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Mouse models for dissecting vertebrate planar cell polarity signaling in the inner ear

Maria F. Chacon-Heszele and Ping Chen*

Department of Cell Biology, Emory University, 615 Michael St., Atlanta, GA 30322, USA

Abstract

Planar cell polarity (PCP) refers to coordinated polarization of cells in the plane of a cell sheet. In *Drosophila*, the stereotypical arrangement of the eight photoreceptor cells in each of the ommatidia of the fly compound eye and the uniform orientation of the hairs in all the wing cells are two representative forms of PCP. Using these powerful *Drosophila* model systems, a set of genes was identified to constitute the invertebrate PCP signaling pathway. In vertebrates, the inner ear sensory organs display distinctive forms of PCP. In particular, the auditory sensory organ in the cochlea, adorned with precisely patterned sensory hair cell arrays and uniformly oriented hair bundles, has served as an excellent model system to complement other vertebrate PCP models and has illustrated a genetic pathway that consists of genes conserved from the *Drosophila* model as well as genes uniquely required for vertebrate PCP regulation. This review will focus on the mouse models that have made valuable contributions to our current understanding of PCP signaling in the vertebrates.

Keywords

Hair cell; Stereocilia; Primary cilia; Convergent extension; PCP gene; Wnt

1. Introduction

The mammalian inner ear consists of one sensory organ, the organ of Corti, in the cochlea for audition and five sensory organs, the saccular and utricular maculae and three perpendicularly positioned cristae, in the vestibule for balance (Fig. 1A). These sensory organs share a common type of sensory cells, known as the hair cells, each of which projects microvilli-derived stereocilia and a single primary cilium to make up a hair bundle on its apical surface (Fig. 1B). In the cochlea, the stereocilia of a hair bundle are arranged in order of increasing height toward the edge of the apical cortex to form a “V”-shaped structure with a single primary cilium, known as the kinocilium, placed near the tallest stereocilia at the vertex of the “V”-shaped stereociliary bundle (Fig. 1B). Hence, each hair cell is intrinsically polarized, and its polarity is displayed by the orientation of the stereociliary bundle and the position of the kinocilium. In addition, hair cells are precisely patterned into one row of inner and three rows of outer hair cells interdigitated with nonsensory supporting cells along

the longitudinal axis of the cochlear duct. The vertices of all the hair bundles point toward the periphery of the cochlear spiral, manifesting a distinct form of planar cell polarity (PCP) parallel to the plane of the epithelial sheet (Fig. 1B). The vestibular sensory organs also display PCP. Hair cells are polarized and are oriented either uniformly or in opposite directions across a line of polarity reversal within each of the vestibular sensory epithelia (Fig. 1C).

The generation of PCP in any tissue requires symmetry breaking in individual cells to direct the formation of intrinsically polarized structures unique for each cell type, and cell–cell communication to ensure coordinated orientation of all the cells across the field. Genetic studies in *Drosophila* identified a set of genes, including Flamingo (*fmi*, also known as Starry night), Strabismus (*stbm*) or Vang-gogh (*vang*), Prickle (*pk*), trimeric G protein Gα0, Fat (*ft*), Wnt receptor Frizzled (*fzd*), Dishevelled (*dsh*), Diego (*dgo*), Dachous (*ds*), Four-jointed (*fj*), Inturned (*in*), Fuzzy (*fy*), and Fritz (*frtz*), for PCP regulation in adult tissues of the fly (Axelrod and McNeill, 2002; Seifert and Mlodzik, 2007; Simons and Mlodzik, 2008; Strutt, 2008). These genes constitute regulatory modules that signal sequentially to integrate directional cues with cellular factors and to direct coordinated polarization of cells. It is known that Fzd-containing (Dsh, Dgo, Fmi) and Stbm/Vang-containing (Pk and Fmi) membrane-bound complexes are sorted on opposite sides of the cells across the tissue to establish the axis of PCP (Lawrence et al., 2007). Subsequently, In, Fz, and Frtz function as effectors to direct cellular morphologic polarization. Two models have been proposed for the initiation of polarized sorting of Fzd and Vang: (1) a gradient of Fzd activity is initiated by Ft, Ds, and Fj and propagated subsequently through intercellular interaction of Fzd and Stbm/Vang on opposing plasma membranes of the two contacting neighboring cells (Wu and Mlodzik, 2008; Ma et al., 2008); or (2) the gradient of Fzd activity is initiated by an unknown mechanism and Ft/Ds/Fj can act independent of Fzd and Stbm/Vang under certain conditions to signal for cytoskeletal polarization (Lawrence et al., 2007).

