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Tissue/planar cell polarity in vertebrates: new insights and new questions

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This review focuses on the tissue/planar cell polarity (PCP) pathway and its role in generating spatial patterns in vertebrates. Current evidence suggests that PCP integrates both global and local signals to orient diverse structures with respect to the body axes. Interestingly, the system acts on both subcellular structures, such as hair bundles in auditory and vestibular sensory neurons, and multicellular structures, such as hair follicles. Recent work has shown that intriguing connections exist between the PCP-based orienting system and left-right asymmetry, as well as between the oriented cell movements required for neural tube closure and tubulogenesis. Studies in mice, frogs and zebrafish have revealed that similarities, as well as differences, exist between PCP in *Drosophila* and vertebrates.

Introduction

The genetic and molecular dissection of what is now referred to as planar cell polarity (PCP) began 25 years ago with the realization by Gubb and Garcia-Bellido (Gubb and Garcia-Bellido, 1982) that a small set of genes controls the polarity of cuticular hairs and bristles in *Drosophila*. Morphologists and embryologists had long appreciated the precise orientation of cuticular structures with respect to the body axes, but Gubb and Garcia-Bellido's work represented a conceptual departure in that it suggested the existence of a genetically defined system dedicated to coordinating these patterns. The genes that they studied are now known to be players in a complex system of developmental regulation that governs cell and tissue movements and patterns in both invertebrates and vertebrates. Although this phenomenon is now commonly referred to as PCP, Gubb and Garcia-Bellido's original and somewhat more general name 'tissue polarity' might ultimately prove more appropriate as its role is revealed in ever more diverse developmental processes.

As is often the case in developmental biology, the vertebrate PCP field owes a large debt to its *Drosophila* counterpart, which has served as the source for many of the components and concepts in this system. However, the numerous differences between vertebrates and invertebrates in anatomy, tissue types and morphogenetic processes, together with the existence of a number of distinct PCP components in vertebrates, have made the study of vertebrate PCP uniquely interesting. In this review, we highlight recent work on vertebrate PCP and discuss several developmental processes in which there is suggestive, but still incomplete, evidence for PCP signaling or for the activity of a subset of PCP components. We have not attempted to cover the PCP field in a comprehensive manner because many excellent and detailed reviews have recently been published in this area [reviews with an emphasis on *Drosophila* PCP (Adler, 2002; Strutt, 2002; Tree et al., 2002; Klein and Mlodzik, 2005; Strutt and Strutt, 2005); reviews on various aspects of vertebrate PCP (Wallingford et al., 2002; Barrow, 2006; Karner et al., 2006; Montcouquiol et al., 2006a); a review on the very different mechanisms of planar polarity in plants (Grebe, 2004)]. Instead, we have focused on those areas that we think are the most exciting and that address interesting unanswered questions. We hope that in the paragraphs that follow we can convey some of this excitement.

PCP in *Drosophila*

In *Drosophila*, the eye and wing have been the favored tissues for studying PCP phenotypes (Fig. 1), and the wing has also been used in most studies of PCP protein localization. In the compound eye, each ommatidium is precisely oriented in a nearly crystalline lattice. Moreover, each ommatidium exhibits one of two possible chiralities, as defined by the asymmetric packing of the eight photoreceptors. In the wild-type (WT) eye, ommatidia of differing chirality are segregated into two mirror image zones by a transverse equator (see Fig. 1A,B). In PCP mutants, the ommatidia are variably oriented and the spatial segregation of ommatidial chirality is lost, with the result that individual ommatidia of the 'wrong' chirality are found in each zone.

The surface of the wing is covered by a nearly crystalline epithelium of hexagonal cells, each of which elaborates a single distally-directed actin-filled protrusion (a wing hair). Both the orientation of the wing hairs and the hexagonal shape and regular packing of the wing epithelial cells are under PCP control (Classen et al., 2005). In the wing, mutations in PCP genes generally do not cause a complete randomization of hair orientation. Rather, hairs tend to be roughly aligned with their immediate neighbors (see Fig. 1D), leading to large-scale patterns in which many hundreds of hairs create whorls and waves that resemble the brushstrokes of an impressionist painting, inspiring the mutant names *Van Gogh* [*Vang*; also known as *Strabismus* or *Stbm* (Taylor et al., 1998; Wolff and Rubin, 1998)] and *starry night* [*stan*; also known as *flamingo* or *fmi* (Chae et al., 1999; Usui et al., 1999)]. As discussed more fully below, this propensity for local order among neighboring polar structures in the context of global disorder strongly suggests the existence of mechanistically distinct systems for controlling global and local orientation.

The *Drosophila* wing has also revealed an interesting feature referred to as 'domineering non-autonomy' in which WT epithelial cells adjacent to a clone of mutant cells exhibit a misoriented phenotype (Vinson and Adler, 1987). In many cases, the misorientation is only observed on one side of the mutant tissue. For example, in the wing, domineering non-autonomy caused by a clone of homozygous *frizzled* mutant cells generally affects only those WT cells that reside distal to the patch of mutant tissue. In the *Drosophila* abdominal epithelium, a wide variety of PCP gene over-expression and loss-of-function clones have been studied in the context of surrounding tissue that is either WT or one of various mutant backgrounds. In these studies, bristles and hairs within the surrounding tissue either turn toward or away from the clonal patch

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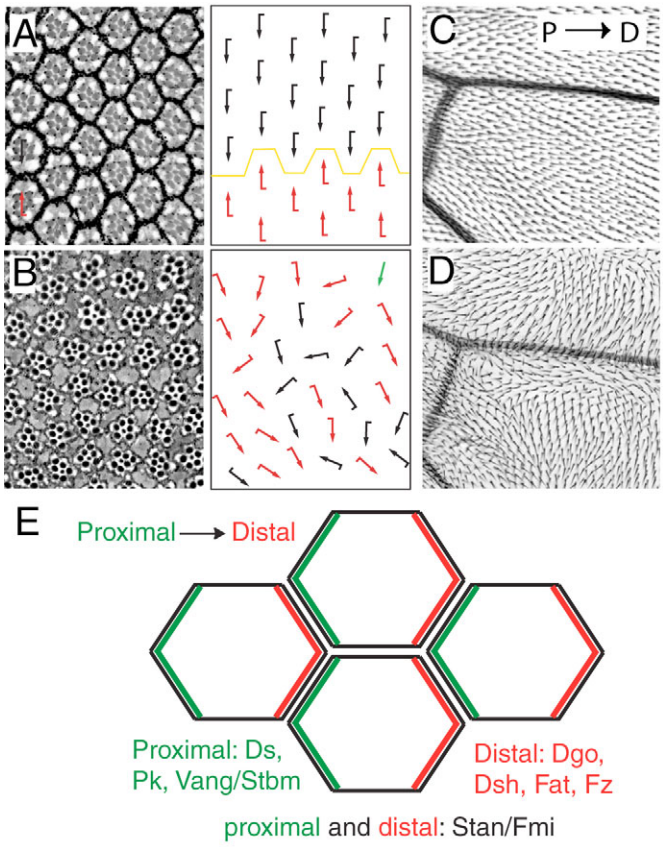

Fig. 1. PCP phenotype in the *Drosophila* eye and wing, and subcellular localization of PCP proteins in wing epithelial cells. (**A**) A WT eye. (**B**) *Vang* mutant eye. The left panels show the arrangement of individual photoreceptor rhabdomeres (the central grey or black circular structures within each ommatidium). The right panels are schematics of the chirality and orientation of each ommatidium (black or red arrows for the different chiralities; green for nonchiral) and of the equator separating the two territories of differing chirality. In the *Vang* mutant eye, the ommatidia show defects in both chirality and rotation. (**C**) WT wing showing the nearly parallel alignment of distally pointing wing hairs. (**D**) *Vang* mutant wing showing aberrant wing hair orientations globally, but substantial alignment locally. Arrow indicates proximal (P) and distal (D); anterior is up. (**E**) The subcellular localization of PCP proteins in the *Drosophila* wing epithelium (Adler, 2002; Strutt, 2002; Klein and Mlodzik, 2005; Strutt and Strutt, 2005). Four adjacent hexagonal wing epithelial cells are shown, with PCP protein accumulation at proximal or distal faces coded in red or green, respectively. A-D reproduced with permission from Jenny and Mlodzik (Jenny and Mlodzik, 2006). Dgo, Diego; Ds, Dachsous; Dsh, Dishevelled; Fz, Frizzled; Pk, Prickle; Vang/Stbm, Van Gogh/ Strabismus; Stan/Fmi, Starry night/Flamingo.

in a manner that is characteristic of the mutant genotype of each clonal patch and the anterior or posterior location of the surrounding tissue (Casal et al., 2006). This asymmetry is most likely to reflect the asymmetric propagation of a signaling molecule and/or the cooperative and asymmetric assembly of cell-surface signaling complexes.

