MICRO- AND NANOMECHANICS OF THE COCHLEAR OUTER HAIR CELL

W. E. Brownell¹, A. A. Spector², R. M. Raphael², and A. S. Popel²

¹Bobby R. Alford Department of Otorhinolaryngology and Communicative Sciences, Baylor College of Medicine, and Department of Bioengineering, Rice University, Houston, Texas 77030
²Department of Biomedical Engineering and Center for Computational Medicine and Biology, Johns Hopkins University, Baltimore, Maryland 21205; e-mail: brownell@bcm.tmc.edu, aspector@bme.jhu.edu, rraphael@bme.jhu.edu, apopel@jhu.edu

Key Words electromotility, cytoskeleton, electromechanical transduction, flexoelectricity, computational models

Abstract Outer hair cell electromotility is crucial for the amplification, sharp frequency selectivity, and nonlinearities of the mammalian cochlea. Current modeling efforts based on morphological, physiological, and biophysical observations reveal transmembrane potential gradients and membrane tension as key independent variables controlling the passive and active mechanics of the cell. The cell’s mechanics has been modeled on the microscale using a continuum approach formulated in terms of effective (cellular level) mechanical and electric properties. Another modeling approach is nanostructural and is based on the molecular organization of the cell’s membranes and cytoskeleton. It considers interactions between the components of the composite cell wall and the molecular elements within each of its components. The methods and techniques utilized to increase our understanding of the central role outer hair cell mechanics plays in hearing are also relevant to broader research questions in cell mechanics, cell motility, and cell transduction.

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1523-9829/01/0825-0169$14.00
INTRODUCTION

Hair cells are found in the balance and hearing organs of the inner ear and are the most sensitive mechano-sensory cells. The mammalian cochlea contains two types of hair cells: inner hair cells and outer hair cells (Figure 1). Twenty years ago, the role of outer hair cells (OHCs) in hearing was unclear. This changed in the mid-1980s, when Brownell and coworkers (1–3) discovered that the OHC changes its length in response to electrical stimulation. Intra- and extracellular receptor potentials with magnitudes similar to those used experimentally are experienced by OHCs under physiological conditions. Fifteen years of experimental studies have established that the OHC is able to generate an active force and that this can be produced at frequencies up to and exceeding 50 kHz (4). A broad range of in vitro and in vivo experiments are consistent with and support the role of OHC electromotility in active cochlear amplification that contributes to the sharp frequency selectivity necessary for localizing sounds in space and for understanding complex speech (see 5–8).

The field of cell mechanics has advanced and increased our understanding of the physiological behavior of cardiac cells, endothelial cells, red and white blood cells, and now OHCs. Computational models based on biophysical measurements describe the mechanics of these cells at both the micromechanical and nanomechanical level. For the purpose of this review, micromechanics refers to effective cellular-level properties on the scale of microns. Nanomechanics refers to the subcellular and molecular organization and properties on the scale of nanometers. Modeling hair cells is especially critical because of the extreme vulnerability of living cochleae to any invasive measurement technique. Modeling establishes the rational basis for the extrapolation of data collected in isolated cells or cochleae to physiological conditions. The analysis and modeling of the OHC micro- and
nanomechanics is an important step toward a better understanding of normal hearing as well as hearing loss and deafness.

The Organ of Corti

The mammalian inner ear or cochlea is a spiral-shaped cavity located in the skull behind the eyes. The length of the cochlear spiral varies with species. Its hair cells and their supporting cells are housed in the organ of Corti (Figure 1), which spirals with the cochlea and contains one row of between 1000–3000 inner hair cells (IHCs) and three to four rows of OHCs. The IHCs are so named because they are closer to the central axis of the cochlear spiral than the OHCs. All hair cells are endowed with a bundle of stereocilia at their apical end. The tips of the tallest OHC stereocilia are firmly inserted into the relatively stiff tectorial membrane. Differential vibrations between the tectorial membrane and the apical end of the hair cells bend the stereocilia and initiate mechanoelectrical transduction [for a review, see Eatock (8a)]. Most, if not all, of the nerve fibers that carry auditory information to the brain contact IHCs. Even though OHCs make the major contribution to the production of cochlear receptor potentials (9), they are innervated by only 5% of the afferent fibers leaving the cochlea to go to the brain (10). Vibrations of the organ of Corti ultimately activate the IHCs that modulate the release of a neurotransmitter onto the 10–18 auditory nerve fibers that innervate them.

The acoustic spectrum is mapped out along the length of the organ of Corti, which vibrates most easily to high frequencies at the cochlea’s basal end and to low frequencies at the apical end. The tonotopic mapping results from systematic differences in the organ of Corti’s geometry, elastic properties, and mass. The width of the basiliar membrane, its compliance, and the size of the hair cells and supporting cells vary in a manner consistent with the frequency mapping (8). Cochlear mechanics has been a subject of intense interest for over 50 years, since von Bekesy made his pioneering measures of basilar membrane vibrations in cadaver ears (11). Mechanical models show that when the acoustic energy of the input and the mass and elastic properties of the vibrating structures (including the hydrodynamics of inner ear fluids) are considered, a broad mechanical tuning results that matches von Bekesy’s measurements. However, even at the time von Bekesy made his observations, it was known that the frequency selectivity of human hearing as well as individual auditory nerve fibers in animals respond over a narrower range than can be accounted for by a passive system.

The Cochlear Amplifier

The poor frequency response of cadaver ears is attributed to viscous damping that increases with frequency, which accounts for the observation that the difference between mechanical filtering in the cadaver ear and behavioral and neural tuning becomes progressively greater above 1 kHz. Gold, an American astrophysicist, was the first to suggest that mechanical filtering by the inner ear could be enhanced by force generation within the hearing organ (12). His suggestion was largely ignored...
until new experimental techniques provided more-sensitive measurements in liv-
ing ears and revealed mechanical tuning that approached the narrow neural tuning
but which returned to the broad tuning on the death of the animal (13–15). Models
of so-called cochlear micromechanics then began to incorporate “negative damp-
ing” to achieve narrower mechanical tuning (16–18). The concept that a source
of mechanical energy exists in the cochlea appeared validated in the late 1970s,
when it was discovered that the living inner ear produces sounds (19) referred
to as otoacoustic emissions. These emissions are now used routinely to diagnose
hearing problems in newborn infants. Shortly thereafter, OHC electromotility was
discovered: Isolated OHCs were observed to elongate when hyperpolarized and
shorten when depolarized (1–3). The function of the OHC in hearing is now per-
ceived as that of a “cochlear amplifier” that refines the sensitivity and frequency
selectivity of the mechanical vibrations of the cochlea (5, 7, 20).

