

Planar cell polarity, ciliogenesis and neural tube defects

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Cilia are microtubule-based protrusions that are found on the surface of most vertebrate cells. Long studied by cell biologists, these organelles have recently caught the attention of developmental biologists and human geneticists. In this review, I will discuss recent findings suggesting a link between cilia and the planar cell polarity signaling cascade. In particular, I will focus on how this interaction may influence the process of neural tube closure and how these results may be relevant to our understanding of common human birth defects in which neural tube closure is compromised.

INTRODUCTION

The normal development of vertebrate embryos, including humans, requires concerted changes in cell fate and cell movement. Cell fate is governed in large part by cellular signaling pathways that signal through the nucleus, impacting the transcription of developmentally relevant genes. Cell movement is governed instead by signals that impact the cytoskeleton. Understanding the mechanisms by which these processes are integrated is central to a comprehensive understanding of development.

A key example of this interconnection is the process of neural tube closure. In the normal development of most vertebrates, the initially flat precursor to the central nervous system (the neural plate) buckles, rolls up and then fuses to form the hollow tube that will become the mature brain and spinal cord (Fig. 1A). Defects in neural tube closure are the second most common human birth defect, and similar defects are observed in genetically modified experimental animals (1–3). Experiments in animal models reveal that failure of neural tube closure can stem either from defects in cell fate or from defects in cell movement (1–3).

Recently, several studies have implicated cilia, the tiny microtubule-based cellular protrusions that decorate most vertebrate cells (4), in the etiology of neural tube closure defects. These studies highlight the tight interconnections between cell fate and cytoskeletal organization. In this review, I will

synthesize data from model animals and from human studies to illustrate this integration.

THE PLANAR CELL POLARITY SIGNALING PATHWAY AND NEURAL TUBE DEFECTS

The planar cell polarity (PCP) signaling pathway was first identified in the fruitfly *Drosophila*, where it controls the uniform orientation of hairs and bristles on the body (Figs 2 and 3A) (5–7). Forward genetic studies have delineated a complex pathway that drives localized actin polymerization, leading to localized hair or bristle outgrowth. This pathway involves several proteins referred to as ‘core PCP’ components. These include transmembrane proteins, such as Frizzled, Strabismus and Flamingo and intracellular proteins, including Disheveled (DVL) and Prickle (5–7). Downstream of the core PCP proteins, additional factors mediate PCP signaling in different tissues. These so-called ‘PCP effectors’ include the novel proteins Inturned, Fuzzy and Fritz (6,7). Mutation of any of these PCP genes results in a failure to polarize hair and bristle outgrowth; hair and bristles form, but their orientation is now random (Fig. 3D). This pathway has recently drawn the attention of vertebrate biologists, and complementary studies in mice and other model animals have demonstrated how this signaling pathway may be directly relevant to human biology.

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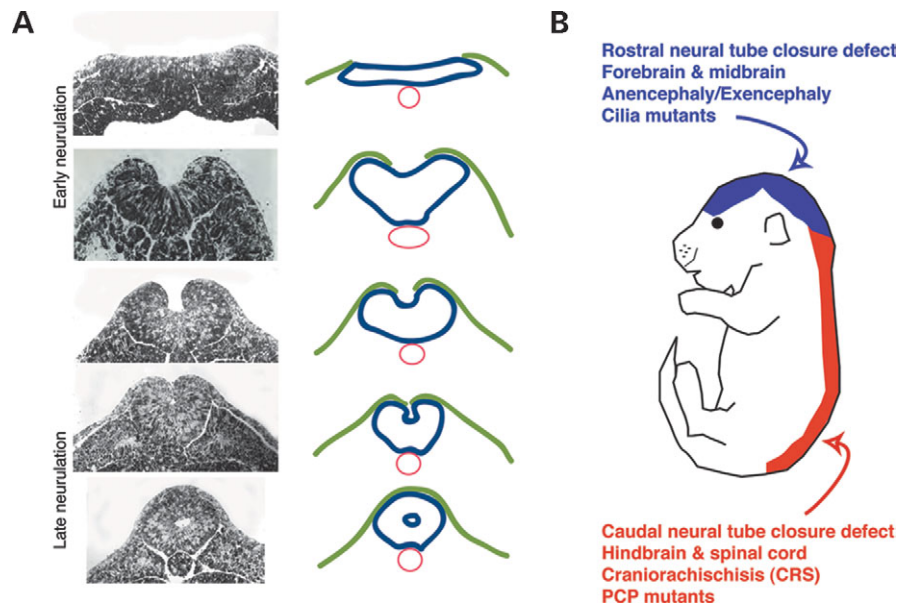


