). There is now experimental evidence that certain hair cells have their own electrical and mechanical tuning mechanisms.

Electrical Tuning

ELECTRICAL RESONANCE The ears of some amphibians (Ashmore 1983; Lewis & Hudspeth 1983a, b; Pitchford & Ashmore 1987), reptiles (Crawford & Fettiplace 1981), and birds (Fuchs & Mann 1986) achieve frequency selectivity largely through the hair cells' electrical properties. For these cells, the relation between the transduction current and membrane potential is that of a sharply tuned, linear filter (Crawford & Fettiplace 1981): A small, sinusoidal current produces a sinusoidal voltage change whose amplitude depends upon the stimulus frequency.

The properties of such a linear filter are characterized completely by its response to a small current step (Figure 5). A positive step evokes an exponentially damped, sinusoidal resonance in membrane potential. At the onset of a positive step, the membrane depolarizes at a rate determined by the cell's capacitance. This depolarization opens Ca^{2+} channels that allow extracellular Ca^{2+} to enter the cell. The elevated intracellular Ca^{2+} concentration and depolarized membrane potential then cooperate to open

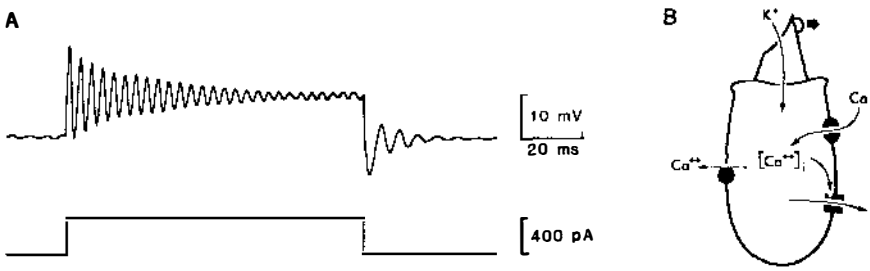

Figure 5 Electrical resonance and its ionic basis. (*A*) When a hair cell of the bullfrog's sacculus was depolarized by a square current pulse, the membrane potential exhibited damped, sinusoidal oscillation at a frequency of 270 Hz. Oscillation at a lower frequency also occurred at the termination of the pulse. (*B*) Positive deflection of a hair bundle allows K^+, the principal cation in the endolymph that bathes the bundles, to enter through transduction channels and to depolarize the cell; the injection of current through a microelectrode has a similar effect. As depolarization activates voltage-sensitive Ca^{2+} channels, the influx of Ca^{2+} further depolarizes the cell. As Ca^{2+} accumulates in the cytoplasm near the plasma membrane, however, it opens Ca^{2+}-sensitive K^+ channels; the efflux of K^+ through these channels repolarizes the membrane and the next cycle of oscillation commences.

K^+ channels, producing an outward K^+ current that repolarizes the cell, but after a delay that allows the membrane potential to overshoot the new steady-state value (Lewis & Hudspeth 1983a, b; Art & Fettiplace 1987; Hudspeth & Lewis 1988a, b). In sharply tuned cells, the membrane potential oscillates through many cycles before a steady state is reached. The membrane's predilection for generating an oscillating potential makes it maximally responsive to sinusoidal receptor currents at frequencies near the resonant frequency; the sharpness of tuning increases with the time constant with which the oscillation is damped.

For resonance to occur, the membrane's resting conductance must be small. Hair cells accomplish this by having, at the resting potential, no open ion channels other than those directly involved in transduction or resonance: transduction channels, voltage-gated Ca^{2+} channels, and Ca^{2+}-activated K^+ channels. The voltage-gated Ca^{2+} channels amplify a small receptor current to produce a large receptor potential. Hair cells in vitro often have spontaneously oscillating membrane potentials (Crawford & Fettiplace 1980; Ashmore 1983; Lewis & Hudspeth 1983b) that probably result from the presence of enough Ca^{2+} channels to preclude a stable resting potential. Because spontaneous oscillations could be abolished by the leakage conductance or decreased Ca^{2+} current caused by recording, it is possible that spontaneous oscillation is more common among hair cells in vivo.

