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From Planar Cell Polarity to Ciliogenesis and Back: The Curious Tale of the PPE and CPLANE proteins

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The genome is a big place, and while many genes are the subject of thousands of papers, many others have yet to receive substantial attention. Indeed, one recent paper coined the term “ignorome” to describe these “unpopular” or still poorly studied genes [1]. Why some genes are more popular than others remains an open question [2], but one example of this phenomenon involves the genes controlling planar cell polarity (PCP), the polarization of cells within a plane of a tissue [3–5].

Many PCP genes have received significant attention due to their key roles in development, homeostasis, and disease. Indeed, the so-called “core” PCP genes such as *dishevelled*, *frizzled*, and *prickle* have been extensively studied both in animal models and by human geneticists [3–5]. By contrast, other genes that influence PCP signaling have received far less attention. Among the latter are *inturned*, *fuzzy*, and *fritz*, but recent work should bring these once obscure proteins into the limelight.

Inturned, Fuzzy, and Fritz are essential for planar polarity in the *Drosophila* wing, which is evident from the oriented projection of actin-rich wing hairs (Fig. 1A). Genetic experiments placed these genes downstream of the core PCP module, leading to their designation as Planar Polarity Effector (PPE) proteins (Fig. 1A). Surprisingly, the vertebrate orthologues of these proteins (called Intu, Fuz, and Wdpcp) were subsequently found to be more important for ciliogenesis than for planar polarity (Fig. 2A), and so have been termed CPLANE proteins (for ciliogenesis and planar polarity effectors)(Box 1). Despite their seemingly divergent functions (Box 2), new data from both *Drosophila* and vertebrates demonstrate that the PPE/CPLANE proteins form a physical and functional complex. Moreover, recent data also suggest that mutation of CPLANE genes underlies human birth defects. Here, we provide a brief history of the PPE and CPLANE proteins, discuss recent advances in understanding their molecular mechanisms of action, and describe their roles in human disease.

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Box 1

Planar polarity, ciliogenesis and the evolution of PPE/CPLANE proteins

Perhaps the most puzzling aspect of PPE/CPLANE biology is the apparent divergence of function for these proteins between flies and vertebrates. In flies, all three PPE proteins play strong roles in PCP, while in vertebrates only Wdpcp has been strongly implicated (see Box 2). By contrast, while all three CPLANE proteins control ciliogenesis in vertebrates, no PPE mutants display the uncoordinated phenotypes associated with cilia defects in flies (PNA, unpublished). Nonetheless, the PPE/CPLANE proteins are strongly conserved across evolution. Using reciprocal BLAST hits, clear orthologues for all three proteins can be found across the metazoan tree of life from cnidarians to humans [51]. This conservation raises important questions about the ancestral function of these proteins: Did they originally control ciliogenesis, planar polarity, or both?

Interestingly, the cnidarian orthologues of Intu, Fuz, and Wdpcp each share greater similarity to their vertebrate counterparts than do the *Drosophila* proteins [51]. In addition, orthologues of the key CPLANE interactors Rsg1 and Jbts17 can be identified by reciprocal BLAST hits in cnidarians, but not in *Drosophila*. These results may argue that the situation in *Drosophila* is evolutionarily derived, in turn suggesting that that the PPE/CPLANE proteins govern ciliogenesis in basally branching animals. This notion is supported by a recent study of the evolution of PCP-related proteins, which identified orthologs of Fuzzy and Inturned (or an Inturned-like protein) as far back as unicellular, ciliated choanoflagellates [74]. Because PCP signaling is an inherently multi-cellular process, the presence of Intu and Fuz in unicellular choanoflagellates, combined with the *absence* of several critical PCP proteins in that organism [74], may also argue for an ancestral and ancient role for Intu and Fuz in ciliogenesis. It will be important now to experimentally assess the function of these proteins in more distantly related organisms and also to further probe the interplay between PPE proteins and PCP in *Drosophila*.

Box 2

CPLANE proteins in vertebrate PCP signaling

In flies, it is abundantly clear that the PPE proteins play a key role in wing hair PCP. The mutants have clear polarity defects, and these genes display genetic interactions with core PCP genes [59]. Moreover, PPE proteins themselves display overt planar polarized localization in wing cells [12, 13]. By contrast, PCP-related phenotypes for vertebrate CPLANE genes are much less clear. Convergent extension (C/E) during gastrulation and neurulation is a key context in which vertebrate PCP has been studied [31], but Intu and Fuz knockdowns elicit only very mild C/E defects [18]. Likewise, PCP-related C/E defects in mice are associated with craniorachischisis (a failure of neural tube closure involving the entire spinal cord and hindbrain), but none of the CPLANE mutant mice display this phenotype [20, 21, 32]. (Note that the excencephalic neural tube closure phenotype in CPLANE mutants is quite divergent from craniorachischisis; see Ref. [75]). Even double mutants of *Intu* and *Fuz* fail to display craniorachischisis [60]. On the other

hand, a looped tail is associated with mild PCP defects in mice (e.g. [76]), and *Fuz* mutants display looped tails [20].

The situation with Wdpcp is quite different. In *Xenopus*, disruption of Wdpcp function has a profound effect on C/E [30], where it acts via septins to control the polarization of the actomyosin contractile machinery [77]. Moreover, while *wdpcp* mutant mice do not display craniorachischisis, they do display overt planar polarity defects in the cochlea [32], another well-defined core PCP phenotype [78]. Moreover, disruption of Wdpcp function disrupts the localization of the core PCP proteins Vangl2 (in the mouse cochlea [32]) and Prickle2 (in *Xenopus* multiciliated cells [79]), strongly arguing that Wdpcp influences core PCP protein function.

More focused study of the links between CPLANE proteins and PCP are warranted. In particular, the cochlear hair cell polarity phenotype of mice lacking Intu or Fuz has not been reported. Likewise, though functional interaction between CPLANE proteins and core PCP proteins has been observed by knockdown in *Xenopus* [18], this interaction has yet to be validated by genetic studies in mice. Ultimately, because the PPE proteins impact *Drosophila* PCP differently in different tissues (e.g. strong in hairs and bristle and weak in ommatidia)[59], understanding the role (if any) of CPLANE proteins in vertebrate PCP will require careful, quantiative assessment of a wide range of PCP-dependent processes.

The PPE proteins control planar cell polarity in *Drosophila*

Three *Drosophila* genes, *inturned* (*in*), *fuzzy* (*fy*) and *fritz* (*frtz*) are generally considered to encode planar polarity effectors that couple the cellular asymmetry of the core planar cell polarity (PCP) pathway to the cytoskeleton. *inturned* was discovered by Bridges in 1926 and *fy* by Ives in 1939 [6]. In both of these cases the original mutations were discovered due to their sensory bristle polarity phenotype that is visible in a stereo microscope. *frtz* was independently discovered in Cambridge by Simon Collier and David Gubb and colleagues and by the Adler lab. In both cases they were discovered in screens for new mutations that altered fly PCP. These two groups were also responsible for the initial molecular characterization of the three genes [7–9].