OUTER HAIR CELL MORPHOLOGY

Basal and Apical Poles

OHCs are cylinders (Figure 2) with a uniform diameter of ~9 µm and a length
that ranges from <15 µm for OHCs from the high-frequency basal cochlea to
>90 µm in the low-frequency apex. The basal end of the OHC is roughly hemis-
spherical and contains the cell’s nucleus, below which are found afferent and
efferent synapses. Postsynaptic cisterna are found adjacent to the postsynaptic
thickening of efferent synapses. Presynaptic dense bodies abut presynaptic thick-
enings of afferent synapses but are fewer in number than those found in IHCs.
OHCs in some species do not normally have presynaptic dense bodies, but they
polymerize when neurotransmission is blocked (21).

OHCs are capped at their apical end by a cuticular plate and stereociliar bundle.
The stereociliar bundle is common to all hair cells and responsible for mechano-
electrical transduction. Stereocilia, essentially enlarged microvilli, are protrusions
of the cell’s plasma membrane (PM) and contain a dense core of actin filaments
(F-actin) that maintains their shape. Stereocilia rootlets are anchored in a matrix
of cytoskeletal filaments called the cuticular plate (CP) (see Figure 2). The OHC
cuticular plate is larger than it is in other hair cells. It spans the width of the cell,
nearly contacting the lateral wall PM and is so thick that the stereocilia rootlets
do not penetrate it (22, 23). In addition to its size, its shape also differs from
that of other hair cells. The OHC CP is concave apically, whereas the CP in all
other hair cells is convex and does not reach the lateral membranes. Cytoskeletal
proteins radiate away from the stereocilia rootlets toward the lateral wall in the
OHC CP, whereas the CP of other hair cells are morphologically isotropic (24).
The OHC stereocilia bundle contains about 130 stereocilia, with an average di-
diameter of 145 nm arranged in four rows in a “W” pattern, with a mean spacing
between rows of 253 nm (25). The tallest stereocilia are embedded in the tectorial
membrane, and their deflection is produced by shear displacement of the tectorial
membrane. Large transducer currents have been recorded in OHCs from the mouse
The Lateral Wall

The OHC lateral wall has a unique nanoscale trilaminate organization that is self-assembling and self-repairing. It is $\sim 100$ nm thick and composed of three layers that form three axially concentric cylinders (Figure 2). The PM is the outermost layer. Another membrane-bound organelle, called the subsurface cisterna (SSC), forms the innermost layer. They are separated by a $\sim 30$-nm–wide extracisternal space (ECiS). Although the SSC structurally resembles the endoplasmic reticulum (ER), it is stained with both ER and Golgi body markers (28) and shows no evidence of belonging to the PM-ER-Golgi membrane pool (29). The SSC may be more closely related to the canalicular reticulum, a structure that occurs selectively in ion-transporting epithelia (30). The molecular composition of the SSC is unknown, in terms of its phospholipid composition, its compliment of integral membrane proteins, and the content of the narrow ($\sim 20$ nm) lumen lying between the inner and outer SSC membranes. Although the function of the SSC is unknown, it partitions the cytoplasm into two domains, the axial core and the ECiS. The axial core is the larger of the compartments and contains only a few structures, such as most of the cell’s compliment of mitochondria, which are located adjacent to the lateral wall. This partitioning may help to maintain the OHCs cytoskeletal organization. F-actin is present in stereocilia at the OHCs apex and near the synaptic structures at the base of the cell but is poorly represented in the cell’s axial core (24, 31, 32). The cytoskeletal proteins that maintain the OHCs cylindrical shape are found...
in the narrow confines of the ECiS, where they constitute a cortical lattice (CL) with a well-defined orthotropic organization. Radially oriented pillars (of unknown molecular composition) tether the PM to parallel bands of F-actin that lie adjacent to the SSC. The actin filaments are spaced about 40 nm apart and cross-linked with molecules of spectrin. Careful examination of the cortical lattice reveals that it is organized in microdomains defined by regions of parallel actin filaments. The orientation of the actin differs between the microdomains but on average is circumferential (33). The mean orientation of the spectrin is longitudinal. The OHC is a cellular hydrostat and its cylindrical shape is maintained by a modest cytoplasmic turgor (~1–2 kPa) (34), which is required for the full expression of electromotility (1, 3, 35, 36). The pressure and the tensile properties of the lateral wall permit shape changes and hydraulic force transmission at high frequencies (5).

It is possible to dissect the lateral wall and observe the individual layers that make up this composite structure. The micropipette aspiration technique, originally developed to study red blood cell (RBC) mechanics, was applied to the OHC (37). The pressure required to deform the OHC lateral wall was an order of magnitude greater that that required for the RBC. Oghalai et al (32) selectively labeled and imaged each of the lateral wall components under confocal microscopy. Interactions among the three layers were evaluated with the micropipette aspiration technique. The PM was tethered to the CL and SSC until, at a critical deformation pressure, the PM separated, allowing visualization of the extracisternal space. Ultimately the PM pinched off and formed a vesicle. After detaching, the stiffness parameter of the PM (length of the aspirated tongue per unit pressure) was ∼20% of that of the intact lateral wall. These results suggest that most of lateral wall’s resistance to local bending derives from the underlying CL/SSC complex. The stiffness parameter of the intact lateral wall is reduced by the anionic amphipath salicylate (38).

The Lateral Wall Plasma Membrane

The appearance of the lateral wall PM differs from SSC membranes under transmission electron microscopy. The PM is rippled or folded (39, 40), whereas the SSC membranes retain the crisp bilayer appearance usually associated with biological membranes. Although rippling can be produced by fixation procedures, its presence nevertheless suggests that the lateral wall PM is structurally unique, regardless of whether it is rippled in vivo. Support for in vivo folding comes from both micropipette aspiration (41) and laser tweezers (42, 43) experiments. Additional evidence comes from the effect of curvature reagents on membrane-associated charge (44), electromotility (45), and lipid lateral diffusion (46). Folding is a common feature of many cell membranes (47), particularly in those cells whose function requires shape changes.