In the vertebrates, early studies of *Drosophila* PCP gene homologs in *Xenopus* and zebrafish revealed a role for vertebrate PCP genes in regulating convergent extension (CE), a cellular process that involves polarization of cells along a planar axis to drive cellular convergence along the same axis and consequent extension of the tissue along a perpendicular axis (Keller, 2002). More recently, the availability of mouse mutants has allowed the assessment of mouse PCP orthologs in mammalian development. In particular, the precise cellular patterning and the distinct structure of polarized hair bundles of the organ of Corti offer an excellent model system to dissect the molecular and cellular mechanisms underlying PCP signaling in vertebrates and identify novel vertebrate PCP genes. Furthermore, the organ of Corti appears to undergo cellular rearrangements characteristic of CE during terminal differentiation of the hair cells when they acquire PCP (Chen et al., 2002), serving as a unique tool to study PCP signaling in both epithelial planar polarity and CE. Collectively, studies of mouse models to date showed (Fig. 1) that mouse genes homologous to *Drosophila fmi* (Shimada et al., 2001), *stbm/vang* (Montcouquiol et al., 2003; Montcouquiol et al., 2006), *fzd* (Wang et al., 2006b), *dsh* (Etheridge et al., 2008; Wang et al., 2005; Wang et al., 2006a), and *fat* (Saburi et al., 2008) are required for PCP processes in the cochlea, and that proteins encoded by some of these PCP genes show asymmetric subcellular

localizations to presumably establish the PCP axis in the cochlea (Jones and Chen, 2007). In addition to these conserved PCP genes, studies of the inner ear PCP also revealed several novel components of the vertebrate PCP pathway. Scribbled (Montcouquiol et al., 2003) and mouse protein tyrosine kinase 7 (PTK7, also known as colon carcinoma kinase-4, CCK-4) (Lu et al., 2004) were found to be required for coordinated orientation of hair bundles, and primary cilia and their associated basal bodies have a role in determination of the intrinsic polarity of hair cells and a role in interacting with polarized membrane-bound PCP protein complexes for morphologic polarization of hair cells (Jones et al., 2008). Furthermore, Wnt molecules, the ligands for Fz receptors appear to be required for inner ear PCP (Dabdoub et al., 2003; Qian et al., 2007), in contrast to their apparently dispensable role in the formation of PCP in the fly (Sato et al., 2006). The vertebrates appear to have evolved a mechanism involving diversification of Wnt molecules and their receptors/co-receptors (Forrester et al., 1999; Green et al., 2007; Green et al., 2008; Oishi et al., 2003) to allow increased complexity and specificity in the regulation of PCP downstream signaling. Here, we review the individual mouse models (Table 1) that lead to the revelation of a molecular network comprising of primary cilia/centrioles for intrinsic polarity of hair cells and a signaling cascade of long-range directional cues, reception and translation of vectorial information via asymmetric segregation of membrane-bound protein complexes, and the concerted action of membrane-bound PCP protein complexes and centrioles on the cytoskeleton for coordinated polarization of all hair cells (Fig. 1).

2. Mouse mutants for a Fmi homolog

Mouse *Celsr1*, 2, and 3 genes encode three large protocadherin molecules that are mammalian homologs of *Drosophila*'s Flamingo/Starry night (*fmi*) (Curtin et al., 2003; Shima et al., 2002). In 2003, Murdoch and colleagues determined that two mouse mutants generated from independent ENU mutagenesis experiments, *spin cycle* (*Scy*) and *crash* (*Crsh*), carry independent mis-sense mutations within the coding region of one copy of the *Celsr1* gene. These two mutants, *Scy* and *Crsh*, exhibit head-shaking behavior, and belly curling and spinning during tail suspension, indicating defects in the vestibular system. Although no obvious phenotypic defect was detected in the vestibular sensory organs from *Scy* and *Crsh* mutants, outer hair cells in both mutant cochleae have small but quantitatively significant misorientation of hair bundles. Less than 4% of the outer hair cells in any given region have misoriented hair bundles (Curtin et al., 2003). This mild phenotype is drastically enhanced in homozygous *Celsr1* mutant mice. Outer hair cells in homozygous *Celsr1* mutants show apparent randomization of hair bundle orientation as soon as they become recognizable during embryonic development, indicating an essential role for *Celsr1* in establishing coordinated cell orientation in the cochlea.

Homozygous *Celsr1* mutant embryos also show a completely open neural tube defect, known as craniorachischisis, and a delay or failure of eyelid closure (Curtin et al., 2003). Neural tube development in mice (Ybot-Gonzalez et al., 2007) and zebrafish involves convergent extension (Ciruna et al., 2006), and zebrafish PCP genes have been shown to be essential for proper convergent extension of the neural tube (Ciruna et al., 2006; Ybot-Gonzalez et al., 2007). The failure of eyelid closure has been observed in other PCP mouse mutants in later studies and presumably is due to defects in cellular polarization during

eyelid development. Together with the study of a vang homolog (Montcouquiol et al., 2003) as described in the next section, the tightly associated PCP phenotypes in the inner ear, the neural tube, and the eyelid in *Celsr1* mutants, provide the first evidence to indicate firmly that a genetically conserved pathway operates in the mammals for PCP processes. A subsequent study further showed that chicken *Celsr1* displays polarized membrane localization along the PCP axis of the chicken auditory sensory organ (Davies et al., 2005), suggesting for the first time that the molecular and cellular mechanism underlying the requirement for *Celsr1* in vertebrate PCP regulation may share common features with its invertebrate homolog.

