Cold Spring Harbour 'Hearing and deafness'

Outer hair cells and electromotility

Short title: Cochlear Outer Hair Cells

Jonathan Ashmore
UCL Ear Institute,
332 Grays' Inn Road,
London WC1X8EE, UK
email: j.ashmore@ucl.ac.uk

Abstract:

Outer hair cells (OHCs) of the mammalian cochlea behave like actuators: they feed energy into the cochlear partition and determine the overall mechanics of hearing. They do this by generating voltage-dependent axial forces. The resulting change in the cell length, observed by microscope, has been termed 'electromotility'. The mechanism of force generation OHCs can be traced to a specific protein, prestin, a member of a superfamily SLC26 of transporters. This short review will identify some of the more recent findings on prestin. Although prestin's molecular structure has yet to be determined, results from the presence of its homologs in non-mammalian species suggest how it may be conformed, how it can act like a transport protein and how it may have evolved.

Introduction

The outer hair cells (OHCs) of the mammalian cochlea are an identifiable group of cells of the inner ear which are responsible for many of the distinct features of our hearing. These features include the absolute sensitivity to low sound levels, the selectivity to sound frequencies over a many octaves and the dependence of their continued performance on the physiological state of the cochlea. These properties have collectively become known as 'cochlear amplification'. Although other orders of terrestrial animals also have cells homologous to the mammalian OHCs, it seems that the evolutionary stages to construct a hearing organ have taken slightly different routes between different species. Thus although analogous molecules can be found shared between many hair cells in all vertebrates, there have been particular specialisations in mammalian hearing to enable and favour high frequency hearing. This chapter will focus specifically on mammalian OHCs. The loss of OHC function is the major cause of hearing loss, particularly with age. Although several reviews have been published (Ashmore 2008; He et al. 2014; Corey et al. 2017; Santos-Sacchi et al. 2017) the emphasis here will be on several outstanding issues.

Electromotility

Mammalian OHCs, as cells of the organ of Corti, are organised in three (and sometimes four) rows along the full length of the cochlear partition. Scrutinising sections of the partition, they are placed so as to mechanically influence the flexure of the basilar membrane.

The original observation that OHCs were cells that were 'motile' is due to Brownell and his coworkers in Geneva (Brownell et al. 1985). They showed, using intracellular recording electrodes, that the cells could be driven to change length when the membrane potential was changed. The term 'electromotility' to describe this behaviour was shorthand for the behaviour (although it might be claimed that 'motile' implies the cells are moving somewhere) but it has stuck. It required the much wider electrical bandwidth possible with patch clamp recording to show that such length changes were rapid and certainly fast enough to claim that OHCs could be force generating elements at acoustic frequencies involved in shaping cochlear mechanics (Ashmore 1987). Since a variety of different techniques have shown that the electromotile mechanism can be driven over the full range of frequencies found in mammalian hearing and certainly up to 80 kHz (Frank et al. 1999).

It is strictly more accurate to describe the OHC as an 'actuator' as the source of the energy for the force generation derives from the potential across the transducer channels: it is not produced seemingly from energy sources within the cell. A short calculation shows that the maintenance of the membrane potential at -50 mV allows sufficient extraction of work from the electric field to explain the work done by the OHC against the constraints of the tissue in which they are embedded.

Mammalian OHCs possess a very characteristic V-shaped bundle of stereocilia , serving to inject current into the cells when deflected. As sensors of the movement of the basilar membrane, OHCs thus form part of a local mechanical-electrical-mechanical feedback loop to control the basilar membrane tuning. The intimate role of OHCs in such feedback has meant that their role in *the in-vivo* cochlea often has to be interpreted by appeal to cochlear models. When studied in isolation a property such as electromotility gives an exaggerated impression of the length change which an OHC exhibits in a fully functioning cochlea.

A number of other features of OHCs should also be considered when considering electromotility. First, although they are neuroepithelial cells, specialised so that the apical surface facing scala media is mechanosensitive and the basal surface contains synaptic machinery, OHCs are relatively sparsely innervated compared to inner hair cells. It is clear that OHCs can generate activity in the Type II auditory nerve fibres (Weisz et al. 2009) but this information may be used in a very different way from the major sensory pathways from the sharply-tuned Type I fibres forming the majority of the auditory nerve. Second, OHCs are the target of a descending pathway, fibres of the medial olivo-cochlear bundle. These fibres, releasing acetylcholine, ACh, act on OHCs via a heteromeric alpha9/10 AChR, and serve to control the membrane conductance of the cell. The net effect is to reduce membrane potential excursions in the cell and thus to reduce the mechanical OHC loop gain and overall cochlear sensitivity. The role of the efferent system is contentious but the effect of their activation on mechanical tuning and distortion product emissions is clear (e.g. (Maison et al. 2007)) and there is the suggestion, from mouse studies where the receptor has been deleted, that the efferent system activity might slow auditory ageing (Liberman et al. 2014).