A second requirement for resonance is that small perturbations from the resting potential produce large changes in the number of open K^+ channels (Ashmore & Attwell 1985). This steep voltage dependence is achieved by the concerted action of three factors: the voltage dependence of Ca^{2+} channels, the highly cooperative Ca^{2+} gating of K^+ channels (Hudspeth & Lewis 1988a), and the direct voltage sensitivity of K^+ channels. A depolarization of only 1.9–2.2 mV is sufficient to double the number of open K^+ channels; for the hair cell's Ca^{2+} channels, the comparable value is 2.2–4.6 mV (Art & Fettiplace 1987; Hudspeth & Lewis 1988a). For comparable responses, axonal K^+ and Na^+ channels respectively require depolarizations of $\sim$3.3 mV and $\sim$2.7 mV (Hodgkin & Huxley 1952).

The general considerations presented above, which place restraints on the membrane's electrical properties that allow resonance, do little justice to the hair cell's remarkable ability to fine-tune its resonant frequency and sharpness of tuning. Hair cells of the turtle's basilar papilla have resonant frequencies that vary continuously from 10 Hz to 350 Hz, depending upon the position along the basilar papilla from which they are isolated (Art & Fettiplace 1987). To take its proper place in this gradient, each cell has an appropriate activation time constant (140–0.7 ms) and number (500–3700)

of K^+ channels. It is not known how a hair cell sets the activation rate constant of its Ca^{2+}-activated K^+ channels; in addition to modification of the channels themselves, other processes, such as the entry, buffering, or extrusion of Ca^{2+}, could be involved. The rate constants for the activation of Ca^{2+} channels also vary with resonant frequency (Roberts et al 1986).

The data obtained from voltage-clamp experiments can be incorporated into models that accurately predict a hair cell's resonant frequency. Art & Fettiplace (1987) used parameter values extracted from the time course of a cell's response to small voltage steps to predict the cell's resonance. Hudspeth & Lewis (1988a, b) included a more detailed description of the steps that precede the opening of K^+ channels: the opening of Ca^{2+} channels, diffusion, buffering, and extrusion of Ca^{2+}, and the binding of Ca^{2+} to the K^+ channels. Both models yield quantitatively accurate predictions of the cell's resonant frequency and sharpness of tuning.

Whether electrical tuning can operate at frequencies >400 Hz, in the range of greatest behavioral significance to mammals and birds, remains a crucial but unresolved question. The resonant frequency could be increased most readily by raising the activation rates or voltage sensitivites of the hair cell's Ca^{2+} and K^+ channels above those reported for the highest-frequency hair cells of turtles. It is possible, however, that the observed channels already operate near physical limits that cannot be exceeded. The activation rates and voltage sensitivities of the ion channels involved in action potentials, which are among the highest values for any known channels, are similar to those of the channels that produce electrical resonance.

If a hair cell's ion channels cannot be made to operate significantly faster, or with higher voltage sensitivity, it is unlikely that electrical resonance can occur at frequencies above a few kilohertz. Although some increase in the resonant frequency could be achieved by increasing the density of K^+ channels in the membrane, packing them as closely as the Na^+ channels at the node of Ranvier (20,000 μm^{-2}) would apparently raise the resonant frequency to <5 kHz.

EFFERENT CONTROL OF ELECTRICAL RESONANCE Neurons in the brainstem extend to make cholinergic synapses onto hair cells (Daigneault 1981). In the turtle's hair cells, an efferent action potential produces an inhibitory postsynaptic potential that involves an increased K^+ conductance and lasts for 100–150 ms; trains of efferent spikes can hyperpolarize the hair cell by up to 20 mV (Art et al 1984). Synaptic inhibition of the hair cell is mimicked by perilymphatic perfusion of acetylcholine. The receptor differs from the ordinary muscarinic acetylcholine receptor in being highly sensitive to curare; it differs from the usual nicotinic receptor in being blocked by atropine (Art et al 1984).