Loss of function mutations in *in, fy* and *frtz* produce quite similar phenotypes that have been best studied in the fly wing (Fig. 1). The adult wing is covered by an array of distally pointing cuticular hairs. These arise in the pupae, where each wing cell produces a cytoplasmic extension at the distal apical edge of the cell that becomes the adult cuticular hair [10]. Many cells in PPE mutants produce two or three hairs (in some wing regions >70%) and most point in an abnormal direction. This phenotype is visible at the time of hair initiation and is a consequence of the PCP system functioning to regulate the subcellular location for activation of the cytoskeleton to produce a hair [10]. This regulation is defective in PPE mutants (Fig. 1).

Hair polarity in mutants is not random and is best described as a complicated abnormal stereotypic pattern [11]. The pattern seen in PPE mutants is quite similar to, but often a bit

more severe, than that of upstream PCP genes such as *fz* [3]. PCP proteins accumulate asymmetrically in wing cells [3, 4] and this is also seen for the PPE proteins, which accumulate on the apical proximal edge of pupal wing cells [12, 13] (Fig. 1A, colors). This asymmetry is lost and PPE protein accumulation is decreased in core PCP mutants. It is interesting that the PPE proteins retain some function even when not properly localized, as the strong multiple hair cell phenotype of PPE mutants is not seen in PCP mutants (Fig. 1A). The asymmetric accumulation of PPE proteins in wing cells appears to be instructed by the upstream core PCP proteins, as when the accumulation of PCP proteins is altered by genetic manipulation the PPE proteins follows the new PCP pattern [12]. The accumulation of PPE proteins on the proximal side of wing cells is informative, as this is the opposite side of the cell from where the hair forms, suggesting the PPE proteins act as inhibitors of hair initiation. Consistent with this hypothesis, temperature shift experiments argue that *in* functions just prior to hair initiation [14].

Most genetic data support the hypothesis that the PPE genes function downstream of the PCP genes, however that is not a universal result, as the overexpression of *frtz* or *in* plus *fy* can repattern the asymmetric accumulation of PCP proteins [15]. That is, they are acting as if upstream of the PCP proteins. This seems likely to be due to increased PPE protein levels interfering in some way with the negative intracellular feedback system that reinforces the formation of the proximal and distal complexes of PCP proteins [16, 17].

The vertebrate CPLANE proteins control ciliogenesis

The vertebrate orthologues of *Inturned* and *Fuzzy* were first described in *Xenopus*, where they were identified by homology to the *Drosophila* genes [18]. *Intu* and *Fuz* were found to be expressed at high levels in ciliated cell types, such as the floorplate and kidneys, as well as in the multiciliated cells of the *Xenopus* epidermis. Curiously, knockdown of Intu or Fuz did not elicit strong PCP-related phenotypes (Box 2), but instead elicited phenotypes associated with defective ciliogenesis, such as craniofacial and neural patterning defects [18]. Moreover, analysis of gene expression revealed severe defects in Hedgehog target gene activation after *Intu* or *Fuz* knockdown [18], consistent with the key role of cilia in Hedgehog signal transduction [19]. Finally, Intu and Fuz disruption was found to severely perturb ciliogenesis, both in mono-ciliated cells of the neural tube and multiciliated cells of the epidermis [18].

Subsequent work suggests that these CPLANE protein functions are conserved in vertebrates, as mice lacking either *Fuz* or *Intu* display neural tube and craniofacial defects, disruption of Hedgehog signaling, and loss of cilia [20, 21]. Mouse studies also revealed additional ciliopathy-related developmental phenotypes such as polydactyly, skeletal defects, encephalocoel, and hydrocephalus [20, 21]. Importantly, studies with mutant mice directly demonstrated that cilia defects in *Fuz* or *Intu* mutants are accompanied by defective processing of the Hedgehog signal transducer Gli3, both in limb buds and in facial mesenchyme [21–23]. More recently, focused studies have further explored the role of Intu and Fuz in craniofacial development [23–25], the axial skeleton [26, 27] and the skin [28, 29].

Wdpcp was also initially identified in *Xenopus*, again by homology to the *Drosophila* gene (*fritz*) [30]. Like *Intu* or *Fuz*, *Wdpcp* was found to be expressed in ciliated cells and to be essential for ciliogenesis. Accordingly, disruption of Wdpcp/Fritz results in ciliopathy phenotypes, including neural tube defects and loss of Hedgehog target gene expression [30]. However, in contrast to *Intu* and *Fuz*, *Wdpcp* knockdown in *Xenopus* also elicits profound defects in convergent extension [30], a collective cell movement that requires core PCP protein function [31]. This dual role for Wdpcp in cilia and planar polarization of cells is conserved across vertebrates, as indicated by experiments with mouse mutants [32].

PPE and CPLANE proteins control the actin cytoskeleton

The cell biology underlying the tissue-level phenotypes for the PPE/CPLANE proteins was first explored in *Drosophila*. The planar polarized hairs that decorate the surface of the adult *Drosophila* wing are formed from cytoplasmic extensions from the distal apical edge of epithelial cells [10]. The first sign of hair initiation is the accumulation of F-actin in a small apical region around the distal most vertex of these hexagonally shaped cells. Multiple thin extensions form and these coalesce to form the growing hair [33] (Fig. 1B). In growing hairs, F-actin is found under the plasma membrane with a central core of microtubules.

Mutations in PCP and PPE genes lead to an increase in the region where F-actin accumulation is seen, and it is no longer seen only in the vicinity of the distal most region [10, 33, 34]. These phenotypes argue the PCP pathway functions to restrict activation of the actin cytoskeleton to a small region of the cell, and one PCP/PPE effector that functions as a cytoskeletal antagonist has been identified (Fig. 1B). It is *multiple wing hairs* (*mwh*), the most downstream member of the pathway (Box 3). The overexpression of *frtz* leads to a dramatic delay in hair initiation and to a general disruption of the apical actin cytoskeleton suggesting that it at least indirectly regulates actin function [15], which may be mediated by over expressed Frtz hyperactivating Mwh.

Box 3

Other *Drosophila* PCP effectors

The *mwh* gene can also be considered to be a PPE gene as it appears to function as a specific downstream component of the PCP pathway [10]. *mwh* mutations are epistatic to mutations in both PCP and PPE genes. Mwh accumulates on the proximal side of pupal wing cells prior to hair initiation and this requires the function of both the PCP and PPE genes [13, 34, 47]. During the process of hair growth, Mwh is recruited to hair where it is enriched proximally [34] and this does not require the function of the PCP or PPE genes. Mwh stands apart from all of the *Drosophila* PCP and PPE proteins as it is only found in insects. It does however contain two conserved sequence motifs (GBD (G protein binding domain) and FH3 (formin homology 3 domain) found in the amino terminal half of diaphanous family formins. In *mwh* mutants several changes in hair morphogenesis were seen. The region where an increase in F-actin is seen more than doubled in size [33] and there was an increase in the number of thin actin filled pre-hairs formed. Next, the process of prehair coalescence that normally ensures only a single hair is formed did not go to completion [33]. Further, hair initiation continues for several hours after the

formation of the initial hair. This leads to the formation of very small ectopic hairs [33]. In vitro biochemistry established that Mwh binds to F-actin, inhibits the elongation of F-actin chains and cross links F-actin chains to promote the formation of thicker F-actin bundles [33]. These activities can explain the *mwh* mutant phenotype. The localization, accumulation and activation of Mwh appears to depend on its direct interaction with In [34] and Rho1 [80] and its phosphorylation, which is reduced in a *frtz* mutant [13].