Identification of the mechanism

A number of hypotheses could explain the phenomenon of OHC electromotility. There are several constraining observations: 1) the lateral membrane of the OHC is packed with a particle about 8 nm in diameter; 2) a change in the OHC membrane potential is accompanied by a gating charge movement, or equivalently the cell membrane capacitance is voltage dependent; 3) the charge movement is blocked by the amphiphilic anion salicylate (the methylated form being aspirin) and 4) OHCs only acquire motile properties progressively during a during a short period of development.

The identification of prestin (Zheng et al. 2000) by using a subtraction cDNA library for isolated hair cells in principle solves most of these problems. Mammalian prestin is a 744 amino acid protein with a molecular weight of 81 kDa, but when expressed in a heterologous system exhbits voltage dependent movements and a non-linear capacitance (NLC), indicative of protein rearrangements when under the influence of the membrane potential. The surprise is that prestin is member of a superfamily of membrane transporters SLC26A5, a family whose other members are chloride-bicarbonate exchangers (Lohi et al. 2000).

There are some differences in the NLC between expression in a cell system (e.g. HEK293, TSA201 or CHO cells) and the behaviour ex vivo. The difference seems to depend on the molecular packing density: in cell systems where the prestin is at a relatively low level (e.g. giving rise to a maximum NLC of 0.4 pF) corresponding to a copy number of about $2.5x10^5$ prestins/cell, the peak capacitance is close to -75 mV (Oliver et al. 2001); in OHCs the voltage at the peak progressively increases during maturation of the cell to reach a steady state at -40 mV in mouse at P12, where the density is estimated to be about 4000 copies/μm^2 (Oliver and Fakler 1999).

An incomplete transporter.

How a transport protein can give rise to a motor was solved soon after the discovery of prestin with the proposal that prestin/SLC26A5 was an incomplete transporter (Oliver et al. 2001). The idea is that the protein acts like mechanoezyme as part of its transport cycle, but that the cycle is incomplete. As a result the binding of an intracellular anion (choride) the conformation change of the proteins is sufficient to produce a length change in the plane of the membrane and hence of the OHC. The maximum electromotile change in and OHC is 4%, so the change in the particle diameter would be 0.04x8 nM = 0.3 nM, not impossibly small but close to the limit currently observable by structure techniques.

Antiporter activity

Even if mammalian prestin/SLC26A5 is an antiporter for chloride and bicarbonate, it has proved more difficult to show that any transport does occur. In the cases of chick and of zebrafish, the prestin homolog certain does act like a transporter (Schaechinger and Oliver 2007). In both these species, although in different orders, transport is electrogenic, with two bicarbonates being exchanged for one chloride, and currents are readily observable.

If other anions are used instead of chloride, then transport can be measured either by electrical methods or by using more classical radioactively labelled ions. Thus prestin transfected cells take up ^{14}C- formate as an ion, but at a rate which was only weakly inhibited by 10 mM salicylate, the inhibitor of NLC (Bai et al. 2009).

Electrophysiological studies have shown that mammalian prestin/SLC26A5 is electrogenic. In the case of the zebrafish prestin homolog, the expressed protein is electrogenic and acts as a SO_4^{2-}: Cl^- antiporter, the sulfate being transported in place of two bicarbonates.

With similar methods to those used by Schaechinger and Oliver, exposure of prestin expressing CHO cells to a low chloride-high bicarbonate gradient produces a change in membrane potentiol compatible with a 2:1 HCO_3^-: Cl^- stoichiometry (Mistrik et al. 2012). It can also be show that the presence of prestin in the cells allows for a faster recovery from an acid load by allowing an influx of bicarbonate; such experiments used prestin, tagged with the fluorescent pH sensor pHluorin, to monitor the influx near the membrane.

The comparable experiments have not been completed fully to monitor choride movement although a chloride movement can be detected using YFP, which is chloride sensitive, when the cells are exposed to low (6mM) chloride and high (23 mM) bicarbonate on the outside favoring exchange. An anion transport current can be detected however with the much more permeant anion thiocyanate, SCN^- (Schanzler and Fahlke 2012) . Both SCN^- and Cl^- give rise to comparable NLC in transfected HEK293 cells, although the reposted extenal thiocynate shifts the curve more negatively from -69 (typical of a cell expression system) to -102 mV. The authors conclude however that there is a transport pathway for anions, and on the basis of parallel studies with zSLC26A5 (from zebrafish) and hSLC26A7 (from human) a pathway which may be conserved between many members of the SLC26 family.

