J. Membrane Biol. 209, 1–16 (2006)
DOI: 10.1007/s00232-005-0833-9

The Journal of
Membrane
Biology

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Tuning in to the Amazing Outer Hair Cell: Membrane Wizardry with a Twist and Shout

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Received: 15 December 2005

From Basilar to Plasma Membrane

Over 40 years ago the auditory community rejoiced that frequency tuning in the auditory periphery had been understood, and was proud that von Bekesy received the Nobel Prize for showing us how the basilar membrane (BM) traveling wave provides for mechanical Fourier analysis (von Bekesy, 1960). However, as soon as new techniques were devised to measure smaller movements of the BM than Bekesy could imagine, curious incongruities arose concerning the significance of Bekesy's measurements. Von Bekesy made much of his measures on preparations that were compromised in a way unknown to him. Thus, the linear extrapolations from his high-intensity measures, which put auditory threshold movements below the diameter of an atom, were becoming unraveled. The problem turns out to be an inherent nonlinearity in the motion of the basilar membrane that is lost upon the slightest insult to the organ of Corti, that sensory epithelium of hair cells which is stimulated by BM movements. This remarkable observation of a compressive nonlinearity was made by Rhode (Rhode, 1971; Rhode & Robles, 1974), and has revolutionized our thinking on the micro mechanics of the inner ear. We now know that BM vibration is as sharply tuned to frequency as the eighth nerve fiber activity it evokes (Narayan et al., 1998). But what drives this nonlinearity? Why is it so vulnerable to insult? The answer is that it depends on the proper functioning of a particularly fragile cell type within the organ, the outer hair cell (OHC) (Dallos & Harris, 1978; Ryan & Dallo, 1975).

The OHC is one of the two types of hair cells present in the organ of Corti. The other hair cell type is the inner hair cell (IHC) which receives up to 95% of the auditory nerve's afferent innervation (Spoendlin, 1988), but is fewer in number than OHCs by a factor of 3–4. Two decades ago, Brownell (Brownell, 1984; Brownell et al., 1985; Kachar et al., 1986) discovered that OHCs are capable of electrically evoked mechanical activity. Again, a revolution in our thinking began. The bottom line, after years of study, is that though both inner and outer hair cells respond to sound by generating receptor potentials (Russell & Sellick, 1978; Dallos, Santos-Sacchi & Flock, 1982), the OHCs alone are both receptor and effector – incredibly fast, at that. (You can view a movie of the OHC dance at www.YaleEarLab.org). Currently, the consensus is that OHCs provide a frequency-dependent boost to BM motion, which enhances the mechanical input to IHCs, thereby promoting enhanced tuning and amplification. Indeed, the term "cochlear amplifier" is commonly used to describe the work of the OHC (Davis, 1983). This review will summarize much of what we know about the OHC's electro-mechanical capabilities — phenomena which largely result from properties intrinsic to its plasma membrane (PM) and associated structures.

OHC Somatic Electromotility and Its Electrical Signature

The OHC is a cylindrically shaped cell whose length varies from short (~10–20 μm) to long (> 80 μm)

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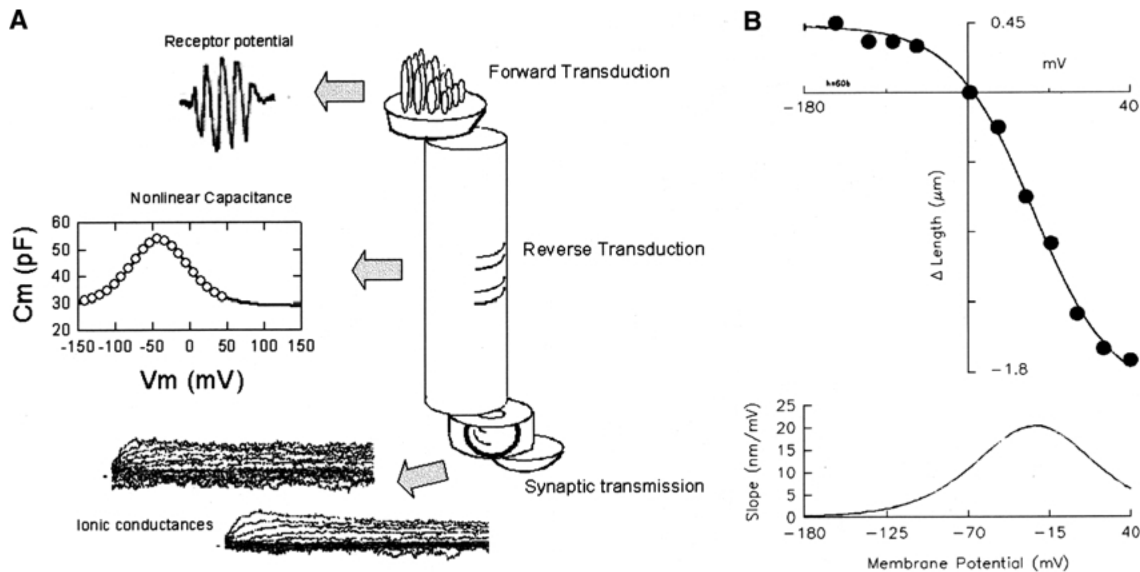

Fig. 1. (*A*) The OHC has a highly partitioned membrane. Apical, lateral and basal membranes possess characteristic integral membrane constituents with measurable electrical correlates. The apical stereocilia channels work to produce receptor potentials; the basal membrane houses the voltage-dependent ion channels; the lateral membrane houses the molecular motors (prestin), whose electrical signature is a nonlinear, voltage-dependent capacitance (NLC). Fits of the capacitance with the first derivative of a two-state Boltzmann provides information on the motors' operating range (characterized by $V_{1/2}$, the voltage at peak capacitance) and the sensitivity of the motors to voltage (an estimate of elementary motor charge, typically about 0.75). (*B*) The mechanical response (*filled circles*) of the OHC can also be fit by a two-state Boltzmann function. The first derivative defines the mechanical gain of the cell at a particular resting potential.