3. Mouse mutants and transgenic mice for *Stbm/Vang* homologs

There are two mouse homologs of *Drosophila stbm/vang* gene, *Vang-like 1 (Vangl1)* and *Vang-like 2 (Vangl2)*. The *Vangl* genes encode four-transmembrane domain proteins with a C-terminal PDZ domain. Kibar et al. in 2001 showed that *Vangl2* is mutated in the *loop-tail (Lp)* mouse, a missense mutation at the C-terminal cytoplasmic domain of *Vangl2* that originated spontaneously in Strong's A mouse strain and may lead to destabilization of the protein (Montcouquiol et al., 2006). The *Lp* mutation causes craniorachischisis in homozygous mutant animals (Kibar et al., 2001), implicating a defect in CE and potentially other PCP processes. In 2003, Montcouquiol and colleagues in Kelley's laboratory studied the function of *Vangl2* in the development of the cochlea using the *Lp* mouse model (Montcouquiol et al., 2003). The *Lp* mice display pronounced polarization defects in the orientation of stereociliary bundles in the inner and outer hair cells of the mouse cochlea (Montcouquiol et al., 2003); thereby, providing one of the first two pieces of evidence supporting the existence of a conserved PCP pathway in the mammals. The characterization of *Lp* mice in the PCP regulation of the cochlea (Montcouquiol et al., 2003) is highly significant and establishes the *Lp* mice as a classic mouse model for PCP study. The *Lp* mice have been used extensively for subsequent PCP studies to test genetic interactions in PCP signaling and to validate novel PCP genes.

Mouse mutants for *Vangl1* have also been generated (Torban et al., 2008). Torban and colleagues were motivated to create a mouse model to study the role of *Vangl1* in mammalian PCP processes since *Vangl1* is the only known *Drosophila* PCP homologous gene that has been linked to human neural tube defects (NTD) (Kibar et al., 2007). The group identified a gene-trap mutant mouse ES cell line in which a β -Geo gene-trap cassette (β -gal and neomycin phosphotransferase fusion) is inserted into *Vangl1*, predicting the production of a severely truncated nonfunctional protein consisting of 63 aa of the N-terminal cytoplasmic portion fused to β -gal. Mutant mice generated using the ES cells, *Vangl1^{gt}* mice, were verified to express a mutant transcript. Capriciously, homozygous *Vangl1^{gt/gt}* mutant mice do not show open neural tube defects and exhibit only subtle alterations in hair bundle polarity. The double heterozygous animals carrying one *Vangl2^{Lp}* copy and one *Vangl1^{gt}* copy, however, show strong defects in both misorientation of hair bundles in the cochlea and craniorachischisis. These observations indicate that *Vangl1* and *Vangl2* function redundantly for mammalian PCP signaling in both the inner ear and the neural tube.

It is worth noting that the study of *loop-tail* mutants by Montcouquiol et al. reported an additional cochlear phenotype that is present in many other PCP mutant mice characterized in subsequent studies. In *Lp* homozygous mutants, the cochlear duct is shortened and the organ of Corti is widened (Montcouquiol et al., 2003). This observation provided the first evidence to support the hypothesis that CE occurs during development of the cochlea.

In addition to loss-of-function of *Vangl1* or *Vangl2* mutant mice, transgenic mice carrying a tagged *Vangl2* gene have been generated to facilitate the analysis of PCP processes and the molecular and cellular role for *Vangl2* in PCP signaling (Qian et al., 2007). A bacterial artificial chromosome (BAC) containing the *Vangl2* genomic sequences mediated the generation of a transgenic mouse strain that expresses a *Vangl2*-GFP fusion protein under the regulation of *Vangl2*-regulatory elements. The fusion protein shows an asymmetric membrane localization that is polarized along the axis of PCP in the cochlea (Jones et al., 2008; Qian et al., 2007), similar to what has been observed using an antibody raised against a *Vangl2* peptide (Montcouquiol et al., 2006). Future studies using this transgenic strain may reveal the dynamic sorting of *Vangl2* during PCP processes and uncover other forms of PCP that are otherwise not apparent.

4. Targeted gene knockout mouse mutants for Fzd homologs

Frizzled (Fzd) proteins are seven transmembrane domain proteins known to act as receptors for secreted Wnt proteins (Wang et al., 1996). There are 10 known mammalian Fzd homologs, amongst which Fzd3 and Fzd6 have been shown to regulate PCP signaling in mice. Knockout mouse models of Fzd3 and Fzd6 were first generated in 2002 (Wang et al., 2002) and 2004 (Guo et al., 2004), respectively. In 2006, Wang et al. (2006b) demonstrated that Fzd3 and Fzd6 have redundant roles in PCP regulation in the inner ear. Neural tube and eyelid defects are also observed in Fzd3 and Fzd6 double knockout mice. The group further demonstrated asymmetric membrane localization of both Fzd3 and Fzd6 in the organ of Corti and the vestibular sensory organs. Moreover, they used an elegant mosaic approach to unequivocally demonstrate the subcellular localization of Fz6. The cellular boundaries in the organ of Corti are formed between hair cells and supporting cells and between different types of supporting cells. Most of the polarized localizations of PCP proteins in the organ of Corti are on the boundaries formed between a hair cell and a supporting cell. It is difficult to assign the localization of these PCP proteins to either the hair cell and/or the supporting cell. Chimera *Fzd6*^{+/+} and *Fzd6*^{-/-} mice were created and used to demonstrate that Fzd6 protein is localized to the medial side of hair cells (Wang et al., 2006b). These data together support a conserved role for Fzd in PCP signaling.