Although SCN^- is used as permeant anion in many transport studies its transport pathway in prestin may differ from that of chloride (Bai et al. 2017). In a tet-inducible prestin expressing HEK cell line, which shows a large NLC, a current carried by chloride is also present whenever the NLC can be measured. Such observations only become apparent with high expression levels, and so possibly missed in early experiments (where the peak NLC was 10-15% of that now found). Evidence presented supports the idea that chloride is able to pass though a separate pathway intimately linked to to the NLC, and most probably linked to a stretch-sensitive chloride channel reported for prestin (Rybalchenko and Santos-Sacchi 2003). Other members of the SLC26 family (SLC26A3 and SLC26A6), as well as some ion channels (manifesting as a so called omega or gatinig-pore currents (Moreau et al. 2015)) also show evidence for a similar sensitive leakage current. Whether this influences the gain and function of OHCs in cochlea mechanics is undecided, but it does suggest that may be several osmoregulating mechanism present in the cell.

Pharmacology.

The pharmacological manipulations of prestin have suffered from a shortage of reagents. The best known is aspirin (methyl salicylate) which is effective at mM levels from the extracellular surface, although the binding site thought to be on the cytoplasmic surface. Ingestion of aspirin is known to (reversibly) elevate auditory thresholds (and lead to a tinnitus as a consequence). As a competitive antagonist to choride salicylates dissociation constant is estimated from the non-linear capacitance to be Ksal =21 µM. (Oliver et al. 2001). Non-steroidal anti-inflammatories have little or no effect.

Genetics: **Mouse hearing**

In mouse there is ample evidence that prestin determines cochlear sensitivity. The first knockouts of prestin clearly showed that in vitro OHCs are non-motile and a 40-60 dB loss of cochlear sensitivity in vivo (Liberman et al. 2002). A cochlear microphonic could still be measured so the OHC transduction was not affected, although there appeared to be a progressive apoptosis of the cells. A more direct measurement of the basilar membrane in the hook region (60-70kHz) of the mouse cochlea also showed that the knockout lost the sharp mechanical tuning characteristic of the wildtypes, but curiously did not lose significant thresholds (Mellado Lagarde et al. 2008). The possible explanation is that prestin contributes to the stiffness of the OHCs, and that removal of prestin alters the sound coupling to the basilar membrane mechanics.

The issue of OHC mechanical changes can be removed by using a prestin mutant mouse where two residues (V499G/Y501H) are replaced near the presumed last transmembrane helix, yielding OHCs which are structurally and biophysically near normal but have a NLC shifted very positively and outside the physiological operating range (Dallos et al. 2008). This mutant indeed shows a basilar

membrane tuning where both frequency selectivity and cochlear sensitivity is the major effect with no alterations in cochlear impedance matching (Weddell et al. 2011).

Genetics: Human hearing

There has been some dispute about the prevalence of prestin/SLC26A5 mutations in humans. An early report suggested that a recessive locus, termed DFNB61, was associated with single nucleotide change in the second intron of SLAC26A5. This splice acceptor site for exon 3 of the 21 exons of the gene, generated a hearing deficit (Liu et al. 2003). However it was subsequently argued that this variation occurs no more frequently in hearing impaired than in controls and therefore precludes a causative role (Tang et al. 2005).

To date, therefore, human prestin mutations appear quite rare. A single case report identifies two profoundly deaf sisters with compound heterozygote mutations, one at p.W70X in the N-terminal region which is expected to inactivate the protein and the other at p.R130S which is within the sulfate transport motif of the SLC26 genes (Matsunaga and Morimoto 2016). In all other respects the subjects were normal. When the latter mutation is made in mouse prestin the resultant has impaired membrane targeting, slowed kinetics (producing a reduced NLC at the measurement frequencies) and enhanced transport for thiocyanate, SCN^- (Takahashi et al. 2016). Apart from one other candidate mutation reported at p.R150Q (Toth et al. 2007) these are the only cases reported so far.

Evolutionary aspects of prestin

Is mammalian prestin really a modifed transporter? Its ubiquitous presence in sensory hair cells for vertebrates and invertebrates suggest that it has a particular role that has been conserved through evolutionary changes. The obvious suggestion is that it plays a role in cellular pH or perhaps osmotic regulation.