The lipid composition of OHC membranes is unknown. Fluorescent labeling studies suggest that lateral wall membranes contain less cholesterol than the apical and basal PM (48) and that the PM does not retain cholesterol (49). The lipid
composition of the membrane can modulate the activity of membrane proteins. Just as low membrane cholesterol is required for rhodopsin to undergo a conformational change in response to light (50), it may be that low cholesterol is required for OHC electromotility. The different functional roles of the apex, lateral wall, and base of the OHC are associated with known differences in their integral membrane proteins. Immunohistochemical studies have shown the presence of a modified anion exchanger AE2 (51) and a sugar transporter in the lateral wall membranes (52, 53). Mechanoelectrical transduction channels and purinergic receptor channels are located at the apex. There is little evidence for voltage-gated K+ channels along the lateral wall. These appear to be concentrated at the base of the cell along with ACh receptor channels. The existence of different functional domains in the OHC PM raises the possibility that the function of integral membrane proteins in the three regions is regulated by differences in lipid composition. It has also been proposed that integral membrane proteins prefer regions of the cell with a specific membrane curvature (54–57). This represents another mechanism by which functional membrane domains may be achieved.

Freeze fracture images of the OHC PM reveal an orderly array of large (9–12 nm), closely packed particles in the cytoplasmic face (7, 58). These particles resemble freeze fracture images of integral membrane proteins, such as sodium channels, and it has been suggested that they represent a protein important to electromotility, possibly the motor itself. Another possibility is that they represent lipidic particles that form as a result of a phase transition when the membranes are frozen. Some biologically important lipids, particularly those that support membrane curvature, can enter an inverted hexagonal phase and form lipidic particles that resemble the particles found in the OHC PM [for a review, see Mouritsen & Kinnunen (59)]. Lipidic particles have not been reported in biological membranes, but lipids that contribute to these phase transitions, if present in OHC lateral wall membranes, would greatly alter their membrane properties.

### OHC PASSIVE MECHANICS

Understanding electromechanical coupling in the OHC wall requires detailed characterization of the cell’s passive mechanics. By passive mechanics we mean a characterization of the mechanical responses of the OHC to mechanical loading. The passive properties of the OHC have been derived from experiments in a number of laboratories and by the development of theoretical models to interpret the experimental results.

#### OHC Stiffness

The overall cell stiffness has the primary importance for the cell behavior as a part of the organ of Corti, where the cell undergoes mechanical loading from the tectorial membrane at the apical end and from the Deiter’s cell/basilar membrane complex at the basal end (Figure 1). In the experimental measurements of
the cell stiffness, one end of the cell is held with a micropipet and the other end is loaded by a thin fiber of high (known) stiffness. Estimates of cell stiffness by different research groups reveal considerable variability. Holley & Ashmore (60) estimated stiffness as 0.544 nN/µm. Russell & Schauz (61) gave an estimate of 1.6 nN/µm. Hallworth (62) found the mean value of stiffness equal to 6.6 nN/µm. Ulfendahl et al (63) estimated stiffness as 1.6 nN/µm. Iwasa & Adachi (64) used a different technique, pulling the movable end of the cell with a probe, and estimated the cell stiffness as 10 nN/µm. He & Dallos (65, 66) showed that cell stiffness is voltage dependent, decreasing and increasing with hyperpolarization and depolarization, respectively. A 30% change was observed for hyperpolarization and 50% for depolarization. The experimental variability of the cell stiffness is primarily determined by differences in turgor pressure and cell wall potential.

Local Elastic Moduli of the Cell Wall

The cell axial stiffness characterizes the resistance of the cell wall and the cell liquid core to axial (along the cell) loading. The cell wall elastic moduli provide complete information on the wall resistance to local loading of arbitrary nature (for small deformations). Iwasa & Chadwick (67) treated the OHC wall as an isotropic membrane and applied their model to the interpretation of an experiment with cell inflation through the micropipet; they estimated cell area expansion modulus $K$ and shear modulus $\mu$ as $6.6 \times 10^{-2}$ N/m and $6.6 \times 10^{-3}$ N/m, respectively. Ratnanather et al (68) applied the model of isotropic membrane to the interpretation of the osmotic challenge of the OHC, which resulted in changes in OHC volume and turgor pressure, and obtained an estimate $\mu/K = 0.04$–0.05. Spector et al (69) found alternative estimates of shear modulus ($\mu = 15 \times 10^{-3}$ N/m) by combining the data from the osmotic challenge experiment with the micropipet aspiration experiment (37). Tolomeo & Steele (70) showed that the data from the cell inflation experiment could be reconciled with the data from the measurement of the axial stiffness only in the framework of a model of anisotropic membrane. Assuming orthotropy of the cell wall, they estimated the local moduli $C(C_{xx}, C_{yy}, C_{xy})$, respectively, as $16 \times 10^{-3}$ N/m, $29 \times 10^{-3}$ N/m, and $56 \times 10^{-3}$ N/m. The assumption of orthotropy of the cell wall is consistent with its composite microstructure, where the cell cytoskeleton consists of almost circumferential actin filaments and longitudinal spectrin cross-links. A more-detailed, molecular-based characterization of the cytoskeleton is described below. Spector et al (71–73) applied the model of orthotropic membrane (shell, see below) to the interpretation of a combination of the osmotic challenge, micropipet aspiration, and axial loading experiments. They represented the orthotropic moduli $C$ as well as Young’s moduli ($E_x$ and $E_y$) and Poisson’s ratios ($\nu_x$ and $\nu_y$) of the wall as functions of the axial stiffness. The obtained Young’s moduli were close to those from Tolomeo & Steele (70), although the corresponding $C$ moduli were several times greater. Iwasa & Adachi (64) estimated orthotropic moduli on the basis of the cell inflation experiment in combination with their method of the cell stiffness measurement.
They found the C moduli equal 45.9 \times 10^{-3} \text{N/m}, 46.2 \times 10^{-3} \text{N/m}, and 68 \times 10^{-3} \text{N/m}. For the same value of the cell stiffness, these moduli are about 2.5 times smaller than those from Spector et al (72). It is interesting to note that the level of anisotropy of the wall measured by the ratio $C_{\theta\theta}/C_{xx}$ (or $E_{\theta}/E_{x}$) was 3.5–3.9, 3.5, and 1.5 in Spector et al (72), Tolomeo & Steele (70), and Iwasa & Adachi (64), respectively. In the orthotropic model, shear modulus is a characteristic independent of the C moduli. Although shear modulus enters the analysis of the micropipet aspiration experiment (71), its effect in that experiment was insignificant. Thus, shear modulus of the orthotropic wall has not been estimated. Because of incompressibility of the cell liquid core, additional pressure created inside the cell under its axial loading can be expressed in terms of the cell wall moduli. As a result, the total resistance of the cell to axial loading (axial stiffness $K_{ax}$) can be expressed in terms of the wall moduli by the equation

