New tunes from Corti's organ: the outer hair cell boogie rules

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The amplification of acoustic stimuli is a feature of hair cells that evolved early on in vertebrates. Though standard stereocilia mechanisms to promote such amplification may persist in the mammal, an additional mechanism evolved to enhance high frequency sensation. Only in mammals, a special cell type, the outer hair cell, arose that possesses a remarkably fast somatic mechanical response, which probably endows the passive cochlea with a boost in sensitivity by a factor of 100 (40 dB), at least. Experiments conducted over the past few years have shed light on many aspects of outer hair cell electromotility, including the molecular identification of the motor, the effects of a knockout, and underlying mechanisms of action. A review of this remarkable progress is attempted.

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Abbreviations

BM	basilar membrane
IHC	inner hair cell
NLC	non-linear capacitance
OHC	outer hair cell
RC	resistor x capacitor
TH	thyroid hormone
TM	tectorial membrane

Introduction

The organ of Corti, the auditory sensory epithelium of the mammal, houses two types of hair cells, the inner (IHC) and outer (OHC) hair cell. Both cell types transduce mechanical stimuli into electrical signals by modulating a standing cationic current in response to stereocilia displacement (forward transduction). This current induces a receptor potential across the basolateral membrane of the cell, the depolarizing phase of which may promote the release of neurotransmitter [1,2]. Evidence accumulated that an interaction between OHCs and IHCs promoted the highly selective and sensitive responses of the mammalian auditory system to high frequency acoustic stimulation [3–5]. However, a potential mechanism for such an interaction remained obscure until Brownell [6] observed the twitching of outer hair cells in response to electrical stimulation. Following his discovery of reverse transduction [6], a re-evaluation of the classical concepts of mammalian hearing has been underway. Current theories that are concerned with the basis of the cochlear amplifier envision an acoustically evoked cycle by cycle feedback process between the OHCs and the basilar membrane (BM) [7–10]. That is, *in vivo*, the acoustically evoked electrical responses of the OHCs (receptor potentials) are assumed to effect rapid mechanical responses (length changes) by these cells, which boost the mechanical input to the IHC — the receptor cell that receives up to 95% of the afferent innervation [11].

