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Review

Prestin at year 14: Progress and prospect

David Z.Z. He [a,b,*], Sándor Lovas [a], Yu Ai [c], Yi Li [a,d], Kirk W. Beisel [a]

[a] Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE 68175, USA
[b] Neuroscience Center, Ningbo University School of Medicine, Ningbo 315211, China
[c] Department of Otolaryngology, Shandong Provincial Hospital, Jinan 250021, PR China
[d] Department of Otolaryngology, Beijing Tongren Hospital, Beijing 100730, PR China

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ABSTRACT

Prestin, the motor protein of cochlear outer hair cells, was identified 14 years ago. Prestin-based outer hair cell motility is responsible for the exquisite sensitivity and frequency selectivity seen in the mammalian cochlea. Prestin is the 5th member of an eleven-member membrane transporter superfamily of SLC26A proteins. Unlike its paralogs, which are capable of transporting anions across the cell membrane, prestin primarily functions as a motor protein with unique capability of performing direct and reciprocal electromechanical conversion on microsecond time scale. Significant progress in the understanding of its structure and the molecular mechanism has been made in recent years using electrophysiological, biochemical, comparative genomics, structural bioinformatics, molecular dynamics simulation, site-directed mutagenesis and domain-swapping techniques. This article reviews recent advances of the structural and functional properties of prestin with focus on the areas that are critical but still controversial in understanding the molecular mechanism of how prestin works: The structural domains for voltage sensing and interaction with anions and for conformational change. Future research directions and potential application of prestin are also discussed.

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1. Outer hair cell motility

In 1985, Brownell and colleagues discovered that isolated outer hair cells (OHCs) from the guinea pig cochlea were able to change their length when the cells were electrically stimulated by passing extracellular current along the cell: Hyperpolarization of the membrane led to elongation of the cell and depolarization resulted in cell contraction (Brownell et al., 1985; Kachar et al., 1986). Subsequently, either “motility” or “electromotility” is used to describe such change in the length of cells. Immediately after motility was discovered, understanding its mechanism and role in cochlear mechanics has quickly become one of the most exciting areas in auditory research for two obvious reasons: First, motility was rapidly recognized as a potential mechanism for cochlear amplification, the phrase that was originally coined by Davis (1983); Second, OHC motility appeared to be operated on a novel mechanism that was completely different from conventional energy-dependent, actin-myosin-based contraction in muscle cells (Brownell et al., 1985; Kachar et al., 1986; Ashmore, 1987; Holley and Ashmore, 1988). The magnitude of motility is close to 4% of the cell length and the motile response is contraction-asymmetric and nonlinear with saturation at the directions of contraction and elongation (Ashmore, 1987; Evans et al., 1989; Santos-Sacchi, 1989; Hallworth et al., 1993). The maximal motility sensitivity (slope) is approximately 20 nm/mV (Ashmore, 1987; Santos-Sacchi, 1989). Motility is driven by transmembrane voltage instead of transmembrane current (Santos-Sacchi and Dilger, 1988; Iwasa and Kachar, 1989) and can be blocked by gadolinium and salicylate ions (Santos-Sacchi, 1991; Shehata et al., 1991; Tunstall et al., 1995). Motile responses of OHCs are accompanied by charge movement, which is reflected in nonlinear capacitance (NLC) (Ashmore, 1989; Santos-Sacchi, 1991), akin to the translocation of gating charges of voltage-gated ion channels (Armstrong and Bezanilla, 1977). NLC of OHCs is characterized by a bell-shaped dependence on membrane potential, with a peak between −70 and −30 mV (Santos-Sacchi, 1991). Further experiments suggested that motility is associated with structures in the lateral wall of the OHCs (Ashmore,

Abbreviations: 3D, three-dimensional; FRET, fluorescence resonance energy transfer; IHC, inner hair cell; MD, molecular dynamics; NLC, nonlinear capacitance; OHC, outer hair cell; SulTP, sulfate transporter; STAS, sulfate transporter and antisigma-factor antagonist; TM, transmembrane

* Corresponding author. Department of Biomedical Sciences, Creighton University School of Medicine, 2500 California Plaza, Omaha, NE 68178, USA. Tel.: +1 4022801409.
E-mail address: hed@creighton.edu (D.Z.Z. He).

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1987; Holley and Ashmore, 1988; Huang and Santos-Sacchi, 1994). The strongest evidence for a plasma membrane-based mechanism came from experiments where motility was still detectable from cells after their cellular content was degradated by internal tryptic digest (Kalinec et al., 1992). Furthermore, patches of membrane withdrawn into the patch pipette responded to hyperpolarized and depolarized voltage change with increases and decreases in membrane area. These lines of evidence strongly suggest that the force generation mechanism is driven by voltage-dependent conformational changes of a molecular motor in the plasma membrane. In fact, Gulley and Reese (1977) already showed that the basolateral wall of OHCs contained a high density of intramembrane particles. These densely populated particles covered as much as 70% of the surface area on plasma membrane. The diameter of the particles was between 12 and 15 nm and the density was as high as 6000 particles per square micrometer (Forge, 1991; He et al., 2010).

