Published in final edited form as:

Curr Opin Cell Biol. 2010 October ; 22(5): 597–604. doi:10.1016/j.ceb.2010.07.011.

Planar cell polarity signaling, cilia and polarized ciliary beating

John B. Wallingford

Howard Hughes Medical Institute, Section of Molecular Cell and Developmental Biology & Institute for Cellular and Molecular Biology, University of Texas at Austin

Abstract

Planar cell polarity signaling governs a wide array of polarized cell behaviors in animals. Recent reports now show that PCP signaling is essential for the directional beating of motile cilia. Interestingly, PCP signaling acts in a variety of ciliated cell types that use motile cilia to generate directional fluid flow in very different ways. This review will synthesize these recent papers and place them in context with previous studies of PCP signaling in polarized cellular morphogenesis and collective cell movement.

Motile cilia play an ancient and crucial role in the movement of materials and cells. Chlamydomonas and other unicellular organisms use motile flagella or cilia to move themselves through watery environs. Conversely, epithelial tissues in metazoan animals frequently employ batteries of cells bearing motile cilia to move fluid inside the animal. Generally, this scenario plays itself out in two key contexts, multi-ciliated cells in differentiated organ systems (Fig. 1A), and mono-ciliated cells in the early embryonic node (Fig. 1B). A steady flow of recent papers has now revealed that the Planar Cell Polarity (PCP) signaling cascade is a central regulator of the orientation of cilia-mediated fluid flow.

Multi-ciliated cells

Multi-ciliated cells generate fluid flow in a variety of epithelial organs. The canonical example is the vertebrate airway, where a role for multi-ciliated cells in generating flow for the clearance of mucus was well described by the 1850's [1,2]. Multi-ciliated cells are also present in the ventricles of the vertebrate brain, where they propel cerebrospinal fluid [3] and in vertebrate oviducts, where they move ova toward the uterus [4].

Curiously, despite their well-known role in mammalian airways, the first connection between polarized beating in multi-ciliated cells and PCP signaling came from studies of an organism with gills rather than lungs. Like the airway, the epidermis of amphibian embryos is a mucociliary epithelium, and the planar polarity of fluid flow across this tissue has been studied for over 100 years [1,5–8]. Recently, the *Xenopus* epidermis has provided a rapid *in vivo* platform for molecular analysis of multi-ciliated cell development and function [9–14].

In multi-ciliated cells, planar polarity is present in two distinct modes, termed rotational polarity and tissue-level polarity (Box 1). The former refers to the alignment of the basal bodies (9+3 microtubule-based organelles that form the base cilia) within each multi-ciliated cell (Box

wallingford@mail.utexas.edu, 1 University Station C1000, University of Texas, Austin, TX 78712, Phone: 512-232-2784.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1; Fig. 2A), and the latter to the coordination of many multi-ciliated cells across the tissue (Box 1, Fig. 2B). PCP signaling controls both types of polarity in the *Xenopus* epidermis.

Box 1

Three modes of planar polarity in ciliated cells

Rotational Planar Polarity (Fig. 2A)

The orientation of each basal body in a multi-ciliated cell is manifested by the positioning of accessory structures such as the basal foot (which points in the direction of effective stroke) and the rootlet (which points in the opposite direction) [73,61,11]. The parallel alignment of all the basal bodies within each multi-ciliated was recently termed “rotational” planar polarity [27].

Tissue-level planar polarity (Fig. 2B)

In addition to the intra-cellular (rotational) polarity, there is also an inter-cellular polarity, which we will refer to as “tissue-level” polarity. This type of polarity is apparent, as all of the multi-ciliated cells within the tissue have their aligned basal bodies oriented in the same direction [14].

Translational planar polarity (Fig. 2C)

In multi-ciliated cells of the airway, oviduct or *Xenopus* epidermis, basal bodies cover essentially the entire apical surface. In ependymal cells, basal bodies are present in a cluster, only partially covering the apical surface and the position of these clusters is planar polarized [27]. Clusters initially form in the center of each cell, and as polarity becomes entrained, the cluster migrates to the posterior apex of each cell. This third aspect of planar polarity (termed translational planar polarity [27]) is also observed in kinocilia in hair cells of the vertebrate inner ear (Fig. 3A)[65,34], in node cilia (Fig. 3B)[41,50,43], and in lens fiber cells (Fig. 3E)[64].

In the wake of early reports that core PCP components were associated with primary cilia [15,16], the cytoplasmic PCP protein Dishevelled (Dvl) was found to be enriched apically multi-ciliated cells [9]. Immunostaining revealed that Dvl localized near the base of cilia in these cells and moreover that it was planar polarized[12], demonstrating that asymmetric localization of a core PCP protein can reflect not only the planar polarity of cells within a tissue, but also of organelles within a cell.

More importantly, disruption of Dvl function by expression of a PCP-specific dominant-negative Dvl results in a randomization of rotational planar polarity within each multi-ciliated cell (Fig. 2A)[12]. High-speed confocal imaging revealed that the defects in basal body polarity was coupled to disorganized beating of cilia and impaired directional fluid flow across the epithelium [12].