Planar cell polarity and the morphogenesis of cellular and subcellular structures require the function of a variety of cellular systems regulate cell structure, the cytoskeleton and protein trafficking machinery. The Diaphanous formin, the Rab11 GTPase and the Gish kinase are good examples of such proteins [81]. Hence, these proteins can also be considered to be PPEs, although their functions are not specific for the PCP/PPE system.

Consistent with the role for PPE proteins in actin regulation in *Drosophila*, disruption of vertebrate CPLANE proteins was found to disrupt apical actin organization in multiciliated cells [18]. The mechanism of action for Fuz in this context remains undefined, but Intu has since been shown to act via the small GTPase RhoA [35] and the formin Daam1 [36]. Both RhoA and Daam1 localization are disrupted after Inturned knockdown [35, 36](Fig. 2A).

The links between Wdpcp and actin in vertebrates are perhaps more clear. Loss of Wdpcp was found to have a profound effect on the dynamic behavior of cell membranes [30, 37], and this effect resembled that resulting from loss of septin function in migrating cells in culture. Indeed, septins are well known modulators of the actin cytoskeleton [38], and further experiments revealed a physical and functional association of Wdpcp with septins during collective cell movement and during epithelial homeostasis in *Xenopus in vivo* [30, 37]. This link between Wdpcp and septin-mediated control of the actin cytoskeleton was also confirmed in mice, where loss of Wdpcp leads to disorganization of septins and actin in cultured embryo fibroblasts [32].

How the function for CPLANE proteins in actin organization relates to their role in ciliogenesis remains an important area for study. In *Xenopus* multiciliated cells, loss of CPLANE proteins leads to both fewer and shorter cilia. The reduced number of cilia stems from defects in basal body docking, a process that requires RhoA and actin [39, 40](Fig. 2A). Interestingly, actin has also been implicated in primary ciliogenesis [41], but the mechanisms of actin function in this context remain unclear. One possibility is that actin acts directly by driving basal body movement in the cytoplasm or by facilitating exocytosis of ciliary vesicles. A second possibility is that the control of actin has an indirect effect; for example, cell cortex tension has been shown to influence basal body docking and ciliogenesis [42], so CPLANE proteins may control actin function at the cell cortex and thereby impact ciliogenesis only secondarily. Finally, in the case of Wdpcp, misregulation of septins may also contribute to the ciliogenesis defect, as septins normally localize to the base of cilia and are essential for ciliogenesis [30, 43–45].

It is clear, then, that both PPE and CPLANE proteins exert an influence on the actin cytoskeleton, and this link to actin may help to explain their effects on vertebrate ciliogenesis. However, it is important to note that like the role for ciliogenesis versus planar

polarization, the functions of PPE and CPLANE proteins have diverged between insects and vertebrates. The CPLANE proteins appear to act as positive regulators of actin assembly [18, 35], and for Intu at least, this function is mediated by the formin protein Daam1 [36], which polymerizes linear actin filaments [46]. By contrast, the PPE proteins act via Mwh, which though it contains the GBD and FH3 domains present in Diaphanous family formin proteins [13, 47], is actually an inhibitor of actin polymerization [15].

CPLANE proteins and intraflagellar transport

In addition to their impact on ciliogenesis via the actin cytoskeleton, it is clear that CPLANE proteins also influence core ciliogenic machinery such as intraflagellar transport (IFT), a deeply conserved mechanism that transports cargo into, along, and out of ciliary axonemes [48]. The first hint of a role for CPLANE proteins in IFT came from the observation that disruption of Fuz specifically disturbed protein localization in the distal, but not the proximal, axoneme in *Xenopus* multiciliated cells [20, 49]. Subsequent analysis of IFT particle movement in axonemes by high-speed confocal microscopy revealed an interesting phenotype. The IFT system is composed of two sub-complexes, IFT-A and IFT-B [48], and after disruption of Fuz function, the normal bidirectional transport of IFT proteins is disrupted. The IFT-B proteins Ift20 and Ift80 forming enlarged and immobile accumulations in the axonemes, while the IFT-A protein Ift43 was simply absent from axonemes [49]. Interestingly, the IFT-A subcomplex is comprised of a three-protein IFT-A “core” and three “peripheral” IFT-A sub-units [50], and more recent analysis has revealed disruption of CPLANE function results specifically in loss of IFT-A peripheral proteins from the axoneme, while the IFT-A core proteins enter the axoneme and traffic bi-directionally at essentially normal speeds [51](Fig. 2A).

Despite the obvious axonemal phenotypes, the CPLANE proteins localize and exert their influence not in axonemes, but rather at the base of cilia, around the basal body. These proteins assemble hierarchically at basal bodies, along with their interactors Jbts17 and Rsg1. All five proteins specifically control the recruitment of IFT-A peripheral proteins [49, 51, 52]. In the absence of CPLANE function, the IFT-A core proteins, lacking the peripheral components enter axonemes and traffic normally, while IFT-B proteins enter axonemes but cannot traffic and thus accumulate [51].

An important goal for future work will be to understand more deeply the molecular mechanisms underlying CPLANE function in ciliogenesis. One possibility is that the CPLANE’s role in actin assembly indirectly impacts IFT, as actin has been shown to control basal body recruitment of IFT in the green algae *Chlamydomonas* [53]. Alternatively, CPLANE may have a more direct role, as suggested by a proteomic study that revealed physical links to ciliopathy proteins, to IFT-A proteins, and to the protein folding chaperones CCT and NudC [51], the latter of which was recently suggested to contribute to IFT-A assembly [54]. Other, more directed studies also provide important insights. For example, Fuz has been shown to be required for recruitment of the vesicle trafficking regulator Rab8 [55]. This finding is of interest both because Rab8 is implicated in ciliogenesis, and also because the related protein Rab11 is implicated both in vertebrate ciliogenesis and in wing hair polarization in *Drosophila* [56, 57]. Because the CPLANE proteins act in the cytoplasm

to control recruitment to basal bodies and assembly of IFT particles [51], it is notable that Rab8 has been linked to IFT particle assembly via the Elipsa protein and mutation of the *C. elegans* homolog of *Elipsa*, *Dyf-11* results in ciliary defects including abnormal accumulation of specific ciliary proteins in the distal axoneme [58].

Finally, though the role for core PCP proteins in ciliogenesis remains murky (Box 4), there is some evidence that, like the PPE proteins in the fly wing, CPLANE proteins also functionally interact with core PCP proteins. Indeed, knockdown of Intu disrupts recruitment of Dishevelled to basal bodies in *Xenopus* MCCs [35], and *Fuz* mutant MEFs likewise display a failure to recruit Dishevelled to the base of primary cilia [55].