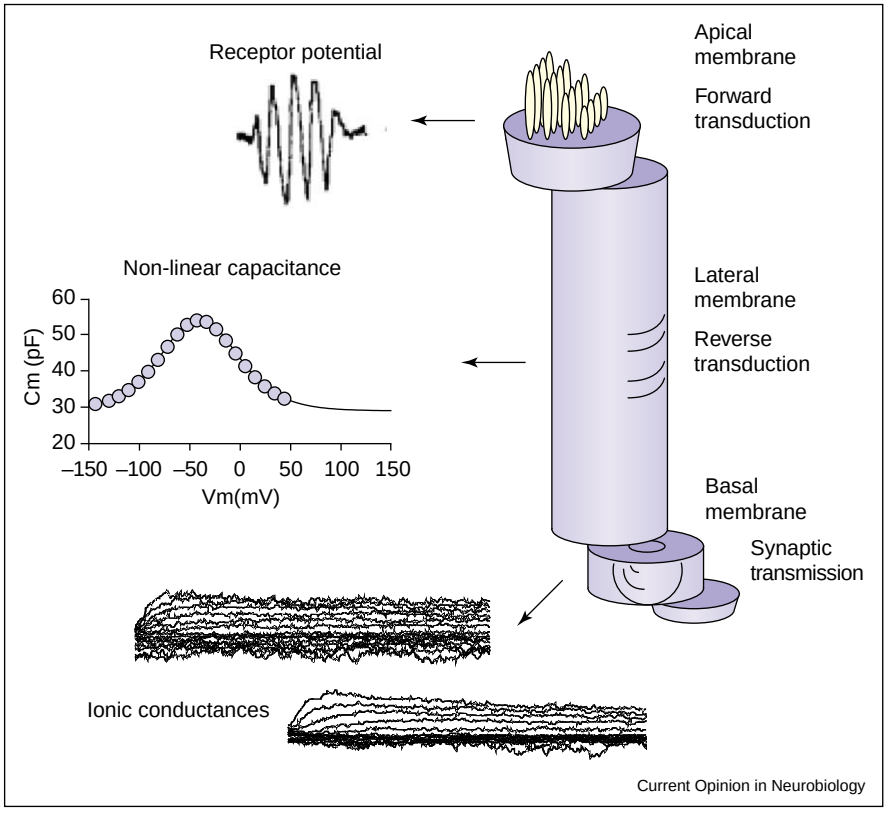
Just three years ago, Dallos and colleagues identified the OHC lateral membrane motor, a protein of 744 amino acid residues that they called prestin, its primary structure classifying it as one of the newer anion transporter family members (SLC26A5) [12]. Subsequently, a flurry of manuscripts have appeared (most of which are review articles! — mea culpa) attesting to prestin's significant role in cochlear physiology. So many questions remain regarding the protein's workings; however, the recent production of a prestin knockout and the demonstration of a human non-syndromic deafness that is linked to a prestin mutation demonstrate the protein's requirement for OHC electromotility, and the devastating effects of its absence on the cochlea amplifier [13••,14•]. The knockout work also raised several more questions, including what proportional contribution does the cellular collection of motors make to the overall cochlea amplifier, and what other cellular and acellular structures direct its force into the organ? For example, in homozygotes, in which prestin was absent, threshold elevations ranged from 40 to 60 dB, but in heterozygotes, roughly a halving of the cellular motor number and activity (3.4 versus 2.3% cell length change) resulted in an apparent proportional (around 6 dB) decline in auditory performance. How might half the motor complement in the OHCs provide only for linear response (e.g. 8th nerve spike rate, BM displacement) growth near threshold, yet the full complement promote non-linear amplificatory growth above that? There are hints in the data that something more than a reduction in the motor number may be occurring. For example, the voltage-displacement function, which can be derived from their figure 3a [13••], seems to show, in addition to the decrease in magnitude, a change in the voltage dependence between wild type and heterozygote. Such a shift denotes an adjustment in the gain of the remaining motor activity, that is, an alteration in the sensitivity of the remaining motors to voltage change. Additionally, could

the change in cell length that accompanied the knockout have compromised normal interactions of OHCs with themselves [15] or the tectorial membrane (TM) superstructure [16]? Indeed, a knockout of a major TM protein, α-tectorin, which resulted in disruption of such interactions, proved detrimental to cochlea amplification [16]. Certainly, a molecular biological trick that could turn on and off prestin, or diminish its activity in a graded way, rather than removing it outright, could help to clarify matters. There is no doubt, however, that understanding the molecular aspects of the cells which form the basis of the cochlea amplifier will be far easier than putting together the whole picture. Here, I first review some basic properties of the OHC lateral membrane motor, and then look at some recent data that relate to the mechanism of OHC electromotility and its effects on cochlear performance.

Biophysical characterization of the outer hair cell lateral membrane motor

The steady-state behavior of the OHC lateral membrane motor is routinely evaluated by fitting a two state Boltzmann function, a sigmoidal function typically used to relate charge displacement and voltage across a membrane, to measures of OHC length change (you can see a video of this length change evoked by a charming voltage stimulus delivered through a patch pipette at the Yale Ear lab website [17]) or non-linear charge movement of the motor's voltage sensor [18,19]. Alternatively, the charge movement is evaluated by fitting the cell's voltage-dependent or non-linear capacitance (NLC; Figure 1) with the first derivative of the same Boltzmann function. In each case, the parameters obtained are V_h (or V_{pkcm}, the voltage at half maximal charge transfer or peak capacitance), z (the unitary charge moved through some portion of the membrane field; otherwise interpreted as the motor's voltage sensitivity) and Q_{max} (the total charge moved across the membrane, indicative of the number of motors possessing unitary charge within the membrane). Typical values for an OHC isolated from the low frequency region of the cochlea are −40 mV, 0.75, and 2.5 pC, respectively. Motor density, derived from non-linear charge density within the cell's lateral membrane has been estimated to be near 8000/μm^2 [20,21]. Although