$$K_{ax} = \frac{a^2}{L} \left( C_{xx} - C_{x\theta} + 0.25 C_{\theta\theta} \right),$$

where $a$ and $L$ are, respectively, the cell radius and length.

**Bending Stiffness of the Cell Wall**

Bending stiffness and bending moduli are important characteristics of the OHC wall properties supplemental to the in-plane moduli discussed above. The geometry of the organ of Corti is such that OHCs are inclined with respect to the basilar and tectorial membranes, both along the cochlea and across the organ (Figure 1). Because the OHCs in vivo are loaded via transverse and radial vibration of these membranes, load applied to the cells has bending and twisting components. In the micropipet experiment, the cell wall undergoes bending localized around the bottom of the pipet. The bending (twisting) resistance of the wall is characterized by bending (twisting) moduli. In some biological membranes, for example RBCs, where the thickness of the PM and the associated cytoskeleton is about 10 nm, a continuum description in terms of the coordinate normal to the membrane surface is considered inadequate (74). In such cases, only two-dimensional (along the membrane surface) description is used, and the bending and twisting moduli are independent of the in-plane moduli. In the theory of thin shells, simplified assumptions regarding through-the-thickness distribution of stresses and strains are used, and it allows expression of the bending (twisting) moduli in terms of the in-plane moduli. The OHC wall is about 100 nm thick (10 times thicker than that of the RBC), and so the shell theory approach seems appropriate for the characterization of the effective properties of the wall. Spector et al (69, 71) applied the shell theory to an interpretation of the micropipet experiment and found the orthotropic bending moduli of the cell wall. The bending $D(D_{xx}, D_{x\theta}, D_{\theta\theta})$ moduli were proportional, respectively, to the in-plane moduli $C(C_{xx}, C_{x\theta}, C_{\theta\theta})$, with the factor $h^2/12$, where $h$ is the wall thickness. The twisting modulus, which is proportional to in-plane shear modulus, has not been estimated.
Modeling the Composite Structure of the Cell Wall

Spector et al (73) proposed a model of the composite elastic shell for the representation of the effective properties of the wall in terms of particular properties of the wall components. In that model, the subsurface cisterna, cytoskeleton, and PM were represented by thin shells. No-slip conditions were assumed between the subsurface cisterna and the cytoskeleton, and the PM was separated from the cytoskeleton by a liquid layer representing the extracisternal space. That space was penetrated by elastic springs that represented the radial pillars. Experimental information that would allow the estimation of particular properties of the major components of the cell wall similar to the analysis of the whole wall is not currently available. An estimation of the relative contribution of the cell wall component to the overall stiffness of the cell wall is a problem important to the understanding of active strain and force transmission throughout the wall.

Analysis of the Cell Cytoskeleton

Holley & Ashmore (60) used the glass fiber technique and estimated the axial stiffness of the demembranated cell to be about 20 times smaller than that of the normal cell. Tolomeo et al (75) measured the orthotropic properties of the cytoskeleton in both directions. They pulled the cytoskeleton along the cell axis to determine the longitudinal stiffness and stretched the cytoskeleton with two inserted probes to measure the circumferential stiffness. The demembranated cell was about six times stiffer circumferentially than longitudinally. They also found that the axial stiffness of demembranated cell is about six times smaller than that of the normal cell. Spector et al (76) developed a computational model of the cytoskeleton that took into account the cytoskeleton nanostructure. They represented the cytoskeleton as a composition of microdomains connected by a hypothetical intermediate material (Figure 3). Each domain was composed of a two-dimensional meshwork explicitly representing the actin filaments and spectrin cross-links. By assuming that the nanostructural parameters, such as the domain size and orientation, cross-link, and filament spacings, are random parameters uniformly distributed within the ranges obtained from the measurements (33, 77), Spector et al (76) estimated the mean values of the $C$ moduli of the cytoskeleton. These moduli were determined by the stiffness of three molecules: the filament, cross-link, and hypothetical molecule comprising the material between the domains. By substituting the stiffness of isolated actin as the filament stiffness and interpreting the measurements of Tolomeo et al (75) in terms of moduli $C_{xx}$ and $C_{yy}$, Spector et al (76) estimated the stiffness of the cross-link (spectrin) and the connective molecule. Spectrin may serve as the molecule connecting the domains because the calculations required that its stiffness be similar to that of spectrin. Another finding was the pattern of deformation of the cytoskeleton. Because the filaments are much stiffer than the connective molecule, the split between the strains of the domains and connective material in the circumferential direction is
extremely uneven: The domains remain almost undeformed, and the intermediate material deformation is much larger to meet the overall circumferential strain of the cytoskeleton. On the contrary, strain split in the longitudinal direction is almost even because the stiffness of the cross-link is close to that of the connective molecule.

OHC Dynamics

Under high-frequency conditions, additional factors enter the models of OHC mechanics. In particular, viscous damping has a major effect on cell motility. The impact of viscous damping increases with frequency and is a result of two processes: interaction between the cell wall and the surrounding fluids, and the relative motion (shear) of the wall elements. Jen & Steele (78) considered dynamic vibration of OHC under the action of a transcellular electric field and took into account the interaction of the cell with the inner and outer fluids. Tolomeo & Steele (79) studied the dynamics of cell motion under the action of both an axial force and transwall potential change. One of the interesting results of that study was that, under viscous damping, the frequency tuning of isolated OHCs is quite small. Ratnanather et al (80) proposed a viscoelastic model for the OHC wall and studied the dynamics of the cell under the action of an axial force. The analysis of the cell dynamics, including the viscous damping in the fluid surrounding the cell wall and within the wall itself, has primary importance for models of the whole cochlea under high-frequency conditions.