The study of Fzd3 and Fzd6 in inner ear PCP signaling (Wang et al., 2006b) also points to the nature of PCP establishment in the cochlea. The first row of hair cells from the medial side of the cochlear epithelium, the inner hair cells, shows a reversed orientation in *Fzd3*^{-/-}; *Fzd6*^{-/-} double knockout mice, and outer hair cells display milder misorientation. In contrast, inner hair cells in mice carrying mutations in *Celsr1* (Curtin et al., 2003) or *Vangl2* gene (Montcouquiol et al., 2003) do not have a reversed orientation while the outer hair cells show stronger levels of misorientation. It is possible that the inner and outer hair cells are

under the influence of different combination of directional cues and that individual PCP genes carry differential weight in regulating the orientation of inner versus outer hair cells.

5. Transgenic mice for Dishevelled

The first Wnt-mediated signaling pathway discovered was characterized as “canonical” Wnt signaling, which comprises of Wnt ligand binding to the Fzd receptor and subsequent stabilization of β -catenin and β -catenin-mediated transcriptional regulation of target genes (Kohn and Moon, 2005). Dishevelled (Dvl) has been known to transduce Fzd activity upon Wnt ligand binding in β -catenin-mediated canonical Wnt signaling (Klingensmith et al., 1994; Krasnow et al., 1995). Its ability to transduce PCP signaling was first identified in *Drosophila* (Klingensmith et al., 1994) and was linked with Fzd-mediated PCP signaling shortly thereafter (Krasnow et al., 1995). Dvl is a multidomain cytoplasmic protein (Boutros and Mlodzik, 1999) and its dual roles in mediating both the canonical Wnt and noncanonical PCP signaling are harbored by separate domains. In *Drosophila*, Dsh (Dvl) asymmetrically localizes at the cell membrane along the planar polarity axis in a Fzd-dependent manner (Axelrod, 2001).

There are three mammalian Dvl homologs: Dvl1, Dvl2, and Dvl3 (Klingensmith et al., 1996; Sussman et al., 1994; Tsang et al., 1996). Wynshaw-Boris’s laboratory has generated Dvl1, Dvl2 and Dvl3 knockout mice. The group first generated a Dvl1 knockout mouse strain in 1997 and described that while viable and fertile, these mice displayed abnormal social behavior and sensory-motor neuron gating abnormalities (Lijam et al., 1997). In 2002 they generated Dvl2 knockout mice which displayed 50% lethality as well as defects in cardiac development and somite segmentation. Furthermore, they also generated Dvl1/2 double knockouts and found that there is functional overlap between Dvl1 and Dvl2 as the majority of Dvl1/2 knockouts display craniorachischisis as opposed to 1–3% of the single knockouts (Hamblet et al., 2002). In a collaborative effort by the Wynshaw-Boris and the Chen laboratories, the team determined that the cochlea shows characteristic PCP defects in Dvl1 and Dvl2 double knockout mice (Wang et al., 2005; Wang et al., 2006a). They found that the cochlear duct is shortened and widened, and there is discernable misorientation of hair bundles in the organ of Corti from Dvl1 and Dvl2 double knockout mice (Wang et al., 2005; Wang et al., 2006a). Similar phenotypes were observed in Dvl2 and Dvl3 double knockout mice (Etheridge et al., 2008). Of particular importance to inner ear studies is the fact that (1) Dvl2 and Dvl3 knockouts display defects in convergent extension and misorientation of the stereociliary bundles in the cochlea; (2) Dvl2 and Dvl3 genetically interact with Vangl2 and; (3) subsequent removal of other Dvl homologs exacerbates the phenotype of any one knockout in a dose dependent manner; therefore suggesting that all three homologs have functional overlap (Etheridge et al., 2008; Wang et al., 2005).

Transgenic mice expressing either GFP-tagged Dvl2 (Wang et al., 2005) or YFP-tagged Dvl3 (Etheridge et al., 2008) fusion protein under the control, respectively, of Dvl2 or Dvl3 regulatory elements have also been generated and used to analyze Dvl2 and Dvl3 subcellular localization in the cochlea. Similar to their *Drosophila* homolog, Dvl2 and Dvl3 show asymmetrical membrane association. However, neither has been co-localized to Fzd3 or Fzd6, two of the Fzd receptors required for PCP signaling in the cochlea. It is not clear

whether there are additional Fzd receptors involved in mediating Dvl membrane association in the cochlea, or if the mammalian PCP pathway utilizes different mechanisms for Dvl membrane association and for transducing Fzd activity during PCP signaling.

6. Four-jointed mutant mice

Four jointed (*ff*) is a transmembrane protein required for anterior–posterior patterning in *Drosophila*; *ff* can be cleaved producing a secreted signal (Villano and Katz, 1995). A role for *ff* in the regulation of PCP signaling was characterized in *Drosophila* ommatidia (Zeidler et al., 1999). It makes up a distinctive group of the *Drosophila* PCP proteins consisting of two other components, the procadherins Fat (Casal et al., 2002; Ma et al., 2003; Simons and Mlodzik, 2008) and Dachous (Ds). In *Drosophila*, Fat suppresses *ff* transcription, and Ds represses Fat activity in PCP. It appears that this group of *Drosophila* PCP genes can act independent of Fz and Stbm to direct cellular polarity in neighboring cells, implicating *fat/ds/ff* as a parallel pathway for PCP signaling.