Figure 1



The OHC has a highly compartmentalized membrane. Apical, lateral and basal membranes possess characteristic integral membrane constituents with measurable electrical correlates. Of interest for the present review is the non-linear capacitance (NLC) that characterizes the lateral membrane motor. The voltage-dependent capacitance, which rides atop the linear (membrane surface area-dependent) capacitance, peaks at a voltage (V_h) near −40 mV that is modulated by a variety of biophysical forces. In addition to information on the state probability of the motor (contracted versus expanded) provided by V_h, measures of NLC also provide estimates of the motor's unitary charge or voltage sensitivity (z), and the total charge moved across the membrane (Q_{max}).

there is a consensus that non-linear charge movement and OHC mechanical activity are inextricably related, contrary to some claims [22], we do not know if charge movements or displacement currents mirror mechanical responses in time, as simultaneous mechanical and charge measures have not yet been made with sufficient time resolution [23–25]. Such measures could provide valuable information on the link between charge movement and OHC mechanical activity, the latter having been measured beyond 70 kHz [26].

Prior to the identification of prestin as the OHC motor [12], several biophysical attributes of the motor had been characterized. These included the effects of membrane tension (turgor pressure), preconditioning voltage, and temperature [27–34]. The main action of these biophysical forces is to set the steady state energy profile of the motor, perturbation effectively and reversibly shifting the operating voltage range over which the motors work in a time-dependent manner. Each of the following perturbations induces a 20–25 mV rightward (depolarizing) shift in V_h: an increase in OHC turgor pressure of 1 kPa (or circumferential membrane tension of 1 mN/m), a step in resting potential from +40 to −120 mV, and a 10°C increase in temperature. The sensitivity of the motor to these biophysical forces is likely to reflect as yet unraveled mysteries of the motor and underscores the susceptibility to insult of the cochlear amplifier. Recently, these same biophysical attributes have been identified in non-auditory cells transfected with prestin [35,36•,37•], confirming the identity of the lateral membrane motor. However, we did note that the effects were smaller in magnitude than those found in the native OHC [36•], and thus, it is possible that prestin requires additional subunit interactions to achieve the desired amplification. Indeed, estimates of the unit magnitude of the motor stroke (area change) are also smaller in prestin transfected cells [38]. One possible interacting species is GLUT-5, an initial motor protein candidate [39].

A motor complex?

Although GLUT-5, a fructose transporter, presented some characteristics that indicated its similarity to the OHC motor [39], this hexose transporter took a back seat following the identification of prestin. GLUT-5 has a different time course of developmental expression than that of electromotility, whereas prestin expression correlates well [40]; indeed, the co-expression of GLUT-5 with prestin apparently has no effect on the activity of prestin [37•]. Whether these observations mean that GLUT-5 is out of the picture or not is debatable, as preliminary indications are that in prestin knockout mice GLUT-5 is absent [41], and expression of prestin may promote fructose transport [42]. Could GLUT-5 and prestin expression be under some common control mechanism? Are they members of a motor complex?

At present we do not know if prestin functions alone or as multimers, or whether additional subunits are required for its activity or not. The initial subtractive screen between IHCs and OHCs that was used to identify prestin produced a large number of unique OHC proteins [12,43]. Presumably, some of these may interact with prestin. There has been one preliminary report of an interacting protein (termed couplin), which links prestin to structural members (pillars) of the OHC sub-membranous cytoskeleton [44]. However, on the basis of estimates of prestin density in the OHC, there are far too few pillars to suggest a one-to-one interaction of couplin with prestin. Couplin's function is unknown, but it is similar to the calponin homology (CH) domain, a superfamily of actin-binding domains. Interestingly, couplin is expressed in several tissues, indicating that potential interacting proteins of prestin may extend well beyond the number of unique OHC proteins identified in the initial differential screen. Indeed, interacting proteins may naturally exist in cell lines used for prestin transfections, possibly confounding our supposed view of prestin in isolation.