A distinct feature of OHC motility is its high speed oscillation. Measurements made from isolated OHCs or from membrane patches both show that the electromotile response occurs at microsecond time scale and works in a cycle-by-cycle mode up to a frequency of 20 kHz (Dallos and Evans, 1995; Gale and Ashmore, 1997; Frank et al., 1999). Although it is still controversial whether cycle-by-cycle OHC motility can occur at high frequencies *in vivo* due to the low-pass filter characteristic of the OHC basolateral membrane (Santos-Sacchi, 1992), it is quite certain that the motor itself has the ability to change conformation at high rate. It was also shown that voltage-driven motility is accompanied by a voltage-dependent change in axial stiffness (He and Dallos, 1999, 2000), although voltage-dependent stiffness was not observed in another study (Hallworth, 2007). Voltage change can modulate cell stiffness over a range of about 10-fold and the overall stiffness of OHCs is reduced to ~1/3 of its normal value when motility is blocked (He et al., 2003). The force produced by a guinea pig OHC ranged from 20 to100 pN/mV (Hallworth, 1995; Iwasa and Adachi, 1997). Finally, OHCs demonstrate piezoelectric properties similar to a piezoelectric transducer (Iwasa, 1993; Gale and Ashmore, 1994; Ludwig et al., 2001; He et al., 2010). The efficiency of conversion from mechanical force to electrical charge is estimated to be ~20 fC nN^{-1}, four orders of magnitude greater than the efficiency of the best man-made piezoelectric material (Dong et al., 2002).

2. Discovery of prestin

Two pieces of information before the year of 2000 played an important role in designing strategies to identify the elusive motor protein: First, electromotility is unique to OHCs, while inner hair cells (IHCs) are not electromotile; Second, the expression of electromotility is functionally detectable in gerbil OHCs starting from 6 to 7 days after birth (He et al., 1994; He, 1997). The onset of motility coincides with a significant increase in density of intramembrane particles in neonatal gerbil OHCs (Souter et al., 1995). These two lines of evidence indicate that the motor protein expression occurs after birth in altricial rodents. Two thousand IHCs and OHCs were isolated from gerbil cochleae and cDNA libraries were constructed for each cell type. An OHC subtracted cDNA library was subsequently produced to identify genes preferentially expressed in OHCs. Fifteen distinct genes were identified. Of these, one corresponded to an open reading frame of a protein containing 744 amino acids with a molecular mass of 81.4 kDa. The ontogenic expression of this cDNA was consistent with development of motility and intramembrane particles. When expressed in human embryonic kidney TSA201 cells, the resulting protein reproduced all hallmarks of the motor protein including voltage-dependent charge movement and cell motility (Zheng et al., 2000). The protein was named “prestin” to reflect the distinct feature of its ability to change conformation at high rate (prestin is from the presto musical notation). Antibodies generated against prestin detected prestin along the basolateral membrane of OHCs, showing a developmental expression pattern coinciding with the development of NLC and motility (Belyantseva et al., 2000). Subsequent experiments using prestin-null mice confirmed that targeted deletion of prestin resulted in loss of OHC electromotility and 40–60 dB loss of cochlear sensitivity (Liberman et al., 2002). Deletion of prestin also led to loss of voltage-dependent stiffness and piezoelectrical property of OHCs as well as significant reduction of the density of intramembrane particles in the plasma membrane (He et al., 2010). Taken together, all the evidence confirms that prestin indeed is the motor protein of cochlear OHCs.

3. Prestin structure

Prestin shares the overall domain structure of the SLC26A protein family: a highly conserved central core of hydrophobic amino acids (~400 amino acid residues) with the N-terminal (~100 amino acid residues) and C-terminal (~240 amino acid residues) located in the cytoplasmic side of the plasma membrane (Fig. 1). The sulfate transporter (SulTP) signature sequence is in the hydrophobic core, while a STAS domain (amino acid residues 510–710) with multiple clusters of charged residues is located in the C-terminal region (Oliver et al., 2001; Zheng et al., 2001). Analysis of the structure of prestin with a number of topology prediction programs all indicates that the molecule has an even number of transmembrane (TM) domains. Based on immunocytochemical techniques using antibodies against different intracellular and extracellular domains of prestin and using hemagglutinin tag, a 12 TM domain topology model was proposed (Oliver et al., 2001; Zheng et al., 2001). However, Navaratnam and colleagues showed that prestin has only 10 TM domains based on their immunocytochemistry studies (Navaratnam et al., 2005). The main difference between the two topology models lies in the TM domains 5, 6, 7, and 8. Amino acids in the SulTP domain are almost completely identical among human, mouse, rat and gerbil prestins (only one amino acid is different). Sequence variations among mammalian species are primarily restricted to both the N- (residues 1–65) and C- (residues 516–744) termini with the C-end exhibiting the greatest variation. A relatively high amino acid sequence similarity is also shared by other mammalian species, including the prototherian platypus and the metatherian opossum (Okoruwa et al., 2008).

The STAS domain shares 10–15% sequence similarity with bacterial anti-sigma factor antagonists such as SpoIIAA of *Bacillus subtilis*. The STAS domain is conserved within related SLC26 gene family members including prestin. The STAS domain has a few structural features of interest that are based on the sequence alignment (Aravind and Koonin, 2000) and crystal/solution structures of SpoIIAA (Kovacs et al., 1998; Matsuda et al., 2004): it consists of four β-strands, five α-helices, a highly conserved loop that contains G/ATP binding sites and serine phospohorylation sites, and a variable loop. The G/ATP binding sites are consistent with the role of intracellular ATP in eliciting conformational changes (Aravind and Koonin, 2000). The serine residue in the conserved loop, a target for kinases and phosphotases for critical regulation of activity of SpoIIAA, is also present in the SLC26-associated STAS domain.

Experiments were performed to examine the importance of the N- and C-termini in prestin function. Deletion of either the N- or C-terminal region was shown to cause loss of membrane expression. There is conflicting evidence of whether the loss of function in truncated proteins is due to a lack of membrane insertion (Zheng