Some progress has been made in understanding the mechanism by which Dvl acts to control rotational polarity. Dvl is well-known to control cell polarity during the collective cell movements of convergent extension [17], and in that setting Dvl-mediated activation of the small GTPase Rho is essential [18–21]. Likewise, in *Xenopus* multi-ciliated cells, Dvl is required for activation of Rho at the apical surface [12]. A second study showed that control of Dvl stability by the anaphase promoting complex C (APC/C) was also essential for rotational polarity in these cells [22]. These results are consistent with elegant transplantation experiments that revealed the role for Dvl in rotational polarity to be cell autonomous [14].

In addition to aligning individual basal bodies within each multi-ciliated cell, PCP signaling also controls the tissue-level polarity of multi-ciliated cells [14]. Interestingly, transplantation experiments revealed that transmembrane PCP proteins, Van Gogh-Like 2 (Vangl2) and Frizzled act non-cell autonomously in this case (Fig. 2B)[14]. This result indicates that, while we have yet to observe it directly, PCP signaling is active in the non-ciliated cells of mucociliary epithelia. Moreover, these experiments revealed the first vertebrate example of the domineering non-autonomy that has long been observed in *Drosophila* (e.g. [23,24]).

In recent months, three very nice papers have elucidated the mechanisms of planar polarization in another multi-ciliated cell type, the ependymal cells of the brain. Arising from radial glia, the ependymal cells develop tufts of planar polarized cilia that propel the cerebrospinal fluid in the central nervous system [3,25]. A fascinating paper has shown that this fluid flow can actually guide the migration of neurons [26].

The first of these studies revealed that ependymal cells have an added component to their planar polarity, termed translational planar polarity [27](Box 1;Fig. 2C). This study shows that motile cilia are not required for *translational* planar polarity in these cells, which was surprising, as *rotational* polarity absolutely requires that the motile cilia be present [27], as is the case in the *Xenopus* epidermis [11]. The translational planar polarity of differentiated ependymal cells instead requires the presence of non-motile primary cilia on the radial glial precursors from which these cells arise [27](Fig. 2C). Indeed, the single primary cilia present on radial glial precursors also display translational planar polarity [27]. Together, these data suggest that radial glial cells possess a planar polarity that is then essential for the planar polarization of their ependymal cell descendants.

Two other papers have now linked the PCP signaling pathway to the planar polarization of ependymal cilia, showing that the core PCP proteins Vangl2, Celsr2, and Celsr3 are required for establishment of rotational and tissue-level polarity and for polarized fluid flow in the mouse brain [28,29]. Importantly, asymmetric Vangl2 localization reflects polarity in these cells [28], as it does in the mammalian cochlea and *Drosophila* wing [30,31]. Curiously, Celsr2 and Celsr3 are essential for Vangl2 localization in ependymal cells [29], though not in the *Drosophila* eye [32]

Finally, loss of cilia by conditional mutation of Kif3a or Ift88 in ependymal cells severely impairs rotational planar polarity, but leaves the asymmetric localization of Vangl2 intact [28]. This result is consistent with previous studies in the cochlea. Like ependymal cilia, kinocilia in the cochlea display rotational planar polarity manifested by the position of mother and daughter centrioles, translational planar polarity manifested by the ultimately lateral position of the kinocilium on the hair cell, and tissue-level polarity manifested by the consistent orientation of hair cells in the cochlea (Fig. 3A)[33,34]. Interestingly, disruption of ciliogenesis by mutation of the Intraflagellar Transport Protein Ift88 or the kinesin microtubule motor Kif3a significantly impairs rotational, translational, and tissue-level polarity of hair cells while leaving the localization of core PCP proteins unaffected [34]. Thus, a role for Ift88 and Kif3 in planar polarization of cells either downstream or independent of the PCP pathway seems to be widely deployed and applies to both motile and non-motile cilia.

Node monocilia

Leftward fluid flow across the developing node is a conserved feature of vertebrates and is essential for left-right patterning [35]. Flow is generated in these tissues by the rotational beating of 9+0 monocilia on the apical surface of node epithelial cells [36,37]. Unlike multi-ciliated cells, where polarity is imparted largely through rotational orientation of the basal bodies, in the node it is translational planar polarity (the posterior positioning of cilia) that

determines the direction of flow (Fig. 1B; Fig. 1D) [36,37], because node cilia beat in a circular manner, rather than with effective and return strokes [36].

The earliest studies intimating a link between PCP signaling and left-right pattern did not focus on cilia *per se*. For example, Inversin and Seahorse were identified as effectors of left/right patterning and were found to interact with Dvl [38,16,39]. More significantly, the cystic kidney disease gene BicC is also essential for left-right patterning [40,41]. In the nodes of mice mutant for BicC, translational planar polarity of nodal cilia is defective such that they do not take on a posterior position, and consequently, leftward flow is disrupted in BicC mutants [41]. Similar results were likewise observed upon BicC knockdown in *Xenopus* [41], where the cilia on the large gastrocoel roof cells are orthologous to node cilia of mice [35,42]. PCP signaling was implicated because BicC physically and functionally interacts with Dvl [41].