Thus SLC26A5 homologs have been found in an remarkably wide range of animals both in chordates (He et al. 2014) as well as in invertebrates such as in drosophila hearing organs (Kavlie et al. 2015). There have been relatively few reports that it gives rise to any form of hair cell motility except in the mammalian cases. Where it has been studied biophysically, these prestin homologs either seem exhibit significantly reduced NLC at acoustic frequencies, as for zebrafish prestin, (Schaechinger and Oliver 2007) and/or are inserted into the membrane at much lower levels than could give rise to a motility, as found only weakly in chick hair cells (Beurg et al. 2013). What many of these homologs do exhibit however is ion transport.

As sequence data for the SLC26 super-family became more common, and for SLC26A5 in particular, a bioinformatics study showed that there are some significant evolutionary differences for mammalian versions of the gene. The main differences between mammalian and non-mammalian prestin is found in the cytoplasmic portion of the resulting protein rather than in those regions implicated in the transmembrane loops (Franchini and Elgoyhen 2006). Predictions of adaptive changes in such genes are based on whether there is a sufficiently large synonymous versus non-synonymous ratio; by the same measure, it also appears that the acetylcholine alpha9 receptor, found in OHCs, has also undergone adaptive evolution in mammals whereas pendrin (SLC26A4), also implicated in cochlear function has not. It is not immediately clear functionally what such changes indicate, although it is tempting to speculate that it is the modification to the C-terminal STAS domain which confers the special role to mammalian prestin.

Within mammals there are examples of adaptive convergence in auditory system. Some species of bat and all the toothed whales use echolocation to find prey. All of these species use ultrasonic sounds with matched requirement to be able to detect such frequencies. Remarkably both dolphin and Doppler-sensitive CF bats exhibit what appears to be an example of convergent molecular evolution of their prestin even though animals have been separated phylogenetically for many tens of millions of years (Liu et al. 2010). Four of these specific convergent amino acid sites (G167S, I384T, A565S and E700D) have particular interest as the latter two are in the STAS domain, which may suggest a special role in prestin function for this domain

Structure

The structure of prestin remains unresolved. The original sequence data suggested that the molecule had 12 transmembrane regions (Zheng et al. 2000; Oliver et al. 2001). Other proposals including a 10 transmembrane structure have been made (Bai et al. 2009). The current best predictions for the structure, based on homology modelling on the uracil transport family, suggest that instead there are 14 transmembrane domains with a duplication of a 7 transmembrane motif (Gorbunov et al. 2014).

Since the size of the particle in the OHC lateral membrane is 8 nm in diameter, is thus highly unlikely that prestin functions as a monomer, but is oligomeric, most probably a tetramer. Some compelling evidence for this conclusion comes from single molecule photo-bleaching experiments. Successive bleaching of GFP tagged prestin expressed in HEK reveals a series of equal amplitude steps which is most consistent with a tetrameric structure (Hallworth and Nichols 2012).

These results are compatible earlier results which suggest that prestin, at least when expressed in mammalian cell lines, acts as a high order oligomer based on a stable dimer where formed by cross linking disulphide bonds between single molecules (Zheng et al. 2006).

The amino acid sequence of prestin also showed that here was likely to be a C terminal region, in the cytoplasm, accounting for nearly 30% of the peptide, which contained a distinguishing 'sulphate transporter and antisigma factor antagonist' (STAS) domain shared with other members of the SLC26 anion transporters. The role of this domain is unclear. The crystal structure of the cytoplasmic domain is known both in mammals and in chick and it is found that there are subtle differences. The mammalian domain contains an unanticipated anion binding site which it is proposed may act a reservoir for anions involved in the rapid prestin conformational change. In distinction the chick homolog contains no such site.

There is no full structure, membrane and cytoplasmic units, for prestin. However, there is a complete structure, with transmembrane and STAS units together, for a bacterial member of the SLC26 family. The structure at 3.2Å resolution of SLC26Dg, the facilitator (via bicarbonate) of the proton coupled fumarate symporter, from the bacterium *Deinococcus gethermalis*, reveals that this molecule forms an obligate dimer (Geertsma et al. 2015). The dimeric feature is shared by all members of the SLC26 family and the NLC evidence based on independent manipulation of the two components suggest that the two units do not function independently (Detro-Dassen et al. 2008). Thus structural studies need to identify the nature of interface between the component proteins.

In support of this proposal the most complete image of the mammalian prestin is obtained by negative staining of prestin particles and reconstructed with a fourfold symmetry at a resolution of 2 nm (Mio et al. 2008). The size of the resulting particle is compatible with the e.m. pictures of the OHC lateral membrane, but surprisingly about 60% of the particle mass appears to be outside the

membrane on the cytoplasmic surface. This therefore is where the STAS domains are assembled. It is therefore tempting to suggest that prestin is a dimer of dimers, with the cytoplasmic STAS domain region perhaps forming a plate against which the membrane bound units distort.