There are roughly ten core PCP genes in *Drosophila*. The principal PCP signaling pathway appears to be the 'noncanonical' Wnt signaling pathway in which a cell-surface Frizzled (Fz) receptor recruits the adaptor protein Dishevelled (Dsh in *Drosophila*; Dvl in vertebrates) to activate, in a manner that is still poorly defined, a Jun kinase-Rac-Rho pathway that controls cytoskeletal dynamics (Strutt et al., 1997; Boutros et al., 1998; Wallingford and Habas, 2006). Genetic gain- and loss-of-function experiments support a model in which Fz-dependent PCP signaling also utilizes the heterotrimeric G-protein Galpha-O (Katanaev et al., 2005), a signaling pathway first identified in zebrafish (Slusarski et al., 1997). With respect to signaling between cells, at present the identities of the global signal that communicates the orientation of the body axes and the local signal that communicates polarity between neighboring cells remain uncertain. One attractive model posits that two atypical cadherins, Dachsous (Ds) and Fat (Ft), together with a transmembrane Golgi complex protein, Four-jointed (Fj), set up a global polarity signal, which is then sensed and propagated by the asymmetric assembly of cell-surface complexes composed of Fz, Vang, Dsh, Stan and an intracellular adaptor like protein, Diego (Dgo) (Fig. 1E) (Wong and Adler, 1993; Adler et al., 1997; Adler, 2002; Strutt, 2002; Yang et al., 2002; Ma et al., 2003; Uemura and Shimada, 2003; Lawrence et al., 2004; Venema et al., 2004; Strutt and Strutt, 2005; Klein and Mlodzik, 2005). However, recent genetic mosaic experiments in the *Drosophila* abdomen argue that these two systems may function in parallel rather than in series (Casal et al., 2006).

Ironically, both the loss of function and the over-expression of Wnts, the only known Fz ligands in *Drosophila*, have failed to implicate any Wnt in PCP signaling (Klein and Mlodzik, 2005; Casal et al., 2006). We note, however, that redundancy among Wnts might mask a defect associated with single-gene, loss-of-function mutations, a situation observed for *frizzled* and *frizzled2* in the context of embryonic patterning (Bhat, 1998; Kennerdell and Carthew, 1998; Bhanot et al., 1999; Chen and Struhl, 1999). The recent identification of a non-Wnt ligand (Norrin) for vertebrate Fz4 (Xu et al., 2004) suggests that the field should be open to the possibility that one or more non-Wnt Fz ligands might regulate PCP.

PCP processes and components in vertebrates

In vertebrates, the definition of what constitutes a PCP process is not entirely clear. One rough operational definition is that PCP is any process that affects cell polarity within an epithelial plane and involves one or more of the core PCP genes (as defined by the PCP phenotype of the *Drosophila* homolog). At present, the developmental processes that meet these criteria are convergent extension, neural tube closure, eyelid closure, hair bundle orientation in inner ear sensory cells, and hair follicle orientation in the skin (Figs 2 and 3). At the edge of this definition are some processes in both vertebrates and invertebrates that involve PCP genes in cell or tissue patterning but which do not involve epithelia. For example, the mutation of the core PCP gene *stan* in *Drosophila* leads to aberrant pathfinding by photoreceptor axons and defective dendritic morphologies in sensory neurons in the embryonic peripheral nervous system (Gao et al., 2000; Lee et al., 2003; Senti et al., 2003; Kimura et al., 2006), and RNAi knockdown of *Celsr2*, one of three vertebrate homologs of *stan*, in rat organotypic cerebellar and cortical slice cultures leads to loss of dendrites (Shima et al., 2004). Since these non-planar and non-epithelial processes are potentially revealing of how PCP components function, several of them are discussed below.

In addition to the core PCP proteins, there is a wider circle of proteins essential for PCP but not solely devoted to it. Included in this group are: proteins, such as Patj, that are involved in the localization of PCP proteins to the apical edge of one or both lateral faces of the cell (Djiane et al., 2005); proteins, such as inversin, that appear to control the balance between canonical and noncanonical Wnt signaling (Simons et al., 2005); and proteins, such as the c-Jun

N-terminal kinase (JNK) (Basket – Flybase) and the small GTPases RhoA (Rho 1– Flybase) and Rac1, that control cytoskeletal dynamics. The recent observation in both *Drosophila* and mammals that there are intracellular (most likely vesicular) pools of some PCP proteins – perhaps serving as a reservoir for the plasma membrane population – suggests that components of the vesicular transport machinery may also play a supporting role in PCP (Shimada et al., 2006; Wang et al., 2006b).

Table 1 lists vertebrate genes that play a role in PCP. This list includes *Vangl2*, *Dvl1*, *Dvl2*, *Celsr1*, *Fz3* and *Fz6*, each of which is homologous to a core PCP gene in *Drosophila*. Each of these genes, when mutated singly or in combination with one another, gives PCP-like phenotypes in vertebrates. The table also includes genes such as *Scribble* (*Scrb1*) [*Scribbled* (*Scrib*)] and *Ptk7*, for which there is no evidence from *Drosophila* regarding a role in PCP, but which generate compelling PCP phenotypes when mutated in vertebrates, both alone and in combination with other PCP genes (Murdoch et al., 2003; Lu et al., 2004). Also included are genes such as *inturned*

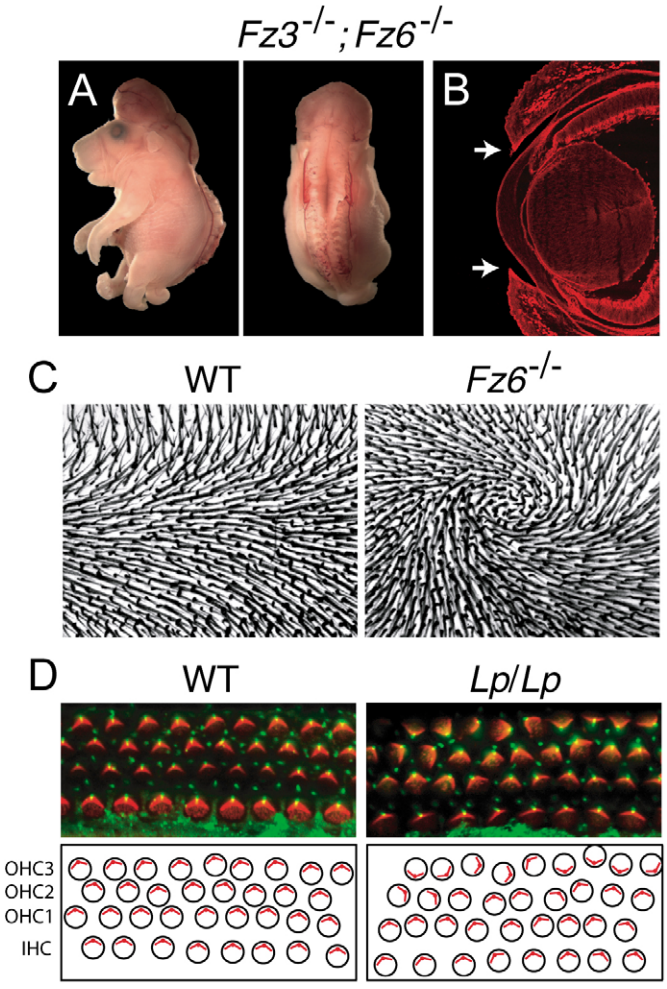

Fig. 2. Mouse planar cell polarity phenotypes. (**A**) Fully open neural tube (craniorrhachischisis) in a *Fz3*$^{-/-}$;*Fz6*$^{-/-}$ fetus at embryonic day (E) 18. (**B**) Open eyelids in a *Fz3*$^{-/-}$;*Fz6*$^{-/-}$ fetus at E18, shown by phalloidin staining of the anterior two-thirds of the eye; the edges of the eyelids are indicated by white arrows. (**C**) Hair follicle orientation defects on the dorsal surface of the *Fz6*$^{-/-}$ paw at postnatal day (P) 8. Proximal (left) and distal (right). The bases of the central digits are immediately beyond the right edge of each image. (**D**) Defects in inner ear sensory hair bundle orientation at E18 in a *Vangl2* mutant (*Lp/Lp*), organ of Corti flat mount. Top, actin-rich stereocilia are labeled with phalloidin (red) and kinocilia are labeled with anti-acetylated tubulin (green). Bottom, diagrams of hair bundle orientations for each image. IHC, inner hair cells; OHC1, inner row of outer hair cells; OHC2, central row of outer hair cells; OHC3, outer row of outer hair cells. Reproduced with permission from Wang et al. (Wang et al., 2006b).