Recently, several papers directly demonstrate roles for PCP signaling in directional beating of nodal cilia. First, in an elegant time-lapse study using cultured mouse embryos, the posterior positioning of basal bodies was shown to be established over time in the node, and positioning correlated with the onset of directional flow [43]. In Dvl mutant mice, which displayed defective left-right pattern, posterior positioning of basal bodies was interrupted, and fluid flow across the node was randomized. As is the case for PCP signaling during convergent extension [44,20], Dvl's role in translational planar polarity of node cilia requires the small GTPase Rac [43]. Curiously, Wnt3a was dispensable for polarization of nodal cilia [43], which was surprising because Wnt3a mutant mice display left-right defects [45]. Finally, and importantly, Dvl-GFP was asymmetrically enriched at the posterior face of mouse node cells (Fig. 1B) [43], consistent with its previously-demonstrated posterior localization during zebrafish convergent extension and its asymmetric distribution in *Drosophila* wing hairs [46,47].

In a second study, Vangl1 and Prickle2 were likewise found to be asymmetrically localized in the mouse node [48]. As is the case in *Drosophila* wing epithelia and vertebrate cochlea [30, 31], Vangl1 and Pk2 localize opposite to Dvl, on the anterior face of node cells (Fig. 1B) [48]. Loss of Vangl1 in the mouse was shown to result in aberrant expression of the left/right marker gene Pitx2 [48]. To link Vangl proteins to cilia positioning, the authors turn to the gastrocoel roof cells of *Xenopus*, where knockdown of Vangl2 disrupted translational planar polarity and perturbed left/right patterning [48]. A third study reports that conditional mutation of both Vangl1 and Vangl2 in the mouse disrupts translational planar polarity in the node, causing a failure of leftward fluid flow across the node. This, in turn, disrupts left/right patterning [49].

A fourth study addresses this issue in zebrafish. Genetic removal of both maternal and zygotic Vangl2 randomized the direction of cilia tilt and fluid flow in Kupffer's vesicle [50], the zebrafish structure that is orthologous to the mouse node [35,51]. In addition, the floorplate of fish larvae are decorated with motile monocilia, and these too display translational planar polarity [50]. The localization of PCP proteins relative to primary cilia in the floorplate was found to be similar to that in the mouse node, as Prickle was asymmetrically localized on the anterior face of these cells [50]. Finally, the polarization of motile cilia in the fish requires glypican4 [50], which likewise controls PCP-mediated convergent extension [52].

In sum, PCP-mediated control of the planar polarity in ciliated cells is clearly a common feature of the frog, fish and mouse. Of particular interest now will be to understand how the PCP signaling mechanisms in each cell type have been adapted to meet the subtly different needs of rotational, translational, and tissue-level planar polarity. Below, I consider four additional outstanding issues.

1. How is initial beat polarity established in ciliated cells?

It is well-established that for multi-ciliated cells, fluid flow itself is crucial for entraining polarized ciliary beating. Experiments with *Xenopus* epidermis first showed that flow was essential for polarity and moreover that experimentally-generated flow could re-orient ciliary beating [11]. That study also revealed that cilia motility is required for re-orienting in response to flow [11]. A subsequent study showed that the same is true for ependymal multi-ciliated cells, and that PCP signaling was essential for the flow-mediated entrainment of polarized beating [28].

How polarity initially arises in these cells is not understood. In the *Xenopus* epidermis, multi-ciliated cells develop with an initially weak rotational planar polarity that is then reinforced by fluid flow until a strong, coherent polarity is established [11]. The existence of such an initial pre-pattern in developing multi-ciliated cells has been demonstrated by tissue reversals in both *Xenopus* epidermis and quail oviduct [53,7].

In the ependymal cells, the issue is less clear. One possibility is that rotational organization of ependymal basal bodies is random initially, and orientation is entirely mediated by fluid flow [28]. In this model, polarized flow is initiated by a pressure gradient arising from asymmetric secretion and re-absorption of cerebrospinal fluid. This flow accelerates as flow-mediated polarization of ciliary beating commences, leading to a positive feedback loop and strong polarization of ciliary beating [28].

However, translational planar polarization of basal bodies has been observed in radial glial cell precursors, even before they begin to differentiate into ependymal cells [27]. This result suggests, on the one hand, the possibility that some form of pre-pattern is at work in ependymal cells, which would be consistent with the case for multi-ciliated cells in quail oviduct and *Xenopus* epidermis [53,7,11]. On the other hand, translational and rotational planar polarity in ependymal cells seem to be, at least in part, independently controlled [27], and the pre-pattern in these cells may apply only to translational polarity. Clearly, more work in this area will be exciting.

In contrast to the situation in multi-ciliated cells, the establishment of translational polarity of node mono-cilia is independent of flow, as translational polarity is normal in mice with immotile node cilia [36]. Indeed, such translational planar polarity clearly can be established in the absence of flow, because PCP signaling also controls translational polarity of non-motile kinocilia of the cochlea and primary cilia in the lens [54,33,55].

2. How is the apical cytoskeleton of planar polarized, ciliated epithelial cells organized?

The regularity and orientation of basal bodies in multi-ciliated cells is striking, and despite these new studies, we still know very little about how this organization is achieved. Older studies of multi-ciliated cells suggest that microtubules, attached to basal feet, link the basal bodies to one another and also to the apical junctions (reviewed in [56]). Interestingly, the classic planar polarity observed in the *Drosophila* wing epithelium is also associated with a web of sub-apical microtubules [57,58]. These microtubules receive only scant attention, but they are planar polarized, with their plus ends slightly enriched at the distal face of cells, where Dvl and Frizzled localize [59].