Organisation of prestin in the lateral membrane.

The high density of prestin in the OHC lateral membrane is quite surprising. The packing is tight so that the lateral membrane particulate density covers about 60% of the surface. This high packing ensures that such molecular crowding amplifies up any small changes in surface area of the protein. Even so, to act as force generating element it is necessary to ensure that the membrane does not buckle allowing all force generation to be in plane only. OHCs have evolved a submembranous cortical network to ensure that the membrane retains a degree of rigidity (Kalinec et al. 1992).

The question of how membrane associated prestin links to the underlying cytoskeleton remains unresolved. The spacing between the plasma-membrane and the cytoskeleton is about 50 nm, and although structures linking the two, termed 'pillars' and apparent in electron micrographs, the nature of the linkage is unknown. The puzzle is that the prestin related particles outnumber the pillers by an order of magnitude. A particular form of spectrin, βV, forms cytoskeletal meshwork with actin, and further builds up at the same time as prestin insertion. during OHC development (Legendre et al. 2008). Although this spectrin interacts directly with F-Actin and band 4.1, it does not interact with prestin. Tantalisingly, an unidentified component in lysates for mature auditory organs does promotes a prestin-spectrin interaction, wherea other lysates from other tissues do not. It has been suggested that this spectrin isoform, like that of prestin itself, has undergone adaptive evolution from a molecular trafficking network to allow it to provide the molecular support for the OHC mechanisms (Cortese et al. 2017).

Concluding remarks

There is sometimes a perception that now we have a molecule that can be assigned the role of the molecular motor that drives electromotility in OHCs that all is solved. Although it is true many of the low hanging fruits have been picked, there remain a number of nagging questions.

The first is the so called 'RC time constant' problem which arises because membrane of an OHC has an electrical filtering effect on any changes of membrane potential driving the prestin. Although many ingenious solutions have been proposed (see (Corey et al. 2017)), it has proved remarkably difficult technically to study OHCs from the basal high frequency end of the cochlea. Not only are electrical recording bandwidths limited (for patch clamp systems to below 10 kHz) but basal hair cells have proved difficult to handle. A partial answer however may be that basal hair cells have a high ionic channel density and therefore a small electrical time constant (Johnson et al. 2011). It also seems likely that OHCs are not driven exclusively by intracellular potentials, but by transmembrane potential (Mistrik et al. 2009) and so the space around the OHCs in the organ of Corti becomes an essential aspect of the function

The molecular basis for the actuator behaviour of prestin/SLC26A5 remains unclear, although inasmuch as all transporters can be considered as mechano-enzymes, a conformational changeof the molecule with a component in the plane of the membrane seems to be the correct starting point. The reason for the uncertainly is that molecular structure of prestin remains unresolved. It clearly seems to form a tetrameric molecule in the membrane, but how it is trafficked and how the interfaces between the monomers are formed has not so far been addressed. A number of studies have endeavoured to identify the 'motor'motif by swapping motifs in prestin. A span of 11 amino

acids (158-168), near the sulfate transport motif. is sufficient for non-linear capacitance and motility in transfected cells compared with prestins where the region has been swapped out (Tan et al. 2012), whereas chimeric prestins can be constructed which show both non-linear capacitance and transport properties (Schaechinger et al. 2011).

A number of fundamental biophysical challenges in cochlear neurobiology arise from hearing performance at the upper end of the auditory range. What sets the upper limit to mammalian hearing? Is it the maximum operating frequency of the hair cell transduction channels? Or is it a structural problem **of** how to evolve an sufficiently high frequency cochlea? Or is it that the feedback loop we have discussed above has an inherent bandwidth, traceable in part to the properties of prestin/SLC26A5? Some of the answers depend on developing tools to explore ultrafast structural processes. For that, the prestin/SLC26A5 system provides a useful laboratory.

Acknowledgements.

The author is supported by grants from the MRC (MR/K015826), BBSRC (BB/M00659) and the Royal Society.