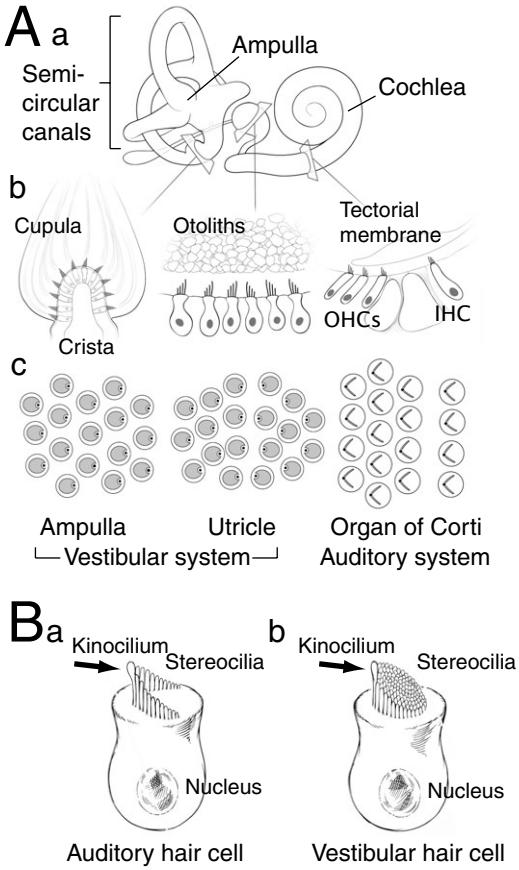

Fig. 3. The mammalian inner ear. (**A**) Location and architecture of sensory structures. (a) The structures of the bony labyrinth of the inner ear showing the locations of the cross-sectional views beneath. (b) Cross-sections through the main types of sensory epithelia, showing sensory hair cells. (c) Face-on views of these sensory epithelia, showing the apical face of the sensory hair cells and the arrangement of hair bundles. Left to right: crista (the sensory epithelium in the ampulla of each semicircular canal), utricle, and organ of Corti (the sensory structure in the cochlea). The saccule (not shown) closely resembles the utricle, except that its hair bundles face away from each other rather than towards each other across the equator (Denman-Johnson and Forge, 1999). In the ampulla of the semicircular canals, the tips of the sensory hair bundles on the apical face of each hair cell insert into a gelatinous structure called the cupula; in the utricle and saccule, sensory hair bundles insert into a gelatinous structure filled with calcium carbonate crystals, the otoliths; and in the organ of Corti, sensory hair bundles insert into the overlying tectorial membrane. Organ of Corti hair cells are arranged in four rows: one of inner (IHC) and three of outer (OHC) hair cells. In c, each kinocilium (black circle) lies adjacent to a group of stereocila (structures filled with actin bundles). In this view, the stereocilia form a V shape on the apical face of hair cells in the organ of Corti and a disc in the utricle, saccule and cristae. (**B**) Schematic of individual hair cells from the auditory system (a) and vestibular system (b). The apical face is at the top and the single kinocilium at the left edge. Panel A is reproduced with permission from Wang et al. (Wang et al., 2006b).

Table 1. PCP genes and PCP-related processes in vertebrate development

Vertebrate gene	Auditory, vestibular hair cell orientation	Neural tube closure	Convergent extension	Eyelid closure	Hair patterning	Axon growth, guidance	Hindbrain neuron migration	Dendritic arborization
Bbs1†	D	–	–	–	–	–	–	–
Bbs4†	D	D	(D)	–	–	–	–	–
Bbs6†	D	–	(D)	–	–	–	–	–
Celsr1	D	D	–	D	–	–	(D)	–
Celsr2	–	–	–	–	–	–	D	D
Celsr3	–	–	–	–	–	D	–	–
Dvl1	D*	D*	D*	–	–	–	–	D
Dvl2	D*	D*	D*	–	–	–	–	–
Dvl3	–	D*	–	–	–	–	–	–
Fuzzy†	–	–	D	–	–	–	–	–
Fz3	D*	D*	D*	(D*)	–	D	D	–
Fz6	D*	D*	D*	(D*)	D	–	–	–
Inturned†	–	–	D	–	–	–	–	–
Inversin	–	–	D	–	D	–	–	–
Prickle-1	–	–	D	–	–	–	D	–
Ptk7	D	D	–	D	–	–	–	–
Scribble	D	D	D	–	–	–	D	–
Vangl2	D	D	D	D	–	–	D	–
Widerborst	–	–	D	–	–	–	–	–

†Implicated in ciliary function.

Phenotypes associated with PCP genes in vertebrates: D, defective; (D), defective, but either low penetrance or observed as an increase in the severity of the phenotype of another PCP mutation; D*, defective only in a double mutant combination (i.e. redundancy between gene family members); –, not reported as abnormal. For some of these genes, one or more of the listed characteristics may not have been examined or examined in sufficient detail to determine whether it is normal; thus, a – entry should be interpreted cautiously.

References: *Bbs1*, *Bbs4*, *Bbs6* (Ross et al., 2005); *Celsr1* (Curtin et al., 2003); *Celsr2* (Shima et al., 2004; Kimura et al., 2006; Wada et al., 2006); *Celsr3* (Tisser et al., 2005; Price et al., 2006); *Dvl1*, *Dvl2*, *Dvl3* (Hamblet et al., 2002; Rosso et al., 2005; Wang et al., 2005; Wang, J. et al., 2006); *Fuzzy* (Park et al., 2006); *Fz3* (Wang et al., 2002; Wang et al., 2006a; Wang et al., 2006b; Wada et al., 2006); *Fz6* (Guo et al., 2004; Wang et al., 2006b; Wang et al., 2006c); *Inturned* (Park et al., 2006); *Inversin* (Otto et al., 2003; Simons et al., 2005); *Prickle-1* (Carreira-Barbosa et al., 2003; Veeman et al., 2003); *Ptk7* (Lu et al., 2004); *Scribble* (Murdoch et al., 2003; Montcouquiol et al., 2003; Wada et al., 2005); *Vangl2* (Greene et al., 1998; Kibar et al., 2001; Murdoch et al., 2001; Bingham et al., 2002; Jessen et al., 2002; Montcouquiol et al., 2003; Torban et al., 2004); *Widerborst* (Hannus et al., 2002).

(*in*) and *fuzzy* (*fy*), which are considered to be PCP effector genes in *Drosophila* and which have been found, by inhibition or over-expression studies in frogs or fish embryos, to give a convergent extension phenotype (Park et al., 2006). Other, miscellaneous genes are included that either by homology or function are likely to play a role in PCP or PCP-like processes.

Oriented cell movements and cell divisions

Neurulation and its associated tissue movements are among the most ancient of vertebrate embryological processes, and they have fascinated embryologists for over a century (Wilson, 1925). In frogs and fish, the process begins with an elongation and narrowing of the embryo, referred to as convergent extension (CE). The elongated neural plate develops a central groove, and the dorsal margins of the two walls of this U-shaped structure ultimately fuse to create the neural tube. In humans, a failure to fuse the neural tube in its entirety occurs at a frequency of 1 in 1000 live births, making it one of the most common congenital defects (Copp et al., 2003).