There is one mouse homolog of *Drosophila ff*: four jointed box 1 (Fjx1) (Ashery-Padan et al., 1999). Its expression is consistent with a role in the regulation of PCP signaling and it appears to be regulated by notch signaling (Rock et al., 2005a; Rock et al., 2005b). To date, two knockout mice of this protein have been generated (Probst et al., 2007; Velocigene, 2008). In 2007, Puschel and colleagues generated a Fjx1 mouse knockout (where endogenous gene was replaced with LacZ-Neo cassette) and determined that Fjx1 regulates dendritic extension; however, no data on cell polarity in the mammalian inner ear was published in this study (Probst et al., 2007). The other knockout mouse model of Fjx1 has been reported in the Mouse Genome Informatics database, but has not yet been published (Bult et al., 2008; Velocigene, 2008).

7. Fat mutant mice

Like Dachous, Fat belongs to a heterogeneous subfamily of protocadherin-related proteins (Tepass et al., 2000). There are four mouse homologs of Fat: Fat1–4 (Bult et al., 2008). Sequence comparison between *Drosophila* and mice, and *in situ* RNA expression in mice (Rock et al., 2005b) suggest that Fats may mediate conserved signals in PCP regulation between *Drosophila* and mammals. Recent work has provided experimental evidence supporting this hypothesis. McNeil and colleagues generated a floxed conditional knockout mouse strain of Fat4 (Saburi et al., 2008). Fat4 mutant mice die soon after birth. The newborns are much smaller in size and possess a curved body and a curly tail. The group showed that loss of Fat4 leads to shortening and widening mouse cochlea and misorientation of the stereociliary bundles in this organ; both phenotypes that are consistent with defects in PCP signaling (Saburi et al., 2008). In addition, genetic inactivation of Fat4 disrupts oriented cell division and tubule elongation during kidney development and Fat4 interacts genetically with Vangl2 in this process. These observations implicate oriented cell division during kidney tubule elongation as an additional process regulated by PCP signaling and the failure in PCP signaling as a potential cause for certain types of cystic diseases.

8. Scribbled mutant mice

Unlike most other PCP genes, *Drosophila* Scribble was first identified not as a PCP gene but as a regulator of apical protein localization (Bllder and Perrimon, 2000). There is one known mammalian homologue of *Drosophila* scribbled, *Scrb1*. *Scrb1* codes for a protein containing a leucine-rich region and four PDZ domains. In 2003, Coop and colleagues demonstrated that in the *circletail* (*Crc*) mouse, *Scrb1* has a single point mutation causing a frame shift and early termination of the protein leading to a loss of two of the four PDZ domains of *Scrb1*. The *Crc* mice display craniorachischisis (Murdoch et al., 2003), a common phenotype in PCP mutations that indicates a defect in convergent extension. Montcouquiol et al. (2003) used the *Crc* mouse to demonstrate that *Scrb1* regulates PCP in mammals. They showed that (1) hair bundles are misoriented in the cochlea of mice homozygous for the *Crc* mutation, and (2) *Scrb1* and *Vangl2* genetically interact to regulate PCP in the cochlea (Montcouquiol et al., 2003). In a follow-up study, the group further showed that the PDZ domains 2, 3, 4 of *Scrb1* bind to the PDZ domain of *Vangl2*, suggesting that *Scrb1* may function in the formation of *Vangl2*-containing PCP complexes (Montcouquiol et al., 2006).

9. Gene-trap mouse mutants for PTK7/CCK-4

In 2004 Tessier-Lavigne and colleagues identified the protein tyrosine kinase 7 (PTK7) gene as a novel regulator of PCP signaling in mammals (Lu et al., 2004). PTK 7 (also known as colon carcinoma kinase 4, CCK-4) encodes for an evolutionarily conserved tyrosine kinase with signal peptide, seven immunoglobulin domains, a single transmembrane domain and an intracellular tyrosine kinase domain. Using a gene trap screen Tessier-Lavigne and colleagues generated a mutant mouse model with no wild type PTK7 expression, expressing instead a chimeric protein consisting of the first 114 amino acids of PTK7 (containing the first immunoglobulin domain) fused in frame with a transmembrane β -geo cassette (PTK7: β geo). These PTK7: β geo mouse mutants (1) display craniorachischisis (2) and disrupted stereociliary bundle orientation. Furthermore, the same study demonstrated that (3) PTK7 genetically interacts with *Vangl2* (*loop-tail mice*) and that (4) *Xenopus* PTK7/CCK4 homolog regulates convergent extension (Lu et al., 2004). The aforementioned phenotypic manifestations support the role of PTK7 as a regulator of vertebrate PCP signaling. A more recent study by Shnitsar and Borchers (2008) also showed that PTK7 regulates Fzd-dependent Dvl localization during neural crest migration in *Xenopus*, thus reinforcing the idea that PTK7 may have a role in vertebrate PCP signaling.

10. Wnt and Wnt co-receptor mutants

Wnts are secreted signaling molecules that regulate a variety of cellular events (i.e. cell proliferation, cell migration, cell polarity, cell differentiation, etc). They bind to Fzd receptors and co-receptors to initiate cascading downstream events. There are 19 known mouse Wnt genes: Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt9a, Wnt9b, Wnt10a, Wnt10b, Wnt11 and Wnt16.