Figure 1. Neural tube closure defects. (A) Images at left show transverse sections through an amphibian embryo at successive stages of neural tube closure, during which the initially flat vertebrate central nervous system rolls up to form the hollow tube that becomes the brain and spinal cord. Schematics at right indicate tissues: green, epidermis; blue, neural; red, notochord (mesoderm). (B) A schematic of a young mouse indicates the regions affected by rostral neural tube defects (purple) and caudal neural tube closure defects (red). Images courtesy of Antone Jacobson.

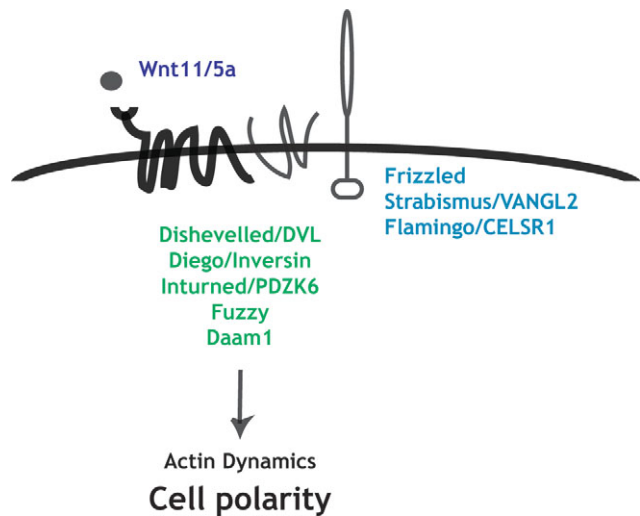


Figure 2. The PCP signaling cascade. The PCP pathway initiates extra-cellularly (purple) signals across the cell membrane (blue components) and influences the dynamics of the actin cytoskeleton (green components), thus controlling cell polarity.

The first demonstration of a role for the PCP pathway in controlling cell polarity in a vertebrate came from work in the frog, *Xenopus*. A *Xenopus* ortholog of DVL was shown to be essential for convergent extension, a morphogenetic process by which cells crawl between one another, forming a longer, narrower array (Fig. 3B) (8–10). During convergent extension, cells form polarized lamellipodia (actin-rich cell feet that generate traction for cell movement) (11). Time-lapse imaging of convergent extension revealed that

the function of DVL in the frog paralleled that in the fly. In *Xenopus* cells lacking DVL function, lamellipodia form, but they fail to polarize; their orientation is now random (12). As a result, convergent extension fails (Fig. 3E) (12). At the same time, work in zebrafish and frogs revealed that a conserved PCP pathway controls hair and bristle polarity in flies and convergent extension in vertebrates (12–14).

These studies became quite relevant to human genetics with the finding that disruption of the PCP signaling pathway in mice causes severe neural tube closure defects. For example, the classical mouse mutant, Looptail, displays a neural tube defect known as craniorachischisis, a failure of caudal neural tube closure (Fig. 1B). This phenotype was found to result from mutation of *Vangl2*, the vertebrate ortholog of the core PCP gene *strabismus* (15,16). Shortly thereafter, it was found that other PCP components, including DVL, Flamingo and Frizzled, were also essential for caudal neural tube closure in mice (17–19). Again, studies in experimentally facile model vertebrates uncovered the mechanism by which PCP signaling is required for neural tube closure. Time-lapse studies in *Xenopus* revealed that PCP-dependent convergent extension was required to narrow the distance between the elevating neural folds, allowing their apposition and fusion (20).

