Dean's Lecture

Planar Cell Polarity Signaling:

A Common Mechanism for Cellular Polarization

ANDREAS JENNY, PH.D. AND MAREK MLODZIK, PH.D.

Abstract

Epithelial cells frequently display—in addition to the common apical-basolateral polarity—a polarization within the plane of the epithelium. This is commonly referred to as planar cell polarity (PCP) or tissue polarity. Examples of vertebrate PCP include epithelial patterning in the skin and inner ear, and also the morphogenetic movements of mesenchymal cells during convergent extension at gastrulation. In *Drosophila*, all adult epithelial structures of the cuticle are polarized within the plane. This review presents recent results and new insights into the molecular mechanisms underlying the establishment of PCP, and compares and contrasts the intriguing similarities between PCP signaling in *Drosophila* and vertebrates.

Key Words: Frizzled, planar cell polarity, tissue polarity, convergent extension, organ of Corti, Drosophila, mouse, zebrafish.

Introduction

EPITHELIAL CELLS are polarized in multiple ways. Apical-basolateral polarity (perpendicular to the plane of the epithelial sheath) enables a cell to directionally transport molecules across a cell layer (e.g., in the gut, kidney and glands) and selectively secrete extracellular matrix components to form a basal lamina (1). To perform many of their functions, epithelia frequently also have to be polarized within the plane of the epithelium. The latter polarization is commonly referred to as epithelial planar cell polarity (PCP) or tissue polarity; it allows cells to form structures that require not only positional, but also vectorial information.

The cellular consequences of PCP signaling range from coordinated organization of cytoskeletal elements in single cells to complex migration of groups of cells. Examples of PCP in vertebrates can be very obvious, as in the ordered arrangement of scales on fish or feathers of birds, and hairs of mammalian skin. Less visible examples are the cilia of the respiratory tract and oviduct, or the stereocilia of the sensory epithelium of the organ of Corti in the vertebrate inner ear. Aberrant PCP of the sensory epithelium in the organ of Corti leads to deafness (2). Furthermore, the complicated movement of mesenchymal cells during gastrulation (called convergent extension), which leads to the elongation and thinning of the body axis, also depends on correct PCP signaling (3–10).

PCP signaling is, however, best studied in *Drosophila melanogaster*, mainly because of the versatility of the fly as a model system. In *Drosophila*, PCP can easily be seen on several external adult structures such as the bristles on the thorax or the precisely aligned hairs on wing cells (Fig. 1A; 11). In addition, the facet eye of insects also shows characteristics of PCP, with its precise arrangement of the building blocks, the ommatidia, with respect to each other and the general axes in each eye (9). Genetic and molecular studies of *Drosophila* led to the identification of a signaling network directing PCP establishment. In recent

From the Brookdale Department of Molecular, Cell and Developmental Biology, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029.

Address all correspondence to Marek Mlodzik, Ph.D., Brookdale Department of Molecular, Cell and Developmental Biology, Box 1020, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029; email: marek.mlodzik@mssm.edu

Presented to the Department of Medicine, Mount Sinai School of Medicine, New York, NY, May 14, 2003 and updated December 2005.

years it has become apparent that the PCP signaling module is highly conserved and is essential for PCP establishment in many vertebrate contexts, including the inner ear and convergent extension during gastrulation and neurulation (6, 12).

Discovery of the mechanism of PCP signaling and its conservation from insects to ascidians and mammals is one of the most exciting topics of developmental biology today (13). In this review we discuss PCP signaling in *Drosophila* and compare it with recent findings in the vertebrate field. In particular, we address intriguing parallels in PCP establishment between fruit flies and vertebrates on the mechanistic level and discuss novel findings that suggest a link between PCP signaling and ciliary diseases.

Planar Cell Polarity in *Drosophila*

Many adult structures of *Drosophila* show an easily visible planar polarization. For example, each cell of the wing blade grows a distally pointing, actin-rich hair close to its distal vertex (Fig. 1A; 11, 14). This process of hair orientation relies solely on directionally driven cytoskeletal rearrangements, without an apparent requirement for a transcriptional response (in contrast to other tissues, see below [15, 16]). In the absence of proper PCP signaling, the wing hairs form in the middle of the cell and are misoriented, or multiple hairs form on a single cell. On the thorax and abdomen, sensory bristles are aligned along the anterior-posterior (A-P) axis and point towards the rear end of the fly body (for details of PCP on the abdomen, see refs. 17–20). The sensory bristles of the thorax develop during pupal stages and consist of 4 different cells that originate from a single sensory organ precursor (SOP) cell in two rounds of asymmetric division. In order for the bristles to be oriented with the body axis, the SOP cell has to divide along the A-P axis. PCP signaling is required to align the spindle and the asymmetrically distributed cell fate determinants Numb and Neuralized with the A-P axis (21–24). In the absence of PCP signaling, the SOP cell still divides asymmetrically; however, the axis of division is randomly chosen and thus the bristle is ultimately randomly oriented.

A less obvious, but nevertheless more complex polarization is found in the facet eye of *Drosophila* (and in insects in general). Within each facet or ommatidium—the modular building block of the eye—the rhabdomeres (light-sensitive organelles) of the six outer (R1–6) and two inner (R7/8) photoreceptors are organized in a trapezoid pattern that is invariable from ommatidium to ommatidium (Fig. 1C; 25, 26). Furthermore, each ommatidium is aligned with the A-P and dorso-ventral (D-V) axes. Such an elaborate geometric organization is required for proper image formation after wiring of the photoreceptor axons in the underlying lamina, medulla and brain lobes. Due to the curvature of the eye surface, photoreceptors of a single facet "see" different points in space, and therefore corresponding photoreceptor neurons of neighboring ommatidia, which "see" the same point in space, project together to represent a single point in space on a topographic map in the brain (27). The precise orientation of each ommatidium and its photoreceptors along the A-P and D-V axes is thus essential for the accurate processing of visual information in the brain. In the absence of PCP signaling, this precise alignment is lost, even though the individual building blocks still form (Fig. 1D).

How is such complex organization established? The adult eye of *Drosophila* develops from the eye imaginal disc, a single-layered epithelium that derives from the embryonic ectoderm. During the third larval instar, a wave of differentiation, the morphogenetic furrow (MF), sweeps from posterior to anterior across the eye disc, leaving in its wake differentiating photoreceptor clusters that mature progressively (28, 29). At the time of PCP signaling, each precluster consists of the R8 founder cell and photoreceptor precursor cells R2/R5 and R3/R4 (Fig. 2A). The preclusters are symmetric with respect to the R3/R4 precursors, with the R3 precursor (orange in Fig. 2A) closer to the midline of the eye (equator) and the R4 precursor adjacent on the polar side (blue). Asymmetry is first seen when the preclusters start to rotate in opposing directions dorsal (clockwise) and ventral (counterclockwise) of the equator from row 4 onward (Fig. 2A). About 9 or 10 rows posterior to the MF, the clusters have rotated by 45°. By that time, the remaining photoreceptor precursors R1/R6 and R7 are recruited as well, and the clusters continue to rotate to complete a 90° rotation with respect to their original position. Ultimately, the opposite direction of rotation leads to mirror-symmetric dorsal and ventral halves of the adult eye with asymmetric positioning of the R3 (anterior, polar position) and R4 cells (posterior, more equatorial) within the trapezoid formed by the 6 outer photoreceptors (Fig. 1C and 2A; 25, 26, 30–34).

The different chiral forms, distinctive in the adult eye, can already be anticipated during the third larval instar. Just prior to the beginning of precluster rotation (about row 4/5 behind the morphogenetic furrow), asymmetries in molecular markers can be detected. A *lacZ* enhancer trap

under control of the Delta (Dl) promoter is expressed more strongly in R3 than in R4 (35–37), while *mδ0.5>lacZ*—a short enhancer fragment of the Notch (N) target gene mδ—is expressed at higher levels in R4 (see below and ref. 38). These differences between the R3 and R4 cells are the result of PCP signaling and direct the subsequent rotation and asymmetric positioning of R3 and R4 in the adult eye.