Many of the Wnt molecules are expressed in the mouse inner ear (Dabdoub et al., 2003). In particular, Wnt7a is expressed in a narrow band of cells within the organ of Corti, consistent with a potential role in the development of the organ of Corti (Fig. 2). An initial study using

an in vitro organ culture system of the developing organ of Corti showed that application of Wnt7a or Wnt inhibitors affects the formation of hair bundle polarity in the culture. However, mice ablated for Wnt7a do not show detectable PCP phenotypes (Dabdoub et al., 2003).

A more recent study by Qian et al. (2007) showed that Wnt5a is expressed in the developing cochlea asymmetrically along the future PCP axis, reciprocal to a Wnt antagonist, Frzb. Furthermore, addition of Frzb to cultured cochleae leads to an extension defect and a hair bundle polarity defect. Examinations of Wnt5a knockout mice confirmed that Wnt5a is essential for PCP regulated processes in the inner ear and in the neural tube (Qian et al., 2007). Wnt5a knockout mice show incomplete penetrance of shortened and widened cochlear duct defects and mild defects in hair bundle polarity. Animals doubly heterozygous for the Wnt5a knockout allele and the *Vangl2* *Lp* allele display neural tube defects and strong hair bundle polarity defects (Qian et al., 2007). These results are consistent with the studies carried out in *Xenopus* and zebrafish where Wnt5 is required for CE during gastrulation. An additional Wnt molecule, Wnt11, has also shown to be essential for CE in the zebrafish (Heisenberg et al., 2000). However, its role in the mammalian inner ear PCP has not been reported.

Together with other vertebrate PCP model systems, such as gastrulation and neurulation in *Xenopus* and zebrafish, it is clear that certain Wnt molecules are essential for PCP-regulated processes. However, the exact mechanisms underlying the requirement for Wnt5 or Wnt11 are not clear. Experiments with members of Fzd receptors indicate that different Wnts have differential activities in activating β -catenin-mediated transcription regulation and that Fzd receptors show different activity toward the canonical Wnt signaling. In addition, recent studies have pointed to the possibility that specific types of co-receptors for Wnt molecules may mediate the role for Wnt5a in PCP signaling. In particular, Ror kinases were initially characterized as orphan receptors with tyrosine kinase activity. An early study showed that the *C. elegans* gene *cam-1* encodes a member of the Ror kinase family that guides migrating cells and orients the polarity of asymmetric cell divisions and axon outgrowth (Forrester et al., 1999), implicating a potential role in cellular polarization processes. Oishi and colleagues subsequently demonstrated that Ror and Wnt5a interact physically and both are involved in CE in *Xenopus*. They further demonstrated phenotypic similarities between Wnt5a and Ror2 knockout mice, suggesting that the role of Wnt5a in CE may be mediated by Ror2 (Oishi et al., 2003). Recently, Green and colleagues in Sternberg's laboratory showed that CAM-1 and the PCP protein VANG-1 mediate an instructive role of a *C. elegans* Wnt molecule, EGL-20 in establishing the polarity axis of cell divisions (Green et al., 2007; Green et al., 2008). Together, these studies suggest that Wnt molecules may utilize Ror receptors to activate downstream events specific for PCP signaling. Indeed, a secreted glycoprotein, Cthrc1, was shown to stabilize Wnt, Fzd, and Ror2 (Yamamoto et al., 2008). Examination of the inner ear from *Ror2*^{-/-} mice revealed misorientation of hair cells in the cochlea, and *Cthrc1* genetically interacts with *Vangl2* for uniform orientation of cochlear hair cells (Yamamoto et al., 2008).

11. Ciliary mutants

In the mammalian cochlea, kinocilia regress prior to the onset of hearing. Furthermore, the formation of a polarized hair bundle is preceded by the migration of the kinocilium to an eccentric position on the apical surface of the hair cell, and the polarity of a hair cell is distinctively marked by the placement of the kinocilium. These observations led to a hypothesized role for the kinocilium in PCP signaling in the inner ear.

The first genetic evidence supporting a potential role for the kinocilium in mammalian PCP signaling came from the study of *bbs* mouse mutants (Ross et al., 2005). Many of the genes implicated in the Bardet-Biedl Syndrome (BBS) are linked to the basal body, a centriole-derived organelle serving as the anchor and the base for primary cilia assembly, and are required for proper ciliogenesis (Klysik, 2008; Pedersen et al., 2008; Tobin and Beales, 2007). *Bbs* gene mutant mice showed abnormal morphology of their stereociliary bundles with the kinocilia showing an apparent loss of their close association with the stereocilia. Mice that were simultaneously mutated for the core PCP gene *Vangl2* and the *bbs* genes showed an exacerbated cochlear phenotype (Ross et al., 2005). However, since kinocilia remain in the *bbs* mutants, it is difficult to use these mutants to assess the specific role of kinocilia in the regulation of PCP in the organ of Corti. Following this pioneering work, recent research collectively has however, unequivocally implicated kinocilia in PCP signaling. In particular, a study using mice carrying a floxed allele of the intraflagellar transport protein (IFT) 88 (Haycraft et al., 2007), an essential component of the IFT complexes required for ciliogenesis showed that in the absence of kinocilia, hair cells are misoriented or have completely lost their innate polarity, even though the asymmetric localization of PCP proteins *Vangl2* and *Fzd3* is not affected (Jones et al., 2008). Similar results were observed for a floxed allele of *Kif3a* (Lin et al., 2003), which encodes a motor protein also required for ciliogenesis (Jones et al., 2008). These observations suggest that ciliary genes have a role downstream of membrane-associated PCP protein complexes and are essential for the intrinsic polarity of hair cells. Additional IFT mutant mice are available to further explore the mechanism underlying the roles of ciliary genes in vertebrate PCP signaling.