Prestin structure

The primary structure of prestin places it in a newly identified anion transporter family, SLC26. Prestin is the fifth identified member, SCL26A5. The nine members identified thus far [45] possess good sequence homology within the probable transmembrane region, but poor homology in the extreme carboxy and amino termini. Most of the SCL26 family members have a clear anion transport function [45–48], but prestin has yet to be fully evaluated, although preliminary evidence indicates that it may transport Cl^- and bicarbonate [42]. The topology of prestin, that is, the number of transmembrane regions and the location of carboxy and amino termini, has received some attention. The carboxy and amino termini are both located intracellularly as determined by antibody staining [37•,49•], indicating that the number of transmembrane domains is even. More detailed topology mapping is lacking, and the results of topology prediction programs are divided among 10 to 12 transmembrane domains. Nevertheless, the favored opinion is that prestin and SCL26A6, arguably prestin's closest relative [46], have 12 transmembrane domains [49•]. This opinion is formed on the basis of the placement of conserved potential N-glycosylation sites within a putative second extracellular loop. The confirmation of prestin topology is important as site directed mutation studies have already been conducted that have observed effects on prestin activity.

Playing with prestin

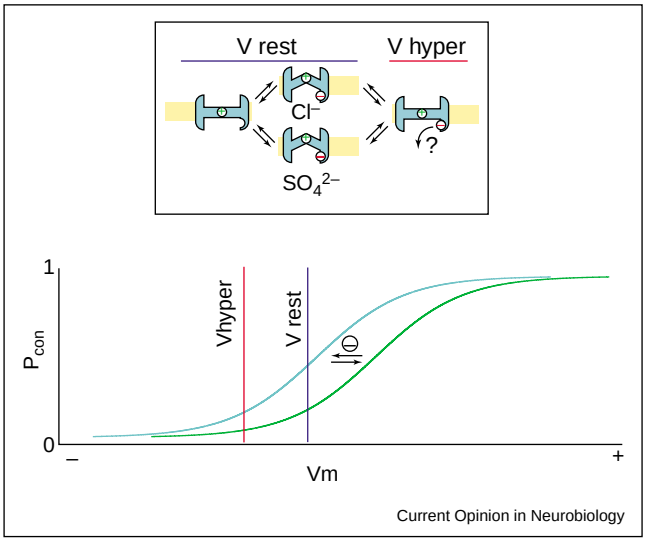
The first structural manipulations of prestin were designed to modify the polarity of non-conserved (among SLC26 members) charged amino acid residues that presumably reside within the voltage sensing transmembrane domain [50••]. Such manipulation failed to

abolish non-linear charge movement under voltage stimulation, but shifted the value of V_h. Subsequently, it was found that full truncation of the carboxy terminus, distal to residue 590, results in absent voltage sensitivity, that is, immeasurable NLC [51]. However, in the absence of a functional protein it is difficult to prove correct plasma membrane targeting and/or folding; thus, it is imperative to also identify similar, yet active truncations. Recently, we [52] were able to produce highly restricted truncations of both carboxy and amino termini at their extreme ends (within 20 residues) that abolished NLC in a graded manner. Additionally, charge reversals of carboxy-terminal charged clusters produced marked shifts in V_h. Therefore, it is clear that the non-conserved (among SLC26 members) carboxy and amino termini of prestin, which are unable to sense the membrane field, are nevertheless important for its sensor function. This importance may relate to allosteric actions that these termini could have on prestin's voltage sensor through prestin–protein or prestin–anion interactions (see below). One other observation that is of interest in light of these data is the resistance of the motor to proteolytic destruction despite the presence of many potential cleavage sites [31,53,54]. Evidently, in the native OHC, the carboxy and amino termini are protected by either their own structural features or interactions with other proteins (or lipids).