Molecular Control of PCP Signaling

Most components of the PCP signaling cassette have been discovered in genetic screens in *Drosophila*. A subgroup of PCP genes—the core PCP genes—are required for PCP signaling in all tissues, while others are required only in certain tissues and therefore probably reflect more downstream effectors (see below). A characteristic common to mutations in core PCP genes is that the structures in question still form correctly, but that their orientation or alignment is perturbed (Fig 1).

In the eye, the cell fate specification of the R3 and R4 photoreceptors is key to the precise ommatidial and tissue polarity in the adult. After recruitment of the R2/R5 and R3/R4 precursors into the precluster—a process requiring the Notch and EGFR signaling pathways—the R3 and R4 precursors are subspecified as such by the action of the transcription factors Seven-up (36) and Spalt (39). PCP signaling is then required to signal the binary switch of R3 versus R4 fate and thereby determines the direction of rotation and positioning of the photoreceptors within the mature cluster. Genetic mosaic analysis of PCP components has revealed that the PCP genes are required for signaling only in the R3 or R4 precursors, but not other photoreceptors (see Table 1 and below; 40–45).

It has been proposed (46, 47) that a PCP signal that has previously been specified emanates from the dorso-ventral midline by a complex signaling interplay between the (canonical) Wingless, JAK-Stat and Notch pathways (ultimately leading to N activation at the equator of the eye [48]). Due to its position closer to the equator at the time of signaling, the R3 precursor receives a slightly higher signal than the more polar R4 precursor. Even though the nature of the ligand is still unknown, it has been shown that the seven-pass transmembrane protein Frizzled (Fz; Figs. 2B and C, and the Table) acts as PCP signal receptor (40, 49, 50). The signal is then transduced to Dishevelled (Dsh, Dvl in vertebrates), which has the structure of a typical adapter protein with three protein-protein interaction domains (DIX, PDZ and DEP domains), of which the PDZ domain has been shown to bind Fz (51–54). The signal then travels via the small GTPases Rho and Rac (15, 55), the Ste20-like kinase Misshapen (Msn; 56) and a JNK/MAPKinase cascade (52, 57) to the nucleus. The elicited transcriptional response specifies the R3 fate. One of the target genes in R3 is Dl, which then signals to its receptor N in the neighboring R4 precursor. N signaling in R4 leads to R4 fate specification (38, 42, 58). The Dl/N signaling thus reinforces an initially small difference in Fz/PCP signaling between the R3 and R4 precursors.

The balance of Fz activity in R3 and R4 appears to be tightly regulated on two levels: First, the golgi-resident, type II transmembrane protein *four-jointed* (*fj*) and the atypical cadherins *fat* (*ft*) and *dachsous* (*ds*) presumably form a system that modulates Fz activity, creating an activity gradient (59–63). Second, Fz activity is actively controlled between R3 and R4 (see below). *fj*, *fat* and *ds* clones in the eye (59–62) lead to chirality inversions at the polar (*fj, fat*) or equatorial (*ds*) border of the clones (and randomization within *ft* and *ds* mutant clones). Genetic and histochemical studies have led to the conclusion that Fj and Ds antagonize Fat, which then in turn acts positively on Fz signaling (61). It is likely that similar scenarios involving *fat*, *ds* and *fj* act in most if not all tissues in *Drosophila*. Evidence for potential upstream input via the Fat, Ds and Fj interactions on Fz signaling is also reported for the wing and the abdomen, although the proposed interactions are not always identical (19, 64, 65). How Fz activity is regulated through potential global cues remains one of the most elusive problems in PCP generation.

Intracellular PCP Factors and Their Interactions

Evidence for a tight regulation of Fz signaling at the level of R3 and R4, or in neighboring wing cells, originates from the analysis of fly mutants. Genetic screens identified four additional core PCP genes (Figs. 2B and C): the atypical cadherin Flamingo (Fmi; aka. Starry-night, Stan; 66, 67), the four pass transmembrane protein Strabismus (Stbm; aka. Van Gogh, Vang; 41, 68) and the cytoplasmic proteins Diego (Dgo; 69) and Prickle (Pk; 70, 71). Dgo can bind Dsh and it stimulates Fz-PCP signaling activity (43, 69). Stbm and Pk both can antagonize Fz-PCP signaling (41, 43, 68, 69, 72). Pk is recruited to the cell membrane by Stbm (44, 73, 74) and also interacts with Dsh (43, 72). Binding of Dgo and Pk to Dsh is mutually exclusive, which is a molecular explanation for the antagonistic effect of Pk and Dgo on Fz-PCP signaling (43).

Genes acting positively on R3 fate specification and promoting Fz activity, such as Fz, Dsh, and Dgo, are genetically required only in the R3 precursor (Table; 40, 43, 75). On the other hand, Stbm and Pk antagonize R3 specification and Fz activity and are genetically required in R4 (41, 44, 75). The only gene known to date that is required in both precursors is Fmi, which—as an atypical cadherin—mediates homophilic adhesion (67), consistent with its early positive requirement for Fz-PCP signaling in R3 and its later function in R4 specification (45). Besides the PCP factors, components of Notch signaling also show specific cellular requirements in the R3/R4 pair: Whereas Dl is required in R3 (as a transcriptional target of Fz/PCP signaling), Notch is only required in R4 (42, 58). Analogous interactions among the PCP factors occur in wing cells, with the exception that Dl and Notch play no role there (see below).

Differences between the Eye and Wing

As mentioned above, PCP signaling in the wing requires a reorganization of cytoskeletal elements and does not require a transcriptional response (15). However, in addition to the core PCP genes, tissue-specific effectors are essential for correct initiation of the actin hairs. Mutations in genes such as *fuzzy*, *inturned*, *tricornered*, *multiple-wing hairs* or *fritz* (which are often referred to as secondary PCP genes) lead to the formation of multiple wing hairs per cell (11, 76). Furthermore, Rho kinase (Drok), an effector of RhoA, also plays a critical role linking Fz-PCP signaling to actomyosin contractility via the regulation of Myosin Regulatory Light Chain (MRLC or Sqh) and Myosin II (MyoII; 77).

In the eye, the specification of the R3 and R4 cell fates also regulates the direction of ommatidial rotation and associated cytoskeletal changes. As with the wing, some aspects of this appear to be governed by common effectors like Drok (77). Ommatidial rotation is further controlled by an eye-specific secondary PCP gene, *nemo* (78), and additional regulatory input via epidermal growth gactor-receptor (EGF-R) signaling (79–81). How the tissue-specific secondary PCP genes regulate the respective downstream processes is poorly understood at best, and their link to the core PCP factors is also unknown. These are some of the most interesting questions to be addressed in the future.

Planar Cell Polarity Signaling in Vertebrates

The highly intriguing parallels between *Drosophila* and vertebrate PCP have recently received considerable attention. Mice lacking mFz6 show irregular hair patterning and swirls on their fur, instead of a regular parallel hair orientation (82). This is intuitively very similar to the aberrant actin hair orientation on *fz* mutant fly wings. Much better studied, however, are PCP establishment in the vertebrate inner ear and the process of convergent extension during gastrulation and neurulation (e.g., 7, 83).