12. Summary

The accessibility of appropriate genetic model systems has facilitated almost all the discoveries of major biological pathways. The distinct and coordinated cellular polarity in the mouse inner ear sensory organs offers an excellent system to study mammalian PCP processes and dissect the underlying mechanisms. Together with other vertebrate model systems, the inner ear studies in the collection of mutant mice included in this review revealed a signaling pathway that is comprised of functionally conserved genes and novel vertebrate components to respond to intrinsic differences in morphogenetic processes and molecular compositions of cells during development. Several genes conserved from *Drosophila*, including homologs of core PCP genes *Vang*, *Fzd*, *Fmi*, *Dvl* are also required for planar polarization in the mouse ear. Moreover, several mammalian PCP genes, *Vangl2*, *Fzd3* and *Fzd6*, and *Dvl2* and *Dvl3* (as well as *Pk2*) (Deans et al., 2007) show polarized membrane association, similar to their invertebrate counterparts during PCP signaling. The

loss of their polarized membrane association accompanies the loss of coordinated cellular polarity, implicating a conserved function for these PCP proteins in establishing the axis of polarity.

The studies of PCP in the mouse inner ear revealed *Scrb1* and *PTK7* as novel PCP genes in vertebrates. In addition, vertebrate Wnts may be bona fide PCP genes signaling through differential co-receptors, and the primary cilia are essential for determination of intrinsic polarity of cells. The study of these new members of the vertebrate PCP pathway, however, is at its initial stages. Future studies focused on vertebrate-specific PCP genes, as well as new mouse models that allow functional inactivation of redundant genes and conditional inactivation of genes at different stages, will not only contribute to the understanding of the formation of the inner ear, but also provide insights into the mechanisms operating in the vertebrates to govern multiple vital cellular polarization processes involving PCP signaling, including cell division orientation, cell migration, gastrulation, neurulation, and epithelial cell polarity.

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Fig. 1.

The planar cell polarity of the inner ear sensory organs. (A) Inner ear isolated from an E18.5 mouse embryo. The embryo expresses GFP under the control of Math1 enhancers in hair cells allowing visualization of the 6 inner ear sensory epithelia: the organ of Corti of the cochlea (CO), the maculae of the saccule (SA) and utricle (UT), and the three orthogonally positioned cristae: the anterior crista (AC), posterior crista (PC) and lateral crista (LC). The white tracing outlines the fluid-filled labyrinth of the inner ear that includes both the cochlear and vestibular systems. (B) Confocal image of the organ of Corti viewed from the apical surface. The actin-rich stereociliary bundles (green) of the 1 row of inner (toward the medial side) and 3 rows of outer hair cells form a “V”-shaped structure and all the “Vs” are uniformly aligned (white arrows, lower panel) with the vertices pointing away from the center, or the medial side, of the spiraling cochlea, showing the distinctive planar polarity of this sensory epithelium. (C) Planar polarity in an isolated utricle from an E18.5 mouse embryo. The panels show a confocal scan of the utricle at a level just below the apical surface. Spectrin staining (red) permits easy visualization of the intrinsic polarity of each hair cell (white arrows, right panel) while cell membranes can be seen by staining for actin (green). The white arrows in the right panel indicate the orientation of hair cells. Hair cells on the two sides of the line of polarity reversal (pink line) are oriented towards each other.

Fig. 2.

PCP signaling in the development of the organ of Corti. (A) Diagram of an apical view of the developing organ of Corti at embryonic day 13 (E13) in mice. The precursor cells of the organ of Corti exit cell cycle around E13 in mice. Cells are irregularly shaped at this stage. No molecular or morphologic differentiation of hair cells is observed at this stage. (B) About one day later, cells become more regularly-shaped under the influence of multiple signals. Wnt molecules may be one class of signaling molecules involved and may utilize co-receptor Ror to activate PCP-specific downstream events. The diagram here shows an apical view of the developing organ of Corti around E14–E15. At this stage, hair cells have just started to express the earliest hair cell-specific markers, and no apical morphologic differentiation is detectable. Notably, membrane PCP proteins, Vangl2 (magenta) and Fz3/Fz6 (blue), display asymmetric localizations that are polarized along the future PCP axis while the primary cilium (the black apical ciliary projection) and its associated basal body (red doublets) of the cell is centrally positioned. It is thought that both Celsr1 (orange) and Scrb1 facilitate the polarized sorting of Vangl2 and Fz3/6 and/or their recruitment of cytoplasmic mediators, such as Dvl (green), Diversin (Di), and Pk. Note that only the localization of Vangl2, Dvl2/3, and Fz3/6 in the mouse organ of Corti has been reported. The localization of Celsr1 in the organ of Corti is assigned based on its chicken homology localization in the chicken cochlea and its *Drosophila* homolog localization. (C) By E18, the morphologic polarization of hair cells is reached. This diagram depicts the apical polarity characteristics of hair cells. Apical hair bundles, consisting of the staircase of stereocilia (dark green apical projections) and a single primary cilium (kinocilium, the black ciliary projection), are uniformly oriented across the organ of Corti by this stage. This is achieved likely through a concerted action of polarized membrane-associated PCP complexes and the primary cilium/basal body on cytoskeleton. The Wnt co-receptor Ror may have a direct role in morphologic polarization since it can activate cytoskeleton regulator JNK. It is worth noting that the space among hair cells is occupied by nonsensory supporting cells (not depicted), and the absolute subcellular localization of PCP proteins, e.g. on hair cell and/or supporting cell membrane, is not determined. The molecular role for PTK7 is not clear. Bb: basal body (Modified from a figure in Jones et al., 2008).

