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Electromechanical Models of the Outer Hair Cell Composite Membrane

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Abstract. The outer hair cell (OHC) is an extremely specialized cell and its proper functioning is essential for normal mammalian hearing. This article reviews recent developments in theoretical modeling that have increased our knowledge of the operation of this fascinating cell. The earliest models aimed at capturing experimental observations on voltage-induced cellular length changes and capacitance were based on isotropic elasticity and a two-state Boltzmann function. Recent advances in modeling based on the thermodynamics of orthotropic electroelastic materials better capture the cell's voltage-dependent stiffness, capacitance, interaction with its environment and ability to generate force at high frequencies. While complete models are crucial, simpler continuum models can be derived that retain fidelity over small changes in transmembrane voltage and strains occurring in vivo. By its function in the cochlea, the OHC behaves like a piezoelectric-like actuator, and the main cellular features can be described by piezoelectric models. However, a finer characterization of the cell's composite wall requires understanding the local mechanical and electrical fields. One of the key questions is the relative contribution of the in-plane and bending modes of electromechanical strains and forces (moments). The latter mode is associated with the flexoelectric effect in curved membranes. New data, including a novel experiment with tethers pulled from the cell membrane, can help in estimating the role of different modes of electromechanical coupling. Despite considerable progress, many problems still confound modelers. Thus, this article will conclude with a discussion of unanswered questions and highlight directions for future research.

Key words: Piezoelectricity — Prestin — Cochlear amplifier — Nonlinear capacitance — Voltage-dependent stiffness — Membrane curvature — Cell mechanics — Hearing — Thermodynamics

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Modeling Outer Hair Cell Electromotility: Goals and Challenges

The construction of mathematical models plays a pivotal role in any scientific field. Models serve to organize disparate experimental observations by relating them to fundamental physical laws, and serve as a guide for the design of new experiments that further increase our knowledge of the system of interest. Modeling is of necessity an approximation to reality, and often requires the introduction of simplifying assumptions. As a prominent membrane biophysicist has recently written: "A good model is a blessing, but it can also be a curse. It may bias our thinking too strongly if we forget that it is just a model and not Nature herself." (Mouritsen, 2005). Over the past decade, diverse modeling efforts have made a major contribution to our current understanding of outer hair cell function. One may even argue that for no other type of cellular motility has the utility of modeling been more necessary for interpreting the diverse experimental phenomena.

The most general goal of theoretical modeling approaches is to characterize the unique electromotile properties of outer hair cells. Located in the organ of Corti in the mammalian cochlea, outer hair cells are cylindrically-shaped cells that exhibit voltage-dependent somatic motility. Outer hair cell (OHC) elec-

tromotility refers to the changes observed in the dimensions of these cells that occur when the transmembrane potential changes. The mammalian cochlea is extremely sensitive to sound, and the existence of a "cochlea amplifier" had been postulated as necessary to explain this remarkable sensitivity (Davis, 1983). However, it was not until the discovery of electromotility by Brownell and coworkers (Brownell et al., 1985; Kachar et al., 1986) that a cellular process for amplification was identified. The unique nonlinear electromotile characteristics exhibited by OHCs and their ideal placement in the organ of Corti makes them the perfect candidates to influence the motion of the basilar membrane (BM) and other cochlear components. (Sound-induced vibrations of the cochlear partition lead to deflection of stereocilia on inner hair cells where mechanosensitive channels are located. The resulting membrane depolarization triggers neurotransmitter release and leads to firing of auditory nerve fibers). Electromotility is now recognized as playing a pivotal role in increasing the sensitivity of the cochlea to low-level sounds and enabling sharp frequency discrimination. However, the presence and recent characterization of active mechanical movements in the stereocilia bundle has re-opened an old debate on the source of the cochlea amplifier (Kennedy et al., 2003; Kennedy, Crawford & Fettiplace, 2005). The current quandary over what cellular mechanisms power the active amplification in the cochlea is still unresolved although a great deal of progress has been made in revealing the capabilities and limitations of the competing hypotheses. What has been shown is that OHC motility is essential for powering amplification in the cochlea (Liberman et al., 2002; Cheatham et al., 2004). Thus, the construction of appropriate models of OHC electromotility has utility in understanding the function of the mammalian cochlea, as macro-cochlear models need accurate descriptions of how the forces generated by outer hair cells influence the vibration of the cochlear partition (e.g., Mountain & Hubbard 1994; Cohen & Furst 2004).

Outer hair cell electromotility involves the conversion of electrical to mechanical energy, and thus thermodynamic approaches have proven to be extremely productive for modeling OHC behavior. In these approaches, an appropriate energy function is constructed and used to derive constitutive equations that describe the observed phenomena (Spector, 1999; Raphael, Popel & Brownell, 2000; Deo & Grosh, 2004). The operative parameters in these models are material parameters, such as mechanical moduli and electromechanical coupling coefficients. Thus an ancillary goal of theoretical modeling is to derive the appropriate material parameters that govern the cell's behavior. What type of empirical behavior needs to be modeled? The primary goal is to describe the voltage-displacement function: that is, the change in the length and radius of the cell as a function of change in the transmembrane potential, which can be considered voltage-dependent strains. Coupled with these strains is a change in the cell's nonlinear capacitance, or voltage-dependent intramembraneous charge movement (Santos-Sacchi, 1991; Iwasa, 1993). This charge movement is analogous to the "gating charges" that accompany the opening of voltage-gated ion channels, but displays a unique dependence on intracellular anions (Oliver et al., 2001; Song et al., 2005a). Furthermore, recent experiments have established that the axial stiffness of the OHC is voltage-dependent. Thus any complete model needs to explain the voltage-dependent changes in the cell's dimensions, capacitance and stiffness. Finally, the model should provide a way of quantifying the active forces produced by the cell, for it is the magnitude and direction of forces which ultimately influence the vibrations of the cochlear partition (Nobili et al., 1998; Cai et al., 2004).

OHCs have been shown to be electromotile up to 70 kHz in vitro in guinea pigs (Frank, Hemmert & Gummer, 1999) and able to induce vibrations of the BM at frequencies up to 100 kHz in vivo (Grosh et al., 2004). The latter experiments were done using bipolar excitation, which is different in level and current path than excitation arising from stereocilia channels. Nevertheless, the vibrations were salicylate-sensitive, and this work implies that OHCs can force the sensory epithelium at acoustic and ultrasonic frequencies. A particular challenge of modeling OHC electromotility is to explain how the cell can operate at such high frequencies. If the outer hair cells are to respond to acoustic stimulation on a cycle-by-cycle basis, they must be capable of functioning at least at 20 kHz in humans and at even higher frequencies in other mammals. The OHC motor is membrane-based (as described below), and two problems arise for high-frequency membrane-based motors. First, they must overcome the effects of viscosity, which become more problematic as the rate of cell deformation increases. Second, they must overcome the effect of the membrane's electrical (RC) time constant, which predicts that sinusoidal changes in membrane voltage start to roll-off around 1 kHz (Santos-Sacchi, 1992), raising questions of how voltage changes are even "seen" by membrane-embedded molecules. In fact, the principal argument against OHC motility as being the force generator has been the high frequency roll-off of the OHC transmembrane voltage which drives the motility (Chan & Hudspeth, 2005).

However, these arguments are based on purely electrical properties of an individual OHC, and several explanations addressing this question have been proposed. The OHC wall is an electromechanical structure, and recent studies (Spector, Brownell & Popel, 2003; Weitzel, Tasker & Brownell, 2003;

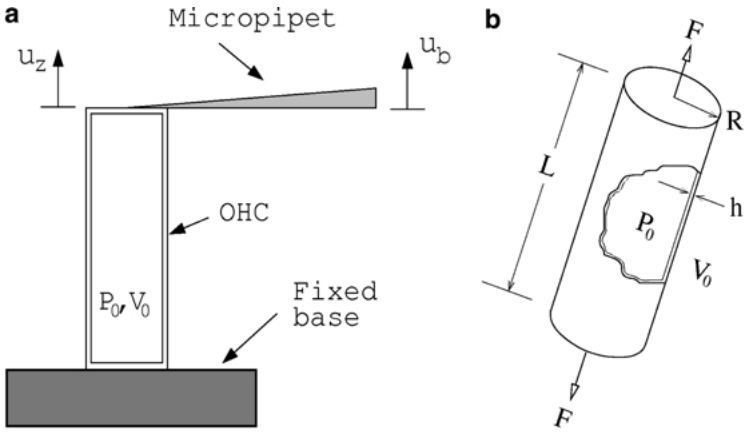

Fig. 1. (*a*) Schematic of an outer hair cell in an in vitro setup. One end of the OHC is fixed (an idealization of the patch-clamp mechanical constraint) while the other end is either free to move or can be subjected to mechanical excitation using a micropipet. Electromechanical characteristics of the OHC are studied through manipulation of the transmembrane potential V_0. u_z denotes displacement of the OHC top while u_b denotes displacement of any pipet used for exciting the OHC. (*b*) Structurally, OHCs are similar to thin fluid-filled cylindrical shells. They have some resting turgor pressure P_0 and the relative ionic content from OHC interior to exterior coupled with selective membrane channels gives rise to a resting transmembrane potential V_0. The figure illustrates an OHC having length L, radius r, and thickness h. F is any external force acting on the OHC.