Figures

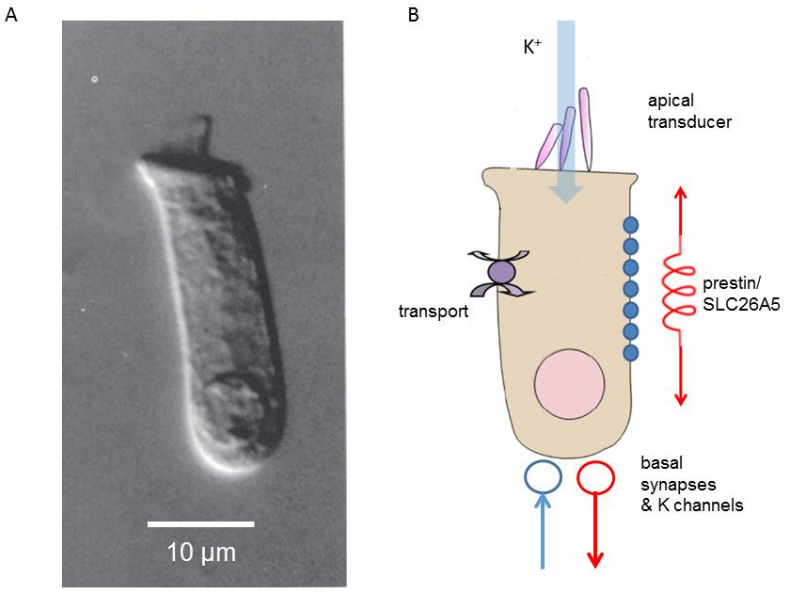

Figure 1: The elements of the mammalian outer hair cell. A) An isolated OHC from turn 2 (approx. 5-10kHz region) of the guinea pig cochlea. The apical stereocilia are apparent at the apical surface. B) Schematic OHC, showing location of prestin/SLC26A5 down basolateral surface, generating longitudinal forces. Transport to regulate pH_i is presumed to be collocated as a parallel property of prestin. K^+ ions from scala media enter through the mechanoelectric transducer channels at the apex and exit through the basally located K^+ channels. Both afferent (red) and efferent (blue) terminals are located at the base of the cell.

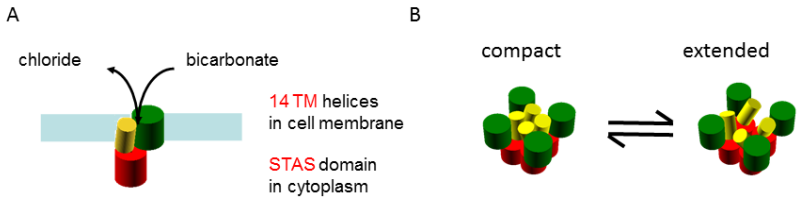

Figure 2 : Models for prestin. A) Organisation of prestin/SLC26A5 in the membrane as a monomer. Two mobile components of the protein are placed in the membrane (Gorbunov et al. 2014; Geertsma et al. 2015) whereas the C-terminal region, containing the STAS domain is cytoplasmic (Mio et al. 2008). B) Hypothetical model for co-assembly into a tetramer. The C-terminal region (red) forms a base against which the oligomer can deform, rapidly shifting between a compact (cell depolarised) and an extended configuration (cell hyperpolarised).

References

Ashmore J. 2008. Cochlear outer hair cell motility. *Physiol Rev* **88**: 173-210.

Ashmore JF. 1987. A fast motile response in guinea-pig outer hair cells: the cellular basis of the cochlear amplifier. *J Physiol* **388**: 323-347.

Bai JP, Moeini-Naghani I, Zhong S, Li FY, Bian S, Sigworth FJ, Santos-Sacchi J, Navaratnam D. 2017. Current carried by the Slc26 family member prestin does not flow through the transporter pathway. *Sci Rep* **7**: 46619.

Bai JP, Surguchev A, Montoya S, Aronson PS, Santos-Sacchi J, Navaratnam D. 2009. Prestin's anion transport and voltage-sensing capabilities are independent. *Biophys J* **96**: 3179-3186.

Beurg M, Tan X, Fettiplace R. 2013. A prestin motor in chicken auditory hair cells: active force generation in a nonmammalian species. *Neuron* **79**: 69-81.

Brownell WE, Bader CR, Bertrand D, de Ribaupierre Y. 1985. Evoked mechanical responses of isolated cochlear outer hair cells. *Science* **227**: 194-196.

Corey DP, O Maoleidigh D, Ashmore JF. 2017. Mechanical Transduction Processes in the Hair Cell. in *Understanding the Cochlea* (eds. GAG Manley, A.W.; Popper, A.N.; Fay, R.R., AW Gummer, AN Popper, RR Fay), pp. 75-111. Springer.