Anion effects on prestin's voltage sensor

Two years ago, Oliver *et al.* [50••] made the key discovery that prestin requires anions to function, notably Cl^- and bicarbonate, but not sulfate. They used membrane patches from prestin-transfected chinese hamster ovary (CHO) cells and rat OHCs to determine that NLC requires either of these physiologically abundant anions. The $K_{1/2}$ (concentration at half maximal response) for chloride and bicarbonate's ability to generate NLC was 6 and 44 mM, respectively. Additionally, they found that salicylate, a known ototoxic agent that modulates NLC on the inner aspect of the OHC plasma membrane [55,56], increased chloride's $K_{1/2}$. It was suggested that these anions function as prestin's extrinsic voltage sensor, in which an incomplete anion transport cycle moves negative charge through the membrane field, causing prestin to reside in the expanded state. Some of these data are consistent with previous work showing that only negatively charged lipophilic ions influenced motility and NLC in intact OHCs [57], and that furosemide, a chloride transporter antagonist, interfered with NLC [58]. Nevertheless, the outcome of more recent studies on the effects of mono and divalent anions on intact OHCs [59••] offers an alternative to the extrinsic voltage sensor hypothesis. Notably, in intact OHCs, sulfate and other sulfonate-containing anions can promote significant non-linear charge movement in the absence of bicarbonate and chloride. Moreover, if sulfates were simply able to substitute for monovalent anions as prestin's extrinsic voltage sensor, the unit charge of the motor and Q_{max} would have doubled, as sulfate is divalent. However, the motor's valence, z, remained the same. Another issue that complicated the simple voltage sensor scheme of Oliver *et al.* [50••] was the identification of large shifts in V_h as a function of chloride concentration, that is, an increase in intracellular chloride shifting V_h to negative potentials [59••,60]. Thus, chloride strongly influences the probability that prestin will reside in the compact or expanded state. In view of these observations, we proposed [59••] that anions serve not as extrinsic voltage sensors but as allosteric modulators of prestin that upon binding shift prestin's voltage dependence into the physiological range, where intrinsic charge senses voltage perturbation (Figure 2). Thus, at any given voltage, the binding of chloride to prestin increases the likelihood of the motor being in the contracted state, which corresponds to cell shortening. Recently, chloride's effect on prestin's operational voltage range has been confirmed [61].

Figure 2



Effect of anions on OHC motor. Boltzmann functions describe the voltage-dependent probability of the motor being in the contracted state (P_{con}). At the fixed resting voltage (V_{rest}, blue line) and in the absence of bound anions (green Boltzmann), a small proportion of motors are in the contracted state. With the delivery and possible binding of Cl^- to a site (or sites) on the intracellular aspect of the membrane motor, the steady state energy profile is altered, resulting in a leftward shift in the probability function along the voltage axis (cyan Boltzmann). Consequently, at V_{rest}, an increased number of motors reside in the contracted state (inset, motors in cyan, membrane in yellow). A hyperpolarizing step to V_{hyper} (red line) restores the original distribution of motor states. Whether chloride or sulfate is bound, the slope (z), which is indicative of the voltage sensor's unit charge, remains the same, showing that the extrinsic anion does not serve as the voltage sensor [60].

Targeting the lateral plasma membrane

The OHC motor is clearly restricted to the cell's lateral plasma membrane, which possesses a surface area of

greater than 2,000 μm^2 in low frequency OHCs. This layout has been revealed through physiological tests of the motor's electro-mechanical activity [53,54,62] as well as its mechano-electrical activity [31]. Indeed, prestin has been mapped exactly to the location identified by those physiological measures by antibody labeling [40]. However, the lateral membrane is not uniform, but instead composed of structural and functional microdomains, in which local forces may impose on motor activity [63,64]. That is, the voltage range over which the motors work, and the sensitivity of the motors may not be uniform along the cell surface [64]. These microdomains can have an impact on the overall mechanical activity of the OHC. For example, movements of the cell along an axis other than the longitudinal one, as has been observed *in vitro* [65] and *in vivo* (*in situ*) [66], could arise from local characteristics of motor microdomains that provide for anisotropic (on the level of the whole cell) electromechanical properties. Thus, the evolving concept that the organ of Corti or parts of it deform in a non-rigid manner [66,67,68•,69] may be substantially derived from the mosaicism of the OHC lateral membrane.