Rabbitt et al., 2005) have shown that electromechanical coupling, including piezoelectricity and mechanosensitive channels, can balance the capacitive filtering of the receptor potential. Also, the electric field across the cell membrane generated by neighboring cells is not filtered by cell capacitance, and it can contribute to high-frequency potentials driving electromotility (Dallos & Evans, 1995; Fridberger et al., 2004). In addition, predictive models of the cochlea that include OHC motility coupled to the mechanics of the other components of the organ of Corti in a feedback loop (Grosh et al., 2005; Liu, 2005) reveal that the coupled mechanics of the system can overcome the response roll-off resulting from membrane capacitance.

At a continuum level, a description of outer hair cell phenomena (voltage-dependent strains, capacitance and stiffness) can be constructed without considering the actual molecular machinery that underlies these phenomena. (One can, for example, describe the bulk electromechanical properties of quartz without reference to its molecular structure.) Several early experiments established that the mechanism of electromotility was membrane-based (Kalinec et al., 1992; Huang & Santos-Sacchi, 1994), meaning it did not reside or even depend upon the presence of the cytoskeleton. Although many speculated that ''molecular motors'' embedded in the plasma membrane were responsible for electromotility, it was not until the discovery of the protein prestin (Zheng et al., 2000) that the molecular identity of any component of the machinery was identified. With the identification of prestin, attention is now focused on understanding how prestin functions and elicits deformation and force at the whole-cell level.

For those wishing to explore the development of this exciting field, several excellent reviews have appeared over the years. A good introduction to the role of outer hair cells in the active cochlea can be found in Dallos, 1992. The relation of outer hair cell electromotility to basilar membrane motion has been recently reviewed by Robles & Ruggero, 2001. A good general overview of OHC structure and the initial characterization of electromotility before the discovery of prestin can be found in Holley, 1996 and Lim and Kalinec, 1998. The development of modeling efforts up to 2001, including the orthotropic model and the membrane bending model, is reviewed in Brownell et al., 2001. A good commentary on the advancements since the discovery of prestin can be found in Santos-Sacchi, 2003 and Ashmore, 2002. Finally, Brownell considers outer hair cell electromotility from an evolutionary context and discusses a universal role for membrane piezoelectricity (Brownell, 2005).

Experimental Observations and Two-State Boltzmann Models

Since the initial discovery and characterization of outer hair cell electromotility (Brownell et al., 1985; Kachar et al., 1986; Ashmore, 1987), there has been a flurry of experimental investigations aimed at unraveling the underlying mechanisms. OHCs have been extensively studied using in vitro preparations and one of the most common preparations used is a patch-clamped OHC (Fig. 1).

In vitro experiments on OHCs have revealed nonlinear transmembrane charge (and therefore capacitance) of the OHC lateral membrane, nonlin-

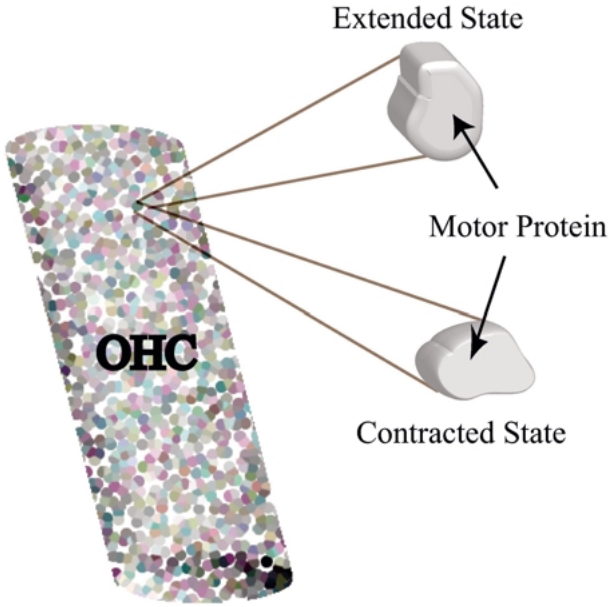

Fig. 2. Cartoon of an OHC with motor proteins uniformly embedded throughout the cell wall. The proteins are hypothesized to exist either in an extended configuration or in a contracted configuration. A change in the configuration is accompanied by some charge moving across the cell wall (*not shown* in figure).

ear motility, and most remarkably nonlinear axial stiffness when the transmembrane voltage was altered. A short review on experimentally observed OHC effects can be found in Deo and Grosh, 2004. The dependence of the transmembrane charge and motility on the transmembrane voltage has been observed to follow a sigmoidal shape, while the capacitance follows a bell-shaped curve, consistent with the capacitance as reflecting the derivative of the charge movement associated with the motility. Based on these observations, a two-state Boltzmann model was suggested to explain OHC behavior (Dallos, Hallworth & Evans, 1993; Santos-Sacchi, 1993; Iwasa, 1994). The underlying assumption of the two-state Boltzmann model is the existence of a protein (hypothetical at the time the model was proposed) in the plasma membrane that could have two stable conformations, one larger in area than the other (Fig. 2). The protein could jump almost instantaneously from one state to the other and this flipping between two configurations was accompanied by some charge transfer across the membrane. The concept of the electromotility-related protein being in a number of distinct conformational states was supported by functional and structural data on many membrane proteins, and the two-state model was chosen to reflect the sigmoidal shapes of the voltage dependence of the electromotile length changes and transferred charge. The resulting model fit the capacitance and motility data reasonably well (Iwasa, 1994) and the discovery of the motor protein prestin (Zheng et al., 2000) further gave credence to the theory. However, the concept of prestin having two stable configurations with different areas has not been proven experimentally. Moreover, the two-state model could not fully explain experimental results on the tension dependence of nonlinear capacitance (Kakehata & Santos-Sacchi, 1995), for which a three-state model was proposed (Iwasa, 1996). The original two-state model also lacked a mechanism that could replicate the voltage-dependent stiffness of the cell (He & Dallos, 1999, 2000). As described below, an extended two-state model has recently been shown to account for the stiffness data (Deo & Grosh, 2005), although issues with the turgor pressure and temperature still remain.

Nonlinear Stiffness and Dynamics Simulations

The discovery of the nonlinear stiffness of the OHC had important implications for cochlear mechanics since the percentage change observed in stiffness was much higher than the strain seen in OHC motility for similar voltage excitations (He & Dallos, 1999, 2000). It also meant that previous experimental interpretations based on the two-state model needed to be studied again, since the original model did not account for the change in stiffness as the voltages were altered. On the other hand, variable stiffness could lead to a way to explain outer hair cell behavior as turgor was changed. This is an aspect of OHC mechanics which still isn't understood well — the effect of stress in the OHC on its behavior (*see* Deo & Grosh, 2004, for a discussion). Analysis of the stiffness results and the two-state model revealed that incorporating state-dependent compliance of the protein in the model would give better results in capturing experimental data. Addition of this feature in the model enabled a good match of the stiffness data without compromising the capacitance and motility results from the original model (Fig. 3).

The other test for the model was provided by experimental results of He and Dallos, 2000 on dynamic experiments performed with OHCs. The OHCs were excited simultaneously with a mechanical and electrical stimulus. Resulting motion of the OHC revealed responses at frequencies corresponding to the sum and difference of the excitation frequencies. Excitation of the OHC with only an electrical stimulus showed OHC motion at the fundamental frequency of excitation and its harmonics. However, excitation using only a mechanical stimulus did not reveal any mechanical harmonics. This was a clear indication of a primarily voltage-dependent OHC nonlinearity. When the experimental conditions were mimicked the extended two-state model showed almost exactly the same harmonic content as the experiments (Fig. 4).