OHC ELECTROMOTILITY

OHC electromotility is a shape change that results from the direct conversion of an electrical potential into a mechanical force. Hyperpolarizing potentials elongate and depolarizing potentials shorten the cell (1–3). The OHC is designed to generate electrically evoked movements at acoustic frequencies (3, 81–85) that do not depend on calcium (3, 86) or intracellular stores of ATP (82, 83, 86). OHC electromotility is influenced by the cell’s turgor pressure. When the pressure is released, the magnitude of the evoked displacements is reduced (35, 87). The evoked displacements of an unrestrained isolated OHC appear to be limited by the passive mechanical properties of the cell. However, when the OHC is loaded and under isometric conditions, it can generate mechanical forces at frequencies >50 kHz. Under these conditions, the frequency response is nearly flat, with minimal phase shift throughout (4). The short time constants for electromechanical force transduction (~10 µs) argue against a mechanism such as that found in cardiac and skeletal muscles requiring cell signaling and chemical cascades. OHC force transduction is voltage and not current dependent (88), which shows that the mechanism on which the force transduction is based is piezoelectric-like, an observation that is consistent with the short time constant.
Force Transduction in the OHC Lateral Wall

The first description of OHC electromotility noted the movements were distributed along the length of the OHC in the region defined by the subsurface cisterna (1–3). This qualitative assessment was verified quantitatively by subsequent experiments that measured the movements of intracellular organelles (86), movements evoked while holding the OHC in a microchamber fabricated from a pipette with an inner diameter slightly smaller than that of the OHC (89) and microbead displacements (90). The microbead displacements were, on average, parallel to the long axis of the cell but differed from point to point on the lateral wall in a manner reminiscent of the cortical lattice microdomains. These experiments demonstrated that the mechanism responsible for electromotility resides in the lateral wall. Further experiments revealed the mechanism resides in lateral wall membranes, different from other forms of cell motility where the motile mechanism is associated with cytoskeletal proteins.

Movement of Membrane-Associated Charge

Displacement currents occur in response to the movement of bound charges through an electric field, such as that across the PM of a cell. The most familiar neuronal displacement current is the gating current associated with the movement of the charged S4 segment of voltage-gated ion channels in response to changes in the membrane potential (91). Voltage-clamp experiments on OHCs reveal currents after all known ion channels are blocked pharmacologically, and these are similar to gating currents. Their time course is similar to that of electromotility. They have a magnitude peak at the maximum gain in the electromotile displacement vs voltage function and are vulnerable (along with electromotility) to external application of 0.5 mM gadolinium ions (87). Huang & Santos-Sacchi (92) used a microchamber together with voltage clamp to electrically amputate the cell and demonstrated that the mobile charges are located along the lateral wall, where the mechanism responsible for electromotility is located. Calculations of the membrane-charge density required to account for the maximal measured displacement currents yield numbers that are similar to the PM membrane particle density described above (93). However, it has recently been demonstrated that the increase in charge density observed for short OHCs is not matched by a proportional increase in particle density (94).

Another unique aspect of the OHC nonlinear charge movement is that it is sensitive to pressure applied across the membrane, as first predicted by Iwasa (95). The amount of the mobile charge is divided by the voltage that drives it, and the results are expressed as capacitance. Experimentally, the voltage dependence of the capacitance has a bell shape. The bell shape is characterized by the voltage at which the capacitance is maximal (Vpk). Iwasa applied stress to the membrane by altering osmotic conditions and found that this altered the Vpk. Gale & Ashmore (96) and Kakehata & Santos-Sacchi (97) applied tension to the membrane by inflating the cell through the patch pipette during whole-cell recording. They both reported a
change in Vpk on application of pressure, confirming Iwasa’s initial observations, but they also observed a reduction in the magnitude of the capacitance. In a careful investigation, Kakehata & Santos-Sacchi (44) further confirmed that a reduction in the magnitude of the capacitance occurs on pressure application.

Many membranes contain ionic channels whose open probability is a function of applied stress or stretch. These stretch-activated channels have ionic conductances that are sensitive to pressure across the membrane [for a review, see Sachs & Morris (98)]. Recently, it was found that membrane mechanics affects the function of the sodium channel (99). Mechanical pressure differences have also been shown to affect ion channel gating currents, but the sensitivity is much lower than in the OHC (100). The effect of pressure on the capacitance in the OHC has been modeled by Iwasa (93, 95, 101). In this model, the free energy for a molecule making a transition is not only a function of the voltage, it is also a function of mechanical energy, analogous to the situation in stretch-activated ionic channels. The mechanical energy is represented as a product of the membrane tension and the membrane area. Iwasa showed that a two-state Boltzmann model can explain the observed shift in capacitance with pressure, but it cannot explain observed changes in peak height.

Pharmacological Effects on Electromotility

The most well-characterized suppressor of electromotility is salicylate, the metabolite of aspirin. Salicylate has been known for centuries to reduce hearing, and in the early 1980s, it was shown to suppress some forms of otoacoustic emissions (102). It was later shown to reduce (35, 87) and at the same time linearize electromotility (44). Recently, Lue et al (45) have demonstrated that chlorpromazine (CPZ) can shift the operating range of OHC nonlinear capacitance and electromotility up to 30 mV in the depolarizing direction. CPZ is a cationic amphipath, and unlike the anionic amphipath salicylate, it does not alter the magnitudes of the depolarizing and hyperpolarizing saturation displacements. Hueck et al (103) have demonstrated that CPZ affects the nanoscale morphology of endothelial cells, and Patel et al (104) show that it alters membrane tension and blocks stretch-activated ion channels. The lanthanides in millimolar concentrations are also powerful inhibitors of electromotility (44). Lanthanides are thought to alter surface charge, and the ionic amphipaths may also alter surface charge, but they may also change the dielectric constant and nanoscale geometry of the membrane.