The distribution of prestin along the lateral membrane, spanning from nuclear to sub-cuticular regions of the cylindrical cell, is apparently under thyroid hormone (TH) control. In TH deficient rats, prestin expression is delayed and the protein is distributed along the whole basolateral extent of the cell, never attaining restricted membrane targeting to the lateral membrane [70••]. Motor function, however, appears unaffected, as the absence of TH in *in vitro* explants of Corti's organ is not required for the development of electromotility [71]. The correct localization may not only depend upon targeting per se, possibly with helper proteins, but also on the limitation of lateral diffusion. In the normal OHC, the diffusion of lateral membrane motors into adjacent basal regions of the OHC membrane, in which ion channels are restricted [72], does not occur, even after cytoskeletal destruction with proteolytic enzymes [31]. The membrane-based barriers to diffusion across these membrane domains are unknown. However, within the lateral membrane proper, lateral diffusion of motors appears to occur at rates typical for untethered integral membrane proteins, namely at around 0.08 $\mu m^2/s$ [73]. The diffusion coefficient of the lipid permeable dye di-8-ANNEPS within the OHC lateral membrane is faster, as expected for lipids, but interestingly, it is voltage-dependent and tension-dependent [74,75]. This may relate to the voltage and tension-dependence of the major protein prestin that is located in that membrane. However, another lipid soluble dye, SP-DiIC18, is relatively immobile but can redistribute within the membrane in a voltage-dependent manner, and this redistribution probably depends on prestin activity [63]. In light of these data, it is possible that the diffusion of prestin itself depends on the protein's voltage-dependent area state, an area that has yet to be explored.

Molecular motions

The whole cell mechanical response of the OHC (which can be seen at the Yale ear lab website [17]) is truly amazing. Movements as large as 30 nm/mV have been measured [76]. One of the perplexing questions about prestin or its complex is how the voltage dependent conformational change, which we are able to observe microscopically through displacement current measures, results in cell movements? A variety of hypotheses have been tested, but two putative mechanisms remain entertained in the field. Following the observation that OHC membrane patches change surface area in response to membrane voltage change [53], molecular motor models incorporating two-state area changes appeared. These mechanical models naturally arose from the two-state Boltzmann models of non-linear charge transfer that preceded them [18,19], and suggested that unitary motors fluctuate among area states with an area difference ranging from 0.4 to 8 nm^2 [27,28,34,77–79]. Given the constraints imposed by a cylindrical shape, caused by the cortical cytoskeleton [80] and the fixed volume (especially expected at high acoustic frequencies), surface area changes are predicted to alter OHC length and diameter in line with physiological observations [77]. Of course, an alteration in surface area should cause a change in the cell's linear (specific) capacitance, which correlates with the number of motors that occupy each state, hyperpolarization favoring an increase in specific capacitance (expanded state). Indeed, such a phenomenon appears to occur [81].

The other motor mechanism currently favored is formed on the basis of membrane curvature and interfacial tension. Proponents of this mechanism maintain that prestin functions not as a motor but simply as an entity that provides the membrane with an enhanced capacitance (charge), which in turn amplifies a basic biophysical process that drives membrane motion [82•]. It has been argued that this flexoelectric property of membranes accounts for such phenomena as salicylate and chlorpromazine's (both amphipaths, which affect membrane curvature) actions on the magnitude and voltage dependence of OHC motility and NLC [83–86]. The theory appears solid, but there is disparity between the 'area motor' and 'membrane bending motor' camps over magnitude effects, polarity, and the precise requirements for helper charge that may point to the superiority of one hypothesis over the other. It is likely that each camp will fight until an experiment unequivocally reveals the truth [22,86]! We should not hold our breath, however, as it took nearly a decade to dissuade supporters of an electro-osmotic mechanism for OHC motility [87].

Another property of the OHC that may account for mechanical responses or help to modify them is its voltage dependent stiffness [88]. Two models have been proposed whereby mechanical activity could accompany a