A variable stiffness model of the OHC (or an experimentally verified model of the isolated

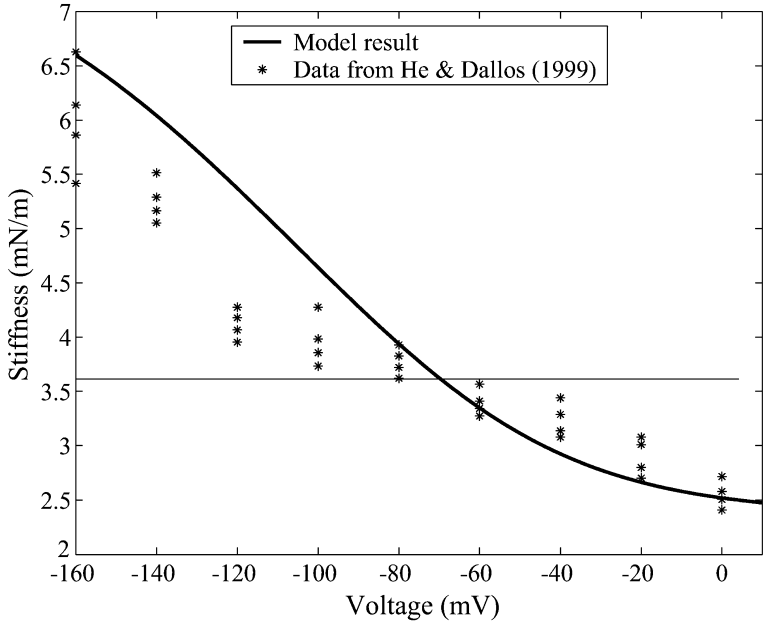

Fig. 3. Stiffness prediction of modified area motor model compared against data from He and Dallos (1999).

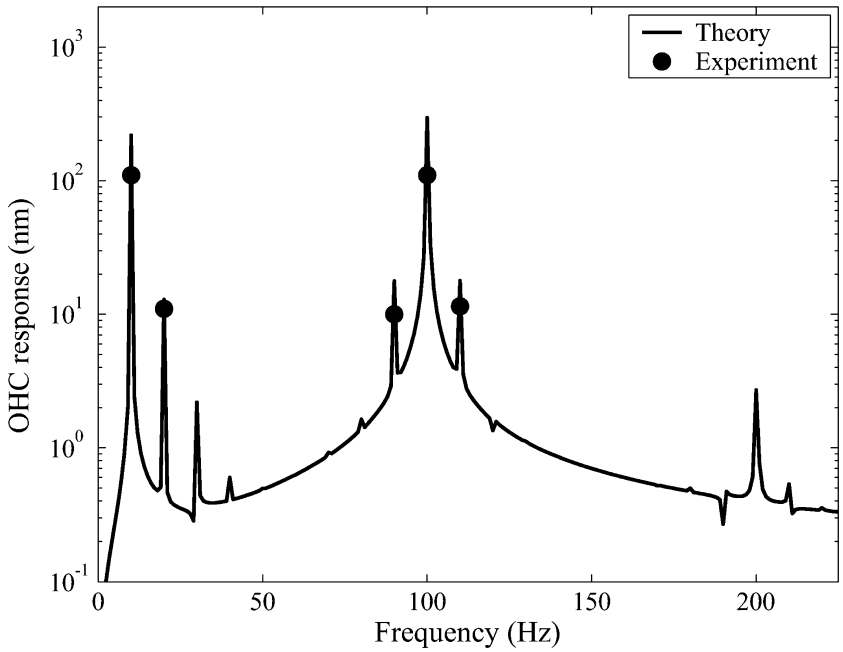

Fig. 4. Frequency spectrum of OHC strain versus time as predicted by the modified area motor model. Electrical excitation was at 10 Hz while mechanical probe excitation was at 100 Hz. Results are compared with the experimental results of He and Dallos (2000).

electromechanical behavior of the OHC) can be used in a nonlinear or linear macroscopic model of the cochlea. Hence these OHC models provide a link between the in vitro measurements of isolated OHCs to models of the global response of the cochlea. In this way, different hypotheses of global cochlear function can be tested in ways that can be verified by combined data from in vivo measurements of cochlear function and the in vitro testing. These models are only valid for low-frequency excitations since viscous and inertial effects are ignored. Further, the properties of the OHC wall are homogenized through the thickness. Modeling the cell's environment and structural details offers avenues to examine certain other aspects of OHC behavior, for instance, ability of the cell to produce/transmit forces at high frequencies. These issues are considered next.

Role of Cell Environment

The outer hair cell's composite wall responds to electrical (Brownell et al., 1985; Ashmore, 1987) and mechanical (Gale & Ashmore, 1994; Iwasa, 1994; Kakehata & Santos-Sacchi, 1995) stimulation, and

the mechanisms of the cellular responses involve the membrane protein prestin (Zheng et al., 2000) and intracellular chloride ions (Zheng et al., 2000; Oliver et al., 2001). The functioning and effectiveness of these mechanisms are modulated by the cell's interaction with its environment. Inside the organ of Corti, cell vibration is constrained by the underlying basilar membrane (BM) + Deiters' cell (DC) and the overlying reticular lamina (RL) + tectorial membrane (TM) complexes. In addition to the structural components, the surrounding fluid also interacts with the cell and generates drag forces acting on the cell wall. Such effects, involving the inertial and viscous forces, increase significantly under high-frequency conditions. Thus, it is a challenge to explain how outer hair cell electromechanics, including the electromotile length changes, active force production, and electric charge transfer, are affected by the cell environment.

The governing equations for the outer hair cell wall can be presented in the following form (Tolomeo & Steele, 1995; Ratnanather et al., 1997; Spector et al., 1999; Spector et al., 2001):

$$N_{\mathrm{x}} = C_{11}\varepsilon_x + C_{12}\varepsilon_\theta + 2\eta s_{\mathrm{x}} + f_{\mathrm{x}}^{\mathrm{a}}(\Psi) \tag{1}$$

$$N_\theta = C_{12}\varepsilon_x + C_{22}\varepsilon_\theta + 2\eta s_\theta + f_\theta^{\mathrm{a}}(\Psi) \tag{2}$$

$$Q = -\frac{\partial f_{\mathrm{x}}^{\mathrm{a}}}{\partial \psi}\varepsilon_{\mathrm{x}} - \frac{\partial f_\theta^{\mathrm{a}}}{\partial \psi}\varepsilon_\theta + h^{-1}G(\Psi) \tag{3}$$

where N_{x} and N_θ are, respectively, the longitudinal (along the cylindrical cell) and circumferential (around the cell) components of the resultant (force per unit length along the θ and x axes, respectively); C_{11}, C_{12} and C_{22} are the orthotropic elastic moduli of the cell wall linearized about the electromechanical operating point of the OHC; ε_{x} and ε_θ are the components of the strain; $f_{\mathrm{x}}^{\mathrm{a}}$ and f_θ^{a} are the components of the active force; Ψ is the cell's transmembrane potential, s_{x} and s_θ are the components of the strain rate, and η is the cell wall viscosity. Also, Q is the transferred charge per unit surface area of the cell membrane; G is a function that characterizes the electrical permittivity of the cell wall; and h is the wall thickness. The functions Q and G are associated with nonlinear capacitance: the voltage derivative of the first function is "effective" specific capacitance, and the same derivative of the second function is proportional to the specific capacitance measured in a fully constrained cell (zero-strain conditions). Below, we have a more detailed discussion of the relationships between different capacitive characteristics of the cell membrane. Under high-frequency conditions, the active force production per unit transmembrane potential, transmembrane potential itself, the nonlinear capacitance of the cell membrane and the viscosity- and inertia-related terms play an important role. The imposed constraints affect the cell wall strains and nonlinear capacitance. Thus, the proposed constitutive relations (1)–(3), involving the cell wall's strain, capacitance, viscosity, etc., are suitable for analyzing the effects of the cell environment.

High-Frequency Force Generation and Cell Viscosity

Frank et al. (1999) did an experimental analysis of outer hair cell electromotility and active force production under high-frequency conditions. The authors electrically stimulated the cell in a microchamber and used an AFM cantilever to measure the produced active force. The main conclusion of this study was that the outer hair cell is capable of generating a constant active force on the order of tens of pN/mV up to tens of kHz. Recently, Scherer and Gummer, 2004, extended the analysis of high-frequency forces by considering the whole cochlea with an excised tectorial membrane and clamped basilar membrane. By stimulating the cells electrically, the authors measured the resulting force within a broad frequency range and found a significant resonance in the area above the local characteristic frequency.

