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Planar cell polarity of the kidney

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Abstract

Planar cell polarity (PCP) or tissue polarity refers to the polarization of tissues perpendicular to the apical-basal axis. Most epithelia, including the vertebrate kidney, show signs of planar polarity. In the kidney, defects in planar polarity are attributed to several disease states including multiple forms of cystic kidney disease. Indeed, planar cell polarity has been shown to be essential for several cellular processes that appear to be necessary for establishing and maintaining tubule diameter. However, uncovering the genetic mechanisms underlying PCP in the kidney has been complicated as the roles of many of the main players are not conserved in flies and vice versa. Here, we review a number of cellular and molecular processes that can affect PCP of the kidney with a particular emphasis on the mechanisms that do not appear to be conserved in flies or that are not part of canonical determinants.

Keywords

Planar cell polarity; Non-canonical Wnt signaling; Morphogenesis; Kidney

What is planar cell polarity?

Planar cell polarity (PCP), also known as tissue polarity, describes the coordinated polarization of cells within the plane of a tissue/ epithelium, which is perpendicular to the apical-basal cell polarity axis. Although PCP is particularly apparent in tissues that give rise to oriented external structures, such as *Drosophila* wing hairs (Fig. 1) and cuticular bristles, mammalian body hair or the stereocilia in the inner ear (Fig. 2), most tissues show some aspect of PCP during their development or in their differentiated state. Examples are directional cell movement and oriented cell divisions during morphogenesis or the uniform orientation of asymmetrically shaped cells observed in many epithelial tissues.

PCP is important in a broad array of developmental and physiological processes in vertebrates, and defects in PCP signaling have been associated with many developmental anomalies and diseases [1]. Although roles for orthologs of the *Drosophila* PCP genes in PCP-like processes have been uncovered in vertebrates, in some cases the phenotypes are extremely mild and great effort must be made to find any sort of defect, suggesting that PCP in vertebrates may be much more complex than in flies. In agreement with this idea, a

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number of vertebrate-specific PCP components have been identified [2], some of which appear to have a more significant role in PCP than members of any of the *Drosophila* cassettes.

Several excellent reviews have recently been written on the topic of PCP, usually focused on one particular process, organism or tissue type. In this review, we will provide a brief overview of the conserved regulators of PCP as well as some vertebrate-specific PCP regulators. Further, we will discuss PCP in the kidney with a particular emphasis on data that we feel indicate novel regulation in this organ.

How is PCP established?

Genetic and molecular studies performed primarily in *Drosophila* have identified three signaling modules: one or more global directional modules that establish polarity with regard to the axes of the entire tissue, a core module that establishes local polarity, and a variety of tissue-specific effector modules downstream of the core and global modules that regulate polarity at the level of individual cells.

The role of the core and upstream groups appears to be largely conserved amongst different species although their precise mechanism may vary. However, it has been suggested that vertebrates also possess a number of regulators not found in flies. Although some of these may play indirect roles, others appear to be bona-fide PCP regulators. In this review, we will discuss the “universal” and “vertebrate specific” regulators as well as the role of morphogenesis in this process. As the tissue specific effectors differ from organism to organism and tissue to tissue, they will not be discussed.

The core module – Fz/Vang/Fmi

The core module consists of the seven-pass transmembrane receptor Frizzled (Fz), the cytoplasmic PDZ-containing protein Dishevelled (Dsh), the tetramembrane-spanning protein Van Gogh (Vang, also called Strabismus/Stbm), the Lim and Pet domain-containing cytoplasmic protein Prickle (Pk), the atypical cadherin Flamingo (Fmi, also called Starry night/Stan), and the ankyrin domain-containing cytoplasmic protein Diego (Dgo). These six core proteins are localized on the apical side of the cell and, in a number of tissues, such as the *Drosophila* wing, show further restriction in their localization to one side of the cell (Fig. 1C). Vang and Pk localize to the proximal side of the cell, while Fz, Dsh, and Dgo localize to the distal side. Fmi is present on both sides [3,4]. Each distal protein complex in one cell interacts with the proximal complex in the neighboring cell, which is hypothesized to further reinforce their asymmetric distribution. The asymmetric localization of the core proteins is easily detectable before the wing hairs emerge and can even be observed in the *Drosophila* wing disk [3,5].