along the length of the basilar membrane, with short cells residing at the basal high-frequency regions of the cochlear spiral. While initial investigations did not identify OHC electrically evoked length changes as resulting from voltage or current flow across the PM, it soon became clear that this robust mechanical response, amounting to about 5% of the cell length, was evoked by voltage. This was indicated by the persistence of mechanical responses in spite of the block of a variety of membrane conductances, and the identification of gating-like currents or a nonlinear capacitance (NLC), which is characteristic of voltage-sensor activity that underlies membrane-based voltage-dependent processes. Consequently, OHCs contract with depolarization and elongate with hyperpolarization (Santos-Sacchi and Dilger 1988; Santos-Sacchi, 1990, 1991; Ashmore, 1989, 1990) Significantly, in isolated OHCs contraction/expansion cycles can be elicited by ciliary displacement (Evans & Dallos, 1993). OHC electromotility is independent of ATP, calcium, and the microtubule or actin system (Brownell et al., 1985; Kachar et al., 1986; Ashmore, 1987; Holley & Ashmore 1988b; Santos-Sacchi 1989; Kalinec et al., 1992). Motile responses can be seen at very high frequencies (Dallos and Evans 1995; Gale & Ashmore 1997; Frank, Hemmert & Gummer 1999). The mechanical response is sigmoidal and corresponds inextricably with those characteristics of the nonlinear charge movement (Fig. 1). Both the mechanical response and its associated charge movement can be fit well with simple two-state Boltzmann functions and each share indistinguishable fitted parameters; namely, slope factors are near 30 mV per e-fold changes in charge or mechanical response, and operating voltage ranges have their midpoints ($V_{1/2}$) near negative 30–40 mV. Maximum charge movement in OHCs ranges up to about 3 pC, and early on it was observed that treatments that reduce this charge transfer or shift its operating voltage range correspondingly affect the mechanical response amplitude and its voltage activation range (Santos-Sacchi, 1991; Wu & Santos-Sacchi, 1998; Kakehata and Santos-Sacchi, 1995, 1996). Most investigators study charge movement in OHCs by measuring the NLC, which is bell-shaped (the first derivative of charge movement) with respect to voltage. Peak NLC can be greater than the cell's linear capacitance, the latter corresponding to the cell's surface area.

There are several perturbations that can influence the OHC motor mechanism, including membrane tension, temperature, and initial (resting) voltage conditions (Iwasa, 1993; Gale & Ashmore, 1994; Takahashi and Santos-Sacchk, 2001; Zhao and Santos-Sacchi 1998; Kakehata & Santos-Sacchi, 1995). In addition, lanthanides and salicylate can block to some extent NLC and somatic electromotility (Tunstall, Gale & Ashmore 1995; Kakehata & Santos-Sacchi 1996; Santos-Sacchi, 1991). The most

common effect on motor activity is a shift of the operating voltage range, indicative of a shift in the state probability of motor molecules residing in a contracted or expanded state. Additionally, maximum charge movement is also susceptible to external perturbations (*see* Santos-Sacchi, 2003, for a more detailed review). It was the electro-mechanical characteristics of the OHC motor that ultimately enabled the identification of the protein responsible for the unique function of the OHC.

The OHC Motor Protein Prestin

Although the existence of a unique motor protein embedded in the OHC's PM was proposed as early as 1992 (Kalinec et al, 1992), it was not until five years ago that the molecular nature of the OHC motor was revealed (Zheng et al., 2000). The basic strategy of this identification was based on the fact that inner hair cells IHCs do not show somatic electromotility but share many similarities with OHCs. An OHC-IHC subtracted cDNA library was created aiming to isolate the OHC-specific genes that are abundantly expressed only in OHCs and not in IHCs (Zheng et al., 2002a). The OHC motor protein was identified in this library and named prestin. Prestin is localized to the PM where the motor was known to be located (Hallworth, Evans & Dallos, 1993; Huang & Santos-Sacchi, 1993, 1994). When prestin was heterologously expressed in several cell lines, the transfected cells exhibited behaviors that are normally observed only in OHCs: voltage-dependent NLC and shape changes (Zheng et al., 2000); electro-mechanical reciprocality (Santos-Sacchi et al., 2001; Dong & Iwasa, 2004); temperature sensitivity (Meltzer & Santos-Sacchi, 2001), and mechanical force generation with constant amplitude and phase up to a stimulating frequency of at least 20 kHz (Ludwig et al., 2001). Ontogenic expressions of the *prestin* gene (Zheng et al., 2000) and prestin protein (Belyantseva et al., 2000a) are the same as that of electromotility (He, Evans & Dallos, 1994). In addition, the electromotile responses in prestin-transfected kidney cells can be blocked by salicylate (Zheng et al., Oliver et al., 2001), an inhibitor of somatic electromotility in OHCs (Shehata, Brownell & Dieler, 1991; Tunstall et al., 1995; Kakehata & Santos-Sacchi, 1996). Furthermore, OHCs from prestin-null mice lack somatic electromotility, and those mice also lose 40–60 dB of hearing sensitivity (Liberman et al., 2002) and lack frequency selectivity (Cheatham et al., 2004). A splicing mutation in *prestin* gene causes non-syndromic deafness (Liu et al., 2003). Collectively, these results are consistent with the idea that prestin is the motor protein of cochlear OHCs.

Prestin's Gene and Its Expression

Prestin belongs to a distinct anion transporter family called solute carrier protein 26 (SLC26) (Zheng et al., 2002b). At present, eleven members of this family have been identified (Mount & Romero 2004). Several proteins in this family, including prestin, are involved in human diseases (for review, *see* Dawson and Markovich, 2005). The human Prestin gene contains 21 exons crossing more than 90 kb in chromosome 7q22 (Liu et al., 2003), a location near to those of other important SLC26 members, such as PDS (SLC26A4) (Everett et al., 1997) and DRA (SLC26A3) (Haila et al., 1998). The mouse Prestin gene has 20 exons spanning nearly 55 kb in chromosome 5 (Zheng et al., 2003). Eighteen of these exons code for prestin amino acids in both hPrestin and mPrestin. Among coding exons, 12 exons are exactly the same sizes as in other members of the SLC26 family, such as Pendrin, DRA and CFEX (Zheng et al., 2003), indicating high homologies among these genes. Several prestin isoforms have also been found in different stages of human cochlear development (Liu et al., 2003). However, these isoforms were not found in mature mouse cochleae (Zheng and colleagues, *unpublished data*). The function of these predicted human prestin isoforms was not further studied. Prestin mRNA was also found in testis, brain and vestibular system through either RT-PCR or in situ experiments (Adler et al., 2003; Zheng et al., 2003). Anti-prestin labeling was found in the cytoplasm of vestibular hair cells, but not in the PM (Adler et al., 2003). The behavior endowed by prestin, somatic motile activity, was never found in vestibular hair cells (Adler et al., 2003). Overall, prestin is abundantly expressed only in OHCs, where somatic motile activity is observed. The time course of prestin mRNA and protein synthesis in OHCs corresponds to the emergence of somatic motile activity in OHCs (Belyantseva et al., 2000a; Zheng et al., 2000). Furthermore, a prominent longitudinal and radial gradient of prestin expression, ranging from base-to-apex and first-to-third row of OHCs, was also observed (Judice et al., 2002). Such tight regulation of prestin mRNA expression is not fully understood. Thyroid hormone was reported to regulate prestin mRNA expression (Weber et al., 2002). In spite of the fact that prestin's promoter and other regulating regions have not been precisely identified (Weber et al., 2002; Zheng, Richter & Cheatham 2003), the prestin gene has been modified to create transgenic mice that are useful for studying hair-cell-specific gene targeting (Tian et al., 2004).