Tolomeo and Steele (1995, 1998) proposed a model of the high-frequency interaction between the outer hair cell wall and the extracellular and intracellular fluids. In that model, the fluids were considered viscous, and the cell wall was assumed to be purely elastic. The authors applied their model to two cases: 1) mechanical excitation normal to the cell surface (model of the water jet experiment of Brundin and Russell (1994) and 2) electrical excitation of an unconstrained cell. As a result of their modeling, the authors computed cellular electromotile responses as functions of frequency and length of the cell. Ratnanather et al. (1997) have introduced the viscosity of the cell and analyzed the cellular responses to the application of an axial force. The authors considered the effect of the wall viscosity and took into account different viscosities of the two fluids interacting with the cell wall.

Liao et al. (2005a, 2005b), on the basis of constitutive equations (1) and (2), have studied outer hair cell motility and active force production under high-frequency conditions, taking into account the cell environment in the experiment and inside the cochlea. In the first of these studies, the authors simulated the high-frequency electrical stimulation of unconstrained cells in the microchamber experiment. Particularly, Liao et al. (2005a) analyzed the effects of the wall viscosity, and, similar to the conclusion of Ratnanather et al. (1997), found that the roll-off frequency decreases with the increase in the wall viscosity. In the second paper, Liao et al. (2005b)

studied the high-frequency active force generation in constrained cells. The authors proposed a model that is applicable to both experimental and physiological conditions. To impose constraints on the cell vibrating within a viscous fluid, two elastic elements were attached to the ends of the cell. To simulate the measurement of the active force in the Frank et al., 1999, experiment, Liao et al., 2005b, used the no-displacement boundary conditions along the interface between the cell wall and the microchamber and assumed that the stiffness of the element attached to the movable end of the cell was equal to that of the AFM cantilever used in the experiment. In their simulation of in vivo conditions, the authors assumed that the two elastic elements have stiffness equal to that of two complexes (BM + DC and RL + TM) constraining outer hair cells in the cochlea. Figure 5 presents the results of simulation of physiological conditions for a cell of 20 μm length. In Fig. 5*a* and *b*, respectively, the active force and the electromotile displacement per unit transmembrane potential are presented. In this simulation, the stiffness of the elastic element representing the RL + TM complex was within a 0.05 N/m – 0.2 N/m range that corresponds to the measurements of several groups (Zwislocki & Cefaratti, 1989; Kolston, 1999; Scherer & Gummer, 2004). Since the stiffness of the BM and DC is much greater than the maximal value of this range, the displacement of the bottom end of the cell was assumed to be negligible. The results show that the cell produces an almost constant force up to 20 kHz, and the roll-off frequency depends on the constraints imposed on the cell. The stronger (stiffer) constraints result in a longer plateau and later roll-off in the active force as a function of frequency. This is consistent with the cell displacements in Fig. 5*b*: the stronger is the constraint, the smaller the corresponding cell displacement, and, as a result of this, the viscous losses in the fluids and the wall associated with cell vibration are smaller.

All components of the composite cell wall (plasma membrane, cytoskeleton, extracisternal fluid space, and subsurface cisternae) contribute to the overall viscosity of the outer hair cell. The viscous properties of the cell stem from relative (shear) motion within and between the cell's wall components. Several experimental analyses have been developed to estimate the viscous properties of the outer hair cell and its components (Ehrenstein & Iwasa, 1996; Li et al., 2002; Zelenskaya et al., 2005). However, the extraction of the effective viscosity of the cell wall is still an open question. Recently, Ermilov et al. (2005) developed a technique to optically perturb a bead adhered to the intact outer hair cell, which can be used to directly estimate the viscosity of the cell wall. In their simulation of the effect of this viscosity on the active force production, Liao et al. 2005a developed a theoretical analysis of viscous properties of the wall and considered a range of the wall viscosity close to that of the spectrin cytoskeleton in the red blood cell, whose properties are known (e.g., Evans & Skalak, 1980). Figure 6 shows the effect of the cell wall viscosity on active force production and demonstrates that the wall viscosity results in a flattening of the force-frequency function near the roll-off frequency as well as in a shorter plateau range for the active force.

Structural and Molecular Models: In-Plane and Bending Modes of Electromotility

In the continuum models thus far described, the voltage-induced strain in the membrane is considered as an in-plane strain. These approaches quite successfully describe and unify various experimental phenomena at the whole-cell macroscopic level. However, at the molecular level, other concerns must enter into the operation of a membrane-based motor. Most cells contain membranes that are highly folded and possess excess area. The plasma membrane is thin and fluctuations in membrane curvature are usually pronounced and can drive many biological processes (Bloom, Evans & Mouritsen, 1991; Jensen & Mouritsen, 2004; McMahon & Gallop, 2005). These considerations raise the question of whether the motor operates strictly "in-plane", or whether it also possesses "out-of-plane" (bending) modes. Interestingly, the OHC is under turgor pressure (described in detail below), and maintenance of turgor pressure is essential for electromotility. Thus, the operation of prestin appears to require a pre-stressed membrane in which curvature fluctuations are diminished.

Some attempts at modeling OHCs have involved details of the OHC structure (Tolomeo, Steele & Holley, 1996; Raphael et al., 2000; Spector, Ameen & Popel, 2001). The OHC is a cylinder with a radius of $\sim$ 4.5 μm and a length that ranges from 20–100 μm, depending on location in the cochlea. The lateral wall is $\sim$100 nm thick and is composed of three layers. The plasma membrane is the outermost layer. The membrane-bound subsurface cisterna (SSC) forms the innermost layer. Sandwiched between the plasma membrane and the SSC is a cytoskeletal matrix called the cortical lattice. This matrix of cytoskeletal proteins, located in the extracisternal space, contains spectrin and F-actin molecules arranged in an approximately perpendicular manner, giving the cytoskeleton a four-fold symmetry (Holley & Ashmore, 1990; Holley, Kalinec & Kacher, 1992; Holley, 1996). Filaments of F-actin are spaced $\sim$ 50 nm apart and cross-linked with molecules of spectrin. The cytoskeleton extends the length of the lateral wall and 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

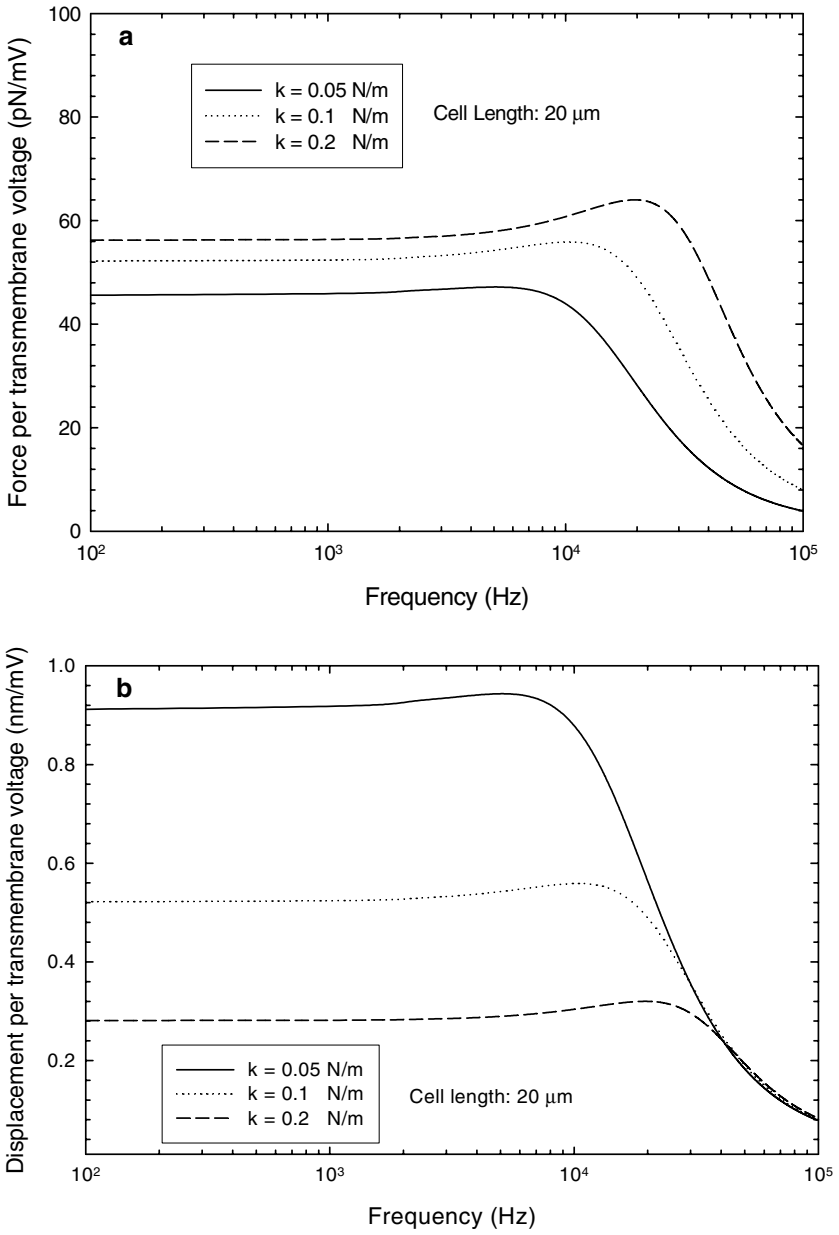

Fig. 5. Effect of the outer hair cell environment on cell's electromotile response. (*a*) Active force production per unit transmembrane potential as a function of frequency for different stiffness k of the constraint imposed on the cell, and (*b*) cell electromotile displacements under the same conditions as in (*a*).