stiffness change [89]; one invokes intrinsic and simultaneous changes in stiffness and the conformation of a motor unit, whereas the other derives from the consequences of a pure stiffness change under constant cellular preload (steady state application of cellular deformation). The results of preload effect (induced by turgor pressure) on OHC motility are contradictory [19,33]. Nevertheless, the conformational changes embodied in the area model of motility are predicted to evoke a voltage dependent compliance that peaks at V_h (corresponding to the voltage at maximal electrical compliance [capacitance], or mechanical voltage sensitivity), much as the stereociliar transduction channels sponsor a maximum bundle compliance when half the channels are open [90]. Iwasa [91] modeled this molecular inevitability and confirmed that motor activity should affect global OHC stiffness. However, as expected, at V_h stiffness is at a minimum, whereas neither the stiffness data nor the models of He and Dallos [88,89] show a minimum at V_h, but instead, a simple (sigmoidal) correspondence between stiffness and motility magnitude. In light of this, it is likely that the modulation of OHC stiffness additionally arises from mechanisms other than direct motor activity. Indeed, it is understandable that global OHC stiffness should derive from the combined structure of the OHC lateral wall (plasma membrane, cortical cytoskeleton, and subsurface cisternae) [92]. Thus, another means to alter global OHC stiffness would be through cytoskeletal effects of second messengers (e.g. cGMP [93], or calcium ions [94–97]) and other signaling pathways (e.g. those employing the small GTPases [98,99]). Neural efferent effects on cochlea mechanics may be mediated partly by modulation of OHC stiffness [100].

The consequences of direct motor effects (viewed through NLC measures) of chemical modifiers (e.g. phosphorylation) have been considered for some time [94,101]. Indeed, there are several sites identified within prestin that could be important targets for functional modification [12,49•], including N-glycosylation and phosphorylation sites. There are some preliminary data of interest with regards to these sites. Mutation of two N-glycosylation sites at residues 163 and 166 produced no changes in NLC [102]. On the other hand, the PKC inhibitors, RO31-8220 and bisindolylmaleimide (BIM), shifted V_h in the depolarizing direction in prestin-transfected cells [103], although the PKC activator phorbol 12-myristate 13-acetate (PMA) had previously been shown to lack effect on OHC motility [93]. The compelling story of prestin's modulation by second messengers and the like is not yet available.

Hop or Hopf?

One of the raging controversies that concerns mammalian cochlea function is the relative importance of stereocilia versus somatic mechanics, each viewed as potential powerhouses for amplification [10,104,105]. The stereocilia bundle may very well sit at or near a point of instability, at a Hopf bifurcation, where oscillation and amplification struggle to prevail [106]; but whether or not it rules in the mammalian system is difficult to reconcile with the obvious need for such a momentous evolutionary stride as the OHC lateral membrane. Certainly, the prestin knockout mouse has strengthened the case for the dominating role of OHC electromotility [13••]. Yet reasonable issues with this experiment remain, as noted above. To highlight one concern, could the structural changes (e.g. shortening) in the OHC that result from a reduction in prestin numbers have modified the interaction of the stereocilia with the TM, ultimately interfering with stereocilia feedback into the organ? That is, could stereocilia mechanics have been altered because, for example, of bundle biasing? Could this possible biasing have also altered their resting potentials? In addition, what is the consequence of missing GLUT-5? In any case, whether or not a Hopf bifurcation underlies the sensitivity, tuning and non-linearity of the mammalian auditory system may not necessarily point to one mechanism over another, as such an instability could conceivably reside within the soma, as well as the stereocilia (see annotation to Julicher *et al.* [107]). As far as the bifurcation concept itself goes, there is some quite heated debate [108••]! However, beyond the underlying principles of amplification, questions of speed persist and may ultimately help to decide which amplificatory mechanism will win. Clearly, stereocilia work at frequencies across the mammalian acoustic spectrum, indicating that the bundle's driving force for amplification is truly wideband; however, can the effector arm follow the input? Using realistic parameters, bundle amplificatory abilities have been modeled up to 5 kHz [105], still far short of mammalian needs. Unfortunately, the lateral membrane mechanism also faces difficulty.

How fast can fast be?