Molecular Basis of Electromotility

Although the phenomenon of OHC electromotility has been well described, the molecular machinery that drives cell motility is only beginning to be identified. Recently, a membrane protein capable of endowing nonmotile cells with motility and nonlinear capacitance has been discovered (105). The protein, Prestin, was found using an OHC-subtracted plasmid library and a combination of suppression subtractive hybridization and differential screening strategies. Portions of the Prestin
gene show significant homology to *Pendrin* and sulfate transporters. TSA201 cells transfected with *Prestin* show electromotile-like oscillations approaching 150 nm that followed extracellular electrical stimulation at 1 kHz. The motility was suppressed by salicylate and was associated with a nonlinear capacitance that was smaller but qualitatively similar to that of OHCs. These important results provide strong evidence that the protein Prestin plays a central role in OHC electromotility.

MODELING OHC ELECTROMOTILITY

Modeling the OHC mechanics and electromotility can be developed on the basis of two different but related descriptions. One phenomenological approach deals with effective, cellular-level characteristics and results in constitutive relations between these characteristics that satisfy major experimental observations of the cell behavior. The other, molecular-level approach explicitly introduces nanoscale mechanisms of the cell behavior. Below, the latter approach is discussed in relation to molecular motors driving OHC electromotility as well as the active strain and force transmission throughout the cell wall. The two descriptions are related because the effective properties can be represented in the form of accumulation of molecular-level effects.

Phenomenological Description

The OHC changes its longitudinal and radial dimensions in response to changes in the transmembrane potential (3), and conversely it exhibits transmembrane charge transfer in response to mechanical loading of the cell (96, 106). These are the major features of the piezoelectric-type behavior of the cell wall material. The dimensional changes and transferred charge are nonlinear functions of the transmembrane potential. Under in vivo and experimental conditions, the strains developed in the cell wall are small. Thus, the material of the cell wall can be considered piezoelectric and linear in terms of strains and nonlinear in terms of the radial electric field or transmembrane potential. The constitutive relations for such a material are obtained on the basis of the thermodynamic potential $W$ (107, 108):

$$W = U + f_x(E)\varepsilon_x + f_\theta(E)\varepsilon_\theta + G(E),$$

where $U$ is a quadratic function of the strain $(\varepsilon_x, \varepsilon_\theta, \varepsilon_{x\theta})$ and curvature (twist) changes $(\kappa_x, \kappa_\theta, \tau)$, and $f_x(E)$, $f_\theta(E)$, and $G(E)$ are nonlinear functions of the transmembrane electric field $E$. Each component of the thermodynamic potential has a clear physiological meaning in terms of a characterization of the passive and (or) active properties of the cell wall. The function $U$ represents internal mechanical energy of the wall element, and it is given by the equation

$$U = 0.5(C_{xx}\varepsilon_x^2 + 2C_{x\theta}\varepsilon_x\varepsilon_\theta + C_{\theta\theta}\varepsilon_\theta^2) + 2G\varepsilon_{x\theta}^2 + 0.5(D_{xx}\kappa_x^2 + 2D_{x\theta}\kappa_x\kappa_\theta + D_{\theta\theta}\kappa_\theta^2) + 2D_\tau \tau^2.$$
The functions \( f_x \) and \( f_\theta \) determine the longitudinal and circumferential projections of the active force that the cell generates under isometric (\( \varepsilon_x = \varepsilon_\theta = 0 \)) conditions. The last term in the thermodynamic potential determines the wall total (linear and nonlinear) capacitance \( C(E) \):

\[
C(E) = G(E)^{''}/h^2
\]

Constitutive relations are obtained in the form of equations,

\[
\begin{align*}
N_x &= \frac{\partial W}{\partial \varepsilon_x}, \quad N_\theta = \frac{\partial W}{\partial \varepsilon_\theta}, \quad N_{x\theta} = \frac{\partial W}{2\partial \varepsilon_{x\theta}}, \quad M_x = \frac{\partial W}{\partial \kappa_x}, \\
M_\theta &= \frac{\partial W}{\partial \kappa_\theta}, \quad M_x = \frac{\partial W}{\partial \kappa_x}, \quad D = -\frac{\partial W}{\partial E},
\end{align*}
\]

where \( N_x, N_\theta, \) and \( N_{x\theta} \) are the components of the resultants, \( M_x, M_\theta, \) and \( M_x \) are the components of the bending and twisting moment, and \( D \) is the electric displacement that can be interpreted as the surface density of the transferred charge.

From the standpoint of continuum-type constitutive relations for a piezoelectric material, \( E \) is the external (applied) electric field and \( C(D), f_x(f_\theta), \) and \( G \) are, respectively, effective elastic, piezoelectric, and dielectric coefficients of the material. An experimental description of the movement and interaction of the motor-related electric charges that accompany OHC electromotility has yet to be developed. However, the local electric field and polarization can be introduced as a part of models of electromotility (this approach is discussed below).

The overall (observable) strains can be represented as the sum of the active (\( \varepsilon^a \)) and passive (\( \varepsilon^p \)) strains

\[
\varepsilon_x = \varepsilon_x^a(E) + \varepsilon_x^p, \quad \varepsilon_\theta = \varepsilon_\theta^a(E) + \varepsilon_\theta^p, \quad \varepsilon_{x\theta} = [C]^{-1}\hat{N}(N_x, N_\theta),
\]

where \([C]\) is the matrix of the \( C \) in-plane moduli of the wall.