As the asymmetric localization of these proteins could play a causal role in PCP, a great deal of work has gone into identifying the mechanisms regulating this process. Models propose that complex negative and positive interactions between the six core proteins are involved in the establishment and reinforcement of their polarized localization. Through these interactions, the PCP core complexes have the ability to self-organize polarity locally

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between adjacent cells. In addition, Fz and Dsh have been shown to be preferentially delivered to the distal side of the cell due to directionally biased trafficking and specific alignment of microtubules [6–8]. Distal complexes can recruit proximal proteins on neighboring cells and vice versa, while they repulse their localization within the same cell.

Interestingly, although the core PCP proteins are conserved in vertebrates/mammals and their mutation leads to defects in PCP in numerous tissues/organs [2] (Fig. 2), in some tissues there is no apparent asymmetry in their localization. Thus, the importance of the planar polarized localization of these factors and/or the level of functional conservation of this pathway in vertebrates is still not completely clear.

The Fat/Ds/Fj module

At the tissue level, PCP is stereotyped. In other words, the bristles on a fly abdomen or the hairs of a mouse always point in the same direction. Genetic mosaic studies in *Drosophila* revealed that clones of some core PCP components can perturb the polarity of neighboring wildtype cells in a non-autonomous manner. Loss-of-function clones of Fz and Vang strongly affect the polarity of neighboring non-mutant cells, a feature referred to as domineering non-autonomy [9]. However, genetic and molecular studies as well as several mathematical models [10–12] investigating the mechanism of the domineering non-autonomy phenomenon have shown that the local polarity of cells resulting from the asymmetric distribution of the core proteins cannot account for global polarity alignment in an epithelial sheet. To orient cells with respect to the tissue axes, long range cues such as gradients of diffusible factors or gradients in the activity of non-soluble factors must exist to propagate polarity across an entire tissue. The strongest evidence for a "global cue" comes from a group of interacting factors referred to as the Fat/Dachsous/Four-jointed group. This module consists of the large atypical cadherins Fat (Ft) and Dachsous (Ds), and the Golgi-associated kinase Four-jointed (Fj). Although this cassette of genes appears to regulate global PCP in *Drosophila* tissues, how this is accomplished is still unclear.

Rather than forming a classical morphogen gradient, this cassette forms an activity gradient. Ft and Ds engage in heterotypic complexes between adjacent cells via their tandem cadherin repeats [13,14]. The binding affinity between Ft and Ds is regulated by Fj, which phosphorylates their extracellular domains. Phosphorylation of Ft increases its affinity for Ds, while phosphorylation of Ds decreases its affinity for Ft [15,16]. Ds and Fj are expressed in opposing gradients across the *Drosophila* wing (Fig. 1C). Ds shows the highest expression in the proximal region and is absent in the distal region of the wing, while Fj has the highest levels at the distal side and fades towards the proximal side [17]. Ft is expressed evenly throughout the wing. The Fj gradient is proposed to result in the graded phosphorylation and activation of Ft across the tissue [14,18,19]. Although this model is supported in some tissues, in others, there does not appear to be a gradient in the expression of any of these molecules and in cases where proposed gradients have been perturbed or even abolished, they do not seem to affect PCP. Thus, it is still not clear how this cassette regulates global tissue polarity.

It is still debated whether the Ft/Ds/Fj module acts upstream of the core module or whether both modules act in parallel (see recent reviews by Peng and Axelrod [9], Lawrence and

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Casal [20], and Thomas and Strutt [21]). In the *Drosophila* wing, the opposing gradients of Ds and Fj were proposed to directly impact the asymmetric localization of the core proteins [3,22–24]. Recently, Matis et al. presented evidence for this proposed hierarchical relationship between the global Ft/Ds/Fj and core PCP module [8]. They showed that in the proximal and central region of the developing *Drosophila* wing, Ds and Fj signal through Ft to polarize the apical microtubule cytoskeleton correspondent to the Ds and Fj gradients. Only in wildtype but not Ft or Ds mutant wings, vesicles containing Dsh were transcytosed along the oriented microtubules towards the distal side of the cell, suggesting that the global Ft/Ds/Fj module is necessary for the directionally biased trafficking of distally located core proteins [8].