Cortese M, Papal S, Pisciottano F, Elgoyhen AB, Hardelin JP, Petit C, Franchini LF, El-Amraoui A. 2017. Spectrin betaV adaptive mutations and changes in subcellular location correlate with emergence of hair cell electromotility in mammalians. *Proc Natl Acad Sci U S A* **114**: 2054-2059.

Dallos P, Wu X, Cheatham MA, Gao J, Zheng J, Anderson CT, Jia S, Wang X, Cheng WH, Sengupta S et al. 2008. Prestin-based outer hair cell motility is necessary for mammalian cochlear amplification. *Neuron* **58**: 333-339.

Detro-Dassen S, Schanzler M, Lauks H, Martin I, zu Berstenhorst SM, Nothmann D, Torres-Salazar D, Hidalgo P, Schmalzing G, Fahlke C. 2008. Conserved dimeric subunit stoichiometry of SLC26 multifunctional anion exchangers. *J Biol Chem* **283**: 4177-4188.

Franchini LF, Elgoyhen AB. 2006. Adaptive evolution in mammalian proteins involved in cochlear outer hair cell electromotility. *Mol Phylogenet Evol* **41**: 622-635.

Frank G, Hemmert W, Gummer AW. 1999. Limiting dynamics of high-frequency electromechanical transduction of outer hair cells. *Proc Natl Acad Sci U S A* **96**: 4420-4425.

Geertsma ER, Chang YN, Shaik FR, Neldner Y, Pardon E, Steyaert J, Dutzler R. 2015. Structure of a prokaryotic fumarate transporter reveals the architecture of the SLC26 family. *Nat Struct Mol Biol* **22**: 803-808.

Gorbunov D, Sturlese M, Nies F, Kluge M, Bellanda M, Battistutta R, Oliver D. 2014. Molecular architecture and the structural basis for anion interaction in prestin and SLC26 transporters. *Nat Commun* **5**: 3622.

Hallworth R, Nichols MG. 2012. Prestin in HEK cells is an obligate tetramer. *J Neurophysiol* **107**: 5-11.

He DZ, Lovas S, Ai Y, Li Y, Beisel KW. 2014. Prestin at year 14: progress and prospect. *Hear Res* **311**: 25-35.

Johnson SL, Beurg M, Marcotti W, Fettiplace R. 2011. Prestin-driven cochlear amplification is not limited by the outer hair cell membrane time constant. *Neuron* **70**: 1143-1154.

Kalinec F, Holley MC, Iwasa KH, Lim DJ, Kachar B. 1992. A membrane-based force generation mechanism in auditory sensory cells. *Proc Natl Acad Sci U S A* **89**: 8671-8675.

Kavlie RG, Fritz JL, Nies F, Gopfert MC, Oliver D, Albert JT, Eberl DF. 2015. Prestin is an anion transporter dispensable for mechanical feedback amplification in Drosophila hearing. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* **201**: 51-60.

Legendre K, Safieddine S, Kussel-Andermann P, Petit C, El-Amraoui A. 2008. alphaII-betaV spectrin bridges the plasma membrane and cortical lattice in the lateral wall of the auditory outer hair cells. *J Cell Sci* **121**: 3347-3356.

Liberman MC, Gao J, He DZ, Wu X, Jia S, Zuo J. 2002. Prestin is required for electromotility of the outer hair cell and for the cochlear amplifier. *Nature* **419**: 300-304.

Liberman MC, Liberman LD, Maison SF. 2014. Efferent feedback slows cochlear aging. *J Neurosci* **34**: 4599-4607.

Liu XZ, Ouyang XM, Xia XJ, Zheng J, Pandya A, Li F, Du LL, Welch KO, Petit C, Smith RJ et al. 2003. Prestin, a cochlear motor protein, is defective in non-syndromic hearing loss. *Hum Mol Genet* **12**: 1155-1162.

Liu Y, Cotton JA, Shen B, Han X, Rossiter SJ, Zhang S. 2010. Convergent sequence evolution between echolocating bats and dolphins. *Curr Biol* **20**: R53-54.

Lohi H, Kujala M, Kerkela E, Saarialho-Kere U, Kestila M, Kere J. 2000. Mapping of five new putative anion transporter genes in human and characterization of SLC26A6, a candidate gene for pancreatic anion exchanger. *Genomics* **70**: 102-112.

Maison SF, Parker LL, Young L, Adelman JP, Zuo J, Liberman MC. 2007. Overexpression of SK2 channels enhances efferent suppression of cochlear responses without enhancing noise resistance. *J Neurophysiol* **97**: 2930-2936.

Matsunaga T, Morimoto N. 2016. The auditory phenotype of children harboring mutations in the prestin gene. *Acta Otolaryngol* **136**: 397-401.