Prestin is a highly conserved protein among different mammalian species. So far, the full-length prestin protein has been identified from four species including gerbil (Zheng et al., 2000), mouse (Adler et al., 2003; Zheng et al., 2003), rat (Ludwig et al.,

2001) and human (Liu et al., 2003). Among these four, 92.7 % of amino acids are identical and 6% show some similarity. Only 10 amino acids (1.3%) were different as determined by the CLUSTALW multiple alignment analysis (Thompson, Higgins & Gibson 1994). Prestin-related SLC26 proteins were also reported in non-mammalian vertebrates and insects (Weber et al., 2002). The closest one (AY278118) related to mammalian prestin was found in the Zebrafish's auditory organ. This prestin-related gene has ~ 15 exons with 49.8% amino acids identical to mammalian prestin. The function of Zebrafish prestin is not fully investigated. The key question, whether zPrestin demonstrates somatic motile activity, has not been answered yet. Therefore, whether non-mammalian prestin has a similar function and role in non-mammalian hearing needs further investigation.

Prestin Protein's Structure and Its Modification

Prestin appears to share the overall structure and specific protein domains of the SLC26 family: a highly conserved central core of hydrophobic amino acids (~ 400 amino acids) predicted to form 10–12 transmembrane domains according to the topology prediction programs chosen for the analysis (Oliver et al., 2001; Zheng et al., 2001; Deak et al., 2005; Navaratnam et al., 2005b). It is generally agreed that Prestin can be divided into three domains: N-terminus (~100 a.a,), hydrophobic core (~400 a.a.) interacting with lipids of the PM, and C-terminus domain (~240 a.a.) as shown in Fig. 2. Through immunofluorescence experiments, the N- and C-termini were shown to be located on the cytoplasmic side of the PM (Ludwig et al., 2001; Zheng et al., 2001). The "sulfate transporter signature" is present in the hydrophobic core, while a STAS (sulfate transporter and antisigma-factor antagonist) motif (Aravind & Koonin, 2000) is located in the C-terminus (a.a. 634–710). There are two predicted N-glycosylation sites in the hydrophobic core region: N163 and N166. While two sets of experiments suggest that these two sites are glycosylated, indicating that prestin is a glycoprotein (Matsuda et al., 2004; Iida et al., 2003), there are contrary data (Navaratnam et al., 2005b).

Aside from possible glycosylation, prestin can also be phosphorylated by cGMP-dependent kinase at S238 and T560 (Deak et al., 2005). Phosphorylation at these sites not only affects the operating voltage range of prestin's NLC (such as S238A), but also significantly changes the slope of voltage dependence (S238D) (Deak et al., 2005). There are several other phosphorylation sites affecting prestin's function, such as PKC-dependent phosphorylation (Matsuda et al., 2003). How these different phosphorylation sites cooperate with each other to regulate motor function in vivo needs further investigation.

Prestin has 744 amino acids. The estimated size of a prestin monomer is too small to form the 10-nm particle originally found in the OHC's lateral membrane (Gulley & Reese 1977; Kalinec et al., 1992; Santos-Sacchi et al., 1998). Hence, the possibility of prestin's oligomerization has been studied through both genetic, biophysical and biochemical methods. Data from FRET and FRAP experiments obtained by several groups demonstrated that prestin can bind to itself (Kural et al., 2004; Greeson, Organ & Raphael 2005; Navaratnam et al., 2005b). In order to investigate the possibility of higher-order oligomers, we examined the quaternary structures of prestin by LDS/PAGE, PFO/PAGE, a membrane-based yeast two-hybrid system, and chemical cross-linker experiments. Our data suggest that prestin forms a high-order oligomer such as tetramer in OHCs (Zheng et al., 2005b). Using a protein density calculation method described by Fischer et al., (Fischer, Polikarpov and Craievich 2004), we estimate a tetramer of prestin transmembrane domains to be on the order of ~11 nm in diameter. Furthermore, 8–13 nm particles (likely to be prestin) were observed through atomic force microscopy in prestin-transfected CHO cells (Murakoshi, 2005). This size is consistent with the motor protein complex observed in the lateral membrane of OHCs (Gulley & Reese 1977; Kalinec et al., 1992; Santos-Sacchi et al., 1998). How prestin forms tetramers and what amino acids are involved in the formation of these structures is still unknown, though N–terminal-based interactions of prestin appear required for such homomeric relations (Navaratnam et al., 2005b).

Molecular Mechanism of Prestin Function

Prestin shows the greatest sequence similarity to PAT1 (putative anion exchanger 1, SLC26A6) (Dallos & Fakler, 2002). Based on early indications that no anion transport was associated with prestin function (Dallos & Fakler 2002), prestin was thought to behave as an incomplete anion transporter. Thus, although prestin demonstrates voltage-dependent charge transfer manifest as NLC, other members of the SLC26A family, such as SLC26A4 and SLC26A6, do not show NLC under similar conditions (Zheng et al., 2000; Oliver et al., 2001). How membrane potential changes across the OHC membrane result in conformational changes in prestin, corresponding to its motor function, is not understood. In order to find the voltage sensor of prestin, the nonconserved charged amino acids (R, K, D and E) between prestin and SLC26A6 in the hydrophobic core region were changed to neutral amino acids as shown in Fig. 2. However, all mutants continued to show NLC even

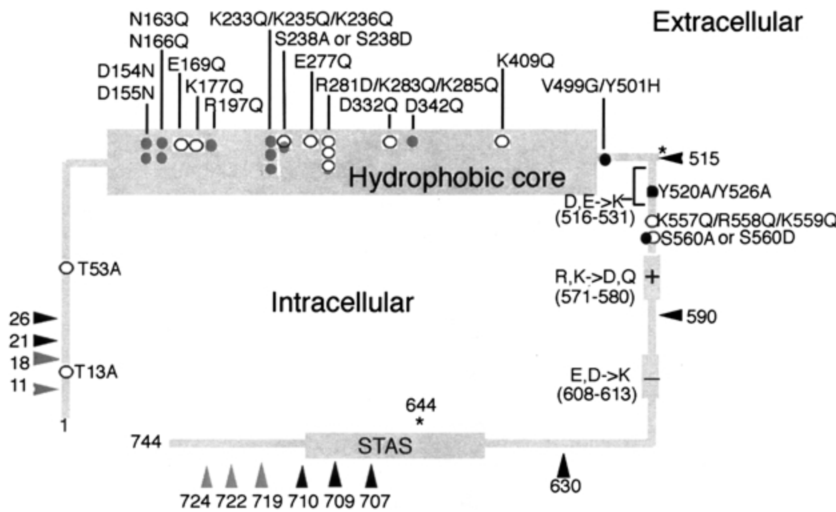

Fig. 2. Effects of mutated amino acids in prestin sequence on prestin's NLC function. *Open circles* are the point mutants that maintain prestin's normal function. *Black dots* are point mutants that produce total loss of prestin's function. *Grey dots* are point mutants that maintain NLC but with significant changes in properties such as voltage dependence. *Grey arrows* indicate truncation mutants (deleted at either the N-terminus or C-terminus end) that maintain prestin's NLC function. *Black arrows* are truncation mutants without NLC. *Asterisks* mark positions where prestin-pendrin, and prestin-pat1 chimeras were switched.