(Holley et al., 1992). The mean orientation of the spectrin is longitudinal. Radially oriented pillars (of unknown molecular composition) tether the plasma membrane to the parallel bands of F-actin that lie adjacent to the SSC. The circumferential bands of F-actin in the CL provide the tensile force that maintains the OHC's cylindrical shape against its elevated cytoplasmic turgor pressure (Brownell, 1990). F-actin is absent in the OHC axial core of the cell (Raphael et al., 1994; Oghalai et al., 1998), which may permit hydraulic force transmission to the ends of the cell without dissipating energy to deforming a rigid cytoskeleton (Brownell, 1990). The mechanics of this trilaminar structure was studied in a number of papers (Spector, Brownell & Popel, 1998; Spector, 1999).

Based on this structure, Raphael et al. (2000) proposed that the membrane between the pillars was organized into "motile units." Application of an electric field was assumed to result in a change in the nanoscale curvature of each "motile unit" which summed to a macroscopic cell deformation. This model was based on the concept of flexoelectricity (Petrov, 2002; Petrov & Sachs, 2002), which is a special type of piezoelectricity well characterized in liquid crystals (de Gennes & Prost, 1993). In flexoelectric materials, there is a relationship between the internal polarization of a material and its curvature. Thus, application of an external electric field can alter internal polarization, leading to changes in curvature. Continuum equations for a flexoelectric membrane

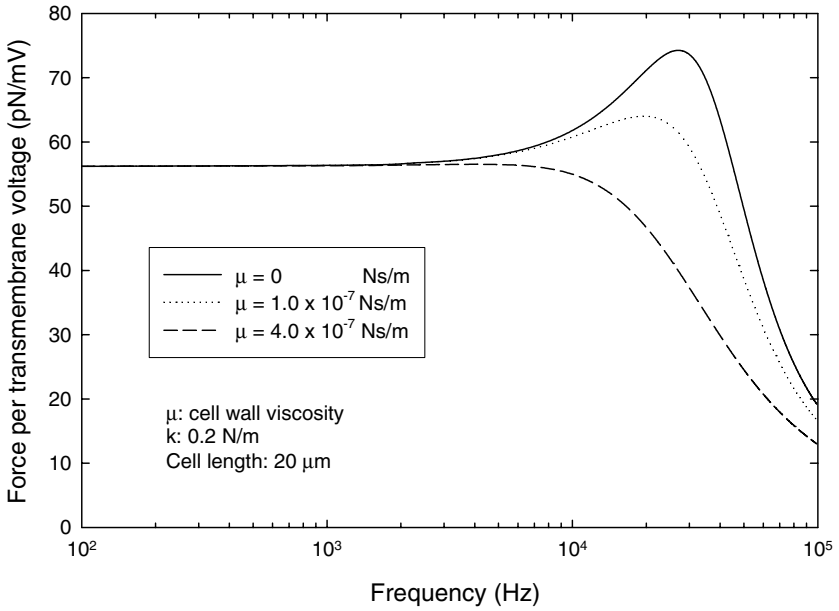

Fig. 6. Effect of the cell wall viscosity η on the active force production.

were derived which were found to explain the outer hair cell's voltage-displacement function (Raphael et al., 2000). As opposed to two-state models, this "membrane-bending" model envisioned a continuum of states and invoked the Langevin function to explain the nonlinear features associated with outer hair cell electromotility. However, the connection between the bending model and the Langevin function should not be viewed too restrictively. It is conceivable that a two-state model could be developed for a curved membrane, in analogy the two-state piezoelectric models (Iwasa, 2001). The difficulty of assessing the number of possible conformational states of prestin from available data has been recently described (Scherer & Gummer, 2005).

Although much theoretical work has been done on the contributions of lipids to membrane flexoelectricity, integral membrane proteins can not only contribute but also dominate the membrane's flexoelectric response (Petrov, Ramsey & Usherwood, 1989; Petrov et al., 1993). In fact, the original membrane bending model predicted the existence of proteins with a large effective dipole moment, and suggested possible scenarios in which conformational changes in integral membrane proteins could result in membrane bending, including voltage-induced protein aggregation (Raphael et al., 2000). Protein aggregation is becoming increasingly recognized as one of several mechanisms cells use to generate curvature changes, as described in a recent review that highlights the salient role that membrane curvature plays in diverse biological processes (McMahon & Gallop, 2005). Despite the ability of the original model to explain experimental data (Raphael et al., 2000), it is likely more accurate to consider biomembranes as analogous to polymer-dispersed liquid crystals that display a very intricate type of electromechanical coupling.

Clearly, further experiments are necessary to measure whether nanoscale changes in membrane curvature occur in response to electrical stimulation. This is a difficult task, but recent experimental and theoretical advances indicate it may be possible. For the past thirty years, experiments involving the formation of thin nanotubes off the surface of the membrane have been extensively utilized to study the mechanical properties of membranes (Hochmuth & Evans, 1982; Hochmuth et al., 1982; Raphael & Waugh, 1996). These nanotubes are called "tethers" and have been formed from outer hair cells (Li et al., 2002). Recent experiments have established that the tether force is voltage-dependent (Qian et al., 2004). Theoretical modeling has established that the tether force depends upon the tension in the membrane and the bending stiffness of the membrane. Hence, voltage-dependent changes in either parameter can affect the tether force. Recent theoretical models that consider the effects of electrical forces indicate that tether experiments may be used to differentiate between in-plane and bending responses of the membrane tether. These models have been developed in detail by Glassinger, Lee and Raphael, 2005 for lipid vesicles and indicate that tether forces are extremely sensitive to flexoelectric effects. Modeling the voltage-response in cells indicates that bending and in-plane effects cause opposite changes in the tether force (Glassinger & Raphael, 2005). The results of recent experiments on outer hair cells (Qian and Anvari, *unpublished observations*) can be fit with the predictions of the flexoelectric model. However, the relevance of these studies to the mechanism of OHC electromotility is debatable. The tether is a very highly deformed

structure, and so the electromechanical properties of this structure may not truly reflect those of the intact hair cell membrane. Further, it has not yet been established whether prestin enters the body of the tether, and piezoelectricity of the OHC membrane is mainly significant because of prestin. Hence, extrapolation of the electromechanical properties of OHC tethers to those in the intact cell should be interpreted with caution. Nevertheless, the strong voltage sensitivity of membrane tethers is an interesting membrane phenomenon that will be further explored in future years as the field of membrane electromechanics matures.

One of the key questions in our understanding the unique structure of the OHC composite wall is the role of its components in electromotility and active force production. To address this problem, Spector et al. 2001 have developed a 3-D computational (finite element) model where the main structural components, including the molecular motors interacting through the cell plasma membrane, were explicitly represented. In that study, the plasma membrane was assumed to be flat and purely elastic. The model was applied to the simulation of the main stages of electromotility, starting from the initiation of the active (motor-related) strain in the plasma membrane, followed by its transmission to the cytoskeleton, and resulting in the active force production by the lateral wall as a whole. The model has demonstrated how the in-plane motor-related active strain can result in the cell deformation and force observed in experiments. Also, the proposed computational approach can be further developed to include other modes of electromotile strain and force in the OHC composite wall.