Table 1

Summary of mouse models for PCP studies in the inner ear

Fly gene	Mouse homologs	Protein	Mouse model(s)	Phenotype	References
<i>fmi</i>	Celsr 1 Celsr 2 Celsr 3	Large proto-cadherin	<i>Spin cycle</i> : missense mutations <i>Crashi</i> : missense mutation	Craniorachischisis, delay or failure in eyelid closure, and random hair cell orientation Same as <i>spin cycle</i> (above)	Curtin et al. (2003)
<i>Stbm/vang</i>	Vangl1 Vangl2	4-TM protein	<i>Vangl^{fl}</i> : gene trap truncation	Neural tube and inner ear PCP defects in <i>Lp</i> × <i>Vangl^{fl}</i> double heterozygotes	Torban et al. (2008)
<i>fz</i>	Fz1–10	7-TM receptor for Wnts	<i>Loop-tail</i> : missense mutation LTAP-GFP	Craniorachischisis and inner ear PCP defects in homozygotes Asymmetric membrane localization along PCP axis in cochlea	Kibar et al. (2001) Qian et al. (2007)
<i>dsh</i>	Dvl1	Multi-domain cytoplasmic proteins	Fz3/Fz6 double knockout	Defects in neural tube closure, eyelid closure and hair cell orientation in Fz3/6 double homozygotes.	Wang et al. (2006b)
	Dvl2		Dvl 1 knockout	Functional overlap with Dvl2 and Dvl3	Lijam et al. (1997); Wang et al. (2005); Wang et al. (2006a)
	Dvl3		Dvl 2 knockout	Functional overlap with Dvl1 and Dvl3 for PCP regulation; genetic interaction with Vangl2.	Hamblet et al. (2002)
<i>Fj</i>	Fjx1	TM protein	Dvl 3 knockout	Perinatal lethality; genetic interaction with Vangl2 (loop-tail).	Etheridge et al. (2008)
			Dvl2-GFP	Asymmetrical membrane localization but no co-localization with Fz3 of Fz6.	Wang et al. (2005)
			Dvl3-GFP Fjx1(LacZ-Neo): Fjx1 knockout Fjx1 knockout	Same as Dvl2-GFP (above). Regulation of dendritic extension; no PCP data reported Reported to Mouse Genome Informatics database but not yet published	Etheridge et al. (2008) Probst et al. (2007) VelociGene (2008)
<i>Ft</i>	Fat1–4	Protocadherin-related proteins	Fat 4 floxed conditional knockout	Inner ear PCP defects; disruption of oriented cell division and kidney tubule elongation; genetic interaction with Vangl2.	Saburi et al. (2008)

Fly gene	Mouse homologs	Protein	Mouse model(s)	Phenotype	References
<i>scrib</i>	Scrb1	Scaffold protein	Ciretail: frame shift point mutation	Hair cell misorientation in homozygotes; genetic interaction with Vangl2 (loop-tail).	Murdoch et al. (2003)
<i>otk</i>	PTK7 (CCK4)	Receptor tyr-kinase	PTK7; β geo; gene trap truncation	Craniorachischisis; hair cell orientation defects; genetic interaction with Vangl2	Lu et al. (2004)
<i>wg</i>	Wnt1/2/2b/3/3a/4/5a/6/7a/7b/8a/8b/9a/10a/10b/11/16	Secreted signaling molecules	Wnt5a knockout Wnt7a knockout	Inner ear PCP defects (incomplete penetrance); interacts with Vangl2 No detectable inner ear PCP defects.	Qian et al. (2007) Dabdoub et al. (2003)
<i>ror</i>	Ror1, 2, 3	Co-receptor for Wnts	Ror2 knockout	Misorientation of cochlear hair cells	Yamamoto et al. (2008)
<i>bbs</i>	Bbs1–12	Various domains	Bbs 1 knockout Bbs 4 knockout Bbs 6 knockout	Neural tube defects, failure to close eyelid, hair bundle morphology defects, genetic interaction with Vangl2	Ross et al. (2005)
<i>nompB</i>	IFT88	IFT protein	Floxed Ifi88 allele	Inner ear PCP defects in spite of asymmetric localization of Vangl2 and Fz3, when inactivated using Foxg1Cre	Jones et al. (2008)
<i>kfp64D</i>	Kif3a	Motor protein	Floxed Kif3a allele	Inner ear PCP defects, when inactivated using CreER induction at E12.5	Jones et al., (2008)