though the parameter values in some mutants were changed (Oliver et al., 2001; Bai et al., 2003). For example, mutant D154N (*grey dot*) has $V_{1/2}$ at −146.7 mV in contrast to −70 mV in wild-type prestin. There are also negative and positive charge clusters in prestin's C-terminus, as shown in Fig. 2. Changing charged amino acids in the C-terminus to either the opposite charge (R, K $\rightarrow$ D, E, D $\rightarrow$ K) or a neutral amino acid (Q), does not abolish NLC function either (Oliver et al., 2001; Bai et al., 2003). It thus appears that these charged amino acids in prestin do not play a primary role as voltage sensors. Subsequent experiments suggested that intracellular anions play a crucial role either as extrinsic voltage sensors (Oliver et al., 2001) or allosteric modulators of the voltage sensor (Oliver et al., 2001; Rybalchenko & Santos-Sacchi 2003). Chloride, in particular, has profound effects on NLC and the motor's operating voltage range (Oliver et al., 2001; Song, Seeger & Santos-Sacchi, 2005; Rybalchenko & Santos-Sacchi, 2003). Most recently, prestin transfected into CHO cells has been shown to exchange extracellular C^{14} formate for intracellular chloride; this transport, which is blocked by salicylate and DIDS, is competitively inhibited by extracellular malate and chloride, but not sulfate (Navaratnam et al., 2005a). Additionally, there is evidence that prestin also provides electrogenic transport of bicarbonate and chloride, and to transport sugar (Chambard & Ashmore, 2003; Ashmore, 2005). This recently observed capability of prestin to transport anions, and the ineffectiveness of anion valence to alter the elementary charge movement underlying NLC (Rybalchenko & Santos-Sacchi, 2003), seriously challenges the extrinsic voltage sensor model (Oliver et al., 2001), and suggests that the effect of anions on prestin activity results from allosteric modulation of the protein's conformation within the lateral membrane. The identity of the charged voltage sensor, however, remains elusive.

Amino acids in the hydrophobic core are near 100% identical among human, mouse, rat and gerbil prestin (only one amino acid is different). Since the function of prestin is directly associated with an increase and decrease of PM area (Kalinec et al., 1992; Santos-Sacchi, 1993; Iwasa, 1994; Santos-Sacchi & Navarrete, 2002), it is logical to assume that the hydrophobic core (embedded in the membrane) is more important for NLC than either the N- and C-termini, since it alone should sense the membrane field. Nevertheless, deletion of N- and C-terminus sections has been shown to be lethal for prestin's function. In fact, deletions of more than 21 amino acids at the N-terminus, or more than 32 amino acids at the C-terminus result in loss of prestin function (*filled arrow* in Fig. 2) (Navaratnam et al., 2005b). There is conflicting evidence whether the loss of function in truncated proteins is due to a lack of membrane insertion (Zheng et al., 2005c) or other causes (Navaratnam et al., 2005b). Replacing the C-terminus of prestin (at position 515 or 644 indicated as "*" in Fig. 2) with the analogous C-terminus portion of one of two closely related SLC26A proteins, Pendrin or PAT1, does not rescue the electrophysiological function of prestin. However, these chimera mutants are not delivered into the PM (Zheng et al., 2005c). In addition, mutating some of the non-charged amino acids in the C-terminus results in loss of electrophysiological function. Some of these mutants, e.g., Y520A/Y52A shown in Fig. 2, lose the ability to reach the PM. Other mutants, e.g., V499G/Y501H, can reach the PM; yet still lose function (Zheng et al., 2005c). These data suggest that the C-terminus plays an important role in both regulating membrane targeting and overall prestin function (Zheng et al., 2005c). Similar results are also found for the N-terminus (Navaratnam et al., 2005b). Together, these data suggest that both N-and C-termini play critical roles for the motor protein's function.

Prestin-Associated Proteins

The PM of OHCs is enriched with protein components. There is also a complicated structural network underlying the PM, including a cortical lattice and cisternal membranes. Although prestin is the key player in somatic electromotility, evidence suggests that other proteins are involved in modifying OHC electromotility. The neurotransmitter acetylcholine (Dallos et al., 1997; Kalinec et al., 2000) and secondary messengers such as cyclic GMP, can modify OHC motor function (Szonyi et al., 1999). An aquaporin-like protein has also been linked to OHC motor function (Belyantseva et al., 2000b). Sugar carrier Glut5 is associated with OHC length changes in a voltage-dependent fashion (Geleoc et al., 1999), Interestingly, Glut5 protein is absent in OHCs of prestin knockout mice (Wu, 2004). The relationship between prestin and Glut5 is still being investigated.

It is of interest to identify the prestin-associated proteins. Several groups used the yeast two-hybrid system to screen prestin-associated proteins. In this system, the C-terminus of prestin was used as ''bait'' to identify prestin-associated proteins. Couplin was first suggested as a novel protein that binds to both prestin and actin (Dougherty et al., 2003). A transcriptional represser, promyleocytic leukemia zinc-finger protein (PLZP) was also identified as a protein interacting with prestin in OHCs (Nagy et al., 2005). PLZP gene produces a POZ/domain Kruppel-type zinc-finger transcription factor reported to have pro-apoptotic and anti-proliferative function. Currently, it is not clear how and where PLZP may affect prestin's function. Other interacting proteins include VCY2-IP1, whose sequence shows homology to MAP1A and MAP1B, and the Na/K ATPase beta 1 subunit (Navaratnam et al., 2005c). Cystic fibrosis transmembrane conductance regulator (CFTR) was reported to activate several of the SLC26 family membrane proteins including DRA, Pendrin and PAT1 via interaction between the STAS domain of SLC26 and the R domain of CFTR (Ko et al., 2004). Prestin contains the conserved STAS domain like other SLC26 family members. Preliminary data also suggest that co-expressing CFTR with prestin in TSA cells could enhance prestin's function (Zheng et al., 2005a). These observations indicate that the motor does not normally act in isolation in the intact OHC and indeed there are elaborate structural details within the sub-membrane spaces. To be sure, the motor must work in concert with these structures.

Development of Structural and Mechanical Characteristics of the OHC

The OHC lateral wall is a unique trilaminate structure consisting of the PM, the cortical lattice, and subsurface cisternae. Like the PM of other cell types, the OHC PM has three main components: the lipids, the proteins, and the glycocalyx. A high density of integral membrane proteins in the lateral PM is one of the distinguishing features of OHCs. When the membrane bilayer is fractured using the freeze fracture technique, a dense array of particles can be seen on the inner surface of the cytoplasmic leaflet (Gulley & Reese 1977). The particles are about 10 nm in diameter and the packing density has been estimated to be from 2500–3000/μm^2 (Saito, 1983; Kalinec et al., 1992; Santos-Sacchi et al., 1998) to as much as 7000/μm^2 (Forge, 1991; Koppl, Forge & Manley, 2004). These values are much higher than those of inner hair cells (1900–2800/μm^2, (Koppl et al., 2004)), non-mammalian hair cells (1880–2360/μm^2, (Koppl et al., 2004)) and axolemma of peripheral myelinated fibers (1300–2500/μm^2). The density of cochlear apical and basal turn cells appears to be the same (Forge & Richardson 1993). These particles in OHCs may include many of the normal membrane proteins, such as non–voltage-dependent ion channels and structural links with the cytoskeleton. However, it was suggested the motor protein might account for a major fraction (Kalinec et al., 1992), and as noted above, it is likely that prestin multimers comprise these structures. Indeed, when the PM of gerbil OHCs between 2 and 16 days after birth (DAB) is examined using replicas of the freeze fracture, the particles are found to be present at low density at 2 DAB and increased in density from 2200/μm^2 at 2 DAB to 4131/μm^2 at 8 DAB, and continue to increase in density until mature values are attained at 16 DAB (Souter, Nevill & Forge 1995). The increase in density of the particles during this time period matches the appearance and development of OHC motility in the same species (He et al., 1994). Since OHC somatic motility is required for normal hearing, it might be expected that development of motility would correspond in time to the development of hearing. Both measures of OHC length changes and NLC have been used to get at this issue.