CE reflects the medial migration and intercalation of mesodermal cells, movements that are directed by lamellipodia on the medial and lateral faces of these cells and by the organized deposition of extracellular matrix (ECM) fibrils, in particular fibronectin (Keller et al., 2000; Wallingford et al., 2002; Goto et al., 2005). CE can be disrupted in zebrafish by mutation of *vangl2* (*trilobite*) (Jessen et al., 2002), *prickle* (Veeman et al., 2003), or *wnt11* (*silberblick*) (Heisenberg et al., 2000) and in *Xenopus* by interfering with any of several PCP proteins, for example by overexpressing a dominant-negative Dishevelled variant that lacks either the DEP or PDZ domains, which in *Drosophila* Dsh are essential for PCP (Wallingford et al., 2000; Wallingford et al., 2002; Wallingford and Habas, 2006). In mice, loss of *Vangl2* (Greene et al., 1998; Kibar et al., 2001; Murdoch et al., 2001), *Celsr1* (Curtin et al., 2003), or *Ptk7* (Lu et al., 2004), or the simultaneous loss of two of the three Dishevelled homologs (*Dvl1* and *Dvl2*) (Hamblet et al., 2002; Wang, J. et al., 2006), or of both *Fz3* and *Fz6* (*Fzd3* and *Fzd6* – Mouse Genome Informatics) (Wang et al., 2006b), all lead to a completely open neural tube and a shortened embryo. It is interesting that many PCP mutants also show an eyelid closure defect (Fig. 2B). Eyelid closure normally occurs at about E16 in the mouse and, like neural tube closure, involves a medial convergence of a pair of flanking epithelial sheets.

A narrowing and lengthening analogous to CE also occurs during development of the organ of Corti in the mammalian cochlea (see Fig. 3A), and this shape change fails to occur in *Vangl2* mutants, *Dvl1;Dvl2* double-mutants, and in *Fz3;Fz6* double-mutants, which all also have neural tube closure defects (Montcouquiol et al., 2003; Wang et al., 2005; Wang et al., 2006b). In the organ of Corti, CE-like movements occur after sensorineural precursors have exited mitosis, indicating that this process does not involve oriented cell division (Wang, J. et al., 2006).

One of the earliest descriptions of the phenotypic consequence of genetic disruption of Wnt-Fz signaling came from studies of the polarity of cleavage planes during early cell divisions in *C. elegans*. Mutations in *mom-2* (a Wnt gene) or *mom-5* (a Fz gene) misorient mitotic spindles in several blastomeres (Rocheleau et al., 1997; Thorpe et al., 1997). Although the evolutionary divergence of Wnt-Fz signaling makes it difficult to establish a clear one-to-one correlation between vertebrate and *C. elegans* signaling pathways, it seems likely that the pathway defined by the *mom* genes includes some elements of PCP signaling (Park et al., 2004). Gong et al. (Gong et al., 2004) have extended this work to vertebrates by imaging zebrafish that ubiquitously express histone H2B-GFP. In normal gastrulating zebrafish embryos, dorsal epiblast cells in all

layers tend to align their cell divisions along the animal-vegetal axis, and this alignment depends on PCP signaling as it is disrupted by the expression of dominant-negative Dvl proteins or by injection of morpholino oligonucleotides that block the synthesis of Vangl2. These observations suggest that, in some contexts, tissue movements and tissue growth may be driven by oriented cell divisions orchestrated by PCP.

New insights into the control of oriented cell division have recently come from the study of mitotic spindle orientation in developing renal tubules (Fischer et al., 2006). It has been hypothesized that developing tubule elongation requires the preferential displacement of pairs of daughter cells along the axis of the tubule, and that cyst formation arises from an excess of transverse, rather than of longitudinal, orientations of pairs of daughter cells (Germino, 2005). Fischer et al. (Fischer et al., 2006) examined individual tubules from the developing kidney in WT controls and in rat and mouse models of cystic kidney disease [polycystic kidney disease (*Pkd*) mutant rats and hepatocyte nuclear factor-1 beta (*Hnf-1*; *Tcf2* – Mouse Genome Informatics) -deficient mice] and found clear evidence supporting both hypotheses. The extent to which PCP signaling plays a role in this process remains to be determined.

In contrast to the observations of Gong et al. (Gong et al., 2004) on zebrafish gastrulation, Ciruna et al. (Ciruna et al., 2006) have shown that coordinating polarized cell division is not the principal function of PCP in zebrafish neural tube closure. Instead, PCP is required for the reintegration of newly postmitotic cells into the neuroepithelium from which they had been transiently extruded. Ciruna et al. observed that loss of *Vangl2* (*trilobite*) leads to an accumulation of apical daughter cells from recent mitoses in the center of the U-shaped, and incompletely closed, neural fold. A striking demonstration that the failure to reintegrate these cells underlies the neural tube closure defect came from the observation that pharmacologically blocking cell division in the *trilobite* mutant late in gastrulation restores neural tube closure, presumably because without cell division there are no extruded cells. By contrast, mitotic inhibitors did not rescue the CE phenotype also caused by the *trilobite* mutation.

The clearest example of PCP control of oriented cell movement is found in the zebrafish hindbrain where facial motor neurons (which form the seventh cranial nerve, nVII) migrate caudally from their birthplace in rhombomere four to rhombomere six (Chandrasekhar et al., 1997). This migration can be visualized in zebrafish that express an *islet1-GFP* transgene, which is expressed selectively in this subpopulation of hindbrain neurons (Higashijima et al., 2000). nVII motor neuron migration is impaired or abolished by mutations in the zebrafish genes *vangl2* (*trilobite*), *prickle1* (also known as *pk1*), *scribble1/landlocked* (*scrb1/llk*), *frizzled3a/off-limits* (*fz3a/olt*; one of two *fz3* homologs in zebrafish), and *celsr2/off-road* [*ord*; one of four *stan* homologs in zebrafish (Bingham et al., 2002; Jessen et al., 2002; Carreira-Barbosa et al., 2003; Wada et al., 2005; Wada et al., 2006)]. The *fz3a* and *celsr2* genes were identified in chemical mutagenesis screens for impaired nVII motor neuron migration (Wada et al., 2006). Genetic mosaic experiments show that each of these genes promotes migration by mechanisms that include cell-nonautonomous components (Jessen et al., 2002; Wada et al., 2005; Wada et al., 2006). The principal role of the PCP system in promoting the caudal trajectory of the nVII motor neurons appears to be to maintain these cells at the pial surface. Loss of PCP gene function leads to the intercalation of the nVII motor neurons into the underlying neuroepithelium with a concomitant switch from caudal to radial migration.

Inner ear development

The vertebrate inner ear is an architectural tour-de-force in which bone, vasculature, fluid-filled chambers, supporting cells, sensory neurons, specialized extracellular deposits, and axons are all arranged with extraordinary precision (Fig. 3A). Three types of sensory epithelia exist in the inner ear: the organ of Corti, which detects airborne vibrations (i.e. sound) following its conversion to a shearing motion of the structures within the central cavity of the cochlea; the utricle and saccule, which detect linear acceleration by sensing the inertial displacement of extracellular calcium-carbonate crystals (otoliths); and the cristae, which detect angular acceleration by sensing the inertial displacement of fluid in three microscopic gyroscopes called semicircular canals. Given the complexities of the inner ear, it is perhaps not surprising that most of the principal developmental signaling systems known in vertebrates have been shown to play a role in its development, including the retinoic acid, Hedgehog, Notch, Neurotrophin, BMP, Wnt and FGF systems (Gao, 2003; Kelley, 2003; Wright and Mansour, 2003; Barald and Kelley, 2004; Fritzsch et al., 2004; Romand et al., 2006).

The structural precision of the inner ear is reiterated subcellularly. In particular, each primary sensory neuron, the hair cell, elaborates on its apical face a set of actin-filled stereocilia adjacent to a single true cilium, the kinocilium (Fig. 3B). This mechanosensory structure, the sensory hair bundle, is precisely oriented with respect to the plane of the epithelium. Hair bundle orientation confers a directional selectivity on the mechanical response of the cell: hair bundle deflection toward the kinocilium opens plasma membrane cation channels; deflection away from the kinocilium closes the channels; and deflections to either side have no effect. When viewed face on, the stereocilia of cochlear hair cells are arranged in the shape of a chevron, with the kinocilium located at the apex of the V (Fig. 3). The stereocilia of vestibular hair cells (i.e. those in the utricule, saccule and cristae) are arranged in a dense cluster, with the kinocilium at one side of the cluster. In all sensory hair bundles, the stereocilia vary in length and are arranged in a precise step-wise manner with the longest stereocilia closest to the kinocilium and the shortest stereocilia furthest from the kinocilium (see Fig. 3B).