Mellado Lagarde MM, Drexl M, Lukashkin AN, Zuo J, Russell IJ. 2008. Prestin's role in cochlear frequency tuning and transmission of mechanical responses to neural excitation. *Curr Biol* **18**: 200-202.

Mio K, Kubo Y, Ogura T, Yamamoto T, Arisaka F, Sato C. 2008. The motor protein prestin is a bullet-shaped molecule with inner cavities. *J Biol Chem* **283**: 1137-1145.

Mistrik P, Daudet N, Morandell K, Ashmore JF. 2012. Mammalian prestin is a weak Cl-/HCO3- electrogenic antiporter. *J Physiol-London* **590**: 5597-5610.

Mistrik P, Mullaley C, Mammano F, Ashmore J. 2009. Three-dimensional current flow in a large-scale model of the cochlea and the mechanism of amplification of sound. *J R Soc Interface* **6**: 279-291.

Moreau A, Gosselin-Badaroudine P, Delemotte L, Klein ML, Chahine M. 2015. Gating pore currents are defects in common with two Nav1.5 mutations in patients with mixed arrhythmias and dilated cardiomyopathy. *J Gen Physiol* **145**: 93-106.

Oliver D, Fakler B. 1999. Expression density and functional characteristics of the outer hair cell motor protein are regulated during postnatal development in rat. *J Physiol* **519 Pt 3**: 791-800.

Oliver D, He DZ, Klocker N, Ludwig J, Schulte U, Waldegger S, Ruppersberg JP, Dallos P, Fakler B. 2001. Intracellular anions as the voltage sensor of prestin, the outer hair cell motor protein. *Science* **292**: 2340-2343.

Rybalchenko V, Santos-Sacchi J. 2003. Cl- flux through a non-selective, stretch-sensitive conductance influences the outer hair cell motor of the guinea-pig. *J Physiol* **547**: 873-891.

Santos-Sacchi J, Navratanam D, Raphael R, Oliver D. 2017. Prestin: Molecular mechanisms underlying outer hair cells electromotility. in *Understanding the Cochlea* (eds. G Manley, A Gummer, A Popper, R Fay), pp. 113-145. Springer.

Schaechinger TJ, Gorbunov D, Halaszovich CR, Moser T, Kugler S, Fakler B, Oliver D. 2011. A synthetic prestin reveals protein domains and molecular operation of outer hair cell piezoelectricity. *EMBO J* **30**: 2793-2804.

Schaechinger TJ, Oliver D. 2007. Nonmammalian orthologs of prestin (SLC26A5) are electrogenic divalent/chloride anion exchangers. *Proc Natl Acad Sci U S A* **104**: 7693-7698.

Schanzler M, Fahlke C. 2012. Anion transport by the cochlear motor protein prestin. *J Physiol* **590**: 259-272.

Takahashi S, Cheatham MA, Zheng J, Homma K. 2016. The R130S mutation significantly affects the function of prestin, the outer hair cell motor protein. *J Mol Med (Berl)* **94**: 1053-1062.

Tan X, Pecka JL, Tang J, Lovas S, Beisel KW, He DZ. 2012. A motif of eleven amino acids is a structural adaptation that facilitates motor capability of eutherian prestin. *J Cell Sci* **125**: 1039-1047.

Tang HY, Xia A, Oghalai JS, Pereira FA, Alford RL. 2005. High frequency of the IVS2-2A>G DNA sequence variation in SLC26A5, encoding the cochlear motor protein prestin, precludes its involvement in hereditary hearing loss. *BMC Med Genet* **6**: 30.

Toth T, Deak L, Fazakas F, Zheng J, Muszbek L, Sziklai I. 2007. A new mutation in the human pres gene and its effect on prestin function. *Int J Mol Med* **20**: 545-550.

Weddell TD, Mellado-Lagarde M, Lukashkina VA, Lukashkin AN, Zuo J, Russell IJ. 2011. Prestin links extrinsic tuning to neural excitation in the mammalian cochlea. *Curr Biol* **21**: R682-683.

Weisz C, Glowatzki E, Fuchs P. 2009. The postsynaptic function of type II cochlear afferents. *Nature* **461**: 1126-1129.

Zheng J, Du GG, Anderson CT, Keller JP, Orem A, Dallos P, Cheatham M. 2006. Analysis of the oligomeric structure of the motor protein prestin. *J Biol Chem* **281**: 19916-19924.

Zheng J, Shen W, He DZ, Long KB, Madison LD, Dallos P. 2000. Prestin is the motor protein of cochlear outer hair cells. *Nature* **405**: 149-155.