To date, OHC motor development has been studied in three species, namely, rat, gerbil and mouse (He et al., 1994; Oliver & Fakler 1999; Belyantseva et al., 2000a; Abe et al., 2005). In these species, slight differences in the development of hearing are found. Hearing onset and maturation in mouse are observed at P10–12 and P18, respectively (Ehret, 1976; Steel & Bock, 1980). In rats and gerbils, these periods are somewhat extended, onset being P12 and P12–l4, and maturation being P25 and P21 for rats and gerbils, respectively. He et al., (l994) reported that the electromotile response elicited using a whole-cell microchamber technique was detectable at P7 in cells from the basal cochlear turn and at P8 from the apical turn. All OHCs tested showed detectable responses at P12 and the threshold of detectable response reached

adult levels at about P12. Percentage ratios of maximal response amplitudes and cell lengths reached adult levels of 1.3% at P15 in the basal turn and at P17 apically. On the other hand, OHC cell length of the apical turn continued to elongate until P17 with decreasing cell width, while in basal cells only minimal changes were found. In apical OHCs, voltage-induced length change increased as the cell length elongated. These results suggest that the density of the motor in the longitudinal direction continued to increase until maturation of somatic motility.

In the rat, NLC is detectable as early as P0 in apical turn OHCs (Oliver & Fakler 1999). Oliver and Fakler measured NLC from P0 to P14, demonstrating that NLC increased up to P14, the oldest day they measured, while linear capacitance (C_{lin}) showed saturation between P3 and P11, but suddenly increased from P13. Thus, the calculated value of charge density displayed saturation after P11 because C_{lin} continued to increase after P11. The authors concluded that motor protein density maturated at P11 and that the increase in NLC after P11 was due to an increase in membrane area containing the same density of motor proteins. They further concluded that characteristics of the OHC motor reach adult level at the onset of hearing, P12, substantially before the maturation of the hearing of P25 in rat. On the contrary, the developmental time course of NLC and intensity of immunolabeling of rat OHC lateral wall with anti-prestin antibodies correlates well and matures at P17 (Belyantseva et al., 2000a).

Marcotti and Kros (1999) studied somatic motility and capacitance of the OHCs from Swiss CD-1 mice. They measured capacitance at a fixed membrane voltage of −84 mV, and therefore could not detect linear and nonlinear capacitance separately. Total capacitance saturated beyond P16. All the cells tested showed detectable motile response at P12 (they recorded somatic electromotility at P6, 8, 9 and 12). In C57BL/6J mice, Abe and coworkers measured NLC to investigate the development of motor protein activity of apical OHCs each postnatal day from P5 to P18 (Abe et al., 2005). It was found that surface area or C_{lin} of OHCs mature at p10, whereas prestin activity – gauged as NLC – increased until p18. These and other data (prestin qPCR) were interpreted as evidence for a completed population of prestin molecules within the PM early on, whereas prestin function continues to mature, possibly through heteromeric formations, changing chloride sensitivity, establishment of other protein interactions or phosphorylation effects. Such maturation may correspond to ultrastructural particle formation in the PM, as noted above. Of course, it should be noted that the attainment of adult cochlear function undoubtedly profits from a concerted developmental contribution from a variety of cellular and acellular structures that work with the motors.

OHC Stiffness, Forces and Lateral Wall Structure

Little is known of the lipid content of the PM in OHCs, but it seems to include high levels of cholesterol that may reduce its flexibility and fluidity (Forge, 1991). Lipid lateral diffusion in the OHC PM is a function of transmembrane potential and bathing medium osmolality. Cell depolarization, hyposmotic challenge, and amphipathic drugs reduce membrane fluidity (Oghalai et al., 2000). The dynamic changes in membrane fluidity may represent the modulation of membrane tension by lipid-protein interactions. This and the high protein content should mean that the PM is relatively stiff. Indeed, the relatively high stiffness of the PM is important for the expression of somatic motility (Holley & Ashmore,1988a; Tolomeo, Steele & Holley. 1996; He, Jia & Dallos 2003).

About 25 nm beneath the PM there is an elaborate and highly structured cortical network called the cortical lattice. The cortical lattice appears to be composed of two distinct types of filament (Holley & Ashmore 1990b; Holley, Kalinec & Kachar 1992). The most obvious of these is the 6–7-nm diameter actin filaments that follow a circumferential path around the cell. Adjacent circumferential filaments are from 30 to 80 nm apart, and they are held in parallel arrays by thinner cross-links (2–3 nm in diameter) that may appear single or branched. These thinner cross-links appear to be spectrin (Holley & Ashmore, 1990b). These arrays form discrete domains that vary from just a few parallel filaments only 200 nm long to at least 10 filaments of up to 1 μm long. Their mean angle to the transverse axis of the cell is about 5°–15° with significant variability ((Kalinec & Kachar 1995; Holley et al., 1992; Holley & Ashmore, 1990a). This wide range of orientations suggests that the mechanical response of the OHC lateral wall could be locally modulated. In this regard, the OHC motor appears to be able to be modulated by local stresses and conditions (Santos-Sacchi, 2002). The Young's modulus of the circumferential filamentous components of the lattice were calculated to be 1×10^7 N/m^2. The axial cross-links, believed to be a form of spectrin, were calculated to have a Young's modulus of 3×10^6 N/m^2 (Tolomeo et al., 1996). The lattice is sufficient to retain the shape of the cell following demembranization and mechanical deformation. The structure of the lattice allows it to be described as a coiled helical spring but with longitudinal stiffness primarily determined by the crosslinks. The axial and circumferential stiffness moduli for the cortical lattice measured in OHCs without PM are 5×10^{-4} N/m and 3×10^{-3} N/m, respectively (Tolomeo et al., 1996). Thus the cortical lattice is a highly orthotropic structure. Its axial stiffness is small compared with that of the intact cell, but its circumferential stiffness is within the same