Wnt ligands

Another candidate for a global orienting cue is provided by the Wnt protein family. Wnts are diffusible ligands that function via binding to Fz receptors (members of the core group of PCP determinants). In the well-studied canonical Wnt signaling pathway, binding of Wnt to Fz and a low-density lipoprotein receptor leads to the activation of Dvl (*Drosophila* Dsh), which, by inhibiting GSK3β-mediated phosphorylation of β-catenin, results in stabilization of β-catenin and the formation of a β-catenin/Lef/Tcf transcriptional complex [25]. However, Wnts can also signal through β-catenin-independent, non-canonical pathways. Here, binding of Wnt ligands to a Fz receptor activates small GTPases including Rho, Rac and Cdc42 and further downstream protein kinases such as JNK or Rho kinase, eventually leading to actin cytoskeleton rearrangements and coordinated polarization of cells resulting in PCP [26]. Indeed, the non-canonical, Rho/Jnk pathway is frequently referred to as the PCP pathway. Wnt5a, Wnt7a, Wnt11 and Wnt9b (kidney) have been shown to control PCP in vertebrates [25,27]. In mammals, zebrafish and frogs, mutations of Wnt/PCP pathway genes lead to phenotypes in kidney tubules and neural tube development once again consistent with PCP defects [28], showing the crucial role of Wnt ligands in PCP during vertebrate morphogenesis.

Given that Fz is a core PCP determinant and that Wnt ligands can affect PCP, it was speculated that Wnts might somehow polarize Fz activity and/or localization. In *Caenorhabditis* elegans worms, this indeed appears to be the case. Wnt expression can direct the planar polarized localization of Frizzled receptors in adjacent cells [29]. Demonstrating this in vertebrates has been more challenging. This is in part due to the fact that there are multiple Wnt ligands and receptors (18 and 10 respectively in mammals) and their biochemical partnering is unclear. However, in melanoma cell lines, Wnt5a has been shown to be able to orient the localization of Frizzled 3 [30], similar to what was observed in worms.

Multiple attempts have been made to categorize Wnts into canonical vs. non-canonical categories. However, there is evidence that pathway activation for a specific ligand can vary from cell type to cell type, depending on the intracellular environment within the receiving cell. This seems to be dependent on the presence of specific co-receptors. For example, Wnt5 and Wnt11 can activate the non-canonical PCP pathway when Fz is in complex with co-receptors such as Ror, Derailed and Ryk [31]. In the developing limb bud, it has been

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shown that Wnt5a induces the formation of a Ror2-Vangl2 receptor complex. Graded expression of Wnt5a leads to a Vangl2 phosphorylation gradient, which controls Vangl2 activity and thereby establishes PCP [32]. Thus, evidence is growing that, depending on the cellular context, Wnts can regulate the planar polarized expression of Frizzled molecules and directly regulate PCP.

Whether the Wnt pathway plays an instructive role in PCP in flies is controversial. Although mutations in Wingless (Wg) were shown to perturb PCP, it has been suggested that this is an indirect effect dependent on β-catenin and transcriptional regulation of Ft and Ds [33]. Other *Drosophila* Wnts (DWnts) so far have not been shown to play a role in PCP [34,35] and were considered as non-essential. However, Wu et al. recently showed that in the *Drosophila* wing margin, Wg and DWnt4 act redundantly to provide directional information by modulating local interaction between Fz and Vang in a dosage-dependent manner, therefore inducing a Fz activity gradient [36]. In addition, Matis et al. recently found that overexpression of DWnt4, but not Wg, led to reorganization of apical microtubules, suggesting a potential role for Wnts in regulating core protein asymmetries in conjunction with the Ft/Ds module [8]. Thus, the precise role of Wnts as determinants of PCP is still being clarified.

PTK7

It is possible that Wnt signaling has evolved as a PCP effector in some organisms and not others. Indeed, protein tyrosine kinase 7 (PTK7), a transmembrane pseudokinase that appears to play a role in Wnt pathway selectivity, has been proposed to be a vertebrate-specific PCP regulator. PTK7 mutants show multiple PCP defects including defective convergent extension (CE) movements in gastrulating *Xenopus*, zebrafish and mice. PTK7 mutant mice show polarity defects of inner ear sensory hair cells and a severe neural tube closure defect [31,37]. The role of PTK7 in Wnt pathway selectivity is not yet fully understood as it has been shown to affect both canonical and non-canonical Wnt signaling [38,39]. PTK7 is able to interact with Fz7, β-catenin and Dvl [31,40]. In *Xenopus* and *Drosophila*, PTK7 (Okt in *Drosophila*) seems to function by recruiting Dvl to the plasma membrane, leading to non-canonical signaling upon co-recruitment of RACK1 (receptor of activated protein kinase C) [31]. In zebrafish, RACK1 also has been identified as an interaction partner of Vangl2 (homolog of *Drosophila* Vang), required for Vangl2 membrane localization [41].