The various modeling approaches have different levels of simplifications and have been designed to simulate specifically chosen features of OHC motility. They bring into prominence the various issues that are important to understand OHCs. Ideally a single model should be able to achieve this and progress made so far will help drive future work to understand the underlying physics behind OHC motility that is needed to build that complete model.

Simplified Models for Cochlear Predictions

One of the common aims of the differing approaches is to derive a model that has enough fidelity to be used in a predictive cochlear model. Models that are derived from an energy basis have the capability to yield simpler expressions which will be valid for a smaller range of voltage changes and strains similar to what OHCs experience in vivo. For instance, a starting point for constructing a simpler model could be the Gibbs free energy function,

$$G_{fe} = G_{fe}(T_z, T_c, \Psi) \tag{4}$$

where T_z is the axial tension in the cell membrane, T_c is the circumferential tension in the cell membrane and Ψ is the transmembrane voltage. Here, bending stresses are assumed to be negligible and due to the high cylindrical radius to shell thickness ratio for the OHC ($\sim$ 200:1, essentially like a thin shell) all radial stresses are also ignored. Second-order expressions for strains and charge can then be derived from the energy expression by taking derivatives of the expression with respect to the corresponding stresses and voltage, respectively. The reason for including first-order nonlinearities is that the linearized expressions have a very limited region of validity — too narrow a range of voltage and strain to be accurate enough for modeling OHC effects in cochlear models. Using this approach a simplified version of the modified two-state model was obtained (Deo & Grosh, 2005). Starting with an energy expression containing axial strain and voltage as the state variables, the following equations were obtained for the charge q and the axial tension t,

$$q = Cv + e\varepsilon + \frac{1}{2}C_2v^2 + C_1v\varepsilon - \frac{1}{2}k_1\varepsilon^2, \tag{5}$$

$$t = k\varepsilon - ev + \frac{1}{2}k_2\varepsilon^2 + k_1\varepsilon v - \frac{1}{2}C_1v^2. \tag{6}$$

Here ε and v represent the fluctuations in axial strain and transmembrane voltage, respectively, about the equilibrium (resting) values. The simpler model described in Eqs. 5 and 6 requires parameters C, k, e, C_1,C_2, k_1, and k_2, which can be obtained from the two-state model, or from experiments, or, for that matter, from any other model (as described in Deo and Grosh, 2005). This model represents the first-order nonlinear expansion of the expressions given in Eqs. 1–3. Since the model includes only first-order nonlinearities, the stiffness and capacitance are linearly dependent on voltage and strain. For small changes in the voltage (up to $\pm$15mV) seen in vivo, the simpler model follows fully nonlinear models and experimental data for capacitance and stiffness quite well. Comparison of the simple model with the modified two-state model for such a voltage range showed a maximum relative error of 1.5% in stiffness and up to 3% error in capturing capacitance at the extreme voltages (Deo & Grosh, 2005). A more important question is whether we miss any important harmonics by limiting ourselves to second-order models. Figure 7 addresses that question, where the same simulation as in Fig. 5 was carried out, but using the simpler model instead. The result shows that the simple model is capable of generating all measurable harmonics.

As mentioned earlier, the simple model is only valid for a limited range of voltages and strains, and

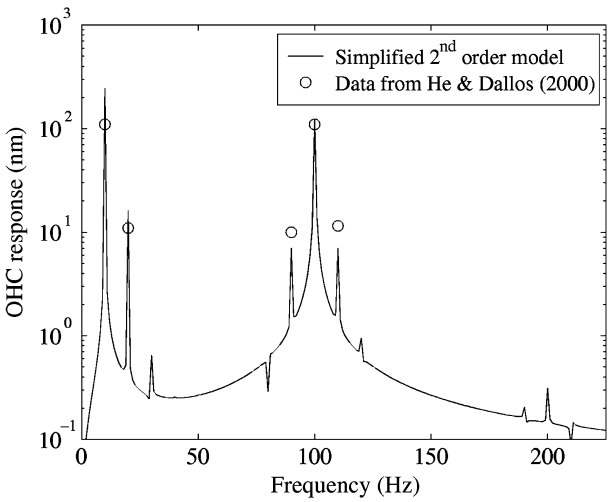

Fig. 7. Frequency spectrum of OHC response: simultaneous excitation by mechanical and electrical input. Voltage stimulus is at 10 Hz while mechanical probe signal is at 100 Hz. Circles represent data from Fig. 3*F* in He and Dallos (2000). Note the match in response at sum and difference frequencies (90 Hz and 110 Hz). Parameters for the simple model are given can be found in Deo and Grosh (2005).

complete models for OHC behavior are still necessary for us to understand the workings of the OHC. One of the uses of OHC models has been to investigate the RC cut-off problem. The next section elaborates on the progress made in solving the RC cut-off paradox through OHC models.

Modulation of the Cell Membrane (Receptor) Potential

In the previously discussed experimental and theoretical results on outer hair cell high-frequency electromotility and active force generation, the transmembrane (command) voltage was prescribed. Thus, these results essentially describe the active force and cell displacement per unit transmembrane potential. Assuming that the changes in the transmembrane potential are relatively small (this is the case for the receptor potential under physiological conditions), the total active force can be presented as the product of the force per unit transmembrane potential and actual transmembrane potential. Analysis of the receptor potential under high-frequency conditions has drawn significant attention: earlier estimates showed that the receptor potential generated via the stereocilia-related transduction current can be severely attenuated by the cell membrane capacitance (e.g., Housley & Ashmore, 1992). To resolve this problem, Dallos & Evans, 1995, suggested that the transmembrane potential is governed by an external electric field rather than by the transduction current. Recently, efforts have been made to better understand the role of the outer hair cell's membrane properties in the frequency modulation of the transmembrane potential. It was shown that several mechanisms involving the membrane electromechanical properties can balance the capacitive filtering of the membrane potential. Santos-Sacchi et al. (1998) found that the nonlinear capacitance and transferred charge are greater in short (high-frequency) cells that can compensate smaller receptor potentials. By using a biophysical model, Ospeck, Dong and Iwasa (2003) showed that fast, multiple-state voltage-gated channels can increase high-frequency membrane potentials in the outer hair cell. Spector et al. (2003) have modified the circuit that determines the outer hair cell receptor potential by taking into account the piezoelectric properties of the membrane. In contrast to predictions by the purely electrical (RC) analysis, this work showed that the resulting receptor potential stays above a certain level of several tenths of a mV throughout the whole frequency range. Weitzel et al. (2003) used a model of the cell that included the piezoelectric and inertial properties, and they predicted resonances in the cell admittance as well as an increase in its roll-off frequency. Early reports of the existence of mechanosensitive channels in the outer hair cell membrane (Ding Salvi & Sachs, 1991; Iwasa et al., 1991) have been recently confirmed (Rybalchenko & Santos-Sacchi, 2003). Rybalchenko and Santos-Sacchi (2003) proposed that such channels associated with the motor protein could provide the charge necessary for the motor activity that is free from the filtering by membrane capacitance. Spector et al. (2005) analyzed the governing equation for the receptor potential, including the effects of piezoelectricity and mechanosensitive channels. The authors have found that if the channels are sensitive to the strain rate in the cell wall, then such properties can result in several-fold gain for the receptor potential in a 3 kHz–15 kHz frequency range. The strain rate sensitivity of the channels in the cell membrane is consistent with the transmission of the gating force via a viscoelastic-type mechanism involving the viscosity of the cytoskeleton and plasma membrane.

Frequency and Constraint Effects on Nonlinear Capacitance

Nonlinear capacitance, one of the major features of outer hair cell electromotility, is also sensitive to the frequency of cell vibration and constraints imposed on the cell. Gale and Ashmore (1997) have analyzed nonlinear capacitance measured for different frequencies via a patch pipette. The authors estimated the roll-off frequency for nonlinear capacitance as being close to 10 kHz, beyond which nonlinear capacitance decreases. This result is consistent with

the later discovered mechanism of extrinsic charges interacting with the motor protein (Oliver et al., 2001). For high frequencies, the mechanism of charge transfer is probably not fast enough. Thus, smaller transferred charges resulted in the observed decrease in nonlinear capacitance.