Nine years ago, Eaton proposed that PCP signaling orients stereociliary hair bundles within the plane of inner ear sensory epithelia (Eaton, 1997). Six years later, Curtin et al. (Curtin et al., 2003) and Montcouquiol et al. (Montcouquiol et al., 2003) simultaneously reported that mutations in *Vangl2* [in the *Looptail* (*Lp*) mouse mutant], *Scribble* [in the *Circletail* (*Crc*) mouse mutant], and *Celsr1* (in the *spin cycle* and *crash* mouse mutants) cause precisely this phenotype in the organ of Corti. Hair bundle orientation defects in the organ of Corti have since been described in *Ptk7* knockout and *Dvl1;Dvl2* and *Fz3;Fz6* (*Fzd6* – Mouse Genome Informatics) double-knockout mice (Lu et al., 2004; Wang et al., 2005; Wang et al., 2006b). Interestingly, the severity of defects and the subsets of hair cells affected vary between mutants, and may also depend on the genetic background. As an example of the latter, in one study, *Vangl2* homozygotes were reported to have the single row of inner hair cells as well as the outermost two of the three rows of outer hair cells severely misorientated (Montcouquiol et al., 2003), whereas the same *Vangl2* allele was reported in another study to cause severe misorientation defects in the outermost row of outer hair cells and milder defects in all other rows of hair cells (Wang et al., 2006b). In contrast to both of these patterns, *Fz3;Fz6* double-mutants have severe orientation defects of the inner hair cells with only mild outer hair cell defects. Some of these differences may reflect the participation of additional and partially redundant family members: as seen in Table 1, many mammalian PCP genes are

members of small, highly homologous gene families. Consistent with this idea, *Fz3* and *Fz6* appear to be completely redundant in inner ear development and are largely redundant in neural tube closure (Wang et al., 2006b). In the first analyses of PCP in the vestibular system, loss of *Vangl2* was found to randomize hair bundle orientation in both the utricle and cristae (Montcouquiol et al., 2006b; Wang, et al., 2006b).

Aside from its importance in the context of hearing and balance, the inner ear sensory epithelium offers a powerful system for studying vertebrate PCP. At present, it is the only place where a mammalian PCP phenotype can be quantitatively scored at single-cell resolution. Moreover, the developing sensory epithelium from the organ of Corti can be cultured in vitro for at least one week, during which time hair bundles refine their orientations (Dabdoub et al., 2003). In this explant system, the application of Wnt7a or of soluble Wnt-binding proteins leads to misoriented hair bundles, implicating Wnt ligands in the orientation process. As described below, the inner ear has also provided a useful system for determining the subcellular localization of vertebrate PCP proteins and the effect of PCP gene mutation on protein localization.

The growth and guidance of axons and dendrites

The inclusion of a section on axon guidance and dendritic patterning in this review is not necessarily meant to imply that neurons and epithelial cells share the same PCP mechanisms. However, the discovery that PCP components function in the context of both epithelial and neuronal patterning suggests that at a molecular level these processes are at least partially related.

In mammals, one of the most dramatic axon growth and guidance phenotypes identified to date is seen in mice that lack either *Fz3* or *Celsr3*, which are homologs of core PCP genes in *Drosophila*. Loss of either of these genes eliminates the major axon tracts that connect the thalamus and cortex, and causes spinal cord sensory axons to stall rather than turn rostrally after midline crossing (Fig. 4) (Wang et al., 2002; Wang et al., 2006a; Lyuksyutova et al., 2003; Tissir et al., 2005; Price et al., 2006; Bovolenta et al., 2006). In both mutants, neuronal proliferation and migration in the forebrain appear to be unaffected. In the $Fz3^{-/-}$ cortex, an analysis of cell morphologies using genetically-directed cell labeling with alkaline phosphatase or YFP shows that projection neurons send their axons into the intermediate zone, the cortical layer in which corticothalamic axon bundles would normally form, but these axons fail to extend and they eventually degenerate (Wang et al., 2006a). Preliminary data point to a similar outcome for $Celsr3^{-/-}$ cortical axons (Price et al., 2006). By contrast, in both mutants thalamic axons extend and fasciculate but fail to exit the thalamus. The near identity of the phenotypes observed in these two mutants, and the known interactions between their *Drosophila* homologs in the context of PCP, argues strongly that they function together in a common axon guidance pathway.

In a search for factors that promote rostral turning of commissural axons, Lyuksyutova et al. (Lyuksyutova et al., 2003) observed that rostral turning in embryonic spinal cord explants is lost if the explants are cut into narrow transverse strips, suggesting that an orienting factor was being lost from the explant by longitudinal diffusion. In testing a series of candidate factors produced by COS cell aggregates cultured adjacent to the spinal cord explant, Lyuksyutva et al. observed that several Wnts stimulate rostral turning. Intriguingly, transcripts encoding Wnt4, a Wnt that promotes rostral turning, show a rostral-caudal gradient of abundance along the spinal cord at midgestation at the time when growing commissural axons cross the midline. Although these data, together with the $Fz3^{-/-}$ rostral-turning defect, suggest that Wnt4

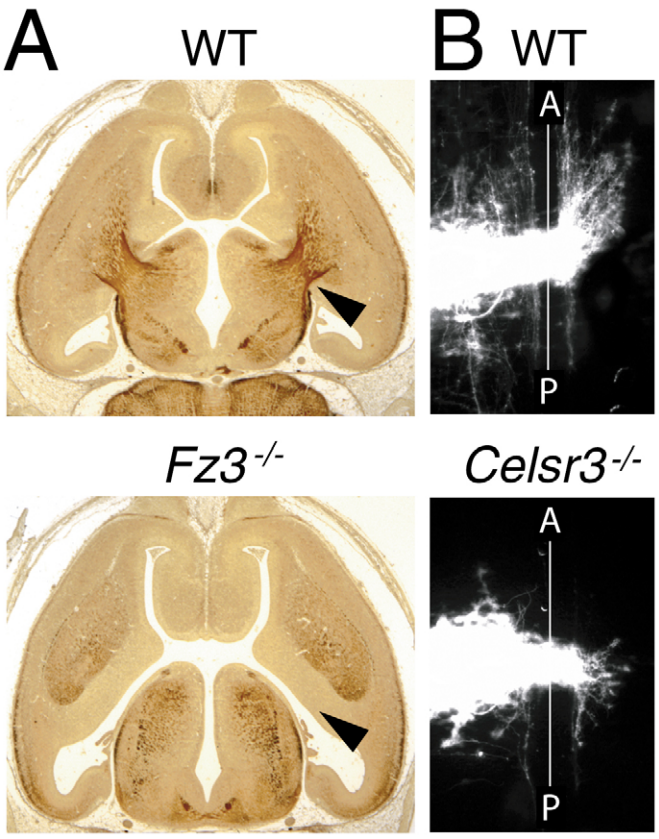

Fig. 4. Axon growth and guidance defects in the developing $Fz3^{-/-}$ and $Celsr3^{-/-}$ central nervous system. (**A**) Anti-neurofilament staining of horizontal sections through E15 WT and $Fz3^{-/-}$ mouse brains shows a complete absence of thalamocortical and corticothalamic fiber bundles in an otherwise normal-appearing $Fz3^{-/-}$ forebrain; the normal location of these fibers is indicated by an arrowhead. (**B**) DiI tracing of commissural axons in E11 WT and $Celsr3^{-/-}$ mouse spinal cords shows a failure of rostral turning by $Celsr^{-/-}$ axons after midline crossing. The spinal cord has been opened at the dorsal midline and flattened (an 'open book' preparation). DiI was placed in commissural cell bodies beyond the left edge of each image. The vertical white line indicates the midline. A, Anterior/rostral; P, posterior/caudal. Panel A is reproduced with permission from Wang et al. (Wang et al., 2002) and panel B is courtesy of Drs Libing Zhou and Andre Goffinet.

promotes growth cone turning by activating Fz3, there is as yet no direct biochemical evidence that these two proteins interact. However, independent support for this general mechanism of axon guidance has come from recent genetic studies in *C. elegans*: EGL-20 (a Wnt) appears to repel axon growth along the anterior-posterior axis via its interaction with MIG-1 (a Fz) (Pan et al., 2006).