The active forces (resultants under zero-strain conditions) and active strains (strains under zero-resultant conditions) give a dual characterization of the cell wall active properties, and these functions are related by the equations

\[
\begin{align*}
f_x &= -C_{xx}\varepsilon_x^a - C_{x\theta}\varepsilon_\theta^a, \quad f_\theta &= -C_{x\theta}\varepsilon_x^a - C_{\theta\theta}\varepsilon_\theta^a.
\end{align*}
\]

The microchamber experiment (89, 109) provides the longitudinal and circumferential components of the electromotile strains under the conditions of minimal resultants. Spector et al (110) interpreted those strains as active strains and obtained the active forces as nonlinear functions of the transwall electric potential. They estimated the active force production per unit of electric potential, \( \psi \), as \( e_x = df_x/d\psi = 4.8 \times 10^{-3} \) N/Vm, \( e_\theta = df_\theta/d\psi = 9 \times 10^{-3} \) N/Vm. Tolomeo & Steele (79) estimates were \( e_x = e_\theta = 1.6 \times 10^{-3} \) N/Vm, and Iwasa & Adachi (64) gave the estimate \( e_x = 3.3 \times 10^{-3} \) N/Vm. The presented approach was extended (107) to the composite wall, with the active PM connected to the passive cytoskeleton and subsurface cisterna. A more comprehensive version of the constitutive relations was given by Spector (111), where the active properties of the cell wall were both voltage and tension dependent.
Molecular-Level Modeling with Conformational Motor

Dallos et al (109, 112) proposed a concept where the active properties of the OHC were determined by uniformly distributed molecular motors that change their conformational states depending on the wall electric potential. Similar to the analysis of voltage-gated channels and some other types of membrane proteins, the probability of the OHC motor changing its conformational state was described by a two-state Boltzmann function. Iwasa (95, 101, 113) and Iwasa & Adachi (64) further developed the molecular motor concept, including the tension effect on the active properties, anisotropy of the passive properties of the wall, and possible three-state motor. Spector et al (76, 114) proposed a three-dimensional computational model that explained the mechanism of the active strain and force transmission throughout the composite wall as a result of the molecular motor activity. All the major components of the wall were explicitly represented in the model, and the motors were associated with the particles embedded in the PM. The conformational changes in the particles were modeled as active longitudinal and circumferential strains, as shown in Figure 4. The parameters of the model were estimated by matching the electromotile strain observed in the microchamber experiment as well as by matching earlier estimates of the cell wall stiffness and the active force production. The results of simulation demonstrated that the PM is the primary generator of the electromotile response. The cytoskeleton and subsurface cisterna are driven by the active PM, and they impose constraints on the active movement of the plasma membrane. This conclusion is in direct correspondence to the data from the experiment with trypsin (33), where the connection of the PM to the rest of the cell was destroyed but the cell was still electromotile. This result is also confirmed by the experiment with Prestin (105), where kidney cells, which do not have an OHC-type cytoskeleton, exhibit electromotility. It has been found (AA Spector, M Ameen & AS Popel, submitted for publication) that the active strains are strongly redistributed by the interaction of the motors with the surrounding material (lipid bilayer) and, to a smaller extent, by the resistance of the passive part of the wall (the cytoskeleton and the subsurface cisterna). In order to match the observed limiting values (5% and −2%) of the electromotile strains, the active motor-related longitudinal and circumferential strains have to be larger and reach, respectively, 11% and −4%. Such strains result in 7% motor-related area change. There are membrane proteins whose conformational changes reach such levels (115, 116).

Membrane-Bending (Liquid Crystal) Model

Raphael et al (117) have developed a theoretical model of electromotility based on the biophysical properties of lipid/protein assemblies and the mechanical properties of membranes. The fundamental starting point of this model is that membranes are thin structures that have a small resistance to bending but a large resistance to in-plane area change (74, 118). Although the OHC appears to change its surface area during electromotility, this observed area change may only be apparent
and the PM may undergo a geometric rearrangement at a level that is not visible in the light microscope. Such a hypothesis would have several experimentally testable corollaries. For one, the membrane would possess excess surface area, and molecules that partition into the membrane and affect its geometric state, such as ionic amphipaths, would affect electromotility. As discussed above, experimental evidence supports these contentions.

Because of the need for efficient force transmission from the membrane to the cytoskeleton, excess membrane surface area is not expected to be distributed haphazardly but would most likely be organized in such a way that geometric changes lead to a concerted deformation of the cytoskeleton. The organization of the lateral wall reviewed earlier suggests a possible mode of nanoscale organization. If the PM is tightly connected to the cytoskeleton at the pillars, then between the circumferential actin filaments, the spectrin cross-links and associated membrane can form a two-dimensional motile unit. The turgor pressure in the cell creates a force that induces nanoscale bending of the PM between the pillars (Figure 5), as predicted for a cylinder supported by circumferential rings under uniform pressure (119). Electron micrographs (39) indicate that the radius of curvature in the longitudinal direction is about 30 nm. Hence, the membrane and associated cytoskeleton can be thought to comprise an “electromechanical flexion motor” or a “nanoscale bending motor.” Mechanically, one can think of the energy stored in membrane bending as being in equilibrium with the energy stored in the cytoskeleton on the length scale of the spectrin filaments (40 nm). Raphael et al (117) have shown that within the experimentally determined values of the material parameters this is indeed the case. The nanoscale curvature of the membrane is determined not only by the turgor pressure but also by the spontaneous curvature. The spontaneous curvature arises from the intrinsic tendency of the constituent protein and lipid molecules to adopt a nonzero curvature in the absence of external forces, which may explain the residual motility observed when the OHC cytoskeleton is digested with trypsin (120, 121). When the cytoskeleton is disrupted with diamide, active force generation is reduced in the cell (122). The motile unit concept accounts for this observation because the disruption of spectrin will decrease the cell’s passive stiffness on which the force generation depends (110).

The proposed model postulates that an applied electric field perturbs the equilibrium of the motile unit by inducing curvature change. The PM of the cell is considered to behave as a liquid crystal composed of molecules that have a net dipole moment parallel to their long axis. The application of an electric field causes reorientation of constituent molecules that is accompanied by out-of-plane deformation related to the asymmetric shape of the molecule (123). This effect, referred to as flexoelectricity, is the dominant mode of electromechanical coupling in liquid crystals (124) and is contrasted with the piezoelectric effect seen in solid crystals (125). Petrov (126) has studied manifestations of flexoelectricity, or curvature-induced polarization, in a great variety of synthetic and biological membranes. Independent experimental confirmation has been obtained by Sun (127) in lipid
membranes and by Mosbacher et al (128) in human embryonic kidney cells. The molecular mechanism of flexoelectricity in cells may involve aggregation of membrane proteins, which an independent theoretical analysis predicts will lead to curvature deformation (55).