Box 4

Core PCP proteins and vertebrate ciliogenesis

As mentioned in Box 2, the CPLANE proteins' critical role in ciliogenesis in vertebrates seems not to be shared by the *Drosophila* PPE proteins (hence the divergent nomenclature). The converse question is also interesting. What roles do core PCP proteins play in vertebrate cilia? Clearly PCP proteins control the *orientation* of cilia beating, as first shown in *Xenopus* MCCs [35, 82], and subsequently for mouse MCCs in the brain [83] and airway [84], and in motile monocilia in mice [85, 86] and zebrafish [87]. But is there a role for core PCP proteins in ciliogenesis? So far, the answer seems to depend on the protein.

The first reports linking core PCP proteins to ciliogenesis came from knockdowns of the core PCP proteins Dishevelled or Frizzled [35, 88]. However, these phenotypes have yet to be confirmed by genetic studies. The situation for Vangl2 is even murkier, as conflicting results on ciliogenesis have been reported even for the same genetic mutant in zebrafish [87, 89]. Clearly, further study is warranted.

The best evidence for a link between core PCP functions and ciliogenesis comes from mice with genetic mutations in *Celsr* and *Prickle* genes. Mice lacking Celsr2 and Celsr3 displayed ciliogenesis defects in MCCs stemming from a failure of basal body docking [90], similar to that first observed after Dishevelled knockdown in *Xenopus* [35]. Interestingly, these *Celsr* mutant mice also display defects in Vangl2 localization [90]. In addition, mice lacking *Prickle2* or *Prickle1* display bulging ciliary membranes in motile cilia on MCCs in the brain or airway, respectively [91, 92]. Tracheal MCCs of *Prickle1* mutant mice also display beat frequency defects, and electron microscopy revealed abnormal organization of axonemal microtubules [92]. Moreover, *Prickle1* mutant embryo fibroblasts in culture also displayed fewer and shorter primary cilia [92], arguing that this role for Prickle2 is not restricted to motile cilia.

Reciprocal interactions among the PPE/CPLANE proteins, a physical and functional complex?

A key hurdle to understanding the biochemical mechanisms of action for PPE/CPLANE proteins is that none harbors any obvious catalytic domain and none closely resembles any

well-studied protein. Indeed, all three PPE/CPLANE genes encode novel proteins of still unknown molecular function. Recently however, biochemical and proteomic approaches are beginning to shed some light on the situation. First, it was noted that the adult phenotypes of PPE double mutants show no additivity [10]; however, double mutants of hypomorphic (weak) alleles interact synergistically and display a strong phenotype [9, 59]. These data suggested the possibility that the encoded proteins may be essential components of a protein complex. Confirmation of this came from both co-immunoprecipitation and yeast two-hybrid experiments [15](Fig. 2B). The data argue that In directly binds to both Frtz and Fy. These three proteins were also found to modulate the accumulation of the others in both loss and gain of function experiments, consistent with the complex being functionally important. Despite the evidence that the three proteins are part of a complex, there is evidence that Frtz can function in the absence of the others as when overexpressed, Frtz can produce a gain of function phenotype that is independent of both the PCP and PPE proteins [15].

Interestingly, despite clear divergence in functions between flies and vertebrates (Box 1, 2), the CPLANE proteins also appear to act as a physical and functional complex (Fig. 2B). As in flies, double mutants of Intu and Fuz in mice display no additive phenotypes [60], though double mutants with Wdpcp have yet to be reported. Likewise, proteomic analysis demonstrated a robust physical association between Intu, Fuz, and Wdpcp [51]. Finally, as is the case in *Drosophila*, Intu, Fuz, and Wdpcp can –to some extent– control one another's localization to basal bodies [51].

These reciprocal interactions between PPE/CPLANE proteins raises the important question of additional interactors. Consistent with the links to actin regulation, *Drosophila* Inturned directly interacts with the formin-related protein Mwh [34], while vertebrate Intu interacts with the formin Daam1 [36]. Regarding ciliogenesis, all three CPLANE protein interact with the poorly characterized GTPase Rsg1, which in turn controls IFT and ciliogenesis [20, 51, 52]. All three CPLANE proteins also interact with Jbts17 [51], which was first identified by its mutation in human patients with cilia-related birth defects [61–64]. Finally, proteomics suggest a link between CPLANE proteins and the Nphp proteins [51], and directed experiments clearly show both physical and functional links between Intu and Nphp4 [36]. Further exploration of the interaction partners for PPE and CPLANE proteins should help us to understand the apparently diverged functions of these proteins during evolution of flies and vertebrates (Box 1).

The CPLANE proteins in human disease

Consistent with the severe phenotype of vertebrate embryos lacking CPLANE function, mutations of human CPLANE genes have now been identified in a number of congenital birth defects. First, mutations in *FUZ* have been associated with neural tube closure defects, consistent with the neural tube closure phenotypes reported after Fuz knockdown in *Xenopus* [18] or *Fuz* mutation in mice [20, 22]. In a study of several hundred Italian patients with neural tube defects, five disease-associated alleles were identified in *FUZ*, and these were distributed throughout the protein [65]. Four of these patients presented with myelomeningocele, and the fifth with caudal regression syndrome; these phenotypes are far milder than the exencephalic phenotype of Fuz null mutant mice, arguing that the alleles

may be hypomorphic. Consistent with this notion, rescue experiments in *Fuz* mutant MEFs reveal that none of the alleles are deficient in the ability to promote ciliogenesis. Rather, one allele induces longer cilia and the others display phenotypes detected only in overexpression assays [65]. Further testing of these alleles will be important, because the relationship between ciliogenesis and neural tube closure remains poorly defined [66]. It will also be important to determine the role, if any, of *INTU* and *WDPCP* in human neural tube defects.

In addition to neural tube defects, mutations in CPLANE genes are also known to be causative for ciliopathies, the diverse spectrum of human disorders arising from defects in the structure or function of cilia [67]. Several alleles of *WDPCP* segregate with ciliopathy phenotypes, including Bardet-Biedl, Meckel-Gruber, and Jeune Syndromes, as well as short-rib-polydactyly, and variants of Oral-Facial-Digital syndrome [30, 51, 68]. Experiments in *Xenopus* have revealed a variety of pathogenic mechanisms for these alleles, including altered protein stability, failure to localize to basal bodies, and inability to recruit IFT machinery [51]. Interestingly, no human mutations have yet been reported in the CPLANE interacting protein RSG1, though mutations in JBTS17 has been associated with diverse human ciliopathies [61–64].

Finally, one study suggests that the CPLANE proteins may be relevant not only to congenital birth defects but also to infectious disease. A genome-wide screen identified Fuz as a key receptor for alpha-virus entry, and loss of Fuz prevented alphavirus entry into cells [69]. Interestingly, this study also implicated Fuz more broadly in clathrin-mediated endocytosis [69], and a recent proteomic survey found broad links between all three CPLANE proteins and the AP1 and AP2 clathrin adaptors [51]. Direct investigation of the role of other CPLANE proteins and their interactors should be of interest, therefore, both in terms of basic cell biology and infectious disease.

