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The role of G protein-coupled receptors in cochlear planar cell polarity

Jinpeng Sun[a,*], Daolai Zhang[a], Yanfei Wang[b], Hal Lin[c], Xiao Yu[a], Zhigang Xu[b,**]

[a] Key Laboratory Experimental Teratology of the Ministry of Education and Department of Biochemistry and Molecular Biology, Shandong University School of Medicine, Jinan 250012, Shandong, China
[b] Shandong Provincial Key Laboratory of Animal Cells and Developmental Biology, Shandong University School of Life Sciences, Jinan 250100, Shandong, China
[c] Department of Medicine, School of Medicine, Duke University, Durham, NC 27705, USA

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ABSTRACT

Planar cell polarity (PCP) is defined as the coordinated alignment of cell polarity across the tissue plane, which is important for the integration of cells into tissues. One of the best examples of PCP is in the cochlear epithelium. Several core PCP proteins have been identified to play important roles in PCP regulation, in which these proteins form complexes and associate with the cell membrane asymmetrically, mediating intercellular PCP signal transduction. Among the core PCP proteins are two G protein-coupled receptors (GPCRs), *Celsr* and *Frizzled*, both of which have been shown to play important roles in cochlear PCP regulation. *Celsr* and *Frizzled* genes are expressed in the cochlear sensory epithelium, and Frizzled1, 2, 3 and 6 show asymmetric localizations on the cell membrane of hair cells or supporting cells. In the animal model, *Celsr1*, *Frizzled2* and *Frizzled3*/*6* mutant or knockout mice have profound cochlear PCP deficits. Downstream of GPCR signaling, Gαi was shown to asymmetrically localize on the apical surface of hair cells, together with LGN and mInsc, Gαi controls cochlear PCP in a cell-autonomous way. Inactivity of Gαi, LGN or mInsc results in PCP deficits in the mouse cochlea. We hypothesize that GPCR-Gαi coupling plays a pivotal role in cochlear PCP regulation via connecting the intercellular PCP signals with cell-autonomous PCP machinery. Further investigations are needed to fully understand the mechanism of cochlear PCP regulation.

1. Introduction

The coordinated alignment of cell polarity across the tissue plane, namely planar cell polarity (PCP), is important for the integration of cells into tissues. First identified in *Drosophila* to control hair orientation on the wing, PCP is largely similar across species, regulating the alignment of fish scales, bird feathers, mammalian inner ear hair cells, etc. (Devenport, 2014; Gubb and García-Bellido, 1982). Mice carrying mutations in PCP regulating proteins exhibit defects in multiple tissues, ranging from axonal growth, hair pattern, eyelid closure, neural tube closure, to cochlear hair cell orientation. Among these tissues, the inner ear cochlear epithelium becomes one of the most popular systems for PCP study because of its unique polarized tissue organization.

During last several decades, researchers have identified quite a few proteins that are involved in PCP regulation. The most important PCP regulators are referred to as "core PCP proteins," which are observed in organisms ranging from insects to mammals (Table 1). Noticeably, among the core PCP proteins are *Flamingo*/*Celsr* and *Frizzled*, which are members of the G protein-coupled receptor (GPCR) protein family known for the sensing of information from background environment (Adler et al., 1990; Curtin et al., 2003; Usui et al., 1999; Vinson et al., 1989; Wang et al., 2006b). GPCRs are comprised of seven transmembrane proteins, which transduce extracellular stimuli into intracellular responses through activating trimeric G proteins and arrestins. Thus, they might play pivotal roles in mediating cell-to-cell communications that define cell polarity. Based on phylogenetic analysis, GPCRs are divided into five subgroups, namely *Rhodopsin*, *Secretin*, *Adhesion*, *Glutamate* and *Frizzled*/*Taste2* (Fredriksson et al., 2003). *Flamingo*/*Celsr* belongs to the *Adhesion* subgroup, whereas *Frizzled* belongs to the *Frizzled*/*Taste2* subgroup.

In this review, we will focus on mammalian cochlear PCP and summarize the current knowledge on the role of *Flamingo*/*Celsr* and *Frizzled* in this process. We will also discuss the possible involvement of GPCR signaling pathways in cochlear PCP regulation, which may provide a new direction for future researches in this field.

* Corresponding author. Tel.: +011 86 15966613591.
** Corresponding author.
E-mail addresses: sunjinpeng@sdu.edu.cn (J. Sun), xuzg@sdu.edu.cn (Z. Xu).

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Table 1
The core PCP proteins are conserved in *Drosophila* and mammals. Core PCP proteins were first identified in Drosophila, and each of them has more than one counterparts in mammals.

Drosophila	Mammals	Subcelular localization	PCP phenotypes in mutant mice
Fizzled (Fz)	Frizzled1-10 (Fz1-10)	Transmembrane	Neural tube closure, hair patterning, cochlear hair cell orientation
Van Gogh (Vang)/ Strabismus (Stbm)	Van Gogh-like1,2 (Vangl1,2)	Transmembrane	Neural tube closure, L-R symmetry, cochlear hair cell orientation
Stary night (Stan)/Flamingo (Fmi)	Celsr1-3	Transmembrane	Neural tube closure, ependymal ciliogenesis, cochlear hair cell orientation
Dishevelled (Dsh)	Dishevelled1-3 (Dvl1-3)	Cytosolic	Neural tube closure, cochlear hair cell orientation
Prickle (Pk)/Spiny leg (Sple)	Prickle-like1-3 (Pk1-3)	Cytosolic	Neural tube closure, epiblast apical-basal polarity
Diego (Dgo)	Inversin, Diversin	Cytosolic	Cystic kidney, L-R symmetry, cochlear hair cell orientation