The work of Petrov only considered linear electromechanical coupling. Raphael et al (117, 129) have extended the flexoelectric formalism to the nonlinear regime by considering that for a collection of molecular dipoles, saturation of the effect is expected when maximum alignment of dipoles is attained. If the dipoles are allowed to rotate freely and continuously, the fractional distribution of dipoles aligned with the field is given by the Langevin function. Raphael et al (117) developed a nonlinear flexoelectric model of OHC electromotility based on the Langevin function and showed that the model is able to predict the electromotility voltage-displacement function using measured values of the elastic constants of the cell membrane. Figure 6 illustrates the ability of the model to fit experimental data in a control cell and a cell treated with salicylate. The salicylate data are fit with a smaller dipole moment, which suggests that the mechanism by which salicylate inhibits electromotility involves a decrease in the dipole moment of the voltage sensor. This is consistent with observations that salicylate intercalates in membranes near the head-group region (130, 131), disrupts the packing of liquid crystals (132), and inhibits sulfate transport (133). The mode of operation of membrane transporters involves reorientational motions of membrane dipoles, and thus, the flexoelectric mechanism is compatible with *Prestin* being the motor protein (105).

**Internal Electric Field and Orientational Order in the Membrane**

Experimental data on OHC electromotility are obtained from patch-clamp recordings in which the transmembrane potential is applied and controlled across the membrane. However, because the membrane is a dielectric and has a large nonlinear capacitance, the field within the membrane (referred to as the internal or polarizing field) will modulate this applied potential in such a way that the potential actually seen by the voltage sensor is not the same as the applied potential. Computing this actual potential requires exact knowledge of the location of all the charges in the membrane and is a difficult task. However, if the electrostatic behavior is dominated by a regular arrangement of charged voltage sensors, the internal field can be written as the sum of the applied field and the polarization using the Lorentz relationship (125). This approach leads to constitutive equations that predict the shift and decrease in nonlinear capacitance experimentally observed with applied pressure (RM Raphael, AS Popel, & WE Brownell, submitted for publication) as shown in Figure 7. Note that this effect arising from the internal field complements, but does not depend on, the membrane bending model discussed above. However, it can be understood qualitatively using the membrane bending illustration (Figure 5), in which the internal polarization of the membrane is related to the curvature of each ripple, which in turn depends on the intracellular pressure. Consequently, the coupling between the applied field
and the internal field will depend on the intracellular pressure. For each different curvature (pressure), there will be a different voltage at peak capacitance ($V_{pk}$) and a different amount of charge that is available to move when an external field is applied. For example, when the pressure is increased, the cell shortens, rippling increases, and the average orientation of the dipoles enters the nonlinear region and approaches saturation. When the membrane is then depolarized from this point, fewer dipoles are available to move into alignment with the field, resulting in reduction in the magnitude of the capacitance. The internal polarization is related to the orientational order of the membrane. Evidence for a change in orientational order with voltage and applied tension comes from experiments in which the diffusion of a fluorescent probe in the OHC PM was found to be a sigmoidal function of membrane potential and osmotic strength (46). Similar behavior has been observed in liquid crystals (135) and reflects the continuous increase in internal membrane order with the applied field. Hence, the molecular basis of electromotility, which enables subtle information to be extracted from broadband acoustic energy, may involve a voltage-induced disorder-order transition occurring within the PM of the OHC (129).

**MOLECULES, MEMBRANES, AND HEARING**

Over the past 15 years, the application of ever more sophisticated experimental techniques and theoretical modeling has provided a more complete characterization of the micro- and nanomechanics of the OHC. Voltage-clamp and pressure-clamp studies have demonstrated that a broad range of passive and active mechanical features are a function of transmembrane potential gradients and membrane tension. Yet for all that has been accomplished, much more remains. What is the precise mechanism by which the motor mechanism senses transmembrane voltage? How is it able to achieve a conformational change? What is the composition of the
membrane particle, how many individual motor molecules can it include, and what kind of electrical and mechanical interaction among the motors takes place? Does the PM undergo nanoscale bending? If so, what is the interplay between the in-plane and out-of-plane active strains?

What about the rest of the cell? The orthotropic mechanics of the OHC lateral wall is based on the structural organization of the cortical lattice. This orderly, almost Cartesian, array of circumferential actin filaments and longitudinal spectrin raises questions about the cell signaling pathways that control its expression and maintenance. Are the pathways that regulate the integrity and mechanics of the OHC cortical lattice (136, 137) the same that modulate cytoskeletal organization in other cell types (138)? What is the function of the SSC and does it deform when the cell changes length? Do the actin filaments slide along the SSC or are they tightly bound?

In addition to these molecular and cellular questions, organ-level questions also remain. Why is the cochlear amplifier effective only in a narrow range around the characteristic frequency? How can OHC electromotility result in negative feedback in the apical turn of the cochlea, whereas its positive feedback provides amplification in the remainder of the cochlea? What is the mechanism of the delivery of the receptor potential under high-frequency conditions? Are mutations in hair cell cytoskeletal proteins that alter cell mechanics responsible for genetic deafness? Just as the original observation of electromotility provided an explanation for von Bekesy’s seminal observations on the whole cochlea, a deeper understanding of OHC micro- and nanomechanics may well provide answers to the remaining questions in the puzzling behavior of the whole organ of Corti.

ACKNOWLEDGMENTS

This work supported by grants DC00354, DC02775, DC0079, and NRSA DC00363 from the National Institutes of Health and grant 9871994 from the National Science Foundation.
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Figure 1  The Organ of Corti. [Modified from Brownell (139).]

Figure 3  Schematic of outer hair cell cytoskeleton. Overview (a) and higher-power view (b) of multidomain unit. Strips between the domains represent connective molecules. [From Spector et al (76).]
Figure 4  (a) General view of the outer hair cell and (b) an element of the composite wall showing deformation of the computational unit as a result of the excitation of the particles/motors. (Dotted lines) Undeformed state; (solid lines) deformed state. Constant displacements along two sides of the unit determine the electromotile response of the unit and the whole cell. The area around one of the particles is discretized with the finite element mesh. [From AA Spector, M Ameen & AS Popel, submitted for publication.]

Figure 5  Illustration of the membrane bending/orientational motor model. Hyperpolarized (a), depolarized (b). [Modified from Oghalai et al (46).]
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