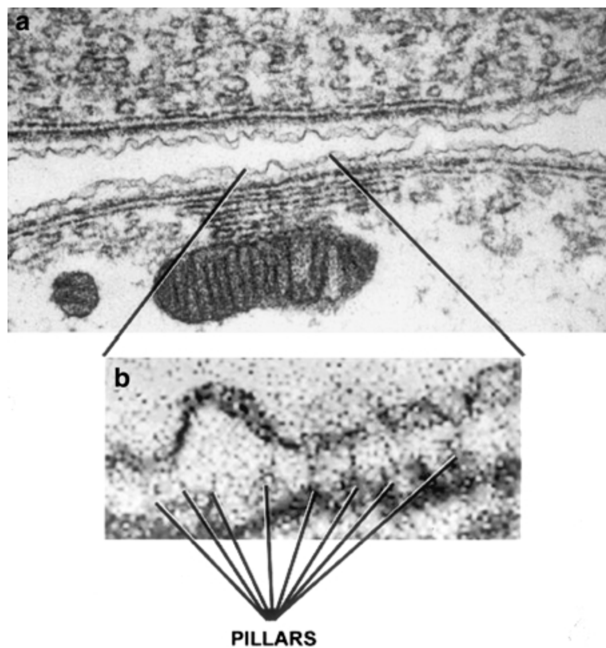

Fig. 3. (*a*) An electron micrograph of a section of a guinea pig organ of Corti showing two neighboring OHCs (separated by the Nuel space), showing a "rippled" plasma membrane. Beneath the PM is the continuous first sheet of subsurface cisternae. In places where the pillars look intact, the PM appears to be flat, whereas its bending is pronounced in regions where the pillars are disrupted (*b*). Note the variability in appearance and number of cytoplasmic cisternea, adjacent to the mitochondrion.

order of magnitude. Direct measurements of longitudinal stiffness indicate that the lattice contributes only a fraction of the overall stiffness of OHCs (Holley & Ashmore, 1988a; Tolomeo et al., 1996; He et al., 2003). These measurements support the theory that the cortical cytoskeleton directs electrically driven length changes along the longitudinal axis of the cell. The cortical lattice is connected to the PM by electron-dense "pillars" 7–10 nm in diameter and 25 nm long (Flock, Flock & Ulfendahl, 1986; Arima et al., 1991) that are attached specifically to the circumferential filaments at intervals of about 30 nm (Holley et al., 1992).

The lateral cisternae are a specialized and substantial fraction of the endoplasmic membrane within the OHC (Gulley & Reese, 1977; Saito, 1983). They form multiple, highly ordered layers that line the lateral cytoplasmic surface of the PM (Gulley & Reese, 1977). The number of layers and the morphology of these membranes vary considerably among different mammals, and between apical and basal turn OHCs, and even at different regions of the same cell (Fig. 3). In the guinea pig, as many as 12 layers have been reported (Evans, 1990; Forge et al., 1993), whereas in rats, only one layer is found in the apical turn OHCs (Raphael & Wroblewski, 1986). Modeling work suggests that the subsurface cisterna may influence the electrical properties of OHCs (Halter et al., 1997) A reduction in the width of the extracisternal space decreases the whole-cell conductance and capacitance. It is unclear whether the lateral cisternae play an important role in the mechanism of somatic motility (beyond that of structural maintenance). Indeed, SSC are not required for normal prestin activity, since disruption of the cisternae with intracellular enzymatic treatments or expression into cells lacking the SSC, shows unmodified NLC (Huang & Santos-Sacchi, 1994; Zheng et al., 2000). Nevertheless, developmental studies show that the appearance of the first layer of the cisternae (Pujol et al., 1991; He, 1997) occurs at the same time when neonatal OHCs begin to exhibit somatic electromotility. The stiffness of the structurally irregular lateral cisternae is very unlikely to contribute significantly to that of the intact OHCs.

Under the influence of the cell's turgor pressure, the PM, the cortical lattice, and the subsurface cisternae contribute to the global axial stiffness of OHCs. The static axial stiffness of OHCs has been measured by compressing the cell with a fine, vibrating glass probe of known stiffness. The stiffness value obtained by different studies from guinea pig OHCs varies from 0.5 to 5 mN/m (Holley & Ashmore, 1990a; Zenner et al., 1992; Gitter, Rudert & Zenner, 1993; Hallworth 1995, 1997; Iwasa & Adachi 1997; Ulfendahl et al., 1998; He & Dallos, 2000). Although the axial stiffness of OHCs varies significantly among different studies, stiffness of short OHCs from the basal turn is found to be much greater than that of long OHCs from the apical turn (Holley & Ashmore, 1988a; Hallworth, 1995; Russell & Schauz, 1996). OHCs are capable of producing an average maximum axial isometric (stall) force of ~5 nN (He & Dallos 2000; Iwasa & Adachi, 1997). From the number of molecules producing this force it can be estimated that the individual molecular stall force is on the order of 2.4 pN. In comparison, the stall force of kinesin is 5–6 pN (Svoboda & Block, 1994). The force generated per mV of command at the cell membrane is estimated to be around 0.1 nN/mV (Hallworth, 1995; Iwasa & Adachi, 1997).

Static stiffness and force of OHCs can also be manipulated by various means (Hallworth, 1997). Axial stiffness and electromotile forces are reduced by 65% when 5 mM salicylate is applied to the cell (Russell & Schauz, 1996). Dallos et al. (1997) demonstrated that delivery of the OHC's efferent neurotransmitter acetylcholine (ACh) to the cell's synaptic pole also decreases the axial stiffness. Quinine at 5.0 mM concentration can also substantially affect the cell mechanics and decrease active force generation in isolated OHCs (Jarboe & Hallworth 1999).

While the axial stiffness of OHCs has been studied, relative stiffness of the lateral wall components is still somewhat controversial. Some studies suggest that the PM appears to be relatively stiff and may be the

dominant contributor to the axial stiffness of intact cells (Holley & Ashmore 1990a; Tolomeo et al., 1996; He & Dallos 1999; He et al., 2003), while a study by Oghalai et al., (1998) indicates that the cortical lattice may account for ~70% of the axial stiffness. In addition, Adachi and Iwasa (1997) also show that diamide can reduce the axial stiffness of OHCs by 65%. Diamide presumably affects spectrin in the cortical lattice. Functionally, the stiffness of the PM must be at least within the same order of magnitude as that of the rest of the cell cortex. If it were not sufficiently stiff, most of the energy associated with conformational change of the motor protein would be stored in the PM itself as strain energy, whereas a stiffer cell cortex would resist the deformation. With the PM dominating the lateral wall stiffness, one hypothesis to explain OHC force coupling is the cytoskeletal spring model proposed by Holley and Ashmore (1988). In this model the molecular motors rest within the PM, and the cortical lattice is less stiff than the PM. When the motors change in dimensions, they push against themselves and their surrounding phospholipids, summating their forces and creating cell length changes. Hence, by this model, force coupling occurs within the PM because the PM has a high stiffness. An alternative possibility, suggested by Iwasa (1994) and further discussed by Tolomeo et al. (1996), is that motors in the PM transmit their force down the pillars to the cortical lattice, which then orients the forces. Because an equal and opposite force would be required to hold the motors in place within the PM, the PM must be stiffer than the cortical lattice in this theory, as well.