Recent experiments have now demonstrated that a similar mode of action underlies neural tube defects in mice lacking PCP function (21), and it seems likely that a similar mechanism is at work in humans. Indeed, human embryos with craniorachischisis are uniformly described to have shorter and wider trunks, consistent with a defect in convergent extension (22–24). One study even tentatively describes a failure of normal cell polarization in the neural epithelium of a human embryo with craniorachischisis (25). So, although

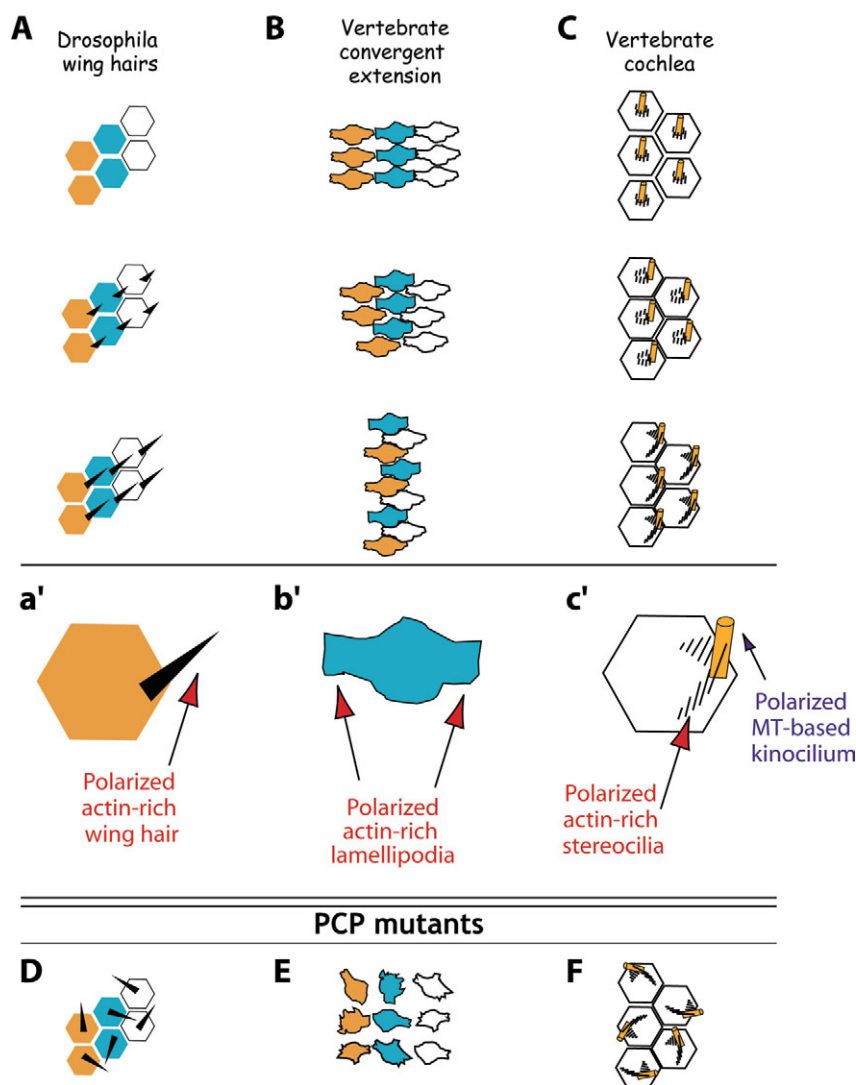


Figure 3. PCP signaling controls polarized cell behaviors in diverse tissues. (A) PCP signals control the positioning of actin-based wing hair in *Drosophila*. (B) PCP signals control the positioning of actin-based lamellipodia (cellular ‘feet’) in vertebrate embryos during convergent extension. (C) PCP signals control the positioning of actin-based hairs and also the positioning of microtubule-based cilia in the vertebrate inner ear. (D–F) Phenotype of PCP mutants in wing hairs, convergent extension and the inner ear, respectively.

mutations in PCP genes have yet to be identified in humans with neural tube defects (26), it is certainly worth looking for them now.