As noted above, the growth and maintenance of dendrites in both the *Drosophila* and mammalian nervous systems involve Stan or its mammalian homolog Celsr2, respectively (Gao et al., 2000; Shima et al., 2004; Kimura et al., 2006). In the WT *Drosophila* embryo, when dendrites of dendritic arborization (da) neurons reach the dorsal midline, they avoid growing into regions that are occupied by arbors from contralateral da neurons, thereby efficiently tiling the surface of the embryo. In *stan* mutants, this dendritic growth inhibition is lost. When expressed in cultured cells, Stan mediates homophilic clustering and, in the *Drosophila* wing, localizes to both the proximal and distal faces of epithelial cells. It is tempting to

speculate that homo- or heterophilic adhesion complexes that contain Stan may signal directly or may localize signaling components in the context of both PCP and dendrite development. Also consistent with a role for PCP-like signaling in dendritic development, cultured hippocampal neurons from *Dvl1*$^{-/-}$ mice show a decrease in dendritic growth and arborization as compared with their WT counterparts, and the increase in growth and arborization that is produced by the transfection of Dvl into WT neurons is insensitive to the cotransfection of the canonical Wnt pathway components glycogen synthase kinase-3 (Gsk-3; Gsk3a – Mouse Genome Informatics) and β-catenin, or to the pharmacological inhibition of Gsk-3 by treatment with lithium chloride or 6-bromoindirubin-3′-oxime (Rosso et al., 2005).

Hair follicle orientation and hair patterning: separate global and local control systems

The mammalian PCP phenomenon that most closely resembles the oriented patterning of hairs and bristles on the *Drosophila* cuticle is the regular and locally parallel arrangement of hairs over the body surface. Hair follicles make an acute angle with the skin, and therefore each follicle and its associated hair has a defined orientation with respect to the body's axes. A principal difference between mammalian hair follicles and *Drosophila* hairs and bristles is one of scale: in *Drosophila*, each wing epithelial cell makes a single actin-rich protrusion (the hair), whereas each mammalian hair follicle is composed of hundreds of cells and is separated from neighboring follicles by tens of cell diameters. In general, the orientation of each follicle closely matches the average orientation of its neighbors. This regular arrangement is defective in *Fz6*$^{-/-}$ mice in the same distinctive manner in which bristle and wing hair orientations are defective in *Drosophila* PCP mutants: the pattern is globally disorganized but locally ordered, giving rise to waves, whorls and tufts, each comprising dozens to hundreds of elements (Figs 1 and 2) (Guo et al., 2004).

A recent analysis of WT and *Fz6*$^{-/-}$ skin during late embryonic and early postnatal development shows that in *Fz6*$^{-/-}$ mutants, hair patterns arise by a process of self-organization from a field of initially misoriented or randomly oriented hair follicles (Fig. 5) (Wang et al., 2006c). This analysis also shows that mammalian hair follicles, despite their large size, possess an unexpected plasticity, reorienting in response to surrounding cues in a matter of days. The data indicate that Fz6 normally functions early in development to set the global orientation of hair follicles with respect to the body axes. The subsequent reorientation is orchestrated by a Fz6-independent system that aligns neighboring follicles. Thus, there appear to be two distinct orienting systems, one that acts early in development and globally, and a second that acts later and locally.

The existence of a local refinement mechanism permits the global signal to produce no more than a rough alignment of immature follicles. In WT mice, the local mechanism efficiently refines initially imperfect follicle orientations to produce orientations that are almost perfectly parallel. As noted above, two-stage models have

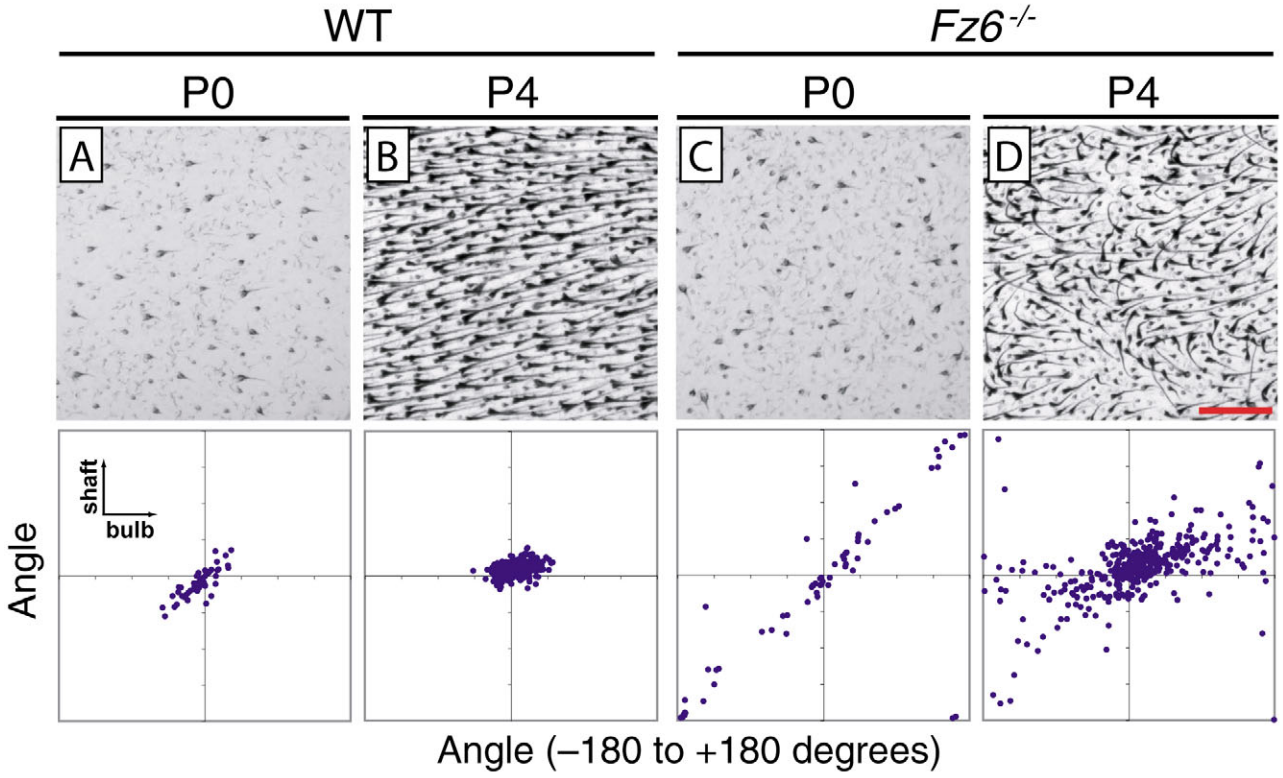

Fig. 5. Hair follicle orientations on the back of WT and *Fz6*$^{-/-}$ mice at P0 and P4. (**A-D**) Posterior is to the right. The scatter plots show the angles of each hair follicle shaft (the region closest to the skin surface) and base (the region furthest from the skin surface) with respect to the anterior-posterior axis (set at zero degrees and corresponding to the central point along each axis). (A,B) WT follicles at P0 have orientations that range from ~ –45 to +45 degrees, a distribution that narrows by P4. (C,D) *Fz6*$^{-/-}$ follicles at P0 have random orientations, which by P4 have partially reoriented to produce spatial domains with imperfect local order. Additional reorientation continues over the next several days (see Fig. 2C for follicle orientation at P8). In both WT and *Fz6*$^{-/-}$ skin, the distribution of shaft angles tightens more than that of bulb angles. Scale bar, 400 μm. Reproduced with permission from Wang et al. (Wang et al., 2006c).

also been proposed in the context of PCP signaling in *Drosophila*, although it is not clear whether the Fz-dependent and Fz-independent processes observed in *Drosophila* are analogous to the ones defined by the *Fz6*$^{-/-}$ hair follicle phenotype. Indeed, the general idea that a Ds, Ft and Fj system acts upstream to set up a global orientation, and a Fz, Vang, Dsh and Stan system acts downstream to refine that orientation, would appear to be at odds with the *Fz6*$^{-/-}$ hair patterning phenotype. Two-stage mechanisms may also exist in the context of other PCP processes in vertebrates. For example, the progressive refinement of sensory hair bundle orientations within the inner ear, a phenomenon observed in both mammals and birds (Cotanche and Corwin, 1991; Dabdoub et al., 2003), is consistent with a two-stage process.