Despite the intense focus on the polarization of cortical membrane domains containing core PCP proteins, it appears that the apical microtubule network is an essential *upstream* regulator of PCP signaling. First, Frizzled-GFP containing vesicles have been observed moving distally along these microtubule tracks, suggesting that these tracks are assembled and polarized prior to asymmetric Frizzled distribution [59]. Moreover, disruption of microtubules perturbs the asymmetric distribution of core PCP proteins [59]. Second, *wiederborst* (*wbd*), a regulatory

subunit of protein phosphatase 2A, is associated with these apical microtubules near the distal edge of wing epithelium cells. This asymmetric localization is surprisingly *not* affected by manipulations of core PCP genes. Strikingly however, disruption of *wbd* function results in disruption of the apical microtubule web and also a failure of asymmetric localization of the core PCP proteins [60]. It is tempting, therefore, to suggest that a similar planar polarized web of microtubules may also influence planar polarity of basal bodies.

Basal bodies in multi-ciliated cells also make complex connections to both actin and cytokeratin networks, and these may certainly be involved in their polarization [61]. Given the link between Dvl and Rho, rotational polarity, and apical actin assembly in multi-ciliated cells [62,12], it is also likely that PCP-mediated control of the actin cytoskeleton influences basal body position and orientation

3. What is the link between polarized localization of PCP proteins, cilia, and cell polarity?

In *Drosophila*, asymmetric localization of core PCP proteins is a hallmark of planar polarized tissues, and the asymmetric localizations of core PCP proteins in ciliated epithelial cells are striking. The posterior localization of Dvl in the node [43] is similar to the posterior Dvl localization in zebrafish gastrula mesoderm [47], and the anterior localization of Vangl2 and Pk in the mouse node [48] reflects that observed in the fish neural tube [63]. However, the link between polarized PCP protein localization and morphological polarization remains quite confusing.

Two issues bear thinking about. First, despite the consistent results laid out above, there are other tissues where localizations differ dramatically. For example, the localization of PCP proteins in lens cells and their placement relative to the translationally planar polarized primary cilia appear to be different from that observed in the node (Fig. 3E)[64]. Moreover, the polarized localization of core PCP proteins relative to the position of hair cell kinocilia differs radically in different regions of the vestibular system (Fig. 3C, D)[65]. The growing variety of cell types in which planar polarity has been described will now provide us with new contexts in which to explore this issue further.

Second, loss of cilia in both multi-ciliated cells and in cochlear inner hair cells results in defective morphological planar polarization but leaves normal asymmetric localization of core PCP proteins intact [34,28]. Thus, cilia seem not to govern the PCP signaling cascade *per se* (as some have suggested), but rather to act in parallel or downstream to confer polarizing information. How these systems are integrated remains unclear.

4. What is the link between PCP proteins and ciliogenesis?

Clearly, PCP signaling can direct cilia-mediated fluid flow by affecting basal body positioning. It also should be noted, however, that some PCP proteins are implicated in the initial assembly of cilia.

For example, manipulations of Frizzled2 and Dvl in zebrafish lead to defective ciliogenesis [66]. Likewise, knockdown of Dvl genes also resulted in defective ciliogenesis in *Xenopus* multi-ciliated cells [12]. In this case, the ciliogenesis defect was linked to a failure of basal body docking at the apical plasma membrane [12]. Similarly, mutation of *Celsr2* and *Celsr3* in the mouse elicits defective basal body docking and ciliogenesis in ependymal cells [29]. Intriguingly, Vangl2 is implicated in ciliogenesis in *Xenopus* epidermis multi-ciliated cells but appears not to be required for ciliogenesis in similar cells in the zebrafish kidney and mouse airway [14,50,49].

Finally, perhaps the most curious case is that of the so-called “PCP effectors,” Inturned and Fuzzy. In *Drosophila*, these proteins play crucial roles in planar polarity in the wing, where

they act downstream of, and are essential for, core PCP protein function [67–70]. The vertebrate orthologues of Inturned and Fuzzy, initially characterized in *Xenopus*, are associated with surprisingly weak convergent extension phenotypes, but display profound defects in ciliogenesis [9]. Inturned has since been shown to act at the level of basal body docking [12], and Fuzzy governs trafficking to the basal body and along axonemes [13]. Most recently, mouse mutants in these genes have confirmed that vertebrate Inturned and Fuzzy are largely dispensable for PCP-mediated processes such as cochlear hair cell alignment and convergent extension, but both proteins are essential for ciliogenesis [13,71,72]. How these proteins evolved to control ciliogenesis in vertebrates and PCP in *Drosophila* is a fascinating open question.

Conclusion

In conclusion, the freshly identified roles for PCP signaling in a variety of ciliated cell types and in relation to new cellular behaviors is exciting. These new examples of PCP-mediated polarization provide new test beds for building a comprehensive understanding not only of PCP signaling specifically, but also of cellular morphogenesis and polarity generally.

Acknowledgments

This work was supported by the NIH/NIGMS, March of Dimes, Burroughs Wellcome Fund, American Asthma Foundation, and Texas Advanced Research Program. J.B.W. is an Early Career Scientist of the Howard Hughes Medical Institute.