A ROLE FOR CILIA IN VERTEBRATE NEURAL TUBE DEFECTS

An interesting facet of neural tube closure is that the mechanisms governing this process in the brain differ significantly from those operating in the spinal cord (Fig. 1B) (1,2,27). Nowhere is this distinction more striking than in the classification of human neural tube defects. ‘Anencephaly’ refers to the human birth defect in which the forebrain and the midbrain fail to close, whereas ‘craniorachischisis’ refers to a failure of closure specifically in the hindbrain and the spinal cord. The most common neural tube defect, ‘spina bifida,’ refers to a

partial failure of closure in the spinal cord. These birth defects are now recognized as having quite distinct etiologies.

Moreover, this classification of neural tube defects based upon rostral-caudal location in the embryo extends to model animals (1,2). For example, the phenotype displayed by mice lacking PCP function is manifested by a failure of the spinal cord and hindbrain to close, but in these animals, the forebrain closes successfully (Fig. 1B). Other mutant mice display prominent defects in the closure of the rostral neural tube, a phenotype referred to in mice as ‘exencephaly’ (Fig. 1B).

Around the time that PCP signaling emerged as a regulator of spinal neural tube closure, a forward genetic screen in mice began uncovering a series of mutants that displayed exencephaly (28). Surprisingly, many of these mutants were found to encode proteins required for the formation of cilia, and in particular, these mutations impacted intraflagellar transport

(IFT) proteins that are responsible for delivery of cargo into and out of cilia (29–31). Because disruption of such proteins results in defective ciliogenesis, the data suggested a requirement for cilia in rostral neural tube closure.

Examination of these mutant mice revealed that the open neural tube defects resulted from a failure in transduction along the hedgehog signaling pathway (30). This pathway acts to control the activity of Gli-type transcription factors and is a critical pathway for patterning of cell fates during embryogenesis (32). Exactly how cilia function in hedgehog signaling remains unknown and is the subject of many recent papers (reviewed in 33). What is clear is that several signal transduction proteins must localize to cilia for hedgehog signal transduction to proceed normally (34–36).

It remains to be seen exactly how hedgehog signaling influences cell movements during neural tube closure. Embryos lacking either hedgehog function or cilia develop with severe defects in the dorsoventral patterning of the neural tube (29–31,37). Because precise patterning of the neural plate is central to its morphogenesis, these patterning defects are likely to underlie the failure of neural tube closure. For example, hedgehog can modify the pattern of cell shape changes during neural plate bending (38). Alternatively, hedgehog may also affect neural cell adhesion (39).

Finally, two recent articles have moved the link between cilia and neural tube closure beyond model animals and into the realm of human disease. Meckel–Gruber syndrome is an autosomal recessive lethal disorder involving neural tube closure defects as well as kidney and limb defects. Two of the genes responsible for Meckel–Gruber have been cloned recently, and both are predicted to be involved in ciliogenesis, though functional evidence is still pending (40,41). MKS1 was previously identified as a component of the basal body proteome, and an ortholog of MKS3 has been predicted to be a ciliary protein in the nematode *C. elegans* (40,41). Given the rostral neural tube defects in humans with Meckel–Gruber syndrome, and the rostral neural tube defects in model animals with defective ciliogenesis (30,42,43), a ciliary basis for Meckel–Gruber syndrome is a very promising hypothesis. If this hypothesis is correct, future studies should be directed at the role of defective hedgehog signaling in the etiology of this syndrome.

PCP SIGNALING AND CILIA: WHAT'S THE CONNECTION?

Among the more recent developments in our understanding of neural tube closure is the emerging link between cilia and PCP signaling. The first evidence to suggest a molecular connection between PCP and cilia came from studies of the ciliary protein inversin. This protein has been studied for some time in the context of cilia function, and mutations in this gene cause renal cystic diseases in humans (44). Inversin has significant primary sequence similarity to the *Drosophila* PCP protein, Diego (45), and recent studies in zebrafish and *Xenopus* embryos revealed that knockdown of inversin disrupts convergent extension (46). Inversin and Diego can each associate physically with other PCP proteins, including DVL and Strabismus/Vangl2 (46–48); and moreover, inversin can control the stability of the DVL protein in vertebrate embryos (46).

