Planar Cell Polarity and the Kidney

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ABSTRACT
Planar cell polarity (PCP) is a form of spatial organization in tissue that was first described in Drosophila melanogaster. PCP plays a critical conserved role in several aspects of mammalian development. Exciting data implicate PCP in normal kidney development and suggest the loss of oriented cell division and convergent extension downstream of defective PCP signaling lead to cystic kidney disease in mouse models. In this review, I first cover the current knowledge of PCP signaling in invertebrate and vertebrate models and then explore how loss of PCP might underlie some forms of cystic kidney disease.


Planar cell polarity (PCP) is defined as the organization of cells in the plane of a tissue that is perpendicular to the apical-basal axis. This organization was first described in the fruit fly, Drosophila melanogaster. Today, there is growing interest in understanding the structural and functional aspects of this perpendicular plane in vertebrates. In the metanephric kidney, there is important evidence of its role in normal development and function.

PCP IN THE DROSOPHILA
Many structures in Drosophila show clear PCP, but the two predominant tissues for studying PCP in the fruit fly are the wing and the eye (Figure 1). PCP in the wing is seen in the organized arrangement of actin-rich extensions called hairs. A single hair extends from the distal portion of each cell in the wing and points distally. In the eye, PCP is seen in the planar organization of ommatidia. Ommatidia are composed of eight photoreceptors and additional accessory cells, which in section form a trapezoidal shape. Normally, all ommatidia in the dorsal half of the eye point dorsally, and those in the ventral half point ventrally. Mutations in PCP genes lead to a loss of planar organization in both the eye and the wing, leaving cell identity and apical-basal cell polarity unaffected (Figure 1).1–3

Genetic studies of Drosophila revealed three major groups of PCP genes (Figure 2). The first to be discovered and the best understood are the core PCP genes. Core PCP genes include frizzled (fz), disheveled (dsf), prickle (pk), vang (vang)/stbm (stbm), flamingo (fmi), and diego (dgo).1–7 Core PCP proteins show a striking asymmetric protein localization in the plane of the epithelium (Figure 3).8–12 Notably, there are two distinct subgroups of localizations: The “distal group,” which includes Dsh, Dgo, and Fz, and the “proximal group,” which includes Pk and Vang Fmi localizes to both proximal and distal sides of the cell. All core PCP proteins are initially localized at the apical cortex and then cleared from the anterior and posterior sides. There are complex biochemical and genetic interactions between core PCP components, with mutually repressive interactions between proximal and distal members that are thought to stabilize the asymmetric distribution of each complex.1–3

Asymmetric localization of core PCP molecules is also seen in the eye; however, there, the situation is more complex, consistent with the more complicated structure of the eye. Whereas in the wing every cell shows asymmetric protein localization, in the eye, only a few cells in each developing ommatidia have polarized localization of core PCP proteins.13,14 Importantly, loss of fz or vang in mutant clones leads to disruptions of polarity in adjacent wild-type tissue. This is thought to reflect communication of spatial information from one cell to the next.1–3 Genetic studies suggested that nonautonomy signals through a separate pathway than autonomous PCP signaling, because loss of dsh, pk, or dgo do not lead to nonautonomous defects in PCP.15

Another distinct group of PCP genes, known as the Fat/Ds PCP group, is composed of fat, dachsous (ds), four-jointed (fj), and atrophin (atro; Figure 4).16–19 Fat and Ds are enormous cadherins that bind each other in what seems to be a receptor-ligand interaction.20,21 The PCP defects of fat or ds mutants can be largely rescued by expression of the cytoplasmic domain of Fat, suggesting the cytoplasmic domain of Fat contains essential information for PCP.20,21 The