Adachi and Iwasa (1999) have proposed a set-up to study the effect of constraints on nonlinear capacitance: the authors trypsinized the cell and imposed constraints by generating turgor pressure in the cell that became spherical. The increase in turgor pressure constrained the motors and resulted in a reduction of nonlinear capacitance. Recently, Spector (2005) developed a mathematical model to analyze the effect of constraints on nonlinear capacitance. Three particular conditions were considered: completely unconstrained cell (zero-resultant conditions), fully constrained cell (zero-strain conditions), and partially constrained cell subjected to electrical stimulation under constant-volume conditions. The first of these conditions is approximately satisfied if a half-included cell electrically stimulated in the microchamber. The second condition is close to that of a cell constrained by a very rigid fiber, and the third condition corresponds to the voltage-clamp experiment. Spector (2005) introduced capacitance of the membrane corresponding to these three conditions and showed that zero-strain capacitance is smaller than zero-resultant capacitance, and the effective (corresponding to electrically stimulated, constant-volume cells) capacitance is between the two. In addition to that, it was shown that the difference between zero-strain and zero-resultant capacitive characteristics is proportional to the useful mechanical work produced by the motor in response to electrical stimulation. The effectiveness of the electromechanical transduction in the cell is defined as the ratio of the useful mechanical work to the energy of the externally applied electric field. As a result, the effectiveness can be expressed in terms of the three capacitive characteristics of the cell. Assuming a uniform distribution of the motors along the cell, the discussed effectiveness can be attributed to an individual motor. Figure 8 shows the effectiveness of the cell and its motor as a function of the membrane potential for different parameters of the cell, and Fig. 9 presents the differences between the capacitive characteristics. The obtained range of the estimated effectiveness, 12%–30%, is similar to that in other motors (myosin, kinesin). As was discussed earlier, the amount of the transferred charge interacting with the motor protein decreases for the frequencies beyond a roll-off frequency of about 10 kHz (in guinea-pig). To reflect this phenomenon, Spector (2005) has proposed a frequency factor modulating the effectiveness of the cell and its molecular motor.

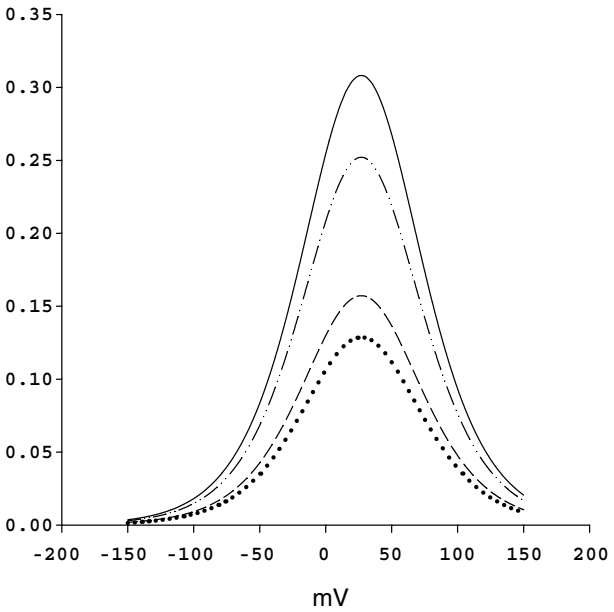

Fig. 8. Effectiveness of the electromechanical transduction by the cell and its molecular motor for different combinations of cellular parameters (cell stiffness γ_a and active strain production per unit transmembrane potential (α). The solid, dashed-dotted, dashed, and dotted lines correspond, respectively, to the following combination of the parameters: ($\gamma_a = 1.2 \times 10^{-2}$ N/m and $\alpha = 1.4$ V^{-1}), ($\gamma_a = 0.6 \times 10^{-2}$N/m and $\alpha = 1.4$ V^{-1}), ($\gamma_a = 0.6 \times 10^{-2}$ N/m and $\alpha = 1$ V^{-1}), and ($\gamma_a = 0.6 \times 10^{-2}$ N/m and $\alpha = 1$ V^{-1}).

Unresolved Questions

There is no single model in the literature that explains all experimentally observed features of outer hair cell electromotility. New molecular information on prestin and its potential interaction partners will need to be interpreted in terms of mesoscale and ultimately molecular models. Unresolved issues relating to boundary conditions in vivo and the effect of amphiphilic compounds and temperature on electromotility need further research. Finally, the origin and significance of the cell's turgor pressure has to be further explored. These important issues are discussed below.

Is Electromotility Both Necessary and Sufficient for Cochlear Amplification?

At the level of the whole hearing organ, we need to understand whether electromotility is sufficient, or whether additional mechanisms contribute to cochlea amplification. Many lower organisms (e.g., reptiles) that do not possess outer hair cells exhibit a phenomenon known as otoacoustic emissions (OAEs) which likely arise from hair bundle motility (Manley et al., 2001; Le Goff et al., 2005). In mammals, OAEs, which are low-intensity sounds that can be recorded in the ear canal, are an indication of a properly

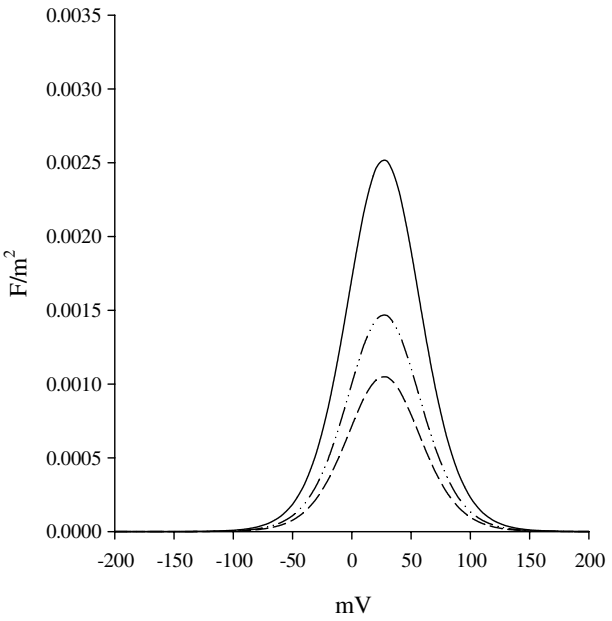

Fig. 9. The differences between three capacitive characteristics as functions of the transmembrane potential. Solid, dashed-dotted, and dashed lines represent, respectively, the differences between zero-resultant and zero-strain, zero-resultant and effective, and effective and zero-strain capacitances.

functioning active mechanism in the cochlea. The question then arises over the relative contribution of hair bundle and electromotility to OAEs and to cochlear amplification in general. Are the two mechanisms active at different frequencies? Or does hair bundle motility work in concert with electromotility? Theoretical models that incorporate both these phenomena will be needed to resolve these important questions related to the origin of various cochlear nonlinearities.

How Does the Outer Hair Cell Produce the Active Force in the Cochlear Environment?

While considerable effort has been expended to model the performance of an isolated OHC, the cell's active force production in the cochlea is still an unresolved question. The cochlear environment affects the cell via its interaction with finite volumes of the viscous fluid and 3-D viscoelastic structures. Getting information on outer hair cells in vivo is very difficult, which means we still have to rely on in vitro data. As discussed above, simpler outer hair cell models can be constructed and used in predictive cochlear models. These models, however, still are of limited use since they also need information on the resting state of the hair cells in vivo. Resolution of this issue is vital for understanding and modeling the contribution of outer hair cells to the functioning of the cochlea.

What is the Mechanism of Action of Prestin?

At the molecular level, we need to understand how the conformational changes in prestin endow the membrane with a motile function. The native structure of prestin is currently unknown, and there is even a debate on whether prestin is a ten-pass or twelve-pass transmembrane protein (Deak et al., 2005; Navaratnam et al., 2005). Putative conformational changes have not yet been explored, but it has recently been reported that deletions of the C terminal regions affect the nonlinear capacitance (Zheng et al., 2005). The nonlinear capacitance is also sensitive to whether or not prestin is phosphorylated (Deak et al., 2005). Fluorescence resonance energy transfer experiments indicate the presence of prestin-prestin interactions (Greeson et al., 2006) that require an intact N-terminus and have functional significance (Navaratnam et al., 2005). Accounting for these and other discoveries will require the development of more sophisticated molecular models that take into account prestin-membrane interactions and prestin-prestin interactions. To correctly model force transmission in the lateral wall, we will need to know whether prestin links to the cytoskeleton or other proteins.

How Do Amphiphilic Agents Affect Electromotility?

Another aspect which has not been addressed by modelers much is the affect of pharmacological compounds on OHC behavior. Many reagents have been shown to influence capacitance curves significantly (Santos-Sacchi, 1991; Santos-Sacchi & Wu, 2004) and while these results may not be important for in vivo mechanics of the cochlea, they do offer an avenue to better understand OHCs function. Similarly, temperature changes have been shown to influence OHC capacitance (Santos-Sacchi & Huang, 1998) and motility (Ashmore & Holley, 1988) but we don't have a good understanding of why this occurs.