References

1. Williams, T. Organs of Respiration. In: Todd, RB., editor. The Cyclopaedia of Anatomy and Physiology. Longman, Brown, Green, Longmans & Roberts; 1859.
2. Knowles MR, Boucher RC. Mucus clearance as a primary innate defense mechanism for mammalian airways. *J Clin Invest* 2002;109:571–577. [PubMed: 11877463]
3. Worthington WC Jr, Cathcart RS 3rd. Ependymal cilia: distribution and activity in the adult human brain. *Science* 1963;139:221–222. [PubMed: 14001896]
4. Lyons RA, Saridogan E, Djahanbakhch O. The reproductive significance of human Fallopian tube cilia. *Hum Reprod Update* 2006;12:363–372. [PubMed: 16565155]
5. Assheton R. Notes on the ciliation of the ectoderm of the amphibian embryo. *Q J Microsc Sci* 1896;38:465–484.
6. Twitty VC. Experimental studies on the ciliary action of amphibian embryos. *J Exp Zool* 1928;50:319–344.
7. Konig G, Hausen P. Planar polarity in the ciliated epidermis of *Xenopus* embryos. *Dev Biol* 1993;160:355–368. [PubMed: 8253269]
8. Nokhbatolfoghahai M, Downie JR, Clelland AK, Rennison K. The surface ciliation of anuran amphibian embryos and early larvae: Patterns, timing differences and functions. *Journal of Natural History* 2005;39:887–929.
9. Park TJ, Haigo SL, Wallingford JB. Ciliogenesis defects in embryos lacking inturned or fuzzy function are associated with failure of planar cell polarity and Hedgehog signaling. *Nat Genet* 2006;38:303–311. [PubMed: 16493421]
10. Hayes JM, Kim SK, Abitua PB, Park TJ, Herrington ER, Kitayama A, Grow MW, Ueno N, Wallingford JB. Identification of novel ciliogenesis factors using a new in vivo model for mucociliary epithelial development. *Dev Biol* 2007;312:115–130. [PubMed: 17961536]
- 11**. Mitchell B, Jacobs R, Li J, Chien S, Kintner C. A positive feedback mechanism governs the polarity and motion of motile cilia. *Nature* 2007;447:97–101. This paper provides the first demonstration that fluid flow governs entrainment of planar polarized ciliary beating. Moreover, the paper was the first to demonstrate that flow-induced polarization of basal bodies requires that cilia be motile. Thus, motile cilia not only generate but also sense fluid flow. [PubMed: 17450123]

- 12*. Park TJ, Mitchell BJ, Abitua PB, Kintner C, Wallingford JB. Dishevelled controls apical docking and planar polarization of basal bodies in ciliated epithelial cells. *Nat Genet* 2008;40:871–879. This paper provides the first demonstration of a role for PCP signaling in polarized ciliary beating. The paper also suggests that some PCP proteins are essential for ciliogenesis prior to their role in oriented beating. [PubMed: 18552847]
13. Gray RS, Abitua PB, Wlodarczyk BJ, Szabo-Rogers HL, Blanchard O, Lee I, Weiss GS, Liu KJ, Marcotte EM, Wallingford JB, et al. The planar cell polarity effector Fuz is essential for targeted membrane trafficking, ciliogenesis and mouse embryonic development. *Nature Cell Biology* 2009;11:1225–1232.
- 14*. Mitchell B, Stubbs JL, Huisman F, Taborek P, Yu C, Kintner C. The PCP pathway instructs the planar orientation of ciliated cells in the *Xenopus* larval skin. *Curr Biol* 2009;19:924–929. This paper reveals that while morphological polarization is not apparent in the non-ciliated cells of a ciliated epithelium, PCP signaling is active in those cells and is essential for polarizing ciliary beating. Moreover, the paper also provides the first demonstration of directional non-autonomy of PCP signaling in a vertebrate. [PubMed: 19427216]
15. Ross AJ, May-Simera H, Eichers ER, Kai M, Hill J, Jagger DJ, Leitch CC, Chapple JP, Munro PM, Fisher S, et al. Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. *Nat Genet* 2005;37:1135–1140. [PubMed: 16170314]
16. Simons M, Gloy J, Ganner A, Bullerkotte A, Bashkurov M, Kronig C, Schermer B, Benzing T, Cabello OA, Jenny A, et al. Inversin, the gene product mutated in nephronophthisis type II, functions as a molecular switch between Wnt signaling pathways. *Nat Genet* 2005;37:537–543. [PubMed: 15852005]
17. Wallingford JB, Rowning BA, Vogeli KM, Rothbächer U, Fraser SE, Harland RM. Dishevelled controls cell polarity during *Xenopus* gastrulation. *Nature* 2000;405:81–85. [PubMed: 10811222]
18. Habas R, Kato Y, He X. Wnt/Frizzled activation of Rho regulates vertebrate gastrulation and requires a novel Formin homology protein Daam1. *Cell* 2001;107:843–854. [PubMed: 11779461]
19. Marlow F, Topczewski J, Sepich D, Solnica-Krezel L. Zebrafish Rho kinase 2 acts downstream of Wnt11 to mediate cell polarity and effective convergence and extension movements. *Curr Biol* 2002;12:876–884. [PubMed: 12062050]
20. Tahinci E, Symes K. Distinct functions of Rho and Rac are required for convergent extension during *Xenopus* gastrulation. *Dev Biol* 2003;259:318–335. [PubMed: 12871704]
21. Ybot-Gonzalez P, Savery D, Gerrelli D, Signore M, Mitchell CE, Faux CH, Greene ND, Copp AJ. Convergent extension, planar-cell-polarity signalling and initiation of mouse neural tube closure. *Development* 2007;134:789–799. [PubMed: 17229766]
22. Ganner A, Lienkamp S, Schafer T, Romaker D, Wegierski T, Park TJ, Spreitzer S, Simons M, Gloy J, Kim E, et al. Regulation of ciliary polarity by the APC/C. *Proc Natl Acad Sci U S A* 2009;106:17799–17804. [PubMed: 19805045]
23. Vinson CR, Adler PN. Directional non-cell autonomy and the transmission of polarity information by the frizzled gene of *Drosophila*. *Nature* 1987;329:549–551. [PubMed: 3116434]
24. Adler PN, Taylor J, Charlton J. The domineering non-autonomy of frizzled and van Gogh clones in the *Drosophila* wing is a consequence of a disruption in local signaling. *Mechanisms of Development* 2000;96:197–207. [PubMed: 10960784]
25. Spassky N, Merkle FT, Flames N, Tramontin AD, Garcia-Verdugo JM, Alvarez-Buylla A. Adult ependymal cells are postmitotic and are derived from radial glial cells during embryogenesis. *J Neurosci* 2005;25:10–18. [PubMed: 15634762]
26. Sawamoto K, Wichterle H, Gonzalez-Perez O, Cholfin JA, Yamada M, Spassky N, Murcia NS, Garcia-Verdugo JM, Marin O, Rubenstein JL, et al. New neurons follow the flow of cerebrospinal fluid in the adult brain. *Science* 2006;311:629–632. [PubMed: 16410488]
- 27*. Mirzadeh Z, Han YG, Soriano-Navarro M, Garcia-Verdugo JM, Alvarez-Buylla A. Cilia organize ependymal planar polarity. *J Neurosci* 2010;30:2600–2610. This paper was the first to define translational planar polarity in ependymal cells and to show that ependymal motile cilia are required for rotational polarity, while primary cilia on radial glial precursors are essential for subsequent translational polarity of ependymal cell descendants. [PubMed: 20164345]