Vertebrate Inner Ear

Within the organ of Corti in the mammalian cochlea, stereocilia bundles on the luminal surface of inner and outer rows of mechanosensory hair cells (Fig. 1E; 91) are stereotypically arranged in a chevron-like pattern. From one cell to the next, the chevrons are precisely aligned. The correct orientation of the hair bundles is a prerequisite for proper hearing, because the bundles are directionally sensitive to the sound waves traveling along the cochlea (for detailed reviews see 2, 7, 8). Strikingly and characteristic for aberrant PCP signaling, mutations in the mouse orthologs of several fly core PCP genes affect the orientation of the cilia chevrons without affecting their actual structure. The best-studied mutation, *looptail* (*lp*), maps to *vangl2* (84, 85), one of the two mouse orthologs of *stbm*/*vang* (see Fig. 1F for its aberrant organization of the stereocilia on its sensory neurons). Similar abnormal hair polarization is also found when *celsr1* (86), one of the *fmi* orthologs, is mutant or in double knock-outs of *dvl1* and *dvl2* (87). The similarity of PCP signaling in mouse and *Drosophila* is further supported by strong genetic interactions between *dvl* and *vangl2* (87). As in the fly, the signal required for cilia orientation is unknown. Even though Wnt7a is expressed in a gradient across the inner and outer hair cells (8) and Wnt7a can affect planar cell polarity in cochlear explant cultures (88), several single *wnt* have no ear PCP phenotype, a fact that has been attributed to redundancy (8, 88). However, it has recently been shown that a mouse Fz3 and Fz6 double knock-out shows typical PCP phenotypes in the inner ear (89).

At least two additional genes have been identified that can affect sensory hair polarity in the inner ear (at least in the outer hair cells): *protein tyrosine kinase 7* (90) and *scribble* (91). However, their fly orthologs, *offtrack* and *scrib*, have not yet been implicated in PCP signaling. It will be important to carefully test them for potential genetic interactions with PCP genes in *Drosophila* in order to determine whether *scribble* and *ptk7* are vertebrate-specific PCP factors.

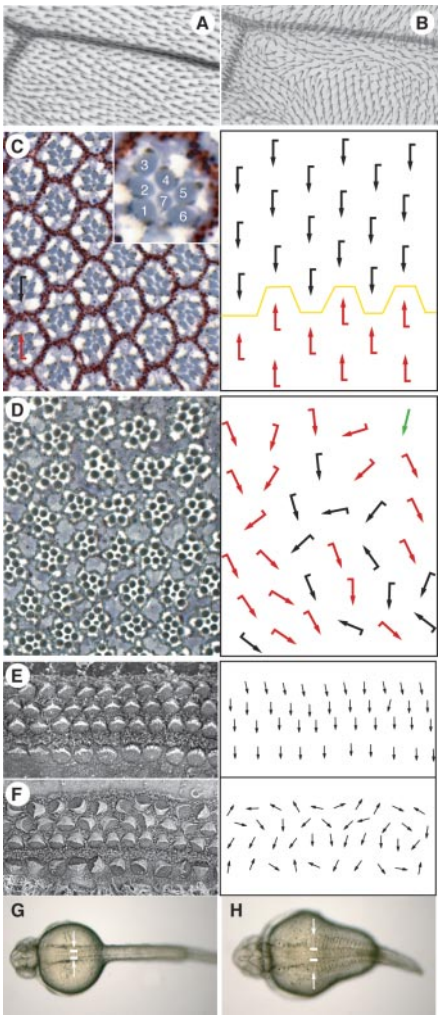

Fig. 1. Characteristics of planar cell polarity phenotypes are conserved between flies and vertebrates. All examples show mutant phenotypes of Stbm/Vang family members. Part of an adult wild-type (**A**) and a mutant wing (**B**). In contrast to wild-type, where wing hairs point towards the distal end of the wing (to the right in both pictures), in the mutant wing the hairs form whorls and waves. **(C, D)** Tangential sections through an adult *Drosophila* eye of wild-type (**C**) and mutant (**D**). In each scheme on the right, the dorsal and ventral ommatidial clusters are represented by black and red arrows, respectively. (**C**) In wild-type, the ommatidia are arranged in a mirror symmetric pattern with respect to the equator (yellow line in scheme on the right) and R3 is positioned at the anterior tip of the trapezoid, while R4 is more posterior (see inset with numbered R-cells; note that R8 is below R7 and thus cannot be seen.). In the *stbm*/*vang* mutant eye, the ommatidia form mostly correctly, but their mirror symmetrical arrangement is lost with both chiral forms forming at random. In addition, symmetrical clusters (green arrow) and misrotated clusters are present. **(E, F)** Scanning electron micrographs of part of the organ of Corti in the inner ear of a wild-type (**E**) and a *vangl2* (the mouse ortholog of *stbm*/*vang*) mutant mouse **(F)**, showing the single inner and three outer rows of sensory hair cells with the characteristic chevrons formed by their stereocilia. The schematic on the right emphasizes the loss of polarity in the *vangl2* mutant. **(G, H)** Dorsal view of a wild-type (**G**) and maternal-zygotic *tri* (*stbm*/*vang* ortholog) (**H**) mutant zebrafish. Compared to the wild type, the shorter, but wider body axis is apparent in the mutant. The lateral borders of the notochord and the somites are marked with bars and arrows, respectively.

Figs. 1E and 1F reproduced with permission from Montcouquiol M, Rachel RA, Lanford PJ, et al. Identification of Vangl2 and Scrb1 as planar polarity genes in mammals. Nature 2003; 423:173–177 (91) and Figs. 1G and 1H reproduced with permission from Ciruna B, Jenny A, Lee D, et al. Planar cell polarity signalling couples cell division and morphogenesis during neurulation. Nature 2006; 439(7073):220–224 (74).

Convergent Extension

During vertebrate gastrulation and neurulation, convergent extension (CE), in which mesodermal and neurectodermal cells migrate towards the midline and intercalate there (6, 10, 83, 92), leads to a significant elongation of the body axis (Fig. 1G; 74). In order to migrate, the cells elongate along the axis of migration and become polarized with high protrusive activity at the medial or both ends. Several zebrafish mutants and morpholino oligonucleotide mediated knock-downs in zebrafish and *Xenopus* have demonstrated the involvement of the PCP signaling pathway in convergent extension. Mutants in the *trilobite* gene, the major ortholog of *stbm*/*vang* (Fig. 1H; 74, 93), as well as in *wnt11* (94), *wnt5* (95) and knockdown of Pk (96–98), Fmi (99), Diversin (a potential ortholog of Dgo), and Jun N-terminal kinase (JNK) (100) all lead to aberrant convergent extension resulting in a shorter axis, with a wider body (e.g., compare Figs. 1G and H). Due to the broadened meso- and neurectoderm, the neural tube frequently fails to close, as with common human birth defects (101, 102). It recently became clear that not all of the extension of the body axis during gastrulation is due to cell intercalation. A significant amount of the extension is due to directional orientation of mitosis along the antero-posterior axis of the dividing mesodermal cells (103). Perhaps like the SOP division in *Drosophila* (see above), the spindle alignment with body axis is controlled by the PCP pathway. In zebrafish embryos injected with Xdd1, a dominant negative form of Dishevelled, and to a lesser extent in *wnt11* and *tri* mutants, the orientation of the axis of cell division is randomized, and thus directional growth contributes less to the extension of the body axis (103).