As first noted by Lewis and Davies (Lewis and Davies, 2002), PCP patterning bears a strong conceptual resemblance to the patterning of electron spins in a ferromagnet. As in PCP, there are both local and global effects: local quantum mechanical interactions favor the alignment of adjacent electron spins, and a global signal, in the form of the external magnetic field, biases the alignment probabilities for all of the individual spins. Both ferromagnetism and the progressive refinement of hair follicle orientations can be modeled with a two-dimensional lattice of uniformly spaced vectors and a local consensus 'rule' (Fig. 6) (Wang et al., 2006c). The rule is applied iteratively, and, with each iteration, it subtly biases each vector's orientation in favor of the most recent average of its neighbors' orientations. This simple lattice model captures the key attributes of hair follicle patterning, including the growth by accretion of hair patterns during development, the spread of oriented WT patterns into adjacent *Fz6*$^{-/-}$ skin (in WT:*Fz6*$^{-/-}$ chimeras), and the ability of a small initial bias in vector orientation to rapidly dominate an otherwise randomly oriented population of follicles.

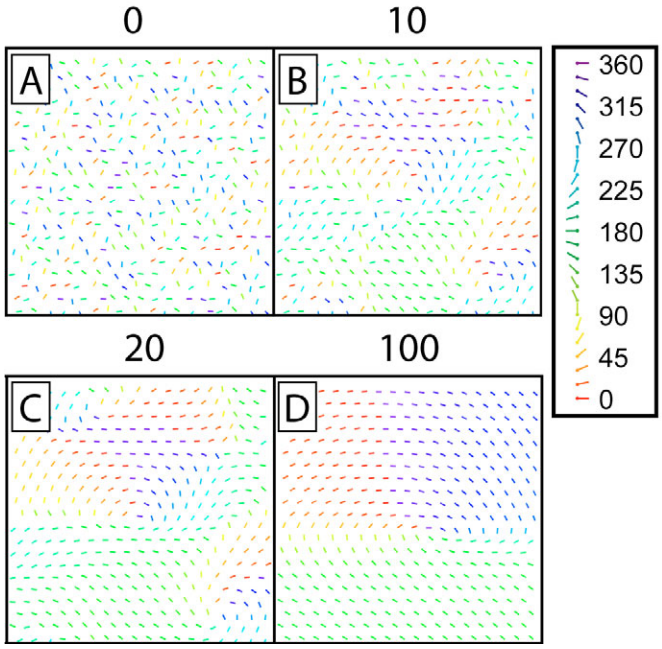

Fig. 6. A two-dimensional lattice model of *Fz6*$^{-/-}$ hair follicle orientation development. Each hair follicle is represented by a unit-length vector placed at one of the vertices of a lattice of equilateral triangles. After the initial vector orientations are set, the lattice develops by repeated application of an updating rule, which specifies that each vector's orientation becomes modified by adding to it the vector sum of its 18 closest neighbors after that sum has been scaled to 2% of its magnitude. This number of neighbors corresponds to two concentric circles of surrounding lattice points. The starting configuration (0) is a set of randomly oriented vectors, and the lattice is shown after 10, 20 and 100 iterations of the local consensus algorithm. Each vector orientation is represented by the angle and color of the corresponding bar, as shown in the key to the right. Reproduced with permission from Wang et al. (Wang et al., 2006c).

PCP and cilia

A fascinating, but still poorly understood, connection has recently emerged between PCP and nonmotile cila based on the observation that several genes that affect vertebrate PCP also affect ciliary structure and/or function (Bisgrove and Yost, 2006; Davis et al., 2006; Singla and Reiter, 2006). In vertebrates, many, if not all, epithelial cells possess a single nonmotile cilium (the primary cilium), which is typically located in the center of the apical face of the cell. By contrast, in *Drosophila* and *C. elegans*, nonmotile cilia have thus far only been found on subsets of neurons. One link between PCP and cilia has come from the study of Bardet-Beidl syndrome (BBS), a genetically heterogeneous human disorder with pleiotropic manifestations including obesity, polydactyly, endocrine dysfunction, cystic renal disease, progressive photoreceptor degeneration and hearing loss (Bisgrove and Yost, 2006; Davis et al., 2006). Bbs genes are structurally diverse but many share the common feature that the encoded proteins localize to the cilium or its cellular anchor, the basal body (Ansley et al., 2003). Targeted disruption of *Bbs1*, *Bbs4* or *Bbs6* (*Mkks* – Mouse Genome Informatics) in mice leads to misorientation of inner ear sensory hair bundles (Ross et al., 2005), and 14% of *Bbs4*$^{-/-}$ mice display an open cephalic neural tube (exencephaly). Moreover, both *Bbs1* and *Bbs6* alleles interact genetically with *Vangl2*, and morpholino oligonucleotide knockdown of *Bbs4* in zebrafish leads to PCP phenotypes, including a failure of embryonic CE. We note that some, and perhaps most, Bbs proteins may also function in the wider context of cytoskeletal regulation – as indicated, for example, by defects in melanosome transport that occur when Bbs genes are knocked down in zebrafish (Yen et al., 2006) – and therefore their effects on PCP may extend beyond their roles in ciliary structure.

A second link between PCP and cilia has come from the identification of the mouse *inversin* (*Invs*) gene, which encodes a large adaptor-like protein with homology to the *Drosophila* PCP protein Diego. *Invs* was discovered as the serendipitous target of a transgene insertion event that produced a *situs inversus* phenotype, apparently the result of a ciliary defect in the embryonic node (Mochizuki et al., 1998; Morgan et al., 1998; Okada et al., 1999). *Invs* mutations also cause cystic renal tubules and progressive renal failure in both mice and humans (nephronophthisis type 2, NPHP2) (Otto et al., 2003; Bisgrove and Yost, 2006). The subcellular localization of inversin is complex and dynamic, but includes the basal bodies, primary cilia and, during metaphase and anaphase, the spindle poles (Morgan et al., 2002; Watanabe et al., 2003; Eley et al., 2004; Nurnberger, 2004). In transfected cells, inversin binds Dvl and accelerates its degradation, and in *Xenopus* embryos it is required for CE (Simons et al., 2005). The data suggest that inversin controls the balance between canonical and noncanonical Wnt signaling, with higher inversin activity favoring noncanonical (i.e. PCP) signaling and lower inversin activity favoring canonical signaling and, with it, misregulated tubule growth and cyst formation (Germino, 2005).

The most recent link between PCP and cilia comes from experiments with *Xenopus* embryos in which homologs of the *Drosophila* PCP genes *fuzzy* and *inturned* were unexpectedly found

to be required for Hedgehog signaling (Park et al., 2006). The earlier discovery, in an unbiased chemical mutagenesis screen in the mouse, that loss of various intraflagellar (i.e. ciliary) transport proteins (IFTs) impairs Hedgehog signaling (reviewed by Huangfu and Anderson, 2006; Huangfu and Anderson, 2005) suggests that Inturned and Fuzzy play a role in ciliary structure or function. Consistent with this hypothesis, cilia in *Inturned* and *Fuzzy* morphants in *Xenopus* are short and often misshapen, and the underlying actin skeleton is of abnormally low density (Park et al., 2006).

The evidence summarized in the preceding paragraphs indicates that cilia or cytoskeletal structures that affect cilia play an important role in PCP. At present, the mechanistic basis of this connection remains obscure.