Activation of the efferent synapse decreases the hair cell's response to sound and broadens its frequency selectivity. By increasing the conductance of the hair cell's membrane, the opening of inhibitory K^+ channels short-circuits the cell's electrical resonance and thus decreases the response at the resonant frequency by as much as a thousandfold. Even though the frequency of resonance depends strongly on membrane potential, the efferent synapse, by shunting in addition to hyperpolarizing, leaves the characteristic frequency little changed. The tonotopic organization of the cochlea, a key to the subsequent processing of auditory information, is therefore preserved (Art et al 1985).

Mechanical Tuning

FREE-STANDING HAIR BUNDLES AS MECHANICAL RESONATORS The auditory organs of mammals (Lim 1976) and reptiles (Mulroy 1974) contain hair cells with free-standing hair bundles that project into the endolymph. These bundles increase in length from high- to low-frequency regions of the epithelium, a correlation that provides compelling circumstantial evidence that the physical properties of hair bundles are involved in frequency selectivity. Perhaps each hair bundle behaves as a mechanical resonator like a tuning fork. Such a resonator is composed of a mass attached to a spring; when the mass is displaced from its equilibrium position and then released, it will vibrate about the equilibrium position at a frequency determined by its mass and the spring's stiffness until friction brings the movement to a halt.

A free-standing hair bundle acts as a cluster of stereociliary rods (a mass) attached to the cuticular plate by an ensemble of tapered, compliant necks (a spring) that restores the bundle to its upright position following a displacement. Each hair bundle could therefore have a mechanical resonance. Were a 5-μm-high bundle struck *in vacuo*, the resonant frequency predicted from its mass ($\sim$50 pg) and the summed stiffnesses of the basal springs ($\sim 1\ mN \cdot m^{-1}$) is $\sim$50 kHz, far above the characteristic frequency of a hair cell of this dimension (Crawford & Fettiplace 1980). However, this calculation ignores the surrounding endolymph, which acts through viscous and inertial forces to damp the resonance and lower its frequency. Inertial forces, which would be important even if the endolymph had no viscosity, arise because the hair bundle sets into motion a substantial volume of the fluid through which it moves; kinetic energy is stored in the endolymph that circulates around the moving bundle. Some hair bundles, such as those from the basillar papillas of lizards, have spatulate shapes that may maximize this circulation (Weiss & Leong 1985; Freeman 1987).

The endolymph's viscosity has a complex effect on hair-bundle motion. Because of viscosity, a hair bundle experiences hydrodynamic damping;

for the bullfrog's saccular hair cell, bundle motion is greatly attenuated at frequencies well above 1 kHz (Howard & Hudspeth 1987b). In addition to damping the resonance, viscosity creates a boundary layer of fluid that moves with the cell's apical surface. A free-standing hair bundle that is too short to extend beyond this boundary layer is immersed in fluid moving at the same velocity as the cell body, and will therefore receive no stimulus. Because the thickness of the boundary layer increases with decreasing frequency, the hair bundles of cells sensitive to low frequencies must extend further into the endolymph than do those of high-frequency hair cells (Weiss & Leong 1985; Freeman 1987).

Theoretical calculations that include the inertia and viscosity of the endolymph (Weiss & Leong 1985; Freeman 1987) predict that hair bundles with sizes and shapes like those in the lizard's basillar papilla have broadly tuned mechanical resonances at frequencies near the experimentally determined characteristic frequencies of these cells (Frishkopf & DeRosier 1983; Holton & Hudspeth 1983).