Yet a different link between cell division and PCP signaling during neural tube formation in zebrafish has recently been described (74). The

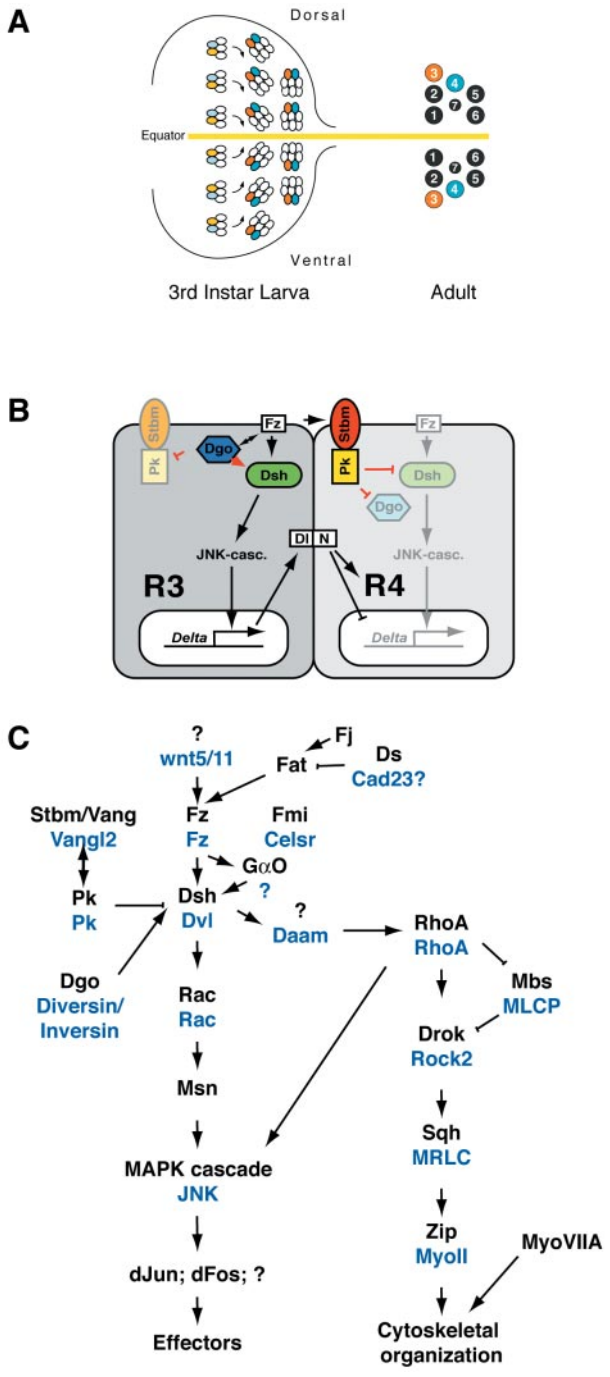

Fig. 2. (**A**) Schematic of third instar *Drosophila* eye imaginal disc with dorso-ventral (D-V) midline or equator, in yellow. Anterior is left and dorsal up. Initially, ommatidial preclusters are symmetrical. PCP signaling leads to the determination of R3 (orange) and R4 (blue), followed by a 90˚ rotation of clusters towards the equator. In the adult, the rhabdomeres of the photoreceptors are positioned in mirror-symmetric trapezoids with R3 anterior to and polar of R4. (**B**) Schematic of PCP signaling in R3 and R4. Signaling of Fz through Dsh and a JNK cascade leads to specification of R3. In R4, Fz signaling is antagonized by Stbm and Pk. In a second step, the signaling difference between R3 and R4 is reinforced by Dl and N (N then specifies the R4 fate). See text for details. (**C**) Schematic summarizing the genes involved in PCP signaling in *Drosophila* (in black) and in vertebrates (compiled from different tissues, blue).

neural plate folds to appose apical surfaces of opposite sides (neural keel). As the cells divide, they round up and lose polarity. After division, both cells repolarize, and while the mother cell reintegrates ipsilaterally, the daughter cell crosses the midline of the embryo and integrates into the neuroepithelium on the contralateral side. In maternal-zygotic *tri* mutant embryos, the daughter cell fails to cross the midline and cannot integrate into the neuroepithelium. Therefore, many unpolarized cells remain in the middle of the future neural tube, which results in an enlarged, unorganized neural anlage. Intriguingly, inhibition of cell division in maternal-zygotic *tri* embryos suppresses this defect, suggesting that PCP signaling is required to coordinate the cellular behavior of newly divided cells and promote their repolarization and integration (74).

Towards a Molecular Understanding of PCP Signaling

Ultimately, PCP signaling leads to polarization of a cell along an axis that is indicated by differential distribution of an activity or molecular marker along this axis. Earliest candidates for asymmetrically localized proteins are the PCP factors themselves. In the fly eye and wing, prior to PCP signaling, the PCP components are localized uniformly around the apical cellular junctions (104–107). In the wing, this apical localization depends on the transmembrane proteins Fz, Stbm and Fmi (107). In these mutant backgrounds, the other proteins, including Dsh, are not targeted to the apical circumference, a prerequisite for PCP signaling (108); in the eye, the interplay between PCP factors contributing to apical localization appears to be more complex (105). As a result of PCP signaling, the subcellular protein distribution becomes further refined. The PCP factors acting positively on Fz/PCP signaling, such as Fz itself, Dsh and Dgo, become localized distally within wing cells and are absent from the proximal side (Fig. 3A and the Table; 104, 105, 107). In contrast, the proteins acting antagonistically to Fz signaling—Pk and Stbm—become enriched at the proximal side of wing cells (106). Based on its requirement for its own proximal and distal enrichment, Fmi is so far the only protein known to become enriched at the proximal and distal ends of the cells (67). Ultimately, PCP signaling thus leads to the asymmetric distribution of its own signaling components across the border of two neighboring wing cells (Fig. 3A). Analogously in the eye, Fz/Dgo are enriched on the apical, polar side of the R3 cell and absent from the equatorial side of R4 (105, 106). In contrast, Stbm shows a complementary localiza-

TABLE
PCP Genes and Their Relationships

PCP gene	Tissues affected in *Drosophila*	Processes affected in vertebrates[#]	Molecular features	localization in *Drosophila* wing cells/eye	R3/R4 require-ment	References
frizzled (fz)	all adult tissues	CE, inner ear, epidermis	seven-pass transmembrane receptor, binds Wnt ligands, binds Dsh, recruits Dsh and Dgo to membrane	distal/polar R3	R3	(49-51, 107, 129-131)
dishevelled (dsh)	all adult tissues	CE, inner ear	cytoplasmic protein containing DIX, PDZ, DEP domains, recruited to membrane by Fz, binds Fz, Pk, Stbm and Dgo	distal/n.d.	R3	(51-53, 94, 104, 132-134)
flamingo (fmi)/ starry night (stan)	all adult tissues	CE, inner ear	Cadherin with seven-pass transmembrane receptor features, homophyllic cell adhesions	proximal + distal/ n.d.	R3 +R4	(45, 66, 67, 86, 99, 135)
diego (dgo)	eye, wing, notum in GOF*	CE	cytoplasmic Ankyrin repeat protein, recruited to membrane by Fz, binds Dsh, Stbm and Dgo	distal/polar R3	R3	(43, 69, 105, 125)
strabismus (stbm)/ Van Gogh (Vang)	all adult tissues	CE, inner ear	novel 4-pass transmembrane protein, binds Pk, Dsh and Dgo, recruits Pk to membrane	proximal/eq. R-	R4	(41, 44, 68, 73, 74, 84, 85, 136-138)
prickle (pk) (a.k.a. *prickle-spiny legs*)	all adult tissues	CE	cytoplasmic protein with 3 LIM domains and PET domain, recruited to membrane by Stbm, physically interacts with Dsh, Stbm and Dgo	proximal/n.d.	R4	(44, 70-73, 96-98)
Fat (Ft)	all adult tissues	?	Proto-cadherin, heterophyllic interaction with Ds, binds Atrophin	uniform (AJ)	R3	(19, 61, 62, 64, 139, 140)
dachsous (ds)	all adult tissues	inner ear (structural)	Proto-cadherin, heterophyllic interaction with Fat	uniform (AJ)	R4	(19, 61, 62, 64, 140)
four jointed (fj)	all adult tissues	?	type-2 transmembrane or secreted peptide possibly functions in Golgi to modify Ds	?		(59-61, 64, 140)
RhoA	eye, wing*	CE	small GTPase, acts downstream of Dsh	n.d.	R3	(55, 141)
rho kinase (drok)	eye, wing	CE	Ser-Thr kinase	n.d.		(77, 142)
misshapen (msn)	eye, wing, notum*	?	STE20-like S/T protein kinase, acts downstream of Dsh	n.d.	R3	(56)
widerborst	wing*	?	B' regulatory subunit of PP2A	diffuse distal/n.d.		(117)

[#] only tested tissues mentioned, combination of analysis in *Xenopus*, zebrafish and mouse
* other tissues were not tested
n.d. not determined

tion pattern and is enriched on the equatorial side of R4 and absent from the polar side of R3 (Fig. 3B; 106; in the eye, the R3/R4 specific distribution of Pk and Dsh remains to be determined). The subcellular localization pattern of the PCP proteins with respect to the R3/R4 cell interface parallels the genetic requirement of their genes (e.g., factors required in R3, like Fz and Dgo, localize to the R3 side of the R3/R4 cell border).