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cytoplasmic domain of Fat binds the transcriptional co-repressor Atro, which has similar PCP defects to Fat, suggesting PCP control by the Fat/Ds group may involve transcriptional regulation of targets. The only known target of Fat and Atro in PCP is fj. Fat and Atro repress the transcription of fj, fj encodes a Golgi-localized kinase, which can phosphorylate the cadherin repeats of Fat and Ds, potentially altering their binding; however, fj mutants have extremely weak PCP phenotypes, indicating there are other, still to be identified targets that mediate Fat/Ds PCP signal. Initial studies suggested the fat/ds genes lay upstream of the core PCP genes; however, more recent studies suggested that these two pathways function in parallel to regulate PCP. The exact relationship between these PCP regulators is unclear and needs further investigation. Loss of ft, ds, atro, or fj leads to clear nonautonomous defects in PCP in surrounding wild-type tissue. Important, loss of ds leads to defects in PCP that are opposite the PCP effects of loss of fat or atro. Ds is expressed in a gradient in the wing and the eye, and generating new gradients of Ds protein expression can repolarize the PCP of tissues. These and other data have led to a model by which Ds binding to Fat inhibits Fat activity in PCP, and this is modified by Fj. Fat is expressed evenly in most tissues; therefore, current models propose that Fat activity is regulated by gradients of Ds and Fj expression, establishing the spatial control of PCP.

Downstream of fat/ds and the core PCP genes are the PCP effectors. These genes encode proteins that are thought to convert the PCP signal into a physical remodeling of cells and often function only in specific tissues. For example, inturned, fuzzy, fritz, and multiple wing hair regulate PCP only in the wing, whereas nemo and unpaired regulate PCP only in the eye. Finally, there are also genes that are now generally termed PCP regulators, such as casein kinase I epsilon (CKIε), protein phosphatase 2A, and the G protein α O, which are not yet well understood in their placement in the PCP pathways.

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Figure 1. In normal flies, wing hairs (top) and photoreceptor clusters, called ommatidia (bottom), show clear organization in the plane of the epithelium. In PCP mutants (right), this precise tissue organization is lost, with hairs on the wing pointing in all directions and ommatidia showing randomized orientation. Aberrant PCP is indicated in red.

Figure 2. Current model of the Drosophila PCP pathway is shown. On the left is a simplified pathway, indicating the genetic relationships between the Fat/Ds cassette, core PCP genes, and the tissue-specific PCP effectors. The Fat/Ds cassette is shown in yellow, core PCP genes in white, and tissue-specific effectors in green. Fat and Ds are cadherins, Four-jointed is a kinase, Frizzled is a seven-pass transmembrane receptor, Flamingo is a seven-pass homophilic cell adhesion molecule, Dsh and Pk are cytoplasmic signal transducers, and Vang is a cell surface protein with four transmembrane domains.

Figure 3. Asymmetric distribution of core PCP proteins in the developing wing at pupal stages is shown. Although core PCP proteins have a nonasymmetric distribution initially, they become asymmetric at pupal stages, with a proximal group (Pk and Vang) and a distal group (Dsh and Fz). Fmi becomes localized to both proximal and distal sides but is cleared from anterior and posterior cell membranes.
Interestingly, protein phosphatase 2A, G protein α O, CKIε, and the core PCP proteins Fz and Dsh also function in Wingless (Wg) signaling.1–3,29 This led to the original hypothesis that PCP is regulated by an unknown Wg-related gene (Wnt) in Drosophila and reference to the PCP pathway as the noncanonical Wnt pathway; however, extensive attempts in the Drosophila PCP field to identify such a Wnt have been unsuccessful. There are seven Wnts in Drosophila. Neither deletion experiments nor overexpression experiments have revealed a direct role for Wnts in PCP signaling. PCP phenotypes do not occur in flies lacking Wnts or even in clones lacking all five Wnt ligands present in the wing disc; neither is the Wg binding domain of Fz required for its PCP signaling capabilities. In addition, overexpression of Wnts does not alter PCP in the wing or the abdomen;23,30 however, Wg controls the expression of Ds, the ligand for Fat.18 Thus, the role of Wnts in PCP signaling in Drosophila may be indirect, potentially through regulation of the Fat/Ds cassette.