Conclusion

In sum, the PPE and CPLANE proteins are essential for normal development and morphogenesis of diverse animals, including humans. Their developmental and cell biological roles are now well defined, but important questions concerning their molecular functions remain. All three PPE/CPLANE genes encode novel proteins, so their primary sequences offer little insight because none encodes any known catalytic or functional domains. Rather, these proteins are characterized by structural motifs such as the predicted beta-propeller in Fritz/Wdpcp [30, 51, 70] and the Longin domain in Fuzzy [20]. The recent insight that the PPE/CPLANE proteins can all interact is informative, because structural modeling suggests that together the CPLANE complex bears some similarity to the COPII vesicle coat complex [51], which plays a crucial role in intracellular cargo transport [71]. Another clue may come from the similarity between the PPE/CPLANE protein structure predictions and other ciliary proteins. Indeed both the IFT complex and the BBSome complex are enriched for structural motifs similar to those observed in PPE/CPLANE proteins. Because IFT complexes and the BBSome are thought to have evolved from pro-coatamer proteins [72, 73], the similar predicted folds in CPLANE proteins may suggest that they followed a similar evolutionary trajectory.

Future work directed at understanding the molecular/biochemical functions of the PPE and CPLANE proteins will be critical and should inform our understanding of the apparent divergence of function between flies and vertebrates. Such exploration should be of great interest, because like the majority of genes [2], the PPE/CPLANE genes have yet to draw that much attention from embryologists, but it is clear that they are terribly important to embryos.

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References

1. Pandey AK, Lu L, Wang X, Homayouni R, Williams RW. Functionally enigmatic genes: a case study of the brain ignorome. PLoS ONE. 2014; 9:e88889. [PubMed: 24523945]
2. Hoffmann R, Valencia A. Life cycles of successful genes. Trends Genet. 2003; 19:79–81. [PubMed: 12547515]
3. Adler PN. The frizzled/stan pathway and planar cell polarity in the Drosophila wing. Curr Top Dev Biol. 2012; 101:1–31. [PubMed: 23140623]
4. Peng Y, Axelrod JD. Asymmetric protein localization in planar cell polarity: mechanisms, puzzles, and challenges. Curr Top Dev Biol. 2012; 101:33–53. [PubMed: 23140624]
5. Gray RS, Roszko I, Solnica-Krezel L. Planar cell polarity: coordinating morphogenetic cell behaviors with embryonic polarity. Dev Cell. 2011; 21:120–133. [PubMed: 21763613]
6. Attrill H, Falls K, Goodman JL, Millburn GH, Antonazzo G, Rey AJ, Marygold SJ, FlyBase C. FlyBase: establishing a Gene Group resource for Drosophila melanogaster. Nucleic Acids Res. 2016; 44:D786–792. [PubMed: 26467478]
7. 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]
8. 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]
9. Collier S, Lee H, Burgess R, Adler P. The WD40 repeat protein fritz links cytoskeletal planar polarity to frizzled subcellular localization in the Drosophila epidermis. Genetics. 2005; 169:2035–2045. [PubMed: 15654087]
10. Wong LL, Adler PN. Tissue polarity genes of Drosophila regulate the subcellular location for prehair initiation in pupal wing cells. J Cell Biol. 1993; 123:209–221. [PubMed: 8408199]
11. Gubb D, Garcia-Bellido A. A genetic analysis of the determination of cuticular polarity during development in Drosophila melanogaster. J Embryol Exp Morphol. 1982; 68:37–57. [PubMed: 6809878]
12. 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]
13. Strutt D, Warrington SJ. Planar polarity genes in the Drosophila wing regulate the localisation of the FH3-domain protein Multiple Wing Hairs to control the site of hair production. Development. 2008; 135:3103–3111. [PubMed: 18701542]
14. Adler PN, Charlton J, Park WJ. The Drosophila tissue polarity gene inturned functions prior to wing hair morphogenesis in the regulation of hair polarity and number. Genetics. 1994; 137:829–836. [PubMed: 8088527]
15. Wang Y, Yan J, Lee H, Lu Q, Adler PN. The proteins encoded by the Drosophila Planar Polarity Effector genes inturned, fuzzy and fritz interact physically and can re-pattern the accumulation of “upstream” Planar Cell Polarity proteins. Dev Biol. 2014

16. Tree DR, Shulman JM, Rousset R, Scott MP, Gubb D, Axelrod JD. Prickle mediates feedback amplification to generate asymmetric planar cell polarity signaling. Cell. 2002; 109:371–381. [PubMed: 12015986]
17. Amonlirdviman K, Khare NA, Tree DR, Chen WS, Axelrod JD, Tomlin CJ. Mathematical modeling of planar cell polarity to understand domineering nonautonomy. Science. 2005; 307:423–426. [PubMed: 15662015]
18. 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]
19. Goetz SC, Anderson KV. The primary cilium: a signalling centre during vertebrate development. Nat Rev Genet. 2010; 11:331–344. [PubMed: 20395968]
20. 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. Nat Cell Biol. 2009; 11:1225–1232. [PubMed: 19767740]
21. 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]
22. 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]
23. Tabler JM, Barrell WB, Szabo-Rogers HL, Healy C, Yeung Y, Perdiguero EG, Schulz C, Yannakoudakis BZ, Mesbahi A, Wlodarczyk B, et al. Fuz mutant mice reveal shared mechanisms between ciliopathies and FGF-related syndromes. Dev Cell. 2013; 25:623–635. [PubMed: 23806618]
24. Zhang Z, Wlodarczyk BJ, Niederreither K, Venugopalan S, Florez S, Finnell RH, Amendt BA. Fuz regulates craniofacial development through tissue specific responses to signaling factors. PLoS ONE. 2011; 6:e24608. [PubMed: 21935430]
25. Tabler JM, Rice CP, Liu KJ, Wallingford JB. A novel ciliopathic skull defect arising from excess neural crest. Dev Biol. 2016; 417:4–10. [PubMed: 27395007]
26. Chang R, Petersen JR, Niswander LA, Liu A. A hypomorphic allele reveals an important role of inturned in mouse skeletal development. Dev Dyn. 2015; 244:736–747. [PubMed: 25774014]
27. Yannakoudakis BZ, Liu KJ. Common skeletal features in rare diseases: New links between ciliopathies and FGF-related syndromes. Rare Dis. 2013; 1:e27109. [PubMed: 25003013]
28. Dai D, Li L, Huebner A, Zeng H, Guevara E, Claypool DJ, Liu A, Chen J. Planar cell polarity effector gene Intu regulates cell fate-specific differentiation of keratinocytes through the primary cilia. Cell Death Differ. 2013; 20:130–138. [PubMed: 22935613]
29. Dai D, Zhu H, Wlodarczyk B, Zhang L, Li L, Li AG, Finnell RH, Roop DR, Chen J. Fuz controls the morphogenesis and differentiation of hair follicles through the formation of primary cilia. J Invest Dermatol. 2011; 131:302–310. [PubMed: 20962855]
30. Kim SK, Shindo A, Park TJ, Oh EC, Ghosh S, Gray RS, Lewis RA, Johnson CA, Attie-Bittach T, Katsanis N, et al. Planar Cell Polarity Acts Through Septins to Control Collective Cell Movement and Ciliogenesis. Science. 2010; 329:1337–1340. [PubMed: 20671153]
31. Wallingford JB. Planar cell polarity and the developmental control of cell behavior in vertebrate embryos. Annu Rev Cell Dev Biol. 2012; 28:627–653. [PubMed: 22905955]
32. Cui C, Chatterjee B, Lozito T, Zhang Z, Lo CW. Wdpcp, a PCP Protein Required for Ciliogenesis, Regulates Directional Cell Migration and Cell Polarity by Direct Modulation of the Actin Cytoskeleton. PLoS Biol. 2013; 11:e1001720. [PubMed: 24302887]
33. Lu Q, Schafer DA, Adler PN. The Drosophila planar polarity gene multiple wing hairs directly regulates the actin cytoskeleton. Development. 2015; 142:2478–2486. [PubMed: 26153232]
34. Lu Q, Yan J, Adler PN. The Drosophila planar polarity proteins inturned and multiple wing hairs interact physically and function together. Genetics. 2010; 185:549–558. [PubMed: 20351219]
35. 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. [PubMed: 18552847]

