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Review

The tip-link molecular complex of the auditory mechano-electrical transduction machinery

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ABSTRACT

Sound waves are converted into electrical signals by a process of mechano-electrical transduction (MET), which takes place in the hair bundle of cochlear hair cells. In response to the mechanical stimulus of the hair bundle, the tip-links, key components of the MET machinery, are tensioned and the MET channels open, which results in the generation of the cell receptor potential. Tip-links are composed of cadherin-23 (Cdh23) and protocadherin-15 (Pcdh15), both non-conventional cadherins, that form the upper and the lower part of these links, respectively. Here, we review the various Pcdh15 isoforms present in the organ of Corti, their localization in the auditory hair bundles, their involvement in the molecular complex forming the tip-link, and their interactions with transmembrane molecules that are components of the lower MET machinery.

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1. Introduction

Auditory mechano-electrical transduction (MET), the conversion of the sound-evoked mechanical stimulus into a membrane receptor potential, takes place in hair cells in the sensory epithelium of the cochlea. In mammals, this epithelium is known as the organ of Corti and consists of one row of inner hair cells (IHCs, the genuine sensory cells), three rows of outer hair cells (OHCs, frequency-tuned mechanical amplifiers), and various types of supporting cells. The MET machinery of the hair cells is located in the hair bundle, an ensemble of stiff microvilli organized in three rows of graded heights. The tip-links, key components of this machinery, are oblique fibrous links that connect the apexes of small and middle row stereocilia and the sides of the taller adjacent stereocilia (see Fig. 1). Mechanical stimulation of the hair bundle in the direction of the tall stereocilia tensions these links, which increases the opening probability of the MET channels (of still largely unknown molecular composition), at the lower tip-link insertion point (Beurg et al., 2009). Two Ca^{++}-dependent transmembrane adhesion proteins, protocadherin-15 (Pcdh15) and cadherin-23 (Cdh23) form the lower and upper parts of this link, respectively (Ahmed et al., 2006; Kazmierczak et al., 2007; Siemens et al., 2004). In transmission electron micrographs of stereocilia, there are electron dense areas at the upper and at the lower insertion points of the tip-link (Furness and Hackney, 1985). These densities presumably contain the proteins of the MET machinery that connect the tip-link to the actin cytoskeleton. Here, we review how the identification of the genes responsible for type 1 Usher syndrome (USH1, which associates congenital deafness, vestibular dysfunction, and prepubertal onset retinitis pigmentosa) unveiled several essential

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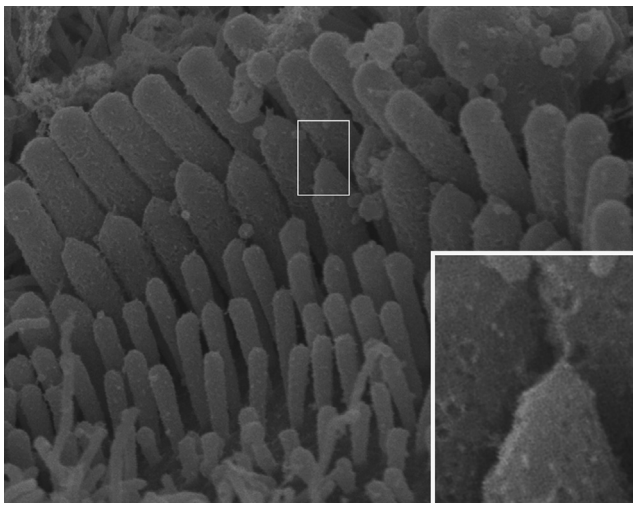

Fig. 1. Tip-links. Tip-links stretch from the apexes of small and middle row stereocilia to the side of the taller adjacent stereocilia (image courtesy of Vincent Michel).

components of the MET machinery. In particular, we will focus on Pcdh15 that makes up the lower part of the tip-link.