Nonetheless, there are many interesting and complicated interactions between the PCP pathway and canonical Wg signaling. Overexpression of negative regulators of canonical Wg signaling, such as Naked, lead to dramatic PCP effects in the eye or the wing. This may be due to overflow effects, that are nonphysiologic, on the basis of the shared components such as Fz and Dsh, that function in both pathways; however, work on vertebrate PCP (see the next section) shows a striking role for Wnts in PCP signaling, suggesting that further investigation is warranted. Interestingly, recent work in mammals revealed novel ligands for Fz receptors that function in Wnt signaling.31 Thus, it remains possible that novel ligands may exist to regulate PCP in Drosophila.

PCP signaling in Drosophila also controls oriented cell division. Oriented cell division in the fly wing is responsible for forming the normal elongated wing shape—without PCP genes such as fat and ds, wings are shorter and rounder.32 PCP signaling by Fz and Dsh also regulates oriented cell division in fly neuroblasts33 and in vertebrate neural tissues.34 How PCP signaling results in a physical orientation of the mitotic spindle is unclear. The mitotic spindle has a population of microtubules, known as astral microtubules, that emanate from the spindle poles toward the cell surface. In budding yeast, astral microtubules are captured at the bud tip cortex and function in positioning the spindle along the mother–bud axis. Astral microtubules are thus prime candidates for regulating spindle orientation downstream of PCP signaling, because they represent a link between the spindle and the cortex.35

**PCP IN VERTEBRATES**

A great deal of excitement accompanied the finding that the PCP genes also function to regulate tissue organization in vertebrates. Homologs of Drosophila core PCP genes function in convergent extension (intercalation of cells to produce linear extension without cell division), neural tube closure, and the organization of hair cells in the inner ear.36–47 Vertebrate PCP can be seen in the coordinate organization of hair cells of the inner ear and hair on the back of the mouse. Loss of core PCP genes such as Vangl2, Celsr1, Fz3/6, and Dvl1/2 disrupts inner ear hair cell organization, and planar organization of fur on a mouse’s back is lost in Vangl2, Celsr1, and Fz6 mutants. Analysis of the PCP pathway in mammals is complicated by the duplication of many PCP genes. For example, defects in neural tube closure are seen only with Fz3,Fz6 double mutants, as a result of redundant functions of the homologs. Neural tube closure defects are also present in mice with mutations in the orthologs of Dsh.46

Notably, work in vertebrates reveals a novel role for PCP genes in the regulation of polarized cell movements during development—most specific, convergent extension. Convergent extension is the lengthening and narrowing of a tissue during development. The best characterized movements of convergent extension involve the intercalation of cells. Muta-
tion of vertebrate homologues of *Drosophila* PCP genes block these cell movements, leading to broader, shorter tissues.\textsuperscript{34,48–50} Defective convergent extension is thought to be the reason for the neural tube closure defects seen in PCP mutants in mice and zebrafish. Interestingly, PCP signaling in both convergent extension and oriented cell division have the same final effect: Lengthening and narrowing tissues during development.

**Vertebrate-Specific PCP**

Although Wg and *Drosophila* Wnts seem to play no role in PCP signaling in the fly, there is mounting evidence that a noncanonical Wnt pathway is involved in PCP in vertebrates. Mammals have 19 Wnt ligands, the majority of which signal predominantly in canonical signaling through binding to Fz receptors and activation of Dsh homologs (Dvl). Activation of Dvl disrupts the β-catenin disruption complex (composed of Axin, APC, GSK3, and CK1), resulting in stabilization of β-catenin. β-Catenin can then translocate to the nucleus, where it activates transcription of target genes in conjunction with TCF/LEF (T Cell Factor/Lymphoid Enhancer Factor) transcription factors.\textsuperscript{51} In contrast, noncanonical signaling is β-catenin independent. A number of Wnts regulate noncanonical signaling. For example, hair cells are misoriented in the cochlea of *Wnt5a* mutant mice,\textsuperscript{52} and both Wnt5 and Wnt11 are required for convergent extension in fish and frogs.\textsuperscript{53–55} Surprising, the convergent extension defects of *Wnt11* mutants are rescued by exogenous expression of Wnt11, suggesting that Wnt11 plays a permissive role in PCP.\textsuperscript{53}