36. Yasunaga T, Hoff S, Schell C, Helmstadter M, Kretz O, Kuechlin S, Yakulov TA, Engel C, Muller B, Bensch R, et al. The polarity protein Inturned links NPHP4 to Daam1 to control the subapical actin network in multiciliated cells. J Cell Biol. 2015; 211:963–973. [PubMed: 26644512]
37. Park TJ, Kim SK, Wallingford JB. The planar cell polarity effector protein Wdpcp (Fritz) controls epithelial cell cortex dynamics via septins and actomyosin. Biochem Biophys Res Commun. 2015; 456:562–566. [PubMed: 25436430]
38. Mostowy S, Cossart P. Septins: the fourth component of the cytoskeleton. Nat Rev Mol Cell Biol. 2012; 13:183–194. [PubMed: 22314400]
39. 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]
40. Lemullois M, Boisvieux-Ulrich E, Laine MC, Chailley B, Sandoz D. Development and functions of the cytoskeleton during ciliogenesis in metazoa. Biol Cell. 1988; 63:195–208. [PubMed: 2904829]
41. Kim J, Lee JE, Heynen-Genel S, Suyama E, Ono K, Lee K, Ideker T, Aza-Blanc P, Gleeson JG. Functional genomic screen for modulators of ciliogenesis and cilium length. Nature. 2010; 464:1048–1051. [PubMed: 20393563]
42. Pitaval A, Tseng Q, Bornens M, Thery M. Cell shape and contractility regulate ciliogenesis in cell cycle-arrested cells. J Cell Biol. 2010; 191:303–312. [PubMed: 20956379]
43. Hu Q, Milenkovic L, Jin H, Scott MP, Nachury MV, Spiliotis ET, Nelson WJ. A septin diffusion barrier at the base of the primary cilium maintains ciliary membrane protein distribution. Science. 2010; 329:436–439. [PubMed: 20558667]
44. Chih B, Liu P, Chinn Y, Chalouni C, Komuves LG, Hass PE, Sandoval W, Peterson AS. A ciliopathy complex at the transition zone protects the cilia as a privileged membrane domain. Nat Cell Biol. 2012; 14:61–72.
45. Zhai G, Gu Q, He J, Lou Q, Chen X, Jin X, Bi E, Yin Z. Sept6 is required for ciliogenesis in Kupffer's vesicle, the pronephros, and the neural tube during early embryonic development. Mol Cell Biol. 2014; 34:1310–1321. [PubMed: 24469395]
46. Moseley JB, Maiti S, Goode BL. Formin proteins: purification and measurement of effects on actin assembly. Methods Enzymol. 2006; 406:215–234. [PubMed: 16472660]
47. Yan J, Huen D, Morely T, Johnson G, Gubb D, Roote J, Adler PN. The multiple-wing-hairs Gene Encodes a Novel GBD-FH3 Domain-Containing Protein That Functions Both Prior to and After Wing Hair Initiation. Genetics. 2008; 180:219–228. [PubMed: 18723886]
48. Taschner M, Bhogaraju S, Lorentzen E. Architecture and function of IFT complex proteins in ciliogenesis. Differentiation. 2012; 83:S12–22. [PubMed: 22118932]
49. Brooks E, Wallingford JB. Control of vertebrate intraflagellar transport by the planar cell polarity effector Fuz. J Cell Biol. 2012; 198:37–45. [PubMed: 22778277]
50. Behal RH, Miller MS, Qin H, Lucker BF, Jones A, Cole DG. Subunit interactions and organization of the Chlamydomonas reinhardtii intraflagellar transport complex A proteins. J Biol Chem. 2012; 287:11689–11703. [PubMed: 22170070]
51. Toriyama M, Lee C, Taylor SP, Duran I, Cohn DH, Bruel AL, Tabler JM, Drew K, Kelly MR, Kim S, et al. The ciliopathy-associated CPLANE proteins direct basal body recruitment of intraflagellar transport machinery. Nat Genet. 2016; 48:648–656. [PubMed: 27158779]
52. Brooks ER, Wallingford JB. The Small GTPase Rsg1 is important for the cytoplasmic localization and axonemal dynamics of intraflagellar transport proteins. Cilia. 2013; 2:13. [PubMed: 24192041]
53. Avasthi P, Onishi M, Karpiak J, Yamamoto R, Mackinder L, Jonikas MC, Sale WS, Shoichet B, Pringle JR, Marshall WF. Actin is required for IFT regulation in Chlamydomonas reinhardtii. Curr Biol. 2014; 24:2025–2032. [PubMed: 25155506]
54. Boldt K, van Reeuwijk J, Lu Q, Koutroumpas K, Nguyen TM, Texier Y, van Beersum SE, Horn N, Willer JR, Mans DA, et al. An organelle-specific protein landscape identifies novel diseases and molecular mechanisms. Nat Commun. 2016; 7:11491. [PubMed: 27173435]
55. Zilber Y, Babayeva S, Seo JH, Liu JJ, Mootin S, Torban E. The PCP effector Fuzzy controls cilial assembly and signaling by recruiting Rab8 and Dishevelled to the primary cilium. Mol Biol Cell. 2013; 24:555–565. [PubMed: 23303251]