ACTIVE MOTILITY OF HAIR BUNDLES Hair cells from the turtle's basilar papilla and bullfrog's sacculus display active mechanical resonance at frequencies of 31–171 Hz (Crawford & Fettiplace 1985; Howard & Hudspeth 1987a). A force step applied at the hair bundle's tip produces an oscillatory bundle movement that is modulated by membrane potential (Crawford & Fettiplace 1985). Because some bundles oscillate spontaneously with an amplitude that is too large to be due to Brownian motion, these cells evidently have an active, force-generating element associated with the hair bundle.

Although the identity of the active component is unknown, the force required to produce the observed movement is modest, similar to that produced by a single cross-bridge in skeletal muscle, and less than that produced by a single motile cilium (Crawford & Fettiplace 1985). The kinocilium, whose axonemal structure resembles that of motile cilia, is a possible force generator; the hair cells in an eel's vestibular organ have 60-μm-long kinocilia that bend when the voltage across the sensory epithelium is altered (Rüsch & Thurm 1986). Because in many organs it transmits force from an accessory structure, the kinocilium is ideally situated to modify the stimulus to the mechanoreceptive stereocilia; whether the kinocilium actively contributes to bundle motility, however, remains enigmatic.

FORCE GENERATORS IN THE MAMMALIAN COCHLEA Mammalian ears produce sounds that can be recorded by a microphone placed outside the eardrum. These acoustic emissions may be evoked in response to loud sounds (Kemp 1978); under pathological conditions, sounds are emitted spontaneously and continuously (Zurek 1981). Some part of the inner ear

can evidently generate forces sufficient to vibrate the eardrum. Other reasons to suspect active mechanical elements within the cochlea include the previously mentioned difficulty with explaining the sharply tuned motions of the basilar membrane by passive mechanical models and the observation that the mechanical properties of mammalian cochleas are metabolically labile (Rhode 1984).

Although there are no direct electrical or synaptic connections between outer and inner hair cells, several lines of evidence suggest that the former somehow influence the responses of the latter. Efferent inhibition of outer hair cells reduces the sensitivity of inner hair cells to as little as 5% of the control level (Brown & Nuttall 1984). Damage to outer hair cells by aminoglycoside antibiotics similarly results in a profound loss of sensitivity and frequency selectivity by apparently undamaged inner hair cells (Dallos & Harris 1978). The interaction between outer and inner hair cells may be mechanical.

LENGTH CHANGES OF OUTER HAIR CELLS Two types of contractions that operate on different time scales and have different energy requirements have been observed in isolated outer hair cells in vitro.

Slow, longitudinal contractions of outer hair cells have been observed in response to depolarization by high-K^+ media (Zenner et al 1985), upon addition of 5 mM Ca^{2+} and 1 mM ATP to detergent-permeabilized cells (Flock et al 1986), or following exposure to Ca^{2+}, ATP, and inositol-1,4,5-triphosphate (Schacht & Zenner 1987). Because these cells require 5–20 minutes to shorten 10–50%, their contraction is too slow to facilitate high-frequency tuning of the basilar membrane, but could play a role in efferent desensitization.

The prominence of a subsurface cisternal system in outer hair cells has led to the speculation that the cisternae play a role in cellular shortening (Flock et al 1986). One to twelve layers of vesicles, tubules, and flattened, fenestrated cisternae line most of the cytoplasmic aspect of the outer hair cell's plasma membrane (Saito 1983; Harada et al 1987). The innermost layers are studded with ribosomes, which suggests that the cisternae are derived from rough endoplasmic reticulum. The membrane of the outermost cisterna, which is not continuous with the plasma membrane (Saito 1983; Ashmore 1987), is connected to it by pillars 25 nm in length (Raphael & Wróblewski 1986) and separated by 30 nm (Flock et al 1986). The P-face of the cisternal membrane facing the plasma membrane contains a dense array ($\sim$1000 per μm^2) of 10-nm intramembrane particles. The P-face of the adjacent plasma membrane also contains 10-nm particles at a density of $\sim$3000 per μm^2 (Saito 1983). The abundance of mitochondria adjacent to the cisternal system (Saito 1983; Harada et al 1987) suggests

a function that requires metabolic energy; perhaps, like the sarcoplasmic reticulum of muscle, this otoplasmic reticulum stores and releases Ca^{2+} that mediates contraction (Flock et al 1986).