Recent reports suggest that the ability of a specific Wnt to signal through canonical versus noncanonical pathways may be due to the presence of specific co-receptors. The tyrosine kinase Ror regulates vertebrate PCP, in part by forming a complex with Fz, converting it from canonical (β-catenin) signaling to noncanonical signaling.\textsuperscript{56} The interaction between Ror and Fz enhances PCP signaling. This interaction is promoted by secreted glycoproteins such as Cthrc1, which modulate the ability of Wnt ligands to activate selectively the PCP pathway upon binding to Fz and the Ror2 co-receptor.\textsuperscript{56,57} Other vertebrate-specific PCP molecules, such as Ptk7,\textsuperscript{58} may reflect the need for more complex regulation of PCP in vertebrates.

A particularly confusing aspect of PCP signaling involves the Jun kinase, JNK. Original overexpression studies in *Drosophila* suggested that JNK plays an important role in PCP signaling;\textsuperscript{59} however, subsequent studies found only very weak PCP defects in JNK mutants. Current models of PCP in *Drosophila* do not include JNK signaling. Vertebrate studies, however, have often found a correlation of JNK phosphorylation with noncanonical Wnt stimulation. A remarkable new study has shown Rac1 activates JNK2 that in turn phosphorylates β-catenin and controls its nuclear translocation,\textsuperscript{59} so although JNK activation in mammals has been taken to indicate that noncanonical signaling is activated, JNK activation may also be an indication of canonical signaling and must be viewed with caution. The need for caution is also suggested by recent studies demonstrating that inhibition of JNK signaling by specific inhibitors does not affect the movements of convergent extension.\textsuperscript{60}

Interestingly, several core PCP proteins, including Vangl2, Dvl2, Pk2, Fz3, and Fz6, have asymmetric localization in vertebrates;\textsuperscript{36,42,47,61} therefore, polarized localization of PCP components is a conserved feature of the PCP signaling cascade. There is not a simple relationship between the protein localization found in *Drosophila* and vertebrate PCP localization, however. For example, although Vang is proximally localized and Fz is distal in *Drosophila*, in mice, Vangl2, Fz, and Fz6 are found on the same side of inner ear sensory cells.\textsuperscript{42}

**PCP in Defective Kidney Development and Cystic Disease**

Recent studies demonstrated that PCP signaling has an important role in normal kidney development and that defective PCP could underlie devastating diseases, such as cystic kidney disease.\textsuperscript{62–75} There are many forms of cystic kidney disease in human.\textsuperscript{66–71} Although many of the genes that are mutated in polycystic kidney disease (PKD) have been identified, the pathogenetic mechanisms initiating cyst formation are still unclear. Autosomal dominant PKD (ADPKD) is caused by mutations in *Pkdi* encoding a large transmembrane protein, PC-1, or by mutations in *Pkd2* encoding a TRP cation channel, PC-2, involved in calcium entry.\textsuperscript{63} In ADPKD, cysts arise as focal outpouches from all segments of the nephron. Cysts eventually separate from the parental nephron and expand through proliferation and accumulation of cyst fluid. Autosomal recessive PKD (ARPKD) is caused by mutations in *PKHD1* encoding a large transmembrane protein, fibrocystin.\textsuperscript{69} ARPKD cysts develop as dilations of collecting ducts and maintain contact with the parental nephron. In contrast to the simple genetics underlying ARPKD and ADPKD, mutations in a large number of other genes can also lead to a recessive cystic kidney disease known as nephronophthisis (NPHP).\textsuperscript{71} In NPHP, cysts are restricted to the corticomedullary border and may be derived from collecting ducts and distal tubules. One of the first indications of a link between cystic kidney disease and PCP was that one of the genes mutated in NPHP, inversin, controls the balance between canonical and noncanonical Wnt signaling and is related to the core PCP protein Dgo.\textsuperscript{75}