This connection between PCP signaling and a known ciliary protein became even more curious with the finding that the PCP proteins Vangl2 and DVL localize at or near the base of cilia in vertebrate cells (42,43).

So, what is the connection between PCP and cilia? As this area of study is only in its infancy, the remainder of this review will focus on the possible inter-connections.

Could PCP signaling be required for ciliogenesis?

The fact that PCP proteins interact physically and functionally with inversin suggested the possibility that PCP signals may govern ciliogenesis, and a recent study of the PCP effector proteins, Inturned and Fuzzy, supports this idea. Knockdown of either Inturned or Fuzzy generated a surprising phenotype. Disruption of Inturned or Fuzzy in *Xenopus* elicited prominent rostral neural tube closure defects in addition to more caudal neural tube defects predicted to result from disruption of PCP signaling. These caudal defects were shown to arise from failure of convergent extension in the neural plate, whereas the rostral defects were shown to stem from a failure of hedgehog signaling (43). Imaging experiments then revealed that Inturned and Fuzzy are essential for normal ciliogenesis in the neural tube and also in other ciliated epithelia (43).

The idea that PCP signals are involved in ciliogenesis may help to explain previous reports of phenotypic spectra encompassing both hedgehog defects and PCP defects (Fig. 4A). For example, manipulations of DVL and Strabismus/Vangl2 have been associated with midline patterning defects in both mice and *Xenopus* (16,20). Given the role of hedgehog in midline patterning (49), such defects could reflect changes in hedgehog signal transduction in embryos lacking PCP function. Moreover, mutations in the receptor tyrosine kinase PTK7 cause obvious defects in PCP signaling and convergent extension and also elicit additional phenotypes including defective kidney development and polydactyly (50). These latter two phenotypes may be attributable to modifications in the hedgehog signaling. Finally, it is tempting to speculate that PCP and hedgehog phenotypes may be linked in humans to neural tube defects. In at least one study, human embryos with craniorachischisis display defects in midline and floorplate organization (24).

There is, however, a key caveat to the findings that support a role for PCP in ciliogenesis. So far, mice bearing disruptions of 'core PCP' signaling components have not been demonstrated to lack cilia, and these mice do not in general display the characteristic rostral open neural tube of mice lacking IFT proteins. The caveat to the caveat here is that these core PCP components (DVL, Vangl, Frizzled and Flamingo) are members of protein families, and redundancies may mask some of their functions. For example, Vangl2 is mutated in Looptail mice, but Vangl1 has not yet been knocked out. Likewise, double knockouts of DVLs have been performed, but the triple knockout eliminating all DVL functions has yet to be reported. Given that rostral neural tube defects similar to those in IFT mutant mice have been reported in a small percentage of DVL mutant mice (17), it seems possible that a role for core PCP components in ciliogenesis will be found in mammals. Additional genetic experiments in the mouse will be required to resolve this issue.