28. Guirao B, Meunier A, Mortaud S, Aguilar A, Corsi JM, Strehl L, Hirota Y, Desoeuvre A, Boutin C, Han YG, et al. Coupling between hydrodynamic forces and planar cell polarity orients mammalian motile cilia. *Nat Cell Biol* 2010;12:341–350. [PubMed: 20305650]
- 29*. Tissir F, Qu Y, Montcouquiol M, Zhou L, Komatsu K, Shi D, Fujimori T, Labeau J, Tyteca D, Courtot P, et al. Lack of cadherins Celsr2 and Celsr3 impairs ependymal ciliogenesis, leading to fatal hydrocephalus. *Nat Neurosci* 2010;13:700–707. This paper demonstrates that the core PCP proteins Celsr2 and Celsr3 play dual roles, governing both ciliogenesis and rotational planar polarity of cilia in mouse ependymal cells. [PubMed: 20473291]
30. Bastock R, Strutt H, Strutt D. Strabismus is asymmetrically localised and binds to Prickle and Dishevelled during *Drosophila* planar polarity patterning. *Development* 2003;130:3007–3014. [PubMed: 12756182]
31. Montcouquiol M, Sans N, Huss D, Kach J, Dickman JD, Forge A, Rachel RA, Copeland NG, Jenkins NA, Bogani D, et al. Asymmetric localization of Vangl2 and Fz3 indicate novel mechanisms for planar cell polarity in mammals. *J Neurosci* 2006;26:5265–5275. [PubMed: 16687519]
32. Rawls AS, Wolff T. Strabismus requires Flamingo and Prickle function to regulate tissue polarity in the *Drosophila* eye. *Development* 2003;130:1877–1887. [PubMed: 12642492]
33. Jones C, Chen P. Primary cilia in planar cell polarity regulation of the inner ear. *Curr Top Dev Biol* 2008;85:197–224. [PubMed: 19147007]
- 34*. Jones C, Roper VC, Foucher I, Qian D, Banizs B, Petit C, Yoder BK, Chen P. Ciliary proteins link basal body polarization to planar cell polarity regulation. *Nat Genet* 2008;40:69–77. This paper was the first to reveal that ciliary proteins play a role in planar cell polarization and polarized cilia positioning and that the role for cilia in this process is independent of asymmetric localization of PCP proteins. [PubMed: 18066062]
35. Essner JJ, Vogan KJ, Wagner MK, Tabin CJ, Yost HJ, Brueckner M. Conserved function for embryonic nodal cilia. *Nature* 2002;418:37–38. [PubMed: 12097899]
36. Nonaka S, Yoshida S, Watanabe D, Ikeuchi S, Goto T, Marshall WF, Hamada H. De novo formation of left-right asymmetry by posterior tilt of nodal cilia. *PLoS Biol* 2005;3:e268. [PubMed: 16035921]
37. Okada Y, Takeda S, Tanaka Y, Belmonte JC, Hirokawa N. Mechanism of nodal flow: a conserved symmetry breaking event in left-right axis determination. *Cell* 2005;121:633–644. [PubMed: 15907475]
38. Mochizuki T, Saijoh Y, Tsuchiya K, Shirayoshi Y, Takai S, Taya C, Yonekawa H, Yamada K, Nihei H, Nakatsuji N, et al. Cloning of inv, a gene that controls left/right asymmetry and kidney development. *Nature* 1998;395:177–181. [PubMed: 9744276]
39. Kishimoto N, Cao Y, Park A, Sun Z. Cystic kidney gene seahorse regulates cilia-mediated processes and Wnt pathways. *Dev Cell* 2008;14:954–961. [PubMed: 18539122]
40. Cogswell C, Price SJ, Hou X, Guay-Woodford LM, Flaherty L, Bryda EC. Positional cloning of jcpk/bpk locus of the mouse. *Mamm Genome* 2003;14:242–249. [PubMed: 12682776]
41. Maisonneuve C, Guilleret I, Vick P, Weber T, Andre P, Beyer T, Blum M, Constam DB. Bicaudal C, a novel regulator of Dvl signaling abutting RNA-processing bodies, controls cilia orientation and leftward flow. *Development* 2009;136:3019–3030. [PubMed: 19666828]
42. Schweickert A, Weber T, Beyer T, Vick P, Bogusch S, Feistel K, Blum M. Cilia-driven leftward flow determines laterality in *Xenopus*. *Curr Biol* 2007;17:60–66. [PubMed: 17208188]
- 43*. Hashimoto M, Shinohara K, Wang J, Ikeuchi S, Yoshida S, Meno C, Nonaka S, Takada S, Hatta K, Wynshaw-Boris A, et al. Planar polarization of node cells determines the rotational axis of node cilia. *Nat Cell Biol* 2010;12:170–176. The first papers (together with 48, 49, and 50) to demonstrate that PCP signaling governs the posterior positioning of node mono-cilia and thus directs left-right patterning. [PubMed: 20098415]
44. Habas R, Dawid IB, He X. Coactivation of Rac and Rho by Wnt/Frizzled signaling is required for vertebrate gastrulation. *Genes Dev* 2003;17:295–309. [PubMed: 12533515]
45. Nakaya MA, Biris K, Tsukiyama T, Jaime S, Rawls JA, Yamaguchi TP. Wnt3a links left-right determination with segmentation and anteroposterior axis elongation. *Development* 2005;132:5425–5436. [PubMed: 16291790]
46. Axelrod JD. Unipolar membrane association of Dishevelled mediates Frizzled planar cell polarity signaling. *Genes Dev* 2001;15:1182–1187. [PubMed: 11358862]