Elegant studies showed that when cells in the straight tubular segments of the outer medulla divide, there is a high degree of oriented cell division;\textsuperscript{62} 95% of cells divide within 34° of the axis of the tubule. This leads to a model in which oriented cell division is essential for the normally thin elongated tubes that make up much of the nephron. An extreme version of a dilated tubule is a cyst. To determine whether cystic kidney disease associates with defective oriented cell divisions, the authors examined two different mouse models of PKD and found a loss of oriented cell division in mutants (Figure 5). Given the role of PCP signaling in oriented cell division, the authors speculated that some forms of cystic kidney disease might be due to defective PCP.

This hypothesis was recently strengthened by the observation that mutants in *Fat4*, an ortholog of *Drosophila Fat*, display...
Ciliary Involvement in PCP and Cystic Kidney Disease

The primary cilium is an antenna-like structure that projects from the surface of most cells.\(^{72}\) Primary cilium are nonmotile and are composed of an axoneme that comprises nine microtubule doublets surrounded by the cilary membrane (9 + 0); motile cilia have two additional microtubules in the center of the surrounding nine (9 + 2) that allow them to beat. The primary cilium is anchored in the cell by the basal body, a microtubule-based structure that also functions as one of the centrioles during cell division. In the kidney, a single primary cilium is found on the apical surface of most tubular epithelial cells. Primary cilia have been implicated in cell-cycle regulation, hedgehog signaling, Wnt signaling, and PCP signaling.\(^{72}\)

Several lines of evidence suggest that cystic disease arises from abnormalities in the structure and/or function of primary cilia.\(^{72,73}\) The proteins mutated in ARPKD and ADPKD are found in primary cilia in renal epithelial cells. The protein products of genes mutated in other cystic kidney diseases, such as NPHP and Bardet-Biedl syndrome, are also found in cilia or in the associated basal body. Importantly, mutant mice that contain shortened or absent primary cilia lose oriented cell division and develop kidney cysts.\(^{74}\)

Several links have also been made between PCP and cilia. Ciliary-associated proteins such as Inversin regulate the balance between canonical Wnt signaling and noncanonical (PCP) signaling, potentially through regulation of Dsh.\(^{65,75,77}\) Loss of the PCP effector genes fuzzy and inturned led to disruption of the cytoskeleton and cilia.\(^{78}\) Interfering with Dsh causes loss of cilia in bronchial epithelial cells as a result of defects in docking of basal bodies.\(^{79}\) In addition, PCP proteins such as Fat4 and Vangl2 localize to the base of cilia in cultured cells.\(^{64,80}\)

Renal cilia project into the tubule lumens and bend in response to fluid flow. Bending of the cilia stimulates an increase in cytosolic calcium concentration.\(^{81}\) These findings suggested that primary cilia function as mechanosensors.
of urine flow in the renal tubules; however, several articles,\(^7,4,8\) challenged the notion that normal renal tubular geometry is maintained by the mechanical bending of cilia in response to flow.\(^8\) These studies demonstrated that loss of cilia does not lead to generation of cysts unless new proliferation is induced by injury. Interestingly, if cell proliferation is blocked in zebrafish, then most of the defects associated with loss of PCP signaling are suppressed.\(^8\) These data highlight the crucial role that PCP has in normal development and regeneration.

**CONCLUSIONS**

It is clear that PCP signaling is essential for normal tissue development in vertebrates and invertebrates and has crucial roles in kidney development and function. It is still unclear how PCP signaling leads to convergent extension movements or regulates spindle orientation. There are also many questions to be answered about the relationship of core PCP genes and the Fat/Ds cassette in both flies and humans. Given the importance of PCP to development and disease, understanding how spatial information is perceived and regulated via PCP signaling will increasingly be the focus of scientists who are interested in cell biology, genetics, tissue patterning, and human disease.

**DISCLOSURES**

None.

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BRIEF REVIEW


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