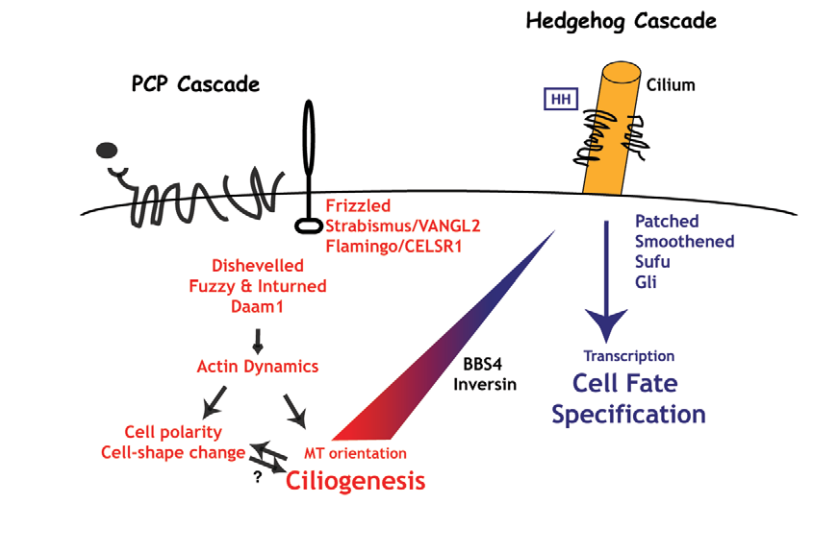
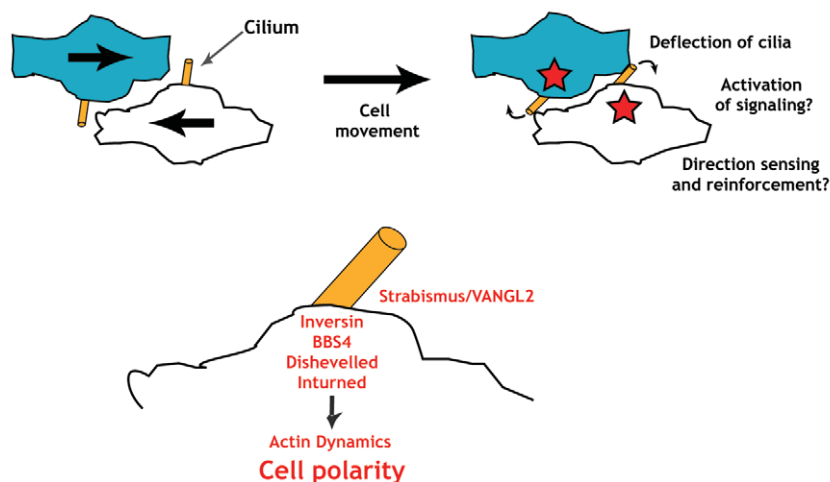
A PCP required for ciliogenesis?**B** Cilia required for PCP signaling?

Figure 4. Models of possible connections between PCP and cilia. (A) PCP is required for ciliogenesis. In this model, PCP signals govern actin dynamics at the base of cilia and are thus required for normal ciliogenesis and in turn for normal hedgehog signaling. (B) Cilia are required for PCP signaling. In this model, cilia act as mechanosensors and act via PCP signals to establish cell polarity and direct cell movement.

Together, these results implicate PCP signaling in ciliogenesis, but the mechanism is unclear. Cilia are microtubule-based cellular protrusions, and PCP signaling is generally implicated in governing the actin cytoskeleton. Indeed, DVL, the protein bound and regulated by inversin (46), also interacts physically and functionally with the formin protein, Daam1 (51), and formins are direct nucleators of actin filaments (52). As it happens, actin filaments are required for proper positioning of ciliary basal bodies, and in cells where the actin cytoskeleton has been disrupted, 'intra-cellular cilia' are formed (53,54). A similar situation was observed in ciliated cells lacking the 'PCP effectors' Inturned or Fuzzy; actin failed to accumulate apically and masses of elongated microtubules accumulated inside these cells (43).

Such results suggest that PCP signals are required not for the nucleation of ciliary microtubules, but instead to govern the actin network that positions ciliary basal bodies (Fig. 4A).

This interpretation may have implications for our understanding of PCP signaling in other contexts. For example, polarized assembly of microtubules is essential for the establishment of planar polarity (55,56), and polarized microtubules have been shown to mediate the directed transport of PCP components such as Frizzled (57). In light of recent reports that DVL can influence microtubule stability (58,59), it is tempting to think that the PCP signaling pathway is generally involved in linking polarized microtubule assembly to polarized actin assembly, and vice versa.

Could cilia be required for PCP signal transduction?

The beguiling result that cilia are essential organelles for transduction of hedgehog signals has sparked an intense hunt for additional signaling pathways that require cilia. The findings that PCP components interact with ciliary proteins such as inversin (46) have focused particular attention on this pathway. The evidence suggesting a requirement for cilia in PCP signal transduction initiated with human genetic studies focussed on the genes mutated in Bardet–Biedl syndrome (BBS). This syndrome, involving obesity, renal disorders and a variety of additional symptoms, has been linked to defective ciliogenesis, and several different genes have been found to be mutated in BBS patients (60,61).