47. Yin C, Kiskowski M, Pouille PA, Farge E, Solnica-Krezel L. Cooperation of polarized cell intercalations drives convergence and extension of presomitic mesoderm during zebrafish gastrulation. *J Cell Biol* 2008;180:221–232. [PubMed: 18195109]
- 48*. Antic D, Stubbs JL, Suyama K, Kintner C, Scott MP, Axelrod JD. Planar cell polarity enables posterior localization of nodal cilia and left-right axis determination during mouse and *Xenopus* embryogenesis. *PLoS One* 2010;5:e8999. See # 43, above. [PubMed: 20126399]
- 49*. Song, ea. Planar cell polarity breaks the bilateral symmetry by controlling ciliary positioning. *Nature*. 2010 In Press. See # 43, above.
- 50*. Borovina A, Superina S, Voskas D, Ciruna B. Vangl2 directs the posterior tilting and asymmetric localization of motile primary cilia. *Nat Cell Biol* 2010;12:407–412. See # 43, above. [PubMed: 20305649]
51. Essner JJ, Amack JD, Nyholm MK, Harris EB, Yost HJ. Kupffer's vesicle is a ciliated organ of asymmetry in the zebrafish embryo that initiates left-right development of the brain, heart and gut. *Development* 2005;132:1247–1260. [PubMed: 15716348]
52. Topczewski J, Sepich DS, Myers DC, Walker C, Amores A, Lele Z, Hammerschmidt M, Postlethwait J, Solnica-Krezel L. The zebrafish glypican knypek controls cell polarity during gastrulation movements of convergent extension. *Dev Cell* 2001;1:251–264. [PubMed: 11702784]
53. Boisvieux-Ulrich E, Laine MC, Sandoz D. The orientation of ciliary basal bodies in quail oviduct is related to the ciliary beating cycle commencement. *Biol Cell* 1985;55:147–150. [PubMed: 2937490]
54. Montcouquiol M, Rachel RA, Lanford PJ, Copeland NG, Jenkins NA, Kelley MW. Identification of Vangl2 and Scrb1 as planar polarity genes in mammals. *Nature* 2003;423:173–177. [PubMed: 12724779]
55. Sugiyama Y, Stump RJ, Nguyen A, Wen L, Chen Y, Wang Y, Murdoch JN, Lovicu FJ, McAvoy JW. Secreted frizzled-related protein disrupts PCP in eye lens fiber cells that have polarised primary cilia. *Dev Biol* 2010;338:193–201. [PubMed: 19968984]
56. Sandoz D, Chailley B, Boisvieux-Ulrich E, Lemullois M, Laine MC, Bautista-Harris G. Organization and functions of cytoskeleton in metazoan ciliated cells. *Biol Cell* 1988;63:183–193. [PubMed: 3060202]
57. Eaton S, Wepf R, Simons K. Roles for Rac1 and Cdc42 in planar polarization and hair outgrowth in the wing of *Drosophila*. *J Cell Biol* 1996;135:1277–1289. [PubMed: 8947551]
58. Turner CM, Adler PN. Distinct roles for the actin and microtubule cytoskeletons in the morphogenesis of epidermal hairs during wing development in *Drosophila*. *Mech Dev* 1998;70:181–192. [PubMed: 9510034]
59. Shimada Y, Yonemura S, Ohkura H, Strutt D, Uemura T. Polarized transport of Frizzled along the planar microtubule arrays in *Drosophila* wing epithelium. *Developmental Cell* 2006;10:209–222. [PubMed: 16459300]
60. Hannus M, Feiguin F, Heisenberg CP, Eaton S. Planar cell polarization requires Widerborst, a B' regulatory subunit of protein phosphatase 2A. *Development* 2002;129:3493–3503. [PubMed: 12091318]
61. Chailley B, Nicolas G, Laine MC. Organization of actin microfilaments in the apical border of oviduct ciliated cells. *Biol Cell* 1989;67:81–90. [PubMed: 2605375]
62. Pan J, You Y, Huang T, Brody SL. RhoA-mediated apical actin enrichment is required for ciliogenesis and promoted by Foxj1. *J Cell Sci* 2007;120:1868–1876. [PubMed: 17488776]
63. Ciruna B, Jenny A, Lee D, Mlodzik M, Schier AF. Planar cell polarity signalling couples cell division and morphogenesis during neurulation. *Nature* 2006;439:220–224. [PubMed: 16407953]
64. Sugiyama Y, Stump RJ, Nguyen A, Wen L, Chen Y, Wang Y, Murdoch JN, Lovicu FJ, McAvoy JW. Secreted frizzled-related protein disrupts PCP in eye lens fiber cells that have polarised primary cilia. *Dev Biol* 338:193–201. [PubMed: 19968984]
- 65*. Deans MR, Antic D, Suyama K, Scott MP, Axelrod JD, Goodrich LV. Asymmetric distribution of prickle-like 2 reveals an early underlying polarization of vestibular sensory epithelia in the inner ear. *J Neurosci* 2007;27:3139–3147. This paper is notable because it reveals the complex interplay between asymmetric PCP protein localization and cilia positioning. In the vestibular system of the mouse inner ear, Prickle2 localizes to the cell face near the translationally polarized kinocilium in