There is no doubt that the OHC motor is fast; as already noted, mechanical activity is measurable above 70 kHz [26], and in some mammals, such as bats, in which the cochlear amplifier presumably works, frequency sensation can extend well beyond 100 kHz. However, in all evaluations of the motor's frequency response, external voltage commands under the frequency and level control of the experimenter were used, thus avoiding one of the enigmatic problems facing the realization of voltage-driven cochlear amplification at high frequencies, the RC (resistor × capacitor) membrane filter [24,109]. The OHC membrane's low pass filter is expected to seriously limit high frequency electromotility, and this difficulty is one of the key arguments employed by proponents of stereocilia-based cochlea amplification in the mammal [105]. The problem arises when one considers that because of the non-linear nature of the OHC electromotility function, the mechanical gain of the OHC is about one-tenth of its maximum (max: around 20 nm/mV) at the

cell's normal resting potential of −70 mV [109]. At this gain, receptor potentials near threshold would result in mechanical responses that fall short of threshold BM movements, making it unlikely that BM motion can be boosted. Since its identification, a few solutions for the apparent RC filter problem have been proposed. Dallos and Evans [110] suggested a mechanism that relies on stimulation of motor activity by wide-band extracellular voltages evoked by remote OHCs within the organ of Corti. Alternatively, we have suggested two cellular-based mechanisms whereby the problem could be alleviated. First, we found that the mechanical gain of the cell at its resting potential could be increased through reductions in turgor pressure [29]. Second, as we found that motor charge density is greater in higher frequency OHCs, we reasoned that the resultant electrical energy ($Q * V$) delivered to the lateral membrane of high frequency OHCs could render the filter-induced drop in receptor potentials less detrimental [111]. The latest suggestions are based on modeling, one positing that the piezoelectric-like properties, that is, reciprocal electromechanical properties, of the OHC lateral membrane can provide for an enhanced receptor potential in high frequency OHCs [112], whereas the other suggests that the apparent inductance of ionic channels may enhance the cell's frequency response [113]. All of these arguments presuppose the existence of an RC filter problem due to prestin's voltage dependence. What if prestin were driven by something other than voltage?

The recent identification of a stretch-activated chloride conductance (termed G_{metL}; [59••]) in the OHC lateral membrane may indicate that the motor can react to currents evoked by mechanical perturbations of the lateral membrane accompanying acoustic stimulation *in vivo*. Indeed, sound induced deformations of the OHC have been observed in the intact cochlea [68•]. In this model, intracellular Cl^- oscillations located near prestin, driven by stretch-activated ac and/or dc Cl^- currents, would directly drive prestin transitions. Because the current or its integral, not the resultant voltage, directly modulates prestin, the mechanism is unencumbered by the RC membrane filter. If this model proves true, then the OHC soma has adopted a mechanism similar to that which stereocilia use to provide cochlea amplification in lower vertebrates. In that case, calcium ion entry through the mechanically-gated forward transduction channels modulates the channels themselves and thus stereocilia bundle mechanics, leading to amplification [104,105]. Nature's capitalization on such a primitive yet well-designed system may possibly have usurped the stereocilia's rule by elevating the OHC lateral membrane to function as both a forward and a reverse transducer.

Conclusions

Despite the advances made in understanding OHC function and its role in cochlear amplification, so many more unknowns remain. In a way, OHC aficionados are faced with the excitement that ion channel biophysicists felt when molecular identifications of their sweetheart proteins were first made. A host of investigations that employ the now well-developed fields of molecular biology, protein biophysics, and the like, which have been successfully employed in other fields, are beginning to surface in our small field. This can be clearly observed when perusing the abstracts of international meetings, some of which have been highlighted here. Although the information gleaned in the past few years has truly been remarkable, I would bet that the next timely review of OHC electromotility will be astonishing by comparison.

Update

Two manuscripts of significant interest have appeared since this review was completed. In a follow-up to the published abstract [42] on sugar transport by prestin, Chambard and Ashmore [114] provide evidence that prestin and another SLC26 family member, Pendrin, can transport fructose. The ability to transport fructose was not assessed in other family members but conceivably could be a general trait. The full significance of the transport is not clear, but certainly it could influence turgor pressure regulation in OHCs.

Weber *et al.* [115] identify a few new SLC26 family members that are homologous to prestin in species that are distant from mammals, namely fish and insects. Nevertheless, the best homology is around 50% (or less) with prestin. It would not be considered unusual to find solute transporters in these species. Furthermore, it is unlikely that motor activity would arise from such homologues, as other members of the SLC26 family that have substantial homology to prestin lack this capacity. Nevertheless, investigation of motor activity (e.g. NLC) is certainly warranted.

Acknowledgements

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