A similar distribution of PCP proteins has been described during the asymmetric cell division of SOP cells, during notum development. Pk and Stbm localize to the anterior cortex, while Fz is enriched at the posterior side of the SOP cell (Fig. 3C; 22, 109). Interestingly, Fmi remains distributed uniformly around the SOP cell cortex (110).

PCP protein localization has recently also been shown to be asymmetric in vertebrate cells. In the inner ear sensory hair cells, GFP-Dsh localizes to the distal side of the cells, where—as in the fly wing—the actin-rich stereocilia are localized (Fig. 3D; 87). Analogously, Vangl2, the mouse Stbm ortholog, localizes to the opposing, proximal side (111). Intriguingly, Fz3 and Fz6 colocalize with Vangl2 and thus show an unexpected difference between PCP signaling in *Drosophila* and vertebrates (89, 111). Furthermore, in the zebrafish notochord and neurectoderm, GFP-Pk localization is enriched on the anterior side of the cells (74). During cell division in the neurectoderm, Pk becomes cytoplasmic and—after division—regains its asymmetry (Fig. 3E). This anterior enrichment is PCP signaling dependent. As in *stbm* mutants in *Drosophila*, in maternal/zygotic *tri* mutants or *wnt11*/*wnt*5/wnt4 triple mutants/knock-downs, Pk membrane localization is lost (74).

Generation of Asymmetric PCP Protein Localization

Using a mathematical model, it has been shown that four components of the PCP system and an initial asymmetry that might be generated by *four-jointed*, *fat* and *dachsous* are sufficient to establish and maintain the asymmetries in protein localization in the fly wing (112). The model is based on Fz recruiting Dsh to the membrane (51, 104, 107), and Pk/Stbm inhibiting Dsh recruitment

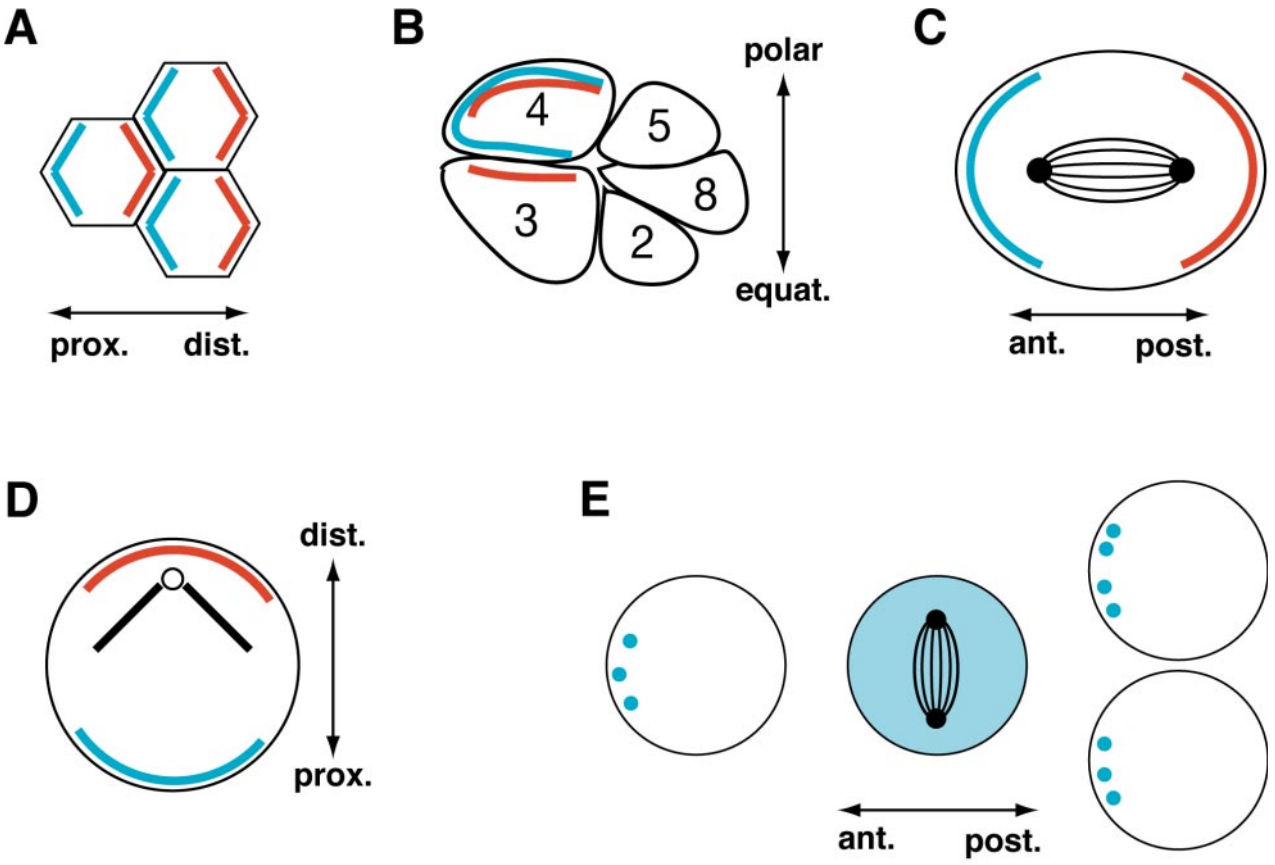

Fig. 3. Subcellular PCP protein distribution in wing cells (**A**), the eye (**B**) and SOP cells (**C**) of *Drosophila*; mouse sensory hair cells of the organ of Corti (**D**); and zebrafish neurectodermal cells (**E**). For each case, the corresponding axis is indicated by arrows. Fz/Dsh/Dgo are indicated in red, while Stbm-Vang and Pk are in blue. Note that, depending on the tissue, only a subset of the respective proteins have been analyzed. (See text for details. In addition, Fz3 and Fz6 colocalize with Vangl2 in the organ of Corti.) (**E**) In zebrafish, Pk is cytoplasmic during cell division, but regains polarity after separation of the daughter cell.

by Fz (44, 72, 73). Furthermore, the model requires an interaction of Fz with Stbm across cell membranes. Under these conditions, the model can explain many phenotypes of PCP mutant clones. However, *in vivo* there are clearly more than four PCP genes required for correct PCP establishment. Thus the model is not complete, and there must be backup mechanisms at work that are required for proper PCP establishment *in vivo*.

To date, many direct protein-protein interactions between core PCP proteins have been described, which has led to some understanding of the intracellular regulation of PCP signaling. The C-tail of Fz can interact with the PDZ domain of Dsh *in vitro* (54), and Fz can recruit Dsh to the apical circumference of cells in *Drosophila* (108) and to the cell membrane in cell culture and in *Xenopus* animal cap explants (72, 113–115). On the other hand, Stbm interacts with Pk, and Stbm is required for membrane recruitment of Pk (44, 73, 74, 109). Animal cap recruitment assays have been used to demonstrate a mutual effect between Stbm and Pk on each other's localization. While GFP-Stbm in the absence of exogenous Pk is localized uniformly in the membrane of animal cap cells, addition of Pk leads not only to Pk membrane recruitment, but also to a redistribution of both proteins into clusters within the membrane (44). It is thus plausible that Pk and Stbm have the potential to promote their aggregation into larger protein complexes. One can speculate that, *in vivo*, initial weak differences in PCP signaling can trigger a process of protein separation in a highly organized way (i.e., Fz/Dsh to one and Pk/Stbm to the other end of a cell). This separation could be enhanced by intrinsic aggregation properties of PCP proteins and positive or negative feedback loops (72, 112). In this regard, it has been shown that Pk can interact with Dsh, which probably reduces Dsh membrane localization where Pk concentration is high, thus explaining the inhibitory effect of Pk on Fz-PCP signaling (72).