2. The tip-link

In the gating-spring model of MET, modulation of the tension of the tip-link changes the opening probability of the MET channels (Assad et al., 1991; Corey and Hudspeth, 1983). The tip-link itself is unlikely to form the spring, so elasticity is thought to be provided by element(s) connected to the tip-link (Sotomayor et al., 2005). Transmission electron microscopy (TEM) reveals these links as amorphous filaments, 5 nm in diameter and 150–300 nm in length (Furness and Hackney, 1985; Pickles et al., 1984). Freeze-etch images indicate that this link is formed by intertwined filaments (Kachar et al., 2000). The biochemical properties of Pcdh15 and Cdh23 and immunogold TEM identified these proteins as tip-link components (Ahmed et al., 2006; Kazmierczak et al., 2007; Siemens et al., 2004). Pcdh15 forms the lower part of the tip-link and Cdh23 forms the upper part in mammalian hair bundles. The extracellular domains of Cdh23 and Pcdh15 form parallel homodimers *in vitro*; homodimers of Pcdh15 interact with homodimers of Cdh23 in an antiparallel manner, and this interaction is Ca^{++}-dependent. The resulting complex *in vitro* is ~180 nm, which is in good agreement with the observed length of the tip-links. These observations led to the conclusion that in mammalian hair bundles the tip-link is formed by cis-homodimers of Pcdh15 that interact in trans with cis-homodimers of Cdh23 (Kazmierczak et al., 2007). This was confirmed in avian vestibular hair bundles (Goodyear et al., 2010). Electrophysiological measurements of MET currents upon EGTA-induced tip-link disruption, showed inhibition of tip-link recovery by application of exogenous Pcdh15 or Cdh23 fragments encompassing the entire extracellular regions; this confirmed that tip-links are indeed composed of these two proteins (Lelli et al., 2010). Indzhykulian et al. used backscatter scanning electron microscopy (SEM) to show that after tip-link disruption, both the lower and the upper part of the newly formed tip-link transiently consist of Pcdh15 only, and that the Pcdh15 at the upper part of the tip-link is subsequently replaced by Cdh23 (Indzhykulian et al., 2013). Cdh23 and Pcdh15 are non-conventional cadherins lacking the N-terminal tryptophan residue involved in canonical cadherin interactions. Crystallographic studies showed that the complex containing the first and second ectocadherin (EC) repeats of both Pcdh15 and Cdh23 forms an overlapping heterodimer (Sotomayor et al., 2012).

Three other proteins encoded by genes responsible for genetic forms of USH1 are components of the tip-link complex: harmonin, sans and myosin-VIIa.

Harmonin is a PDZ domain-containing scaffold protein that interacts directly with the cytoplasmic region of Cdh23 *in vitro* (Bahloul et al., 2010; Boeda et al., 2002; Pan et al., 2009). Q3 Harmonin-b, an isoform of harmonin with F-actin bundling activity (Boeda at al., 2002), is present at the tip-link upper insertion point (Grillet et al., 2009; Michalski et al., 2009), where it is believed to bridge between the cytoplasmic region of Cdh23 and the stereocilia actin core. Consistent with this view, recordings of the MET currents in mutant mice defective for the harmonin-b isoform only, showed its involvement in the process of MET adaptation (Michalski et al., 2009). Both sans and myosin-VIIa have also been detected at the tip-link upper insertion point (Grati and Kachar, 2011), and Cdh23, myosin-VIIa, and harmonin can form a tripartite complex *in vitro* (Bahloul et al., 2010).

The tip-link lower insertion point is where the Pcdh15 molecules making up the tip-link penetrates the membrane. Sans has been found at this site in immature hair bundles (Caberlotto et al., 2011). Conditional knockout of the gene, leading to the disappearance of sans after the full morphogenesis of the hair bundle (see below), demonstrated that sans is an essential component of the MET machinery: although the tip-link forms in the absence of sans, it cannot persist (Caberlotto et al., 2011). The direct involvement of myosin-VIIa in the function of the MET machinery has not yet been demonstrated.

3. Protocadherin-15

3.1. Pcdh15 isoforms

Twenty-four different Pcdh15 transcripts resulting from alternative splicing have been identified in the organ of Corti, and classified as CD1, CD2, CD3, or SI according to the presence of the following exons: exon 35 (CD1), exon 38 (CD2), exon 39 (CD3), or exon 26a (SI) (Ahmed et al., 2001, 2006; Haywood-Watson et al., 2006). Most CD1, CD2 and CD3 transcripts encode predicted integral membrane proteins, consisting of (up to) 11 EC repeats, a single transmembrane domain, and a cytoplasmic domain with a C-terminal PDZ-binding motif (PBM). A few transcripts classified as CD1, CD2 or CD3 according to this nomenclature are not predicted to encode transmembrane proteins, and are therefore unlikely to form the tip-links. The 24 transcripts of *Pcdh15* and predicted encoded proteins classified as transmembrane, cytosolic, or secreted proteins are presented in Fig. 2. It is not known whether all the protein isoforms predicted by transcriptomic analysis are indeed produced.

3.2. Distribution of Pcdh15 isoforms in the cochlear hair bundles

Pcdh15-CD1 was initially described in the ankle link region of immature IHC and OHC hair bundles and all along the stereocilia in mature IHCs (Senften et al., 2006). Later, when the three different splice isoforms were reported, their distribution was analyzed using isoform-specific antibodies directed against the sequences encoded by the last exon of the corresponding transcript (exon 35, 38, and 39 for Pcdh15-CD1, Pcdh15-CD2, and Pcdh15-CD3, respectively). In the immature organ of Corti, the Pcdh15-CD2 immunoreactivity was stronger at the cochlear apex than at the cochlear base, whereas for Pcdh15-CD1 and Pcdh15-CD3, the intensity of labeling followed the maturation gradient (the labeling was more intense in the more mature hair cells towards the

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