some cells but localizes to the opposite face (away from the polarized kinocilium) in neighboring cells. [PubMed: 17376975]

66. Oishi I, Kawakami Y, Raya A, Callol-Massot C, Izpisua Belmonte JC. Regulation of primary cilia formation and left-right patterning in zebrafish by a noncanonical Wnt signaling mediator, *duboraya*. *Nat Genet* 2006;38:1316–1322. [PubMed: 17013396]
67. Park WJ, Liu J, Sharp EJ, Adler PN. The *Drosophila* tissue polarity gene *inturned* acts cell autonomously and encodes a novel protein. *Development* 1996;122:961–969. [PubMed: 8631273]
68. Collier S, Gubb D. *Drosophila* tissue polarity requires the cell-autonomous activity of the *fuzzy* gene, which encodes a novel transmembrane protein. *Development* 1997;124:4029–4037. [PubMed: 9374400]
69. Lee H, Adler PN. The function of the *frizzled* pathway in the *Drosophila* wing is dependent on *inturned* and *fuzzy*. *Genetics* 2002;160:1535–1547. [PubMed: 11973308]
70. Adler PN, Zhu C, Stone D. *Inturned* Localizes to the Proximal Side of Wing Cells under the Instruction of Upstream Planar Polarity Proteins. *Curr Biol* 2004;14:2046–2051. [PubMed: 15556868]
71. Heydeck W, Zeng H, Liu A. Planar cell polarity effector gene *Fuzzy* regulates cilia formation and Hedgehog signal transduction in mouse. *Dev Dyn* 2009;238:3035–3042. [PubMed: 19877275]
72. Zeng H, Hoover AN, Liu A. PCP effector gene *Inturned* is an important regulator of cilia formation and embryonic development in mammals. *Dev Biol* 2010;339:418–428. [PubMed: 20067783]
73. Frisch D, Farbman AI. Development of order during ciliogenesis. *Anat Rec* 1968;162:221–232. [PubMed: 5726142]

Figure 1.

A. Schematic of planar polarized multi-ciliated cells. B. Schematic of planar polarized mono-cilia on node epithelial cells. Red = basal body; green = rootlet.

Figure 2.

A. Normal rotational planar polarity in a multi-ciliated cell. A'. Defective rotational polarity. B. Normal translational planar polarity in multi-ciliated cells. b'. Defective translational polarity. C. Normal tissue-level planar polarity in a field of multi-ciliated cells. c'. Defective tissue-level polarity. Red = basal body; green = rootlet.

Figure 3.

A. Schematic of planar polarity in a cochlear hair cell. B. Planar polarity in a node epithelial cell. C & D. In the saccule of the mouse inner ear, a line of reversal splits cells into medial and lateral populations; the relative position of the kinocilium to asymmetrically localized Prickle2 is reversed in the two populations [65]. E. Planar polarity in a mouse lens fiber cell [64].