Dgo has been shown to act positively on Fz signaling in the eye and wing and to colocalize

with Fz/Dsh (see above; 43, 69, 105). Dgo can compete with Pk for binding to Dsh and thus prevent the inhibitory action of Pk (43). Furthermore, Dgo membrane recruitment in the eye strictly requires Fz, and co-transfection of Dsh with Dgo (in 293T cells) leads to Dsh phosphorylation. It is thus possible that Dgo stabilizes a complex between Dsh and Fz and thereby increases Fz-PCP signaling activity.

If Stbm/Pk clusters prevent Fz/Dsh complex formation in cis, but promote it on the abutting side in the neighboring cell, there has to be communication across the cellular interfaces. This could be mediated via a soluble relay molecule (factor X; 46, 47) or via a postulated protein-protein interaction between Fz and Stbm (112). However, to date no such interaction has been reported. On the other hand, it has been shown that the atypical cadherin Fmi interacts with itself, mediating a homophilic interaction between neighboring cells (67) and thus could also mediate a "functional" Fz/Stbm interaction. It will therefore be important to test for potential interactions of Fmi with either Fz or Stbm in cis.

The core PCP proteins are not the only factors that are asymmetrically distributed in the apical cellular cortex. The GαO subunit of heterotrimeric G proteins that is required for canonical Wnt and Fz-PCP signaling shows a more diffuse, but still proximally enriched expression pattern (116). Conversely, the B' subunit (Widerborst) of protein phosphatase 2A (PP2A) thought to target PP2A to substrates and thus to confer substrate specificity, localizes to a diffuse distal web of microtubules at the level of apical junctions (16, 117). Apparently, the distal localization of Widerborst precedes the asymmetric localization of the PCP proteins. In the absence of *widerborst* function, PCP proteins remain uniform and show no asymmetric distribution (117). It is not known how the asymmetry of Widerborst is achieved. It is tempting to speculate that PP2A is required to keep Fz in a dephosphorylated state and thus to prevent Fz inactivation by phosphorylation. During PCP establishment in the eye, Fz is selectively activated in the R3 and R4 cell by Par3 (Bazooka), which prevents dPatj and aPKC from phosphorylating and inactivating Fz (118). An asymmetric PP2A mediated dephosphorylation and thus activation of Fz could lead to initial biasing of Fz activity towards the distal end.

PCP Signaling and Ciliary Diseases

Recent publications report potential links of PCP signaling and ciliary diseases (119, 120). The elongation of kidney tubules depends on the alignment of the axis of cell division parallel to the direction of extension of the tubules, and perturbation of the mitotic pattern leads to cystic growth (121). Mutations in the gene coding for Inversin, a component of the primary cilia-basal body complex, are associated with left-right axis defects and severe renal cystic disease in mice (122, 123). In humans, *inversin* mutations result in an infantile form of nephronophthisis (NPHP2; 124). Inversin has been shown to suppress Dsh induced axis duplication in *Xenopus*, a phenotype caused by overactivation of canonical Wnt signaling. On the other hand, Inversin is also required for convergent extension. It has been proposed that Inversin acts as a switch between canonical Wnt/Fz and non-canonical Fz/PCP signaling (119), similar to the related Diversin (125).

Inversin, Diversin and Diego are structurally related and are all composed of N-terminal Ankyrin repeats followed by C-terminal regions of different lengths (69, 122, 123, 125). These C-terminal regions of Dgo and Inv both physically interact with Dsh (43, 119) and both act positively on Fz-PCP signaling (note that *dgo* mutations do not show a phenotype reminiscent of overactivation of canonical Wnt/Fz signaling [69]). Loss of Inversin leads to cystic kidneys (dilated tubules), a phenotype which, in contrast to the left-right axis defects, cannot be prevented by inversin lacking the Dvl binding site (126). It has been proposed that Inversin might be required for oriented cell division and thus correct elongation of renal tubules (127).

The most recent link of PCP signaling with a ciliary disease has been found in Bardet-Biedl syndrome (BBS) (120). BBS is a complex disease associated with—among others—renal dystrophy, reproductive tract abnormalities, polydactyly and weak hearing impairment (120, 128). All eight genes that have been linked to BBS appear to be involved in ciliary and basal body functions (120 and references therein). Interestingly, several knockouts of BBS genes in mice show a misorientation of the stereocilia chevron in the organ of Corti characteristic of abnormal PCP signaling (see above). Furthermore, BBS1 and BBS6 knock-out mice also genetically interact with vangl2 mutants: double heterozygous animals show defects in the orientation of inner ear sensory cilia. Similarly, morpholino oligonucleotide mediated knock-down of zebrafish BBS4 can enhance the convergent extension phenotype of mutations in the *tri* gene, the zebrafish ortholog of *vangl2*/*stbm*. However, the exact mechanistic link between the BBS or other ciliary proteins and PCP signaling needs to be examined in detail before a direct link between ciliary function and PCP signaling can be established.

Conclusions

In recent years, amazing parallels between PCP signaling in *Drosophila* and vertebrates have been discovered. These parallels include not only Fz and Dsh, but also their interactions with the other regulatory PCP factors and the downstream effectors Rho Kinase and JNK. Thus, this pathway that is so critical for the function of diverse organs is highly conserved. However, many puzzling questions remain to be answered. For example, what is or are the signal(s) that initially break the symmetry? Even though there is evidence from vertebrates that Wnts are important for PCP establishment, it is still unclear what their exact contribution is. Another question that needs to be addressed experimentally is how the atypical cadherins Fat and Ds feed into the Fz-PCP pathway on a mechanistic level. Thus, despite the recent significant leap in our understanding of the process, many more aspects of PCP establishment remain unresolved and will keep the scientific community busy in the future.

Acknowledgements

We wish to thank all members of the Mlodzik lab for many helpful and stimulating discussions, and Jessica Reynolds-Kenneally and Thomas Klein for critically reading the manuscript. We are grateful to Brian Ciruna, Mireille Montcouquiol and Matthew Kelley for the pictures shown in Fig. 1 and for communicating unpublished data. We apologize to all authors of original work in the field that could not be incorporated or cited due to space limitations. Work in the lab is supported by the NIH with grants from the NEI and NIGMS (to MM) and NIDCD (to AJ).