Much faster changes in the lengths of outer hair cells can be elicited by electrical stimulation. Kachar and associates (1986) reported that the cells shorten by as much as $\sim 1\%$ when placed in an electric field between two extracellular electrodes. The movement, which was visible during sinusoidal stimulation at frequencies up to 30 Hz, persisted in the presence of the metabolic blockers dinitrophenol and iodoacetic acid, which suggests that the energy for contraction is derived from the electric field. Outer hair cells also shorten when depolarized by intracellular current injection (Brownell et al 1985; Ashmore 1987). A maximum shortening of 4% is produced by strong, low-frequency stimulation; smaller movement has been observed at frequencies >1 kHz. The speed of this response and its persistence after ATP is depleted by metabolic blockers or intracellular perfusion suggest that this movement is not myosin based (the actomyosin reaction is not involved). Because shortening ensues regardless of the position of the intracellular electrode on the cell body, it appears that the change in voltage across the plasma membrane, rather than the longitudinal voltage gradient, triggers the response.

Contraction might require the flow of ions across the plasma membrane, in which case experimental tests of the ionic requirements for shortening would aid in elucidating its mechanism. If the energy for contraction is instead derived from the transmembrane electric field, shortening should be accompanied by an increase in the cell's apparent membrane capacitance (Armstrong & Bezanilla 1974). A 100-mV step depolarization requires ~ 150 fJ to charge the membrane's capacitance of ~ 30 pF. The resulting contraction probably requires ~ 1 fJ to produce the observed velocity of shortening in the viscous medium (Ashmore 1987); additional energy may be required to stretch elastic elements within the cytoplasm. By measurement of the capacitance associated with contraction, it might be possible to estimate the energy of contraction.

Although it is unknown whether rapid contractions of outer hair cells occur in vivo, it is tempting to suppose that they augment the oscillation of the basilar membrane. This hypothesis suffers two weaknesses. First, for outer hair cells to do useful work on the basilar membrane, their shortening must be mechanically transmitted to the membrane. Deiter's cells intervene between the outer hair cells and basilar membrane (Figure 2); it remains to be demonstrated that they are firmly bonded to the outer hair cells and that their cytoskeletons are suitable for the transmission of forces. The second difficulty is that the outer hair cells are so oriented that their contraction should have little effect on the shear between the tectorial

membrane and the apical surfaces of the hair cells, the effective stimulus to the inner hair cells. Side-to-side movement or a change in stiffness of the outer hair cells' bundles would seem more directly to affect the stimulation of inner hair cells than would a change in length of the outer hair cells. The mechanism by which the outer hair cells could power the tuning of the cochlea thus remains a mystery.

AFFERENT SYNAPTIC TRANSMISSION

Hair cells have no axons. Instead, they form chemical synapses with primary afferent neurons, which carry the sensory information to the brainstem and cerebellum. Signals are either relayed to higher brain areas where they lead to perception, or are fed back to the periphery via efferent pathways that inhibit hair cells directly or by alteration of their mechanical input.

The unusual signalling requirements for the perception of sound and acceleration mandate exceptional precision in the operation of the afferent synapse. The azimuthal direction of a sound source is computed by the brain from the difference in the arrival time of sound at the two ears; in humans, for example, sound originating directly at the left reaches the left ear ~ 500 μs before it reaches the right ear. To localize sound with the observed accuracy of a few degrees requires that interaural time differences as small as a few tens of microseconds be resolved. Timing information must then be faithfully transmitted across the hair cell's synapse so that the time difference between the two ears can be compared in the brain. In the barn owl, neurons that mediate this perceptual task preserve timing information; they fire action potentials in a fixed phase relation with sounds at frequencies up to ~ 9 kHz (Sullivan & Konishi 1986). Although it may introduce substantial delays, the afferent synapse must minimize the jitter in the delay from one cycle of sound to the next.