He and Dallos (1999, 2000) discovered that the axial stiffness of isolated OHCs is also voltage-dependent. They showed that cell stiffness increases upon hyperpolarization and decreases when the cell is depolarized. The stiffness change can be quite large — on the order of 100%. The stiffness change and somatic electromotility appear to co-vary if manipulated (He & Dallos, 2000). The general covariance of stiffness and length changes suggests that they may arise from a common mechanism, the motor protein (He et al., 2003). Existing standard area models (Iwasa, 1994, 2000) of OHC somatic electromotility do not automatically yield stiffness changes of the type that He and Dallos (1999, 2000) observed. Two generic models (Dallos & He, 2000) have been proposed to explain both phenomena via a single mechanism. The first model assumes that molecular motors change both their axial dimension and stiffness upon a voltage-dependent conformational shift. In the second model, only a stiffness change is associated with elementary motors. Corresponding cell-length change is obtained if the quiescent cell is preloaded so that in its resting state it is shorter than its natural length. This model suggests that somatic motility may simply be a consequence of stiffness change. This would then render voltage-dependent stiffness the primary mechanism, with length change being an epiphenomenon (He & Dallos, 1999).

Honing in on the Role of the Cortical Actin-Spectrin Cytoskeleton in OHC Somatic Motility

A well-known paradigm establishes that even the simplest support system must be adapted to resist tension, compression and bending, three very different kinds of forces (Wainwright et al., 1982). The relative amount of each type of force, together with its intended function, will determine the particular support system for a given cell type. Cell's shape, in turn, will largely be an expression of this mechanical support system. Cochlear OHCs are a unique example of this paradigm.

OHCs possess a hydraulic support system consisting of a fluid under pressure (the cytoplasm), which acts as a compression-resisting component, and the container, which only resists tension (Brownell, 1990). Like OHCs, organisms with a fluid support system are either cylindrical or subcylindrical (Wainwright et al., 1982). However, while the walls of plant cells are rigid enough to support the cylinder at zero pressure, animal hydrostats are flabby and collapse without a positive internal pressure (Wainwright et al., 1982). For a cylindrical vessel closed at its ends — like an OHC— the stress in the longitudinal direction is

$$\sigma_L = pR/2t \quad (t = \text{membrane } \textit{thickness}; \; p = \text{external } \textit{cell radius}).$$

The stress in the circumferential direction, in turn, is

$$\sigma_H = pR/t = 2\sigma_L$$

This means that if slight increases in internal pressure are not to cause disproportionate increase in body diameter, the cylinder wall must be reinforced in order to control the change of shape with changes in pressure. Because of that, lateral walls in pressurized cells are usually reinforced by helically oriented fibers. This is precisely the case in OHCs.

As noted above, OHCs posses a membrane skeleton — the cortical lattice (CL) — composed of relatively long, helically oriented actin filaments cross-linked by spectrin tetramers (Holley & Ashmore 1990a; Holley et al., 1992; Holley, 1996). The cortical actin-spectrin cytoskeleton is a structure common to every known cell population, although its molecular organization, extension and function are cell-specific.

Red blood cells (RBCs) and OHCs, in particular, expose two extreme cases of the functional adaptation of this structure. RBCs, like OHCs, possess a very well developed cortical actin-spectrin cytoskeleton (Bennett, 1990; Matsuoka, Li & Bennet, 2000; Bennet & Gilligan 1993). However, because of the particular

mechanical requirements in each case, they are structurally different. Healthy RBCs are highly deformable, a crucial requirement for cells that need to flow smoothly along very narrow capillary vessels. Consequently, the cytoskeleton is composed of five or six spectrin molecules linked to very short actin filaments, forming a highly flexible ''spider-web'' sheet underlying the plasma membrane. This arrangement ensures that any stress, in any direction, will actually be resisted by the highly deformable spectrin molecules rather than the practically inextensible actin filaments. In OHCs, in contrast, the cytoskeleton must primarily provide mechanical stability to the cells, and deformations are desirable only in the longitudinal direction and in a controlled way. This is provided by orienting the very long actin filaments in a near circular pattern and the spectrin molecules near parallel to the OHC's longitudinal axis, facilitating the cell's changes in length while maintaining the cylindrical shape. Interestingly, the connection between spectrin abundance, localization and particular spatial orientation in OHCs and one of the non-structural functions of these molecules has not been explored yet. Spectrin molecules have been described as ''protein-sorting machines'' that sort the proteins to be incorporated in plasma membranes (Beck & Nelson 1996), as well as ''protein accumulators'' because they can trap and stabilize particular protein species at specific points on cell surfaces (Hammarlund, Davis & Jorgensen, 2000; Moorthy, Chen & Bennet, 2000; Dubreuil et al., 2000). Thus, spectrin could be involved in the sorting, accumulation and organization of prestin molecules in the lateral plasma membrane of OHCs and, consequently, in the genesis of the OHC's motor action.

The orientation and mechanical properties of the circumferential filaments and their cross-links could determine the nature of the shape changes observed in isolated cells (Holley and Ashmore 1988a). Wainwright and co-workers (Wainwright et al., 1982) mentioned as one of the ''design principles for biological structural systems'' that: *''Thin-walled cylinders are most effectively reinforced against explosion and buckling by crossed-helically wound fibers. As the cylinder changes shape, the fiber angle changes. For instance, if the cylinder becomes short and fat the angle increases, and vice-versa.''* In a closed, fiber-wound cylinder like an OHC, the circumferential and longitudinal stresses will be balanced when the fiber angle is 35° 16′ with respect to the transversal axis of the cell (55° 44′ with respect to the longitudinal axis (Clark & Cowey 1958; Wainwright et al., 1982)

$$\sigma_H/\sigma_L = 2 = [\tan^2(35°16')]^{-1}$$

For angles smaller than 35°16′, a given increase in pressure would produce an even larger increase in σ_H than in σ_L. For angles bigger than 35° 16′, the opposite will be true. Interestingly, cell volume would be maximum for a fiber angle of 35° 16′, and would decrease for smaller or larger angles (Clark & Cowey, 1958; Wainwright et al., 1982). Therefore, the near inextensible actin filaments should be forming an angle smaller than 35° with the transversal axis of the cell in order to keep the cell cylindrical. It should be noted that a mechanism capable of controlling the angle of the helically oriented actin filaments could preserve the OHC's cylindrical shape and at the same time modulate the amplitude of OHC motility by regulating the longitudinal component of the membrane-generated forces, cell stiffness, and membrane tension.

The question, of course, is which could be the mechanism capable of dynamically controlling the angle of actin filaments in the OHC cortical cytoskeleton. The answer could be in the modular organization of this structure.

The pillars have an important role in the recently proposed ''membrane bending model of outer hair cell motility'' (Raphael, Popel & Brownell 2000; Oghalai et al., 2000; Brownell et al., 2001; Morimoto et al., 2002). This model is based on the following three considerations (Raphael et al., 2000):

1. Membranes are thin structures in which bending deformations play a pivotal role in the response to external forces.
2. Membranes are liquid crystals and exhibit flexoelectricity.
3. The plasma membrane and the cytoskeleton are tightly associated.