References

1. Eaton S, Simons K. Apical, basal and lateral cues for epithelial polarization. Cell 1995; 82:5–8.
2. Hudspeth A. Mechanical amplification of stimuli by hair cells. Curr Opin Neurobiol 1997; 7:480–486.
3. Klein TJ, Mlodzik M. Planar cell polarization: an emerging model points in the right direction. Annu Rev Cell Dev Biol 2005; 21:155–176.
4. Strutt D. Frizzled signalling and cell polarisation in Drosophila and vertebrates. Development 2003; 130:4501–4513.
5. Keller R, Davidson L, Edlund A, et al. Mechanisms of convergence and extension by cell intercalation. Philos Trans R Soc Lond B Biol Sci 2000; 355(1399):897–922.
6. Wallingford JB, Fraser SE, Harland RM. Convergent extension: the molecular control of polarized cell movement during embryonic development. Dev Cell 2002(6); 2:695–706.
7. Dabdoub A, Kelley MW. Planar cell polarity and a potential role for a Wnt morphogen gradient in stereociliary bundle orientation in the mammalian inner ear. J Neurobiol 2005; 64:446–457.
8. Dabdoub A, Montcouquiol M, Kelley MW. Planar cell polarity in the vertebrate inner ear. In: Mlodzik M, editor. Planar cell polarization during development. Vol. 14. San Diego: Elsevier; 2005. pp. 107–130.
9. Mlodzik M. Planar cell polarity in the Drosophila eye: Cell fate and organization. In: Mlodzik M, editor. Planar cell polarization during development. Vol. 14. San Diego: Elsevier; 2005. pp. 15–38.
10. Mlodzik M. Planar cell polarization: do the same mechanisms regulate Drosophila tissue polarity and vertebrate gastrulation? Trends Genet 2002; 18:564–571.
11. Adler P. Planar polarity in the Drosophila wing. In: Mlodzik M, editor. Planar cell polarization during development. Vol. 14. San Diego: Elsevier; 2005. pp. 1–14.
12. Keller R. Shaping the vertebrate body plan by polarized embryonic cell movements. Science 2002; 298:1950–1954.
13. Lawrence P. Q & A. Curr Biol 2003; 13(3):R82.
14. Adler PN. The genetic control of tissue polarity in Drosophila. Bioessays 1992; 14(11):735–741.
15. Fanto M, Weber U, Strutt DI, Mlodzik M. Nuclear signaling by Rac abd Rho GTPases is required in the establishment of epithelial planar polarity in the Drosophila eye. Curr Biol 2000; 10(16):979–988.
16. Eaton S. Cell biology of planar polarity transmission in the Drosophila wing. Mech Dev 2003; 120:1257–1264.
17. Lawrence PA, Casal J, Struhl G. Cell interactions and planar polarity in the abdominal epidermis of Drosophila. Development 2004; 131:4651–4664.
18. Lawrence PA, Casal J, Struhl G. Towards a model of the organisation of planar polarity and pattern in the Drosophila abdomen. Development 2002; 129:2749–2760.
19. Casal J, Struhl G, Lawrence PA. Developmental compartments and planar polarity in Drosophila. Curr Biol 2002; 12:1189–1198.
20. Strutt H, Strutt D. Long-range coordination of planar polarity patterning in Drosophila. In: Mlodzik M, editor. Planar cell polarization during development. Vol. 14. San Diego: Elsevier, 2005; pp. 39–58.
21. Roegiers F, Younger-Shepherd S, Jan LY, et al. Two types of asymmetric divisions in the Drosophila sensory organ precursor cell lineage. Nat Cell Biol 2001; 3:58–67.
22. Bellaiche Y, Gho M, Kaltschmidt JA, et al. Frizzled regulates localization of cell-fate determinants and mitotic spindle rotation during asymmetric cell division. Nat Cell Biol 2001; 3(1):50–57.
23. Gho M, Schweisguth F. Frizzled signalling controls orientation of asymmetric sense organ precursor cell divisions in Drosophila. Nature 1998; 393:178–181.
24. Le Borgne R, Schweisguth F. Unequal segregation of neuralized biases Notch activation during asymmetric cell division. Dev Cell 2003; 5:139–148.
25. Wolff T, Ready DF. The beginning of pattern formation in the Drosophila compound eye: the morphogenetic furrow and the second mitotic wave. Development 1991; 113:841–850.
26. Wolff T, Ready DF. Pattern formation in the Drosophila retina. In: Martinez-Arias MBA, editor. The development of Drosophila melanogaster. Cold Spring Harbor (NY): Cold Spring Harbor Press; 1993. pp. 1277–1326.
27. Clandinin TR, Zipursky SL. Making connections in the fly visual system. Neuron 2002; 35:827–841.
28. Heberlein U, Moses K. Mechanisms of Drosophila retinal morphogenesis: the virtues of being progressive. Cell 1995; 81:987–990.
29. Treisman JE, Heberlein U. Eye development in Drosophila: formation of the eye field and control of differentiation. Curr Top Dev Biol 1998; 39:119–158.

30. Tomlinson A. Cellular interactions in the developing Drosophila eye. Development 1988; 104:183–193.
31. Tomlinson A, Ready DF. Cell fate in the Drosophila ommatidium. Dev Biol 1987; 123:264–275.
32. Reifegerste R, Moses K. The genetics of epithelial polarity and pattern in the Drosophila retina. Bioessays 1999; 21:275–285.
33. Mlodzik M. Planar polarity in the Drosophila eye: a multifaceted view of signaling specificity and cross-talk. EMBO J. 1999; 18(24):6873–6879.
34. Mollereau B, Domingos PM. Photoreceptor differentiation in Drosophila: from immature neurons to functional photoreceptors. Dev Dyn 2005; 232(3):585–592.
35. Mlodzik M, Hiromi Y, Weber U, et al. The Drosophila seven–up gene, a member of the steroid receptor gene superfamily, controls photoreceptor cell fates. Cell 1990; 60:211–224.
36. Fanto M, Mayes CA, Mlodzik M. Linking cell-fate specification to planar polarity: determination of the R3/R4 photoreceptors is a prerequisite for the interpretation of the Frizzled mediated polarity signal. Mech Dev 1998; 74:51–58.
37. Hiromi Y, Mlodzik M, West SR, et al. Ectopic expression of seven-up causes cell fate changes during ommatidial assembly. Development 1993; 118:1123–1135.
38. Cooper MTD, Bray SJ. Frizzled regulation of Notch signalling polarizes cell fate in the Drosophila eye. Nature 1999; 397:526–529.
39. Domingos PM, Mlodzik M, Mendes CS, et al. Spalt transcription factors are required for R3/R4 specification and establishment of planar cell polarity in the Drosophila eye. Development 2004; 131:5695–5702.
40. Zheng L, Zhang J, Carthew RW. frizzled regulates mirror-symmetric pattern formation in the Drosophila eye. Development 1995; 121:3045–3055.
41. Wolff T, Rubin GM. strabismus, a novel gene that regulates tissue polarity and cell fate decisions in Drosophila. Development 1998; 125:1149–1159.
42. Tomlinson A, Struhl G. Decoding vectorial information from a gradient: sequential roles of the receptors Frizzled and Notch in establishing planar polarity in the Drosophila eye. Development 1999; 126:5725–5738.
43. Jenny A, Reynolds-Kenneally J, Das G, et al. Diego and Prickle regulate Frizzled planar cell polarity signalling by competing for Dishevelled binding. Nat Cell Biol 2005; 7:691–697.
44. Jenny A, Darken RS, Wilson PA, et al. Prickle and Strabismus form a functional complex to generate a correct axis during planar cell polarity signaling. EMBO J 2003; 22:4409–4420.
45. Das G, Reynolds-Kenneally J, Mlodzik M. The atypical cadherin flamingo links frizzled and notch signaling in planar polarity establishment in the Drosophila eye. Dev Cell 2002; 2:655–666.
46. Wehrli M, Tomlinson A. Epithelial planar polarity in the developing Drosophila eye. Development 1995; 121:2451–2459.
47. Wehrli M, Tomlinson A. Independent regulation of anterior/posterior and equatorial/polar polarity in the Drosophila eye; evidence for the involvement of Wnt signaling in the equatorial/polar axis. Development 1998; 125:1421–1432.
48. Singh A, Lim J, Choi K. Dorsoventral boundary for organizing growth and planar polarity in the Drosophila eye. In: Mlodzik M, editor. Planar cell polarization during development. Vol. 14. San Diego: Elsevier; 2005. pp. 59–90.
49. Vinson CR, Adler PN. Directional non-cell autonomy and the transmission of polarity information by the frizzled gene of Drosophila. Nature 1987; 329:549–551.
50. Vinson CR, Conover S, Adler PN. A Drosophila tissue polarity locus encodes a protein containing seven potential transmembrane domains. Nature 1989; 338:263–264.
51. Axelrod JD, Miller JR, Shulman JM, et al. Differential recruitment of Dishevelled provides signaling specificity in the planar cell polarity and Wingless signaling pathways. Genes Dev 1998; 12(16):2610–2622.
52. Boutros M, Paricio N, Strutt DI, et al. Dishevelled activates JNK and discriminates between JNK pathways in planar polarity and wingless signaling. Cell 1998; 94:109–118.
53. Klingensmith J, Nusse R, Perrimon N. The Drosophila segment polarity gene dishevelled encodes a novel protein required for response to the wingless signal. Genes Dev 1994; 8(1):118–130.
54. Wong HC, Bourdelas A, Krauss A, et al. Direct binding of the PDZ domain of Dishevelled to a conserved internal sequence in the C-terminal region of Frizzled. Mol Cell 2003; 12(5):1251–1260.
55. Strutt DI, Weber U, Mlodzik M. The role of RhoA in tissue polarity and Frizzled signalling. Nature 1997; 387:292–295.
56. Paricio N, Feiguin F, Boutros M, et al. The Drosophila STE20-like kinase Misshapen is required downstream of the Frizzled receptor in planar polarity signaling. EMBO J 1999; 18:4669–4678.
57. Weber U, Paricio N, Mlodzik M. Jun mediates Frizzled induced R3/R4 cell fate distinction and planar polarity determination in the Drosophila eye. Development 2000; 127:3619–3629.
58. Fanto M, Mlodzik M. Asymmetric Notch activation specifies photoreceptors R3 and R4 and planar polarity in the Drosophila eye. Nature 1999; 397:523–526.
59. Zeidler MP, Perrimon N, Strutt DI. Multiple roles for four-jointed in planar polarity and limb patterning. Dev Biol 2000; 228:181–196.
60. Zeidler MP, Perrimon N, Strutt DI. The four-jointed gene is required in the Drosophila eye for ommatidial polarity specification. Curr Biol 1999; 9:1363–1372.
61. Yang C, Axelrod JD, Simon MA. Regulation of frizzled by fat-like cadherins during planar polarity signaling in the drosophila compound eye. Cell 2002; 108:675–688.
62. Rawls AS, Guinto JB, Wolff T. The cadherins fat and dachsous regulate dorsal/ventral signaling in the Drosophila eye. Curr Biol 2002; 12:1021–1026.
63. Simon MA. Planar cell polarity in the Drosophila eye is directed by graded Four-jointed and Dachsous expression. Development 2004; 131:6175–6184.
64. Ma D, Yang CH, McNeill H, et al. Fidelity in planar cell polarity signalling. Nature 2003; 421:543–547.
65. Matakatsu H, Blair SS. Interactions between Fat and Dachsous and the regulation of planar cell polarity in the Drosophila wing. Development 2004; 131:3785–3794.
66. Chae J, Kim MJ, Goo JH, et al. The Drosophila tissue polarity gene starry night encodes a member of the protocadherin family. Development 1999; 126:5421–5429.
67. Usui T, Shima Y, Shimada Y, et al. Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of Frizzled. Cell 1999; 98:585–595.
68. Taylor J, Abramova N, Charlton J, et al. Van Gogh: a new Drosophila tissue polarity gene. Genetics 1998; 150:199–210.
69. Feiguin F, Hannus M, Mlodzik M, et al. The Ankyrin repeat protein Diego mediates Frizzled-dependent planar polarization. Developmental Cell 2001; 1:93–101.
70. Gubb D, Green C, Huen D, et al. The balance between isoforms of the prickle LIM domain protein is critical for planar polarity in Drosophila imaginal discs. Genes Dev 1999; 13:2315–2327.
71. Heitzler P, Coulson D, Saenz-Robles MT, et al. Genetic and cytogenetic analysis of the 43A-E region containing the segment polarity gene costa and the cellular polarity genes prickle and spiny-legs in Drosophila melanogaster. Genetics 1993; 135:105–115.