56. Knodler A, Feng S, Zhang J, Zhang X, Das A, Peranen J, Guo W. Coordination of Rab8 and Rab11 in primary ciliogenesis. Proc Natl Acad Sci U S A. 2010; 107:6346–6351. [PubMed: 20308558]
57. Gault WJ, Olguin P, Weber U, Mlodzik M. Drosophila CK1-gamma, gilgamesh, controls PCP-mediated morphogenesis through regulation of vesicle trafficking. J Cell Biol. 2012; 196:605–621. [PubMed: 22391037]
58. Omori Y, Zhao C, Saras A, Mukhopadhyay S, Kim W, Furukawa T, Sengupta P, Veraksa A, Malicki J. Elipsa is an early determinant of ciliogenesis that links the IFT particle to membrane-associated small GTPase Rab8. Nat Cell Biol. 2008; 10:437–444. [PubMed: 18364699]
59. 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]
60. Heydeck W, Liu A. PCP effector proteins inturned and fuzzy play nonredundant roles in the patterning but not convergent extension of mammalian neural tube. Dev Dyn. 2011; 240:1938–1948. [PubMed: 21761479]
61. Alazami AM, Alshammari MJ, Salih MA, Alzahrani F, Hijazi H, Seidahmed MZ, Abu Safieh L, Aldosary M, Khan AO, Alkuraya FS. Molecular characterization of Joubert syndrome in Saudi Arabia. Hum Mutat. 2012; 33:1423–1428. [PubMed: 22693042]
62. Lopez E, Thauvin-Robinet C, Reversade B, Khartoufi NE, Devisme L, Holder M, Ansart-Franquet H, Avila M, Lacombe D, Kleinfinger P, et al. C5orf42 is the major gene responsible for OFD syndrome type VI. Hum Genet. 2014; 133:367–377. [PubMed: 24178751]
63. Shaheen R, Faqeih E, Alshammari MJ, Swaid A, Al-Gazali L, Mardawi E, Ansari S, Sogaty S, Seidahmed MZ, AlMotairi MI, et al. Genomic analysis of Meckel-Gruber syndrome in Arabs reveals marked genetic heterogeneity and novel candidate genes. Eur J Hum Genet. 2013; 21:762–768. [PubMed: 23169490]
64. Srour M, Schwartzentruber J, Hamdan FF, Ospina LH, Patry L, Labuda D, Massicotte C, Dobrzeniecka S, Capo-Chichi JM, Papillon-Cavanagh S, et al. Mutations in C5ORF42 cause Joubert syndrome in the French Canadian population. Am J Hum Genet. 2012; 90:693–700. [PubMed: 22425360]
65. Seo JH, Zilber Y, Babayeva S, Liu J, Kyriakopoulos P, De Marco P, Merello E, Capra V, Gros P, Torban E. Mutations in the planar cell polarity gene, Fuzzy, are associated with neural tube defects in humans. Hum Mol Genet. 2011
66. Murdoch JN, Copp AJ. The relationship between sonic Hedgehog signaling, cilia, and neural tube defects. Birth Defects Res A Clin Mol Teratol. 2010; 88:633–652. [PubMed: 20544799]
67. Hildebrandt F, Benzing T, Katsanis N. Ciliopathies. N Engl J Med. 2011; 364:1533–1543. [PubMed: 21506742]
68. Saari J, Lovell MA, Yu HC, Bellus GA. Compound heterozygosity for a frame shift mutation and a likely pathogenic sequence variant in the planar cell polarity-ciliogenesis gene WDPCP in a girl with polysyndactyly, coarctation of the aorta, and tongue hamartomas. Am J Med Genet A. 2014
69. Ooi YS, Stiles KM, Liu CY, Taylor GM, Kielian M. Genome-wide RNAi screen identifies novel host proteins required for alphavirus entry. PLoS Pathog. 2013; 9:e1003835. [PubMed: 24367265]
70. Wang Y. Characterizing the activity of PPE proteins in the frizzled/PCP signaling pathway. 2016
71. Jensen D, Schekman R. COPII-mediated vesicle formation at a glance. J Cell Sci. 2011; 124:1–4. [PubMed: 21172817]
72. van Dam TJ, Townsend MJ, Turk M, Schlessinger A, Sali A, Field MC, Huynen MA. Evolution of modular intraflagellar transport from a coatomer-like progenitor. Proc Natl Acad Sci U S A. 2013; 110:6943–6948. [PubMed: 23569277]
73. Jekely G, Arendt D. Evolution of intraflagellar transport from coated vesicles and autogenous origin of the eukaryotic cilium. Bioessays. 2006; 28:191–198. [PubMed: 16435301]
74. Schenkelaars Q, Fierro-Constain L, Renard E, Borchiellini C. Retracing the path of planar cell polarity. BMC Evol Biol. 2016; 16:69. [PubMed: 27039172]
75. Wallingford JB, Mitchell B. Strange as it may seem: the many links between Wnt signaling, planar cell polarity, and cilia. Genes Dev. 2011; 25:201–213. [PubMed: 21289065]
76. Kibar Z, Vogan KJ, Groulx N, Justice MJ, Underhill DA, Gros P. Ltap, a mammalian homolog of Drosophila Strabismus/Van Gogh, is altered in the mouse neural tube mutant Loop-tail. Nat Genet. 2001; 28:251–255. [PubMed: 11431695]

77. Shindo A, Wallingford JB. PCP and septins compartmentalize cortical actomyosin to direct collective cell movement. Science. 2014; 343:649–652. [PubMed: 24503851]

78. Rida PC, Chen P. Line up and listen: Planar cell polarity regulation in the mammalian inner ear. Semin Cell Dev Biol. 2009; 20:978–985. [PubMed: 19508855]

79. Butler MT, Wallingford JB. Control of vertebrate core planar cell polarity protein localization and dynamics by Prickle 2. Development. 2015; 142:3429–3439. [PubMed: 26293301]

80. Yan J, Lu Q, Fang X, Adler PN. Rho1 has multiple functions in Drosophila wing planar polarity. Dev Biol. 2009; 333:186–199. [PubMed: 19576201]

81. Lu Q, Adler PN. The diaphanous gene of Drosophila interacts antagonistically with multiple wing hairs and plays a key role in wing hair morphogenesis. PLoS ONE. 2015; 10:e0115623. [PubMed: 25730111]

82. 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. [PubMed: 19427216]

83. 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]

84. Vladar EK, Bayly RD, Sangoram AM, Scott MP, Axelrod JD. Microtubules enable the planar cell polarity of airway cilia. Curr Biol. 2012; 22:2203–2212. [PubMed: 23122850]

85. Hashimoto M, Shinohara K, Wang J, Ikeuchi S, Yoshiba 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. [PubMed: 20098415]

86. Song H, Hu J, Chen W, Elliott G, Andre P, Gao B, Yang Y. Planar cell polarity breaks bilateral symmetry by controlling ciliary positioning. Nature. 2010; 466:378–382. [PubMed: 20562861]

87. 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. [PubMed: 20305649]

88. 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]

89. May-Simera HL, Kai M, Hernandez V, Osborn DP, Tada M, Beales PL. Bbs8, together with the planar cell polarity protein Vangl2, is required to establish left-right asymmetry in zebrafish. Dev Biol. 2010; 345:215–225. [PubMed: 20643117]