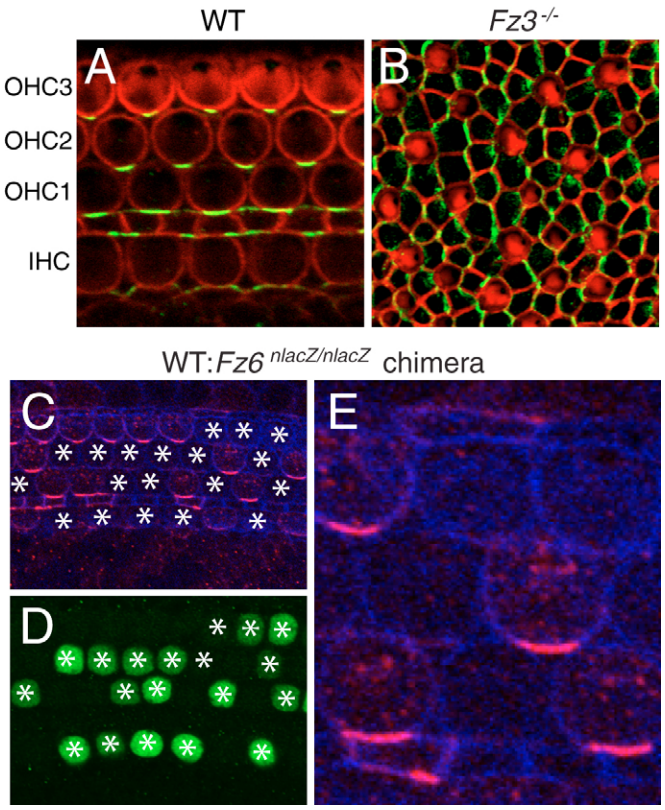

Fig. 7. Fz3 and Fz6 localization in mouse inner ear sensory epithelium. (**A**) Immunolocalization of Fz3 (green) in the organ of Corti at P0. Actin bundles are stained with phalloidin (red). IHC, inner hair cells; OHC1, inner row of outer HCs; OHC2, central row of outer HCs; OHC3, outer row of OHCs. (**B**) In the vestibular system, Fz6 (green) is expressed in supporting cells and sensory neurons. In the *Fz3*$^{-/-}$ crista, sensory hair bundle kinocilia orient to the right, as indicated by the hole on the actin-rich cuticular plate (phalloidin, red), which covers much of the apical face of the sensory neurons. Fz6 localizes preferentially on lateral faces that orient perpendicular to the axis of the sensory hair bundles. (**C-E**) WT:*Fz6*$^{-/-}$ mouse embryo chimeras and Fz6 protein localization. (C,D) Organ of Corti from a WT:*Fz6*$^{-/-}$ embryo chimera immunostained with anti-Fz6 (red) and anti-β-galactosidase (green) to visualize nuclear β-galactosidase encoded by *Fz6*$^{-/-}$ (*lacZ* knock-in) cells (asterisks). Phalloidin (blue) stains actin bundles. β-galactosidase expression varies among *Fz6*$^{-/-}$ cells. The nuclei (D) are present in a deeper plane than the apical edge of the cells, where Fz6 accumulates (A). (E) An enlargement of the right corner of C, showing Fz6 punctate intracellular accumulation only in WT cells. Reproduced with permission from Wang et al. (Wang et al., 2006b).

PCP protein localization

One of the most striking and mechanistically significant observations to emerge from the study of PCP in *Drosophila* is that several PCP proteins are asymmetrically distributed on the proximal or distal faces of wing epithelial cells and on a subset of the lateral faces of R3 and R4 photoreceptors (Usui et al., 1999; Axelrod, 2001; Shimada et al., 2001; Strutt, 2001). In genetically mosaic pupal wings, Ds, Prickle (Pk), and Vang localize to the proximal face of each cell; Dsh, Dgo, Ft and Fz localize to the distal face; Stan localizes to both proximal and distal faces; and all of these proteins are under-represented on the remaining (i.e. anterior and posterior) faces (see Fig. 1E). Further experiments examined the effect on PCP protein localization of juxtaposing clones of mutant and WT cells and of mutating various PCP genes (reviewed by Adler, 2002; Strutt, 2002; Klein and Mlodzik, 2005). Together with the co-precipitation of overexpressed PCP proteins and protein fragments, these studies have provided evidence that several of the genetically defined PCP proteins interact with each other either directly or indirectly at the surface of the same cell (i.e. in cis), or with PCP proteins at the surface of the adjacent cell (in trans).

Analogous studies of PCP protein localization in vertebrates are only just beginning. As in *Drosophila*, PCP protein complexes accumulate at the apical edge of the lateral faces of epithelial cells. In the chicken inner ear, Celsr1 localizes asymmetrically in both hair cells and supporting cells in the sensory epithelium of the basilar papilla (Davies et al., 2005). In the mouse organ of Corti, Dvl2-EGFP expressed from a BAC transgene localizes asymmetrically at the surface of hair cells and supporting cells, and this localization is lost in a *Vangl2* (*Lp*) mutant (Wang et al., 2005; Wang, J. et al., 2006). Similarly, Fz3 and Fz6 colocalize asymmetrically at the surface of hair cells and supporting cells in all inner ear sensory epithelia, and this localization is also lost in the *Vangl2* mutant (Fig. 7A,B) (Wang et al., 2006b). The assembly of PCP protein complexes appears to be highly sensitive to the orientation of the cell's sides with respect to the global axis of the epithelium.

The spatial resolution of light microscopy does not permit a distinction to be made between the localization of PCP proteins to one or the other (or both) surfaces of neighboring cells. In the case of Fz6 localization in the inner ear, it has been possible to make this distinction by immunostaining WT:*Fz6*$^{-/-}$ chimeric tissue (Fig. 7C-E) (Wang et al., 2006b). Since Fz3 is fully redundant with Fz6 in the inner ear, the asymmetric localization of Fz6, where WT and *Fz6*$^{-/-}$ cells interface, takes place in the context of phenotypically normal tissue. This analysis shows that Fz6 accumulates apically in both supporting cells and hair cells, and that the polarity of Fz6 localization occurs with respect to the polarity of the epithelium. Fz3, which has the same immunostaining pattern as Fz6 in non-chimeric sensory epithelia and performs the same function in the inner ear, is presumed to have the same subcellular localization.

Interestingly, in the inner ears of *Fz3*$^{-/-}$ mice, the intensity of Fz6 immunostaining at the cell surface is increased relative to WT. Fz3 immunoreactivity exhibits analogous behavior in the *Fz6*$^{-/-}$ inner ear. These observations are consistent with the genetic redundancy of *Fz3* and *Fz6*, and they suggest the existence of an intracellular pool of Fz (and perhaps other PCP proteins) that is in equilibrium with the protein at the cell surface. Consistent with this model, a complete absence of Fz3 and Fz6 at the cell surface in a *Vangl2* mutant is associated with little or no change in the total abundance of the Fz3 and Fz6 proteins (Wang et al., 2006b).

What form might the intracellular pools of Fz take? Careful inspection of confocal images of sensory epthithelia immunostained for Fz3 or Fz6 reveals numerous punctate immunoreactive

structures, presumably vesicles, within the cytoplasm. These puncta cannot be ascribed to background staining or other artefacts because they are absent in tissue from which the corresponding *Fz* gene has been deleted, as shown in Fig. 7C-E. Polarized vesicular transport of Fz has recently been described in *Drosophila* (Shimada et al., 2006), and it seems plausible in both *Drosophila* and mammals that the trafficking of vesicular pools may regulate the abundance of PCP components at the cell surface.

The immunolocalization of Vangl2 in the inner ear shows a pattern very much like that of Fz3, whereas Scribble (a large cytosolic protein with multiple PDZ domains) localizes uniformly along the circumference of the cell (Montcouquiol et al., 2006b). Binding experiments between Vangl2 and Scribble and between Vangl2 and Fz3 expressed in transfected cells indicate a direct interaction for each (Montcouquiol et al., 2006b). These data have been interpreted to imply that a fundamental difference exists between *Drosophila* and mammalian PCP: in *Drosophila*, Fz and Vang localize to opposite sides of wing epithelial cells, whereas in the mouse inner ear Montcouquiol et al. (Montcouquiol et al., 2006b) suggest that Fz3 and Vangl2 colocalize.

Conclusions

The study of vertebrate PCP is still in its infancy, and there are currently many more questions than answers. What is the nature of the global orienting signal? How are local orienting signals sent and received? What are the compositions of cell-surface PCP signaling complexes? How are these complexes selectively localized to some but not other plasma membrane regions? How do Fz3 and Celsr3 function in axon guidance? Which other tissues or developmental processes utilize PCP signaling? Which human diseases arise from defects in PCP? Addressing these and other questions should make this an exciting area of inquiry for many years to come.

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