BBS genes have now been studied in experimental animals, where they are implicated in PCP-dependent processes, such as convergent extension. For example, mice lacking the microtubule-organizing protein BBS4 sometimes develop with a failure of neural tube closure in the midbrain (42). Although this neural tube defect presents somewhat rostral to those observed in PCP mutant mice, BBS4 mutants also develop with open eyelids, a phenotype observed consistently in PCP mutant mice (18,42,50). To probe for inter-connections between BBS4 and PCP signaling, experiments moved to the zebrafish. Indeed, a functional interaction between Vangl2 and BBS4 was established; simultaneous disruption of these two genes causes a more severe axis elongation defect than does the mutation of either one alone (42). Additional experiments have also implicated the chaperonin-like protein BBS10 in convergent extension; simultaneous disruption of BBS4 and BBS10 causes a severe defect in zebrafish axis elongation (62).

If cilia were required for PCP signaling, what would this look like? One tantalizing model has been proposed in light of studies into the role of cilia in polycystic kidney disease in humans. In that context, cilia act as mechanosensors, sensing movement and activating intracellular signaling. The PC1 protein, which is mutated in autosomal dominant polycystic kidney disease, localizes to cilia. When cilia bend in response to extracellular fluid flow, PC1 is proteolytically cleaved (33), and the cleaved PC1 enters the nucleus to control transcription together with STAT6 (63). This result is particularly intriguing given that STAT proteins are implicated in convergent extension and PCP signaling (64,65). It may be, then, that PCP signaling is activated downstream of mechanical stimuli sensed by cilia. In the kidney, cilia may sense fluid flow; in convergent extension, perhaps cilia sense the movement of neighboring cells sliding by as they interdigitate (Fig. 4B). Indeed, cells engaged in convergent extension can alter their polarity in response to changes in mechanical tension (66).

Together, these data present the tantalizing possibility that cilia help to transduce PCP signals. That said, none of the IFT mutant mice reported so far display phenotypes that would be consistent with a role for cilia in PCP signaling (33). So, given the role of microtubules in PCP signaling (mentioned earlier), it is possible that the BBS proteins could be important effectors of PCP signals, even if the cilium itself turns out not to be. Alternatively, it may be that PCP phenotypes are masked by other defects in IFT mutant mice or by genetic redundancies. Further work on this topic,

including genetic interaction experiments between IFT and PCP proteins and targeted disruption of diverse ciliary proteins, should be of extreme interest.

PCP signaling and cilia orientation in the vertebrate inner ear

Although this review focusses on neural tube defects, it is important not to overlook another key link between PCP signaling and cilia. In the vertebrate inner ear, sensory cells are decorated with regularly patterned bundles of actin-rich hairs that transduce extracellular, mechanical signals into the cell by opening ion channels. These actin-based hairs (called 'stereocilia') are organized around a single microtubule-based kinocilium. Both kinocilia and stereocilia display a prominent planar polarity (Fig. 3C) (67,68). During development, the kinocilium is formed first and its position directs the organization of the stereocilia (69,70).

The kinocilium is a true microtubule-based cilium, and the disruption of ciliary proteins such as BBS4 causes defects in the morphology of the kinocilium, but such disruptions also impact the actin-based stereocilia (42). Intriguingly, disruption of BBS4 also disrupts the regular positioning of the kinocilium (42), raising the question yet again of the role for BBS proteins in planar polarity establishment.

Experiments in mutant mice have also demonstrated that several 'core PCP' genes are required for normal planar polarity in the inner ear, and these include DVL, Frizzled, Strabismus/Vangl2 and Flamingo/Celsr1 (18,19,71,72). Other PCP players, such as PTK7, are also required for this planar polarity (50). In all of these mutants, the actin-based stereocilia maintain their position relative to the kinocilium, but the position of the kinocilia becomes random. These data provide additional important evidence for a link between PCP signaling and cilia.

CONCLUSION

In this review, I have highlighted recent studies suggesting an intimate relationship between PCP signaling and cilia. The nature of this relationship remains unclear. What is certain however is that the cilium, an organelle long ignored in developmental biology, is an important nexus for cellular signaling. It is apparently a critical junction between signals that influence cell fate and signals that influence cell movement. This area of research is only just beginning, and its continued study should yield important new insights into the mechanisms of human neural tube defects.

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