90. Tissir F, Qu Y, Montcouquiol M, Zhou L, Komatsu K, Shi D, Fujimori T, Labeau J, Tyteca D, Courtoy P, et al. Lack of cadherins Celsr2 and Celsr3 impairs ependymal ciliogenesis, leading to fatal hydrocephalus. Nat Neurosci. 2010; 13:700–707. [PubMed: 20473291]

91. Sowers LP, Yin T, Mahajan VB, Bassuk AG. Defective motile cilia in Prickle2-deficient mice. J Neurogenet. 2014; 28:146–152. [PubMed: 24708399]

92. Gibbs BC, Damerla RR, Vladar EK, Chatterjee B, Wan Y, Liu X, Cui C, Gabriel GC, Zahid M, Yagi H, et al. Prickle1 mutation causes planar cell polarity and directional cell migration defects associated with cardiac outflow tract anomalies and other structural birth defects. Biol Open. 2016; 5:323–335. [PubMed: 26883626]

Outstanding Questions Box

What was the ancestral role for the PPE/CPLANE proteins? Did this complex evolve initially to control ciliogenesis or PCP or both? The ultimate answer to this question is not knowable, but a deeper analysis of PPE/CPLANE function in diverse organisms, including basally branching animals will be informative.

What are the biochemical activities of the PPE/CPLANE proteins? These genes all encode novel proteins with no obvious catalytic domains. While it is now clear that the proteins interact, the activity of this complex remains entirely unknown.

What are the other components of the PPE/CPLANE complex, and are these cell-type specific? A few interactors have been described for the PPE/CPLANE proteins, but no clear picture of this complex has yet emerged. It is possible that by interacting with different proteins in different cell types, the PPE/CPLANE complex can execute diverse cell biological processes.

Trends Box

The PPE/CPLANE proteins are evolutionarily conserved proteins that govern planar cell polarity (PCP) in *Drosophila* but predominantly control ciliogenesis in vertebrates.

At least one CPLANE protein also controls PCP in vertebrates.

PPE/CPLANE proteins strongly interact in both *Drosophila* and in vertebrates, arguing that they function as a complex.

PPE and CPLANE proteins are implicated in control of actin assembly.

CPLANE proteins are required for normal intraflagellar transport.

CPLANE proteins are mutated in human birth defects.

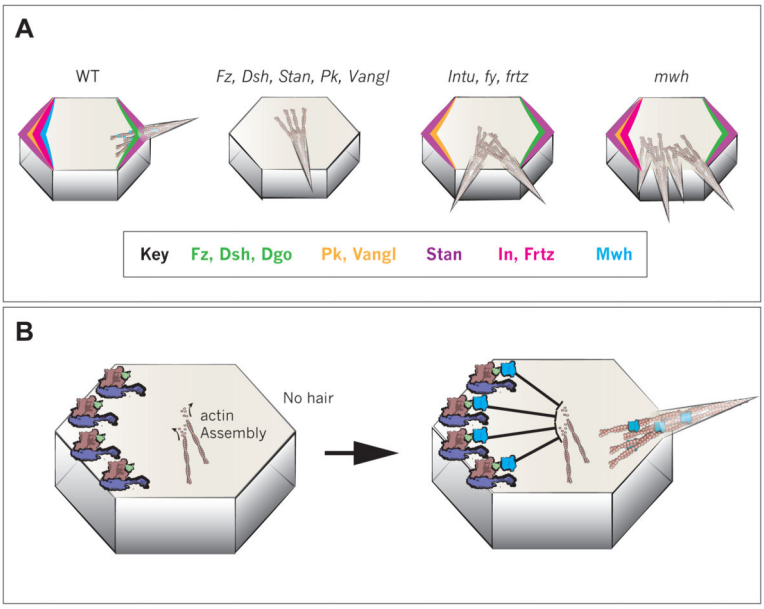

Figure 1.

A. Planar cell polarity in the *Drosophila* wing. Wild type wing cells extend a single distally-directed actin-based hair under the control of asymmetrically localized PCP/PPE proteins (see colors). Mutation of core PCP genes disrupts both polarity of wing hairs and PCP/PPE protein localization. Mutation of PPE genes leads to disrupted polarity as well as multiple wing hairs, but core PCP protein localization is unaffected. Mutation of Mwh disrupts polarity and elicits multiple wing hairs, while core PCP and PPE protein localization is unaffected. **B.** The PPE proteins act via Mwh to restrict actin assembly to the distal vertex of wing cells.

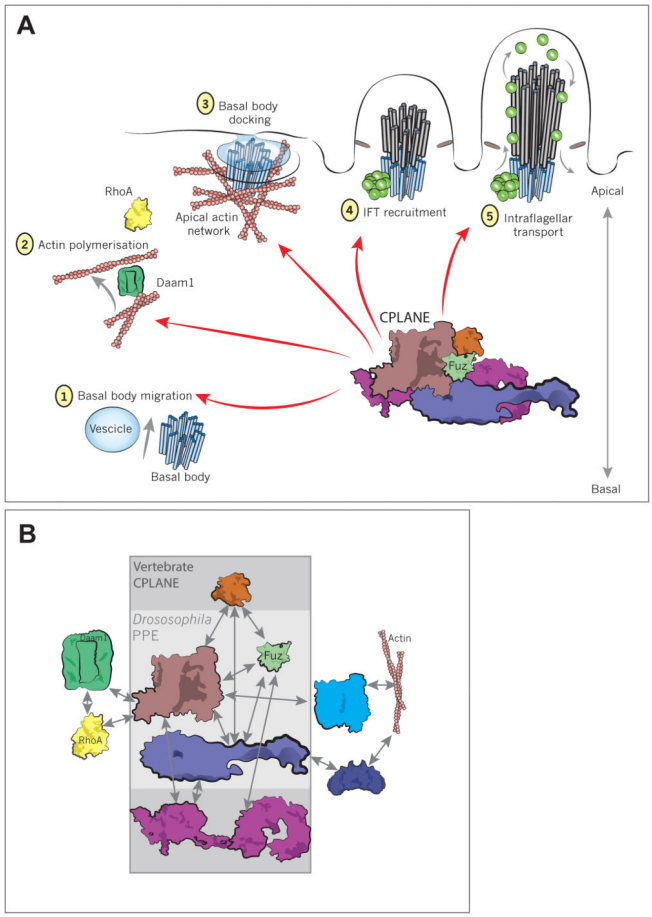

Figure 2.

A. CPLANE proteins control multiple steps of ciliogenesis, acting with Daam1 and RhoA to control actin-dependent apical migration and docking of basal bodies and also recruitment of IFT-A proteins to the base of cilia. The protein structures show here are based on computational modeling reported in [51]; the interaction interfaces in the assemblage depicted are speculative,. **B.** Both *Drosophila* PPE proteins and vertebrate CPLANE proteins reciprocally interact in biochemical and proteomic assays. CPLANE proteins also interact with Jbts17 and Rsg1, proteins which appear not be encoded in the Drosophila genome. PPE proteins interact with the formin-related protein Mwh, CPLANE proteins act via the formin Daam1 and the GTPAse RhoA