2. PCP in mammalian cochlear epithelia

About a decade ago, the inner ear epithelia, especially the cochlear epithelia, became one of the most popular models in studying PCP in mammals (Curtin et al., 2003; Montcouquiol et al., 2003). In contrast to most tissues mentioned above, which mainly consist of homogeneous cells, the mammalian inner ear epithelia are made up of sensory and supporting cells of distinct shape and size. In the cochlear epithelia, a single row of inner hair cells (IHC) and three rows of outer hair cells (OHC1-3) are separated by several types of supporting cells, including inner phalangeal cells (Iph), inner and outer pillar cells (Ipc and Opc), and Deiter cells (Dc1-3) (Fig. 1A). All these cells are apico-basally polarized; the best example is hair cells, which are equipped with hair bundles on the apical surface and synapses at the basal pole. The hair bundles consist of one single microtubule-based kinocilium and hundreds of actin-based stereocilia arranged in precise rows of increasing height, forming a staircase pattern. All the V- or W-shaped hair bundles point in the abneural (lateral) direction, hence establishing the PCP in cochlear epithelia. The microtubule-based kinocilium, which degenerates later during development in cochlear hair cells, is believed to be important for hair cell PCP.

Six core PCP proteins have been identified in *Drosophila*, including three transmembrane proteins (*Frizzled*, *Van Gogh* and *Flamingo*) and three cytoplasmic proteins (*Dishevelled*, *Prickle* and *Diego*). It's believed that under the direction of extracellular clues, transmembrane core PCP proteins localize to the cell membrane asymmetrically, which is then stabilized by cytoplasmic core PCP components as well as other proteins. This asymmetric distribution of core PCP proteins, in turn, changes the cell cytoskeleton and morphology through affecting the activity and/or localization of regulators of the cytoskeleton. Core PCP proteins are well conserved during evolution, and each of them has more than one counterpart in mammals (Table 1). Many core PCP proteins (such as *Vangl2*, *Diversin*, *Dvl2* and *Fz3/6*) have been shown to localize in mammalian inner ear epithelia asymmetrically (Fig. 1B), and the malfunction of these proteins results in severe cochlear PCP phenotype changes (Curtin et al., 2003; Montcouquiol et al., 2003; Wang et al., 2005, 2006b; Yu et al., 2010).

Besides core PCP proteins, several other PCP-related proteins have been identified, such as *Scrb1*, *PTK7*, *Cthrc1*, *Ror2*, *Fat4*, *Smurf1/2*, *Sec24b*, and *Dchs1*. Usually these proteins neither associate with the core PCP protein complex, nor asymmetrically localize in the cells. Malfunction of these proteins often result in mild PCP phenotype changes (including cochlear PCP phenotype) (Lu et al., 2004; Mao et al., 2011; Merte et al., 2010; Montcouquiol et al., 2003; Narimatsu et al., 2009; Saburi et al., 2008; Wansleeben et al., 2010; Yamamoto et al., 2008).

3. *Frizzled* and *Celsr* localization in cochlear epithelia cells

The asymmetric localizations of core PCP proteins are important for their functions in PCP regulation. However, as mentioned above, the mammalian inner ear epithelia consist of sensory and supporting cells of distinct shape and size, which hampered the

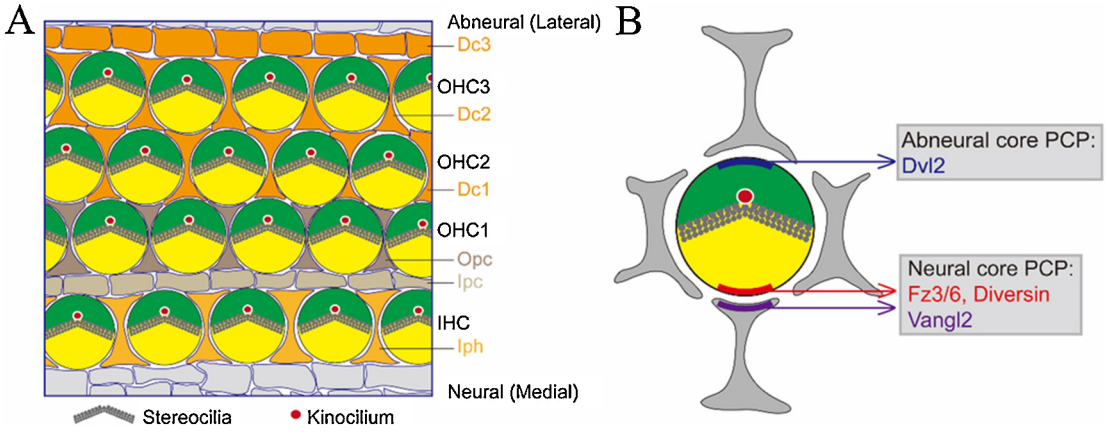

Fig. 1. Schematic drawing of cochlear PCP and asymmetric localizations of core PCP proteins in hair/supporting cells. (A) Schematic drawing of cochlear PCP. Cochlear sensory epithelium consists of a mosaic of hair cells and supporting cells. Hair cells include a single row of inner hair cells (IHC) and three rows of outer hair cells (OHC1-3). Supporting cells include inner phalangeal cells (IPh), inner and outer pillar cells (Ipc, Opc), and Deiter cells (Dc1-3). On the apical surface of the hair cells lies the hair bundle, which consists of the actin-based stereocilia (dark gray) and the tubulin-based kinocilium (red). (B) Schematic drawing of asymmetric localizations of core PCP proteins in hair/supporting cells in the cochlea. Dishevelled2 (Dlv2, blue) localize on the abneural side of hair cells. Frizzled3/6 (Fz3/6) and Diversin (red) localize on the neural side of hair cells. Van Gogh-like 2 (Vanl2, purple) localize on the abneural side of supporting cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)