The afferent synapses from hair cells of the turtle's basilar papilla and the bullfrog's sacculus perform a related function. These hair cells possess such a powerful electrical resonance that their membrane potential can oscillate continuously in the absence of stimulation; the spontaneous activity of the afferent fibers is highly regular as a result (Crawford & Fettiplace 1980; Koyama et al 1982). The effect of vibration or sound is to alter the phase of the oscillation rather than its amplitude; it is this phase information that the synapse must convey. This is a conceptually different type of transmission from that usually performed by a synapse that receives a graded input.

Electron microscopy indicates that the hair cell's afferent contact is a chemical synapse. At its basal end, each hair cell contains a few synaptic bodies, osmiophilic rods or ovoids 200–400 nm in diameter, which abut

the plasma membrane (Gleisner et al 1973). A synaptic body is surrounded by a hundred or so clear, spherical synaptic vesicles, each 30–60 nm in diameter. In aldehyde-fixed material, the fusion of presynaptic vesicles with the plasma membrane is occasionally observed. As at other chemical synapses, a cluster of intramembrane particles distinguishes the presynaptic membrane (Hama 1980); these particles may be the voltage-sensitive channels through which Ca^{2+} enters the cytoplasm and mediates exocytosis.

Although there has been only limited physiological investigation of the afferent synapses of hair cells, the results support the ultrastructural evidence for chemical transmission. Excitatory postsynaptic synaptic potentials of quantized amplitude are recorded from afferent nerve terminals (Furukawa et al 1978). As at conventional chemical synapses, the hair cell's contacts display synaptic delay, facilitation, and depression. It is therefore inferred that synaptic vesicles exocytotically release a transmitter that diffuses across the 20-nm synaptic cleft to the postsynaptic membrane, where it binds to and opens ion channels.

The identities of the transmitter and its receptor are unknown. Because there are reasons to believe that none of the usual candidates—acetylcholine, glutamate, aspartate, ATP, GABA, glycine, or a catecholamine—is the hair cell's transmitter (Guth et al 1981), several groups have initiated searches for a novel transmitter. The perilymph extracted from the inner ears of guinea pigs and bullfrogs during sound stimulation, but not in silence, excites afferent axons when perfused into the bullfrog's ear (Sewell et al 1978). Cation-exchange and gel-permeation chromatography of tissue homogenates from goldfish inner ears separates two fractions that excite afferent neurons of the amphibian lateral-line organ (Sewell & Mroz 1987a). The identity of an active component of low molecular weight, $\sim$200 Da, has not been established. A fraction of molecular weight $\sim$4 kDa has been identified as calcitonin-gene–related peptide (CGRP) on the basis of molecular weight, tissue distribution, and biological activity (Adams et al 1987; Sewell & Mroz 1987b). Hair cells of mammalian cochleas and of the frog's lateral-line organ do not, however, react with antibodies to CGRP (Adams et al 1987; Lu et al 1987); the axons and terminals of the efferent neurons instead exhibit immunoreactivity. It thus appears that the effect of CGRP on afferent neurons is mediated either by a direct synaptic connection between efferent and afferent nerve terminals (Smith 1961) or via excitation of hair cells.

CONCLUSION

This article has focused upon the contributions of hair cells to mechano-electrical transduction, frequency selectivity, and synaptic transmission.

Hair cells are also important, however, because their failure—through genetic abnormalities, infections, aging, and exposure to noises and ototoxic drugs—is responsible for most of the serious clinical problems with the auditory and vestibular systems. Hearing impairment, a condition that afflicts ~22 million Americans (~9% of the population; Pepper 1987), is largely due to deterioration of hair cells, especially their mechanosensitive organelles. An appreciation of the way in which various lesions interfere with transduction by hair cells should ultimately prove of value in preventing or reversing hearing loss and disequilibrium.

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