Recent studies in the mouse inner ear have shown that PTK7 is involved in the regulation of myosin II-based contractile forces to orient PCP independent of the non-canonical Wnt pathway [42]. Modulation of junctional contractility and adhesive strength is achieved by stimulation and stabilization of Src in its active conformation along cell–cell contacts, thereby controlling ROCK2 activity/phosphorylation [43]. Hence, PTK7-mediated signaling may have direct impact on PCP via cytoskeleton remodeling.

PTK7 is regarded as a vertebrate-specific regulator because a role for Okt in the regulation of *Drosophila* PCP has so far not been established. However, Okt has been shown to function as a co-receptor for DWnt4 [31], a Wnt only recently implicated in the regulation of

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PCP in the *Drosophila* wing [8,36]. Hence, further investigation will reveal whether PTK7/Okt is truly a vertebrate specific effector of PCP.

Primary cilia

The primary cilium is another example of a proposed vertebrate specific PCP effector. Interactions between primary cilium-associated proteins and PCP signaling have been observed in many biological contexts including mouse inner ear, embryonic node, ependyma, tracheal and kidney epithelium, *Xenopus* epidermis and zebrafish floor plate ([44] and references therein). Most quiescent cells in vertebrates contain at least one nonmotile primary cilium. Located at the apical plasma membrane, this cilium is composed of the centriole-derived basal body, which acts as a microtubule-organizing center (MTOC), and an array of microtubules, called the axoneme, which forms a projection extending from the apical membrane. Primary cilia play a role in the development, function and maintenance of most organs. Although their precise function is unknown, they have been implicated in detecting fluid movement (mechanosensors) or changes in chemical factors (chemosensors) as well as foci of receptors for morphogens or growth factors produced by the surrounding environment [45]. Defects in ciliogenesis, cilia function and signaling are the basis of a variety of human diseases and developmental abnormalities collectively referred to as "ciliopathies". Ciliopathies can manifest themselves in any organ, but predominantly affect the kidney, eye, liver and brain [46]. Ciliopathic syndromes of the kidney include polycystic kidney disease (PKD), nephronophthisis (NPHP), and renal dysplasia [46].

Primary cilia have been shown to regulate Hedgehog signaling, and also to affect Wnt pathway usage and PCP. Components of the core PCP machinery such as Fz, Vangl, Dvl and Inversin (homolog of *Drosophila* Diego) are present in the cilium or in the base of the basal body. Mice with mutations in genes necessary for ciliogenesis show pathologies that have been linked to PCP defects (for more details [47]). Furthermore, loss of the PCP effector genes Fuzzy and Inturned leads to disruption of the cytoskeleton and defects in cilia formation [48]. Ciliogenesis is a dynamic process during which cilia are constantly being formed and resorbed during the cell cycle. In this process, the basal body is converted back to the centriole, which eventually gives rise to the mitotic spindle poles. Hence, ciliogenesis and the basal body possibly influence the orientation of cell division [45], which is one of the features underlying vertebrate PCP. Thus, the cilia may affect PCP through multiple distinct mechanisms. Alternatively, mutations that disrupt the cilia may affect other aspects of cell polarity which secondarily affects PCP.

Morphogenesis as a global clue

Recent studies revealed that epithelial morphogenesis itself can serve as a mechanism to reorganize and orient global PCP patterns [5,12]. In the developing *Drosophila* pupal wing, PCP domains visualized by Fz/Vang localization are initially oriented toward the wing margin. Contraction of the wing hinge subjects wing-blade epithelial cells to anisotropic tension, inducing specific patterns of oriented cell elongation, cell rearrangement and cell division leading to the elongation of the wing blade proximo-distally. At the same time, PCP

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is realigned to a pattern that points distally [12]. Severing the hinge from the wing blade disturbs the proximal–distal wing elongation and the realignment of the PCP pattern. Since reorientation of the PCP pattern occurs at the same time as the wing blade is reshaping through hinge contraction, these events seem to be interdependent [12]. Recently, studies in the wing disk determined that PCP patterns develop very early and are oriented with regard to organizer regions expressing Notch/Wg, Hedgehog and Ds/Fj [5]. Perturbing any organizer region led to specific alterations in both growth and the polarity pattern, suggesting that each morphogen system independently contributes to the establishment of a global polarity pattern in the wing disk [5]. Sagner et al. suggest that PCP is not directly responding to morphogen gradients on a cellular level, but that the morphological changes drive global polarity patterning from a very early state onwards, which is then propagated during development [5].