One of the key reagents which affects OHCs and does have physiological significance is salicylate, which is an active metabolite of aspirin. It has been long known that salicylate decreases OHC motility and capacitance (Shehata et al., 1991; Kakehata & Santos-Sacchi, 1996) and this is touted as an explanation for salicylate-induced hearing loss. The mechanism of action on OHCs is unclear, but important new research has shed light on the effect of salicylate on membranes. Zhou and Raphael (2005) showed salicylate causes a dramatic decrease in the bending stiffness of phospholipid membranes in the same concentration range in which it affects OHCs. Oliver et al. (2001) obtained electrophysiological evidence that salicylate competes with chloride and inhibits the nonlinear capacitance. Oliver et al.

interpret this competition as being for a binding site on prestin, but recent molecular dynamics simulations have shown that salicylate can displace chloride from the membrane interface (Song et al., 2005b). If charged salicylate molecules absorb to the membrane, alterations in surface potential would be expected, as originally argued by McLaughlin (1973) and supported by recent experiments in HEK cells (Farrell et al., 2006). However, all these effects may be interrelated, as the function of membrane proteins is known to depend on the physical state of the bilayer.

Another agent known to alter the OHC mechanics and electromotility is an antipsychotic drug, chlorpromazine (CPZ). It has been previously found in other cells that CPZ affects curvature of the cell membrane. Lue, Zhao and Brownell (2001) found a shift in the electromotility curves resulting from the application of CPZ. Recently, Murdock et al. (2005) have observed changes in the membrane viscoelastic properties after the application of CPZ. Understanding how reagents affect the mechanical properties of outer hair cells has in vivo significance, as modulation of the cell's stiffness by calcium ions is postulated to be a mechanism for dynamic adaptation of electromotility (Batta et al., 2003).

What is the Role of Turgor Pressure and Hydraulic Conductivity?

Finally, it is well known that turgor, and in general stress in the membrane, affect OHC behavior. The level of OHC sensitivity to stress and the importance of this effect in vivo has to be better understood. The current two-state model still is not capable of addressing the turgor pressure issue. A mathematical analysis of the model suggests that the ability to model turgor pressure changes requires that the charge movement depend on the membrane stress (Deo & Grosh, 2004). An attempt at verifying whether this happens experimentally in the OHC has not been made. More generally, the question of how the OHC's turgor pressure is established and maintained is unresolved.

Like bacterial or plant membranes, the OHC's maintenance of high turgor pressure of 1–2 kPa (Ratnanather, Brownell & Popel, 1993; Chertoff & Brownell, 1994) may be associated with a low water permeability of $P_f = 3 \times 10^{-4}$ cm/s (Ratnanather et al., 1996) through its plasma membrane. This value is on the low side of values reported for different lipid bilayers and is 2 orders of magnitude lower than that for red blood cells or membranes possessing water-mediated channels such as aquaporins (Verkman & Mitra, 2000). When salicylate is added, a value of $P_f = 3.5 \times 10^{-4}$ cm/s is obtained experimentally (Zhi et al., 2006).

As mentioned earlier, the trilaminate structure of the lateral wall of the OHC has been extensively examined (*see* references in Holley, 1996). The extracisternal space (ECiS) is the narrow space between the plasma membrane and the SSC. Although the SSC appears highly fenestrated in some electron microscopy preparations (Furness & Hackney, 1990), rapid freezing followed by freeze substitution appears to reveal an unfenestrated structure (Slepecky & Ligotti, 1992). Also in salicylate-treated OHCs, the SSC has been observed to dilate and vesiculate, i.e., become more fenestrated (Dieler et al., 1991). The unchanged water permeability of salicylate-treated OHCs suggests that water flows mainly through the ECiS with little or negligible flow across the SSC, as predicted by a model of the hydrodynamics of the ECiS (Ratnanather, Popel & Brownell, 2000). Salicylate has recently been shown to stabilize pores in pure phosphatidylcholine membranes (Zhou & Raphael, 2005), and thus the failure of salicylate to change the water permeability of the OHC may indicate an unusual lipid composition of OHC membranes.

Water permeability can be increased by a couple of orders of magnitude when the osmotic challenge is applied via a fluid-jet (Brownell et al., 1994; Belyantseva et al., 2000; Morimoto et al., 2002). This may be attributed to the presence of mechano-sensitive channels (Ding et al., 1991; Iwasa et al., 1991; Rybalchenko & Santos-Sacchi, 2003) or an increase in spontaneously formed meta-stable water defects (Marrink et al., 2001) or pore-like structures observed in the plasma membrane (Le Grimellec et al., 2002). Belyantseva et al., 2000 obtained $P_f \approx 9.7\text{–}11.1 \times 10^{-2}$ cm/s and argued that the lower value may be attributed to a combination of regulatory volume decrease mechanism due to internal osmolytes and duration of the osmotic challenge but it was shown that P_f is independent of the generation of internal osmolytes (Ratnanather et al., 1996).

However, a low value of $P_f = 2 \times 10^{-4}$ cm/s has been obtained from prestin-transfected HEK-293 cells (Chambard & Ashmore, 2003) when the osmotic challenge was applied relatively far from the cell to make the fluid environment near the cell wall akin to that of a constant pressure flow chamber (Chertoff & Brownell, 1994, and Ratnanather et al., 1996), in contrast with rapidly varying pressure and stresses on the OHC wall within a highly localized region as in the fluid jet; *see* Zhi et al. (2006) for discussion. It should be noted, however, that the density of prestin in OHC is much higher than in transfected cells, so it can be argued that water permeability is not associated with prestin.

It is likely that strain-dependent water permeability affects the electromotile properties of the OHC. Belyansteva et al. (2000) noted an increase in the order of magnitude of water permeability during postnatal development of rat OHCs that is consistent with the expression of electromotility over the same period for rat OHCs (Oliver & Fakler, 1999; Belyantseva et al.,

2000). Also, reduced osmotic flow concomitant with depolarization was observed and attributed to the presence of aquaporin-like proteins that co-localize or interact with prestin. Since Chambard and Ashmore (2003) suggested that water may co-transport with fructose, it is possible that the co-transporter protein may coincide with the aforementioned mechanosensitive channels, meta-stable defects, or pores.

The presence of pores is underscored further by the work of Morimoto et al. (2002) in which strain-dependent water permeability was reduced by the presence of amphipathic drugs such as salicylate and chlorpromazine. This may be attributed to the ability of the amphipathic drugs to change membrane tension and thus antagonize the energy for pore formation. Additional evidence for pore formation comes from the fact that membrane stretching leads to the formation of pores that admit mono- and disaccharides, but not raffinose, indicating a maximal pore diameter of 4 nm (Brownell et al., 1994). A possible explanation is that when pores open, calcium ions enter the OHC and interact with the circumferentially-oriented actin and roughly longitudinally oriented spectrin, and thus affect structural changes in the cortical lattice which are in turn transmitted to the plasma membrane. The actual contribution of passive and strain-dependent water permeability to OHC mechanics needs to be explored further via computational and biophysical modeling as well as thorough experimentation.

Conclusions

Outer hair cell electromotility was first reported in the mid-1980s (Brownell et al., 1985). Experiments in the following decade further characterized and established the voltage-dependent strains and capacitance, and led to the first model of electromotility, the two-state area motor models (Dallos et al., 1993; Santos-Sacchi, 1993; Iwasa, 1994). The next ten years saw the development of orthotropic models (Tolomeo & Steele, 1995; Adachi & Iwasa, 1997; Spector, 1999), the characterization of voltage-dependent stiffness of the cell (He & Dallos, 1999, 2000), the discovery of prestin (Zheng et al., 2000), and membrane-based models (Raphael et al., 2000). Most recently modeling efforts have suggested plausible mechanisms that enable the cell to operate at high frequencies (Spector et al., 2005). The debate among the proponents of various models has at times been lively, but this is in keeping accord with Mouritsen's remarks: "Models must constantly be scrutinized and questioned, even the most successful ones, not least because models reflect fashion among scientists" (Mouritsen, 2005).

What will the third decade of research on OHC electromotility bring? It is never safe to predict the future, but we can confidently expect that just as the development of models over the past decade has raised new questions and dramatically increased our understanding of how the OHC functions, further developments in theoretical modeling are expected to drive major advances and reveal more of the subtleties of Nature's most unique membrane-embedded motor molecule.

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