Clearly, consideration number 3 is absolutely dependent on the presence as well as the structural and functional properties of the pillars. In addition, there are two points that, in our opinion, deserve a more detailed discussion. The first and third considerations should always be put in the context of the molecular scale of the OHC lateral wall. In many publications, either the membrane is represented thinner than the CL and/or the separation between the pillars is exaggerated. Although a conventional lipid bilayer may be modeled as ~8 nm-thick, a normal plasma membrane also includes a high concentration of integral membrane proteins and glycoproteins — with entire hydrophilic regions protruding from both surfaces of the bilayer — as well as plenty of extrinsic proteins adsorbed to the cytoplasmic side of the bilayer with strong interactions with plasma membrane lipids, proteins or both. Without considering the membrane glycocalyx — which in OHCs could be at least 100–200 nm thick — the actual thickness of a typical plasma membrane should be realistically assumed to be ~ 25 nm. This value is more than twice the pillar's diameter, thrice the CL thickness, and nearly identical to the average separation between pillars along the actin filaments! From that point of

view, plasma membranes are hardly "thin structures", and membrane bending would not be an easy task.

However, a significant increase in the distance between pillars — together with a positive cell turgor — could make a significant difference in the mechanical properties of the OHC lateral wall. For instance, as already emphasized by Brownell and coworkers, electron microscopy images of the OHC lateral wall frequently show a plasma membrane "rippling" (Fig. 3 *a*). A careful study of this membrane rippling, however, suggests that it is associated with regions of the lateral wall where the pillar-mediated connection between the CL and the plasma membrane is disrupted (Fig. 3*b*). Moreover, experiments with activators and inhibitors of Rho GTPases, which control the dynamics of the cytoskeleton, indicate that the extension and size of membrane rippling is correlated to the integrity of the pillar system (Kalinec et al., *unpublished*). Thus, not only the transmission but even the generation of forces in the OHC plasma membrane by prestin molecules and/or flexoelectricity could be somewhat regulated by the association between the plasma membrane and the CL via pillars.

It is clear, then, that some data suggest that the structure of the CL, the orientation of actin and spectrin molecules, and the integrity of the pillars could be important for the OHC electromotile response. We did not mention, however, how the CL structure, actin and spectrin molecular orientation, and pillar-mediated PM-CL connection — and consequently OHC somatic motility — could be regulated. From numerous studies in other cellular systems, it is currently accepted that the organization and dynamics of the cytoskeleton and associated molecules is mainly regulated by small GTPases of the Rho family (Van Aelst & D'Souza-Schorey,1997; Hall, 1998). The existence of a cellular mechanism of homeostatic control of OHC motility involving small GTPases of the Rho family and Rho-mediated cytoskeletal changes has been already suggested (Kalinec et al., 2000). It has also been demonstrated that the molecular machinery underlying this mechanical homeostatic process requires the activation of Rho-Kinases (ROCK) as well as ROCK-dependent and ROCK-independent signaling pathways downstream RhoA and Racl (Zhang et al., 2003).

A potential problem with experiments aimed at elucidating the role of the cytoskeleton in the regulation of OHC somatic electromotility is that the results could be "contaminated" with slow, prestin-independent motile responses. For example, since electromotile amplitude is proportional to OHC total length, changes in the performance of the prestin-dependent mechanism could be exacerbated — or masked — by changes in total cell length. Recently reported studies, however, described a simple approach — based on continuous measurement of changes in cell length and longitudinal section area — to evaluate the individual contribution of prestin-dependent and prestin-independent mechanisms to the total motile response of OHCs (Matsumoto & Kalinec, 2005). It was demonstrated that if the relative change in OHC length (L) during the motile response is expressed as $L = A^2 \times V^{-1}$ (with A and V being the relative changes in longitudinal section area and volume, respectively), A^2 will describe the contribution of the prestin-dependent, while V^{-1} will describe the contribution of the prestin-independent mechanisms. Thus, relative changes in any two of these cellular morphological parameters (L, A or V) would be necessary and sufficient for characterizing any OHC motile response. This simple approach may become an important tool for increasing our understanding of the cellular and molecular mechanisms of OHC somatic motility.

Putting the OHC into Action

It is certain that the evolution of the OHC was designed to enhance basilar membrane motion in order to amplify the mechanical input to the IHC, thereby providing improved hearing capabilities for mammals, which must register high-frequency sounds. The apparent voltage-dependent nature of OHC somatic electromotility necessarily encumbers the effectiveness of the presumed stimulus — voltage — in driving high-frequency mechanical activity. This conundrum derives from the low-pass filter effect of the cell's plasma membrane (Santos-Sacchi, 1992; Santos- Sacchi, 1989). Consequently, receptor potentials generated by stereociliary transduction elements will be reduced at high acoustic frequency, resulting in diminished feedback into the basilar membrane. In addition to the several proposals advanced to explain how the OHC might deal with this difficulty (Santos-Sacchi et al., 1998; Kakehata and Santos-Sacchi, 1995; Dallos & Evans, 1995; Spector et al., 2003), a stretch-and voltage-activated conductance in the lateral membrane of the OHC, G_{metL}, was recently shown to be permeable to chloride anions (Song et al., 2005; Rybalchenko & Santos-Sacchi, 2003). Because the state of prestin is sensitive to the concentration of chloride near the inner leaflet aspect of prestin, chloride itself by fluctuating across the membrane could "gate" motor activity in a manner which circumvents the low-pass membrane characteristic (Rybalchenko & Santos-Sacchi, 2003; Santos-Sacchi, 2003). Whether such a scheme works in vivo remains to be determined. Nevertheless, we have recently found that modulating extracellular chloride levels in the perilymph bathing OHCs in the living animal (or other manipulations that alter the chloride gradient across the OHC membrane) can drastically alter cochlear amplification in a reversible manner (Nuttall, Zheng

& Santos-Sacchi 2005, Santos-Sacchi et al., 2006). Because intracellular chloride activity is below 10 mM (Santos-Sacchi & Song 2005), and perilymph is about 140 mM, modulation of the chloride gradient could alter the gain of the cochlear amplifier. In fact, changes in intracellular chloride concentration (Song et al., 2005) shift the operating voltage range of somatic electromotility. However, independent of how modulation occurs, it is clear that anions play a powerful physiological role in vivo.

Summary

In an attempt to expand on the pioneering work of the latter part of the last century, a feverish quest continues to uncover the events that lead to cochlear amplification in mammals. The role of the OHC is certainly paramount, and we now have identified many of its membrane constituents that form the basis of the cell's unique contribution. As this review intimates, the motor protein prestin and its interaction with other players within and beneath the plasma membrane drive the cell's augmentation of mammalian hearing acuity. Nevertheless, we are far from understanding the full complement of cellular elaborations that define the cell's mechanical capabilities, Surely, the next quarter century will tell more.

This work was supported by grants NIDCD DC006412 (JZ), NIDCD DC 006496 (DZZH), NIDCD DC05220 and House Ear Institute (FK), Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (SK), NIDCD DC00273 (JSS). We thank Peter Dallos and MaryAnn Cheatham for their comments on manuscript.

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