The question arises as to whether morphogenesis is the cause or consequence of planar polarity. Arguments speaking for orientation of PCP patterns as a consequence of morphogenetic changes are that most mutants for core PCP genes only show minor changes in their wing shape. Although Ds mutants develop shorter and broader wings in part due to perturbed oriented cell elongation and divisions, contraction of the hinge still occurs [12]. Also, in the gastrulating *Drosophila* embryo, several core PCP genes are not required for convergent extension [49]. On the other hand, the global Ft/Ds/Fj module seems to play an active role in driving morphogenesis by affecting anisotropic tension. Ds polarizes the unconventional myosin Dachs, which has been shown to promote anisotropy of junction tension, thereby affecting oriented cell divisions in the wing disk and cell rearrangements in the pupal notum [50–52]. It is also feasible that anisotropy of junction tension could lead to asymmetries in cell shape, which are also a characteristic of PCP. Independent from PCP signaling, spatial differences in proliferation rates can lead to anisotropies in tissues, which will drive epithelial patterning and therefore affect future cell division orientations and tissue shape [53]. However, since Ft and Ds do not only play a role in PCP but also in controlling tissue size via the Hippo/Warts signaling cascade, Ft/Ds may take part in the regulation of proliferation rates.

How mechanical forces and global cues such as the Ft/Ds module or Wnt ligands (inter)act to direct PCP and tissue morphogenesis is an interesting question that still requires further investigation.

PCP in the kidney

One vertebrate tissue that is growing in popularity as a model for studying PCP is the kidney. The kidney consists of numerous (up to one million per kidney in humans) epithelial tubules known as nephrons connected to another tubular network known as the collecting ducts. These two types of tubules form from distinct processes. Vertebrate kidney development initiates when an epithelial structure known as the ureteric bud (UB) emerges from the Wolffian duct and begins to undergo reiterative branching morphogenesis within a population of mesenchymal cells known as the metanephric mesenchyme (MM). As the ureteric bud continues to branch, the more distal elements undergo convergent extension like movements that result in the tubule becoming longer and thinner [54,55]. Once the diameter

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is established, oriented cell divisions (OCD) result in tubule lengthening while diameter is maintained, eventually forming the collecting duct network. The nephrons, on the other hand, form when small clusters of the MM aggregate and transition into an epithelium. It is believed that these newly formed renal vesicles then undergo CE and/or OCD to establish and maintain their diameter; however, the convoluted, twisted nature of the nephron has inhibited the detailed characterization of this process. As the nephrons and collecting ducts are undergoing their normal morphogenetic movements, they do so in an environment of surrounding fibroblast-like cells referred to as “stroma” as well as large and small blood vessels.

Defects in OCD were shown to occur in kidney epithelium that lacked Hnf1b [56]. Hnf1b mutant tubules become extremely dilated reminiscent of a common syndrome in humans known as polycystic kidney disease (PKD). Indeed, interest in the vertebrate cilium as an essential organelle really was promoted by findings that the proteins mutated in the most common forms of PKD were localized to the cilia and indeed kidneys that are not able to form cilia due to specific ablation of the kinesin family member 3A (Kif3a) formed polycystic kidneys [57]. As OCD and CE both rely on PCP and the cilia have been linked to PCP, it was hypothesized that defects in PCP would result in PKD. Although this may be the case, the mechanisms underlying cyst formation cannot simply be attributed to defects in PCP.

Multiple paralogs of the *Drosophila* PCP determinants are expressed in the kidney although their expression domains do not provide insight into how PCP may be regulated in this tissue. Ds1 is expressed in the metanephric mesenchyme while Fat4 is primarily expressed in the adjacent stromal cells (Fat4 may also be expressed at low levels in the MM). Fjx on the other hand is expressed in early stages of the forming nephron, a cell type that does not express significant levels of Fat4 or Ds1. Multiple Fz paralogs are expressed in all cell types of the developing kidney. Dvl1, 2 and 3 are also expressed broadly. Vangl2 is expressed in most epithelial cell types at all stages of development while distinct flamingo paralogs show much more restricted epithelial expression. The two Diego paralogs, Diversin and Inversin, are expressed in the stroma and collecting duct epithelia respectively [58].

Although the expression domains are complex, a simple model of a Ft/Fj/Ds gradient acting to polarize the expression of the core determinants does not seem likely. It is not even clear that paralogs of each core determinant are co-expressed at any one time within either the nephron or collecting duct epithelium when they could be interacting to regulate PCP. Further, polarized expression of core determinants has not been observed although admittedly, planar polarized distribution is most convincingly demonstrated in tissues in which the protein of interest has been mosaically deleted, experiments that have not been performed in the kidney.

Nonetheless, PCP defects have been observed upon deletion of some PCP determinants [59–61]. In Fat4 and Ds1 mutants, cystic epithelia are observed in collecting ducts and loops of Henle although the cystic phenotype is relatively mild. For Vangl2 mutants, kidneys have slightly wider epithelia with more cells in their circumference but no cysts form. Thus is it difficult to construct a straightforward mechanism for cystogenesis based on PCP defects.

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Compound mutants carrying null alleles of Fat4 and Vangl2 show an enhanced cystic phenotype although what this means in terms of linear versus parallel pathways is unclear [59].

Multiple Wnts are expressed in the developing kidney and, based on their described expression patterns, they could be acting as long range determinants of PCP. Wnt7b and Wnt9b are expressed at high levels in the stalks of the collecting ducts and lower levels at the tips while Wnt11 is expressed only in the branching tips of the UB. Wnt4 is the only Wnt expressed in the nephron and it is expressed only on the proximal side of the renal vesicle. Several Wnts including Wnt4, 11 and 5a are expressed in the medullary stroma surrounding the epithelia as they elongate.

Ablation of Wnt9b and 7b both lead to PCP defects in the developing kidney although through different mechanisms. Wnt9b mutants have cystic nephrons. The collecting duct epithelium is broader (although not cystic until much later in life) and shows defects in both CE and OCD [54]. Also, the cells of the collecting duct, normally elongated along an axis parallel to the proximal distal axis of the tubule (a form of PCP), are randomly oriented [54] (Fig. 2C). Interestingly, Wnt9b expression in the collecting ducts appears to be directly regulated by Hnf1b, at least partially explaining the PCP defects in Hnf1b mutants [15,16].

Wnt9b mutants show decreased levels of activated Rho and Jnk, although it is not clear whether this is the result of defects in non-canonical Wnt signaling or a reduction in the cell types where this signaling pathway is most active, the nephrons. Characterization of Wnt9b mutants reinforces the concept that defects in PCP alone are not sufficient to result in cysts as the collecting ducts show clear PCP defects but are not cystic.

As Wnt9b is only expressed in the collecting ducts but cysts arise in both the collecting ducts and the nephrons, the effect of Wnt9b on PCP of the nephrons is occurring through a non-autonomous mechanism. Indeed, previous studies showed that Wnt9b signals to the MM and is necessary for the formation of nephrons from this cell type. However, in the case of cyst formation, the target cell is not clear. It is possible that PCP must be actively established and/or maintained within the MM prior to or at the time of its epithelialization or as the epithelium elongates and that Wnt9b signals to these cells to regulate one (or both) of these processes. Alternatively, as the nephron fuses to the collecting duct shortly after it forms, it is possible that PCP is passed on to the nephron by the already polarized collecting duct. In this case, Wnt9b may only directly regulate the PCP of the collecting duct, which then indirectly regulates the PCP of the nephron. A final, related possibility is that Wnt9b signals to a third cell type which then secondarily regulates PCP of the epithelium. One candidate cell type is the adjacent stroma. As mentioned, several of the PCP determinants are expressed in the stroma indicating this cell type may have a function. Further support for this idea comes from characterization of the Wnt7b mutants [62].

Wnt7b mutants fail to form the most medullary portion of the kidney, a region referred to as the renal papilla. This phenotype is the result of deficits in the elongation of both the collecting ducts and the medial region of the nephron caused by improper OCD and potentially CE movements as well as defects in cell division rates [62]. As in the Wnt9b and

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Vangl2 mutants, the collecting ducts of these mutants do not form cysts. This observation is complicated by the fact that Wnt7b mutant epithelia do not proliferate at the same rate as wildtype. In contrast to the case with Wnt9b mutants, the target cell for the Wnt7b ligand appears to have been identified and it is the stroma. Interestingly, Wnt7b appears to signal in a canonical manner (through beta-catenin) to the stromal cells, which subsequently signal back to the epithelium to regulate PCP. As the target cell of Wnt9b has not been identified, it is possible that it too is the stroma. However, if this is the case, Wnt9b must operate through a distinct mechanism as characterization revealed no overlap in Wnt7b and 9b target genes and, as mentioned, the phenotypes are quite distinct.

The manner in which the stroma regulates the planar polarity of the adjacent epithelium is unclear although there are a number of possibilities. In the case of Wnt7b deletion, it has been shown that the mutant stroma shows decreased expression of Wnt4, 5a and 11. These stromally produced ligands may signal back to and regulate the planar polarity of the adjacent epithelial cells. A second possibility is that the stroma, which sits on the basal side of the epithelium, regulates some aspect of apical-basal polarity of the epithelium and proper A/B polarity is necessary for PCP. Indeed, mutation of several A/B polarity determinants results in PCP defects suggesting the two processes are connected [58]. A third possibility is that, as the stroma at least partially produces the extracellular matrix (ECM) that the epithelia will migrate on during development, defects in ECM production can lead to PCP defects. Indeed both the stiffness and the orientation of the ECM have been implicated in PCP [63,64]. Given the studies in flies relating to organizers, anisotropic tension, morphogenesis and PCP, it is quite tempting to speculate that PCP in the kidney is a consequence of morphogenesis.

Conclusions

The molecular and cellular processes regulating cell and tissue polarity have long fascinated biologists. In the last several years, their popularity has grown given their importance in human disease processes such as spinal cord defects, cancers and polycystic kidney disease. However, as we begin to study these processes in greater detail in vertebrates, caution must be taken not to assume that what happens in a fly wing also happens in a kidney tubule. Biologists have a tendency to seek order and conservation even when such conservation does not always exist. Although many of the factors regulating PCP in flies are conserved in higher vertebrates and in some cases, their roles in regulating PCP are also conserved, the mechanisms through which they function appear distinct. For example, although in the mouse kidney many PCP determinants are expressed, trying to match their expression patterns with conserved functions (based on mechanisms in flies) has been difficult. Further, the severity of the defects observed in some cases is quite mild. Although there are trivial explanations for these findings (e.g. insensitive detection methods, molecular redundancy), it seems quite possible that there are distinct mechanisms and certainly distinct regulators of PCP in mice. Indeed, even in flies, the mechanisms regulating PCP during gastrulation and germ band extension appear to be independent of the classical PCP determinants discussed above. It is clear that PCP can be regulated by cell adhesion, cell shape and cell tension and in some tissues, this may be the major determinant. In the frog kidney, recent data shows that PCP dependent processes in tubule elongation are more similar to those in fly gastrulation

than in wing hair orientation [65]. It may be important for biologists studying PCP to start concentrating on the differences rather than the similarities as these may be more informative.

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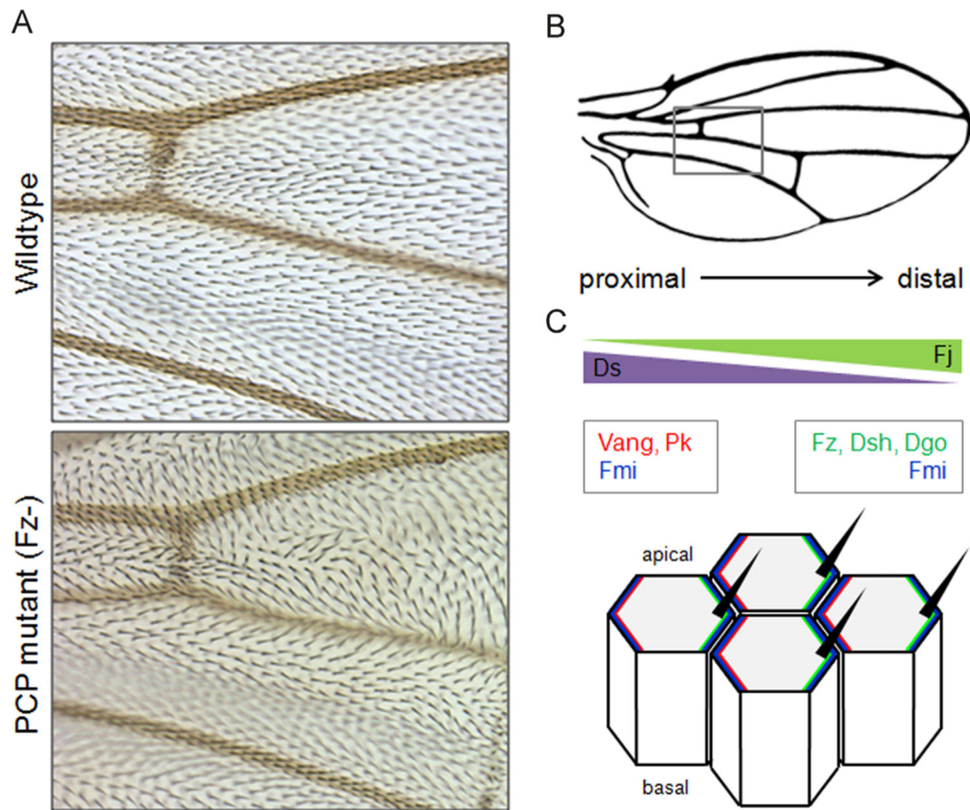

Fig. 1.

PCP signaling in the *Drosophila* wing. A) Epithelial cells in the wing blade generate an actin hair pointing distally in wildtype flies, while mutants lacking Fz show disturbed hair polarization with swirls and waves. The original images were kindly provided by Marek Mlodzik and Jun Wu. B) Schematic illustration of a wing. The grey box indicates the region shown in A. C) Global PCP components Ds and Fj are expressed in opposing gradients across the wing, while the core PCP proteins are asymmetrically localized at the cell junctions between neighboring cells. The asymmetries of global and core proteins generate tissue polarity.

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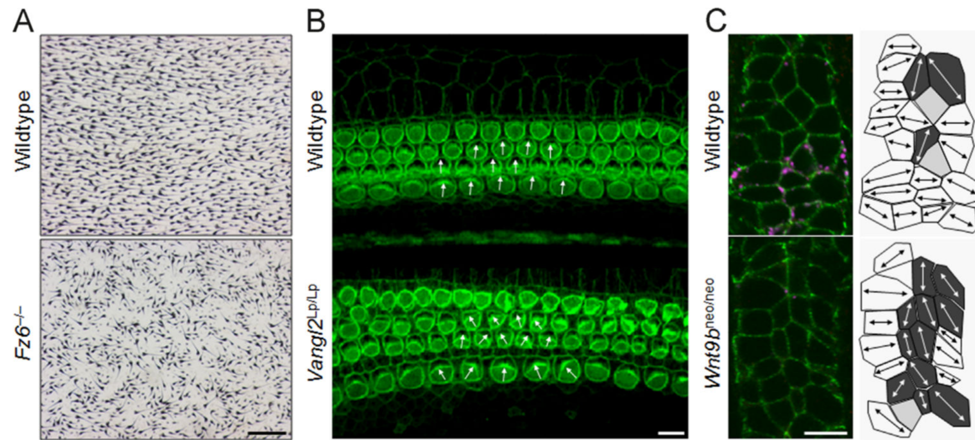

Fig. 2.

Examples of vertebrate PCP. A) Hair and hair follicles in dorsal skin of wildtype and *Fz6*−/− mutant mice at P3, visualized with melanin pigmentation. Mice are oriented with anterior to the left and posterior to the right. In PCP mutants, hairs do not point distally as in but lose their uniform polarity. Scale: 0.5 mm. Images were kindly provided by Jeremy Nathans and Hao Chang. B) Orientation of sensory hair cells of the cochlea (inner ear) of E18.5 wildtype and Vangl2 mutant mice. Polarized bundles of stereocilia are uniformly oriented in wildtype mice, while their orientation becomes randomized in the PCP mutant (direction indicated by white arrows). Scale: 10 μm. Original images were kindly provided by Matthew Kelley. C) Polarized orientation of tubule cells perpendicular to the axis of extension is disturbed in *Wnt9b* mutants. Confocal images (single focal plane from Z-stack) show collecting ducts in E15.5 wildtype and *Wnt9b*neo/neo kidneys, immunostained for E-cadherin (green), DBA (collecting duct marker; magenta) and Par3 (apical membrane marker; red). Chosen areas represent regions just basal to the apical membrane, identified by absence of Par3. Cell outlines: white cells are perpendicular to the axis of elongation (45–90%), dark gray cells are parallel (0–45%). Scale: 10 μm. See Karner et al. [54] for quantification.