72. Tree DR, Shulman JM, Rousset R, et al. Prickle mediates feedback amplification to generate asymmetric planar cell polarity signaling. Cell 2002; 109:371–381.
73. Bastock R, Strutt H, Strutt D. Strabismus is asymmetrically localised and binds to Prickle and Dishevelled during Drosophila planar polarity patterning. Development 2003; 130:3007–3014.
74. Ciruna B, Jenny A, Lee D, et al. Planar cell polarity signalling couples cell division and morphogenesis during neurulation. Nature 2006; 439(7073):220–224.
75. Tomlinson A, Struhl G. Delta/notch and boss/sevenless signals act combinatorially to specify the Drosophila R7 photoreceptor. Molecular Cell 2001; 7:487–495.
76. Adler PN. Planar signaling and morphogenesis in Drosophila. Dev Cell 2002; 2:525–535.
77. Winter CG, Wang B, Ballew A, et al. Drosophila Rho-associated kinase (Drok) links Frizzled-mediated planar cell polarity signaling to the actin cytoskeleton. Cell 2001; 105:81–91.
78. Choi K-W, Benzer S. Rotation of photoreceptor clusters in the developing Drosophila eye requires the nemo gene. Cell 1994; 78(1):125–136.
79. Gaengel K, Mlodzik M. Egfr signaling regulates ommatidial rotation and cell motility in the Drosophila eye via MAPK/Pnt signaling and the Ras effector Canoe/AF6. Development 2003; 130(22):5413–5423.
80. Brown KE, Freeman M. Egfr signalling defines a protective function for ommatidial orientation in the Drosophila eye. Development 2003; 130:5401–5412.
81. Strutt H, Strutt D. EGF signaling and ommatidial rotation in the Drosophila eye. Curr Biol 2003; 13:1451–1457.
82. Guo N, Hawkins C, Nathans J. From The cover: Frizzled6 controls hair patterning in mice. Proc Natl Acad Sci U S A 2004; 101:9277–9281.
83. Jessen JR, Solnica-Krezel L. Morphogenetic movements shaping the zebrafish gastrula. In: Mlodzik M, editor. Planar cell polarization during development. Vol. 14. San Diego: Elsevier; 2005. pp. 131–166.
84. Murdoch JN, Doudney K, Paternotte C, et al. Severe neural tube defects in the loop-tail mouse result from mutation of Lpp1, a novel gene involved in floor plate specification. Hum Mol Genet 2001; 10:2593–2601.
85. Kibar Z, Vogan K, Groulx N, et al. 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.
86. Curtin JA, Quint E, Tsipouri V, et al. Mutation of Celsr1 disrupts planar polarity of inner ear hair cells and causes severe neural tube defects in the mouse. Curr Biol 2003; 13:1129–1133.
87. Wang J, Mark S, Zhang X, et al. Regulation of polarized extension and planar cell polarity in the cochlea by the vertebrate PCP pathway. Nat Genet 2005; 37:980–985.
88. Dabdoub A, Donohue MJ, Brennan A, et al. Wnt signaling mediates reorientation of outer hair cell stereociliary bundles in the mammalian cochlea. Development 2003; 130:2375–2384.
89. Wang Y, Guo N, Nathans J. The role of Frizzled3 and Frizzled6 in neural tube closure and in the planar polarity of inner-ear sensory hair cells. J Neurosci 2006; 26:2147–2156.
90. Lu X, Borchers AG, Jolicoeur C, et al. PTK7/CCK-4 is a novel regulator of planar cell polarity in vertebrates. Nature 2004; 430:93–98.
91. Montcouquiol M, Rachel RA, Lanford PJ, et al. Identification of Vangl2 and Scrb1 as planar polarity genes in mammals. Nature 2003; 423:173–177.
92. Keller R. Cell migration during gastrulation. Curr Opin Cell Biol 2005; 17:533–541.
93. Jessen JR, Topczewski J, Bingham S, et al. Zebrafish trilobite indentifies new roles for Strabismus in gastrulation and neuronal movements. Nat Cell Biol 2002; 4:610–615.
94. Heisenberg CP, Tada M, Rauch GJ, et al. Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. Nature 2000; 405:76–81.
95. Kilian B, Mansukoski H, Barbosa FC, et al. The role of Ppt/Wnt5 in regulating cell shape and movement during zebrafish gastrulation. Mech Dev 2003; 120:467–476.
96. Wallingford JB, Goto T, Keller R, et al. Cloning and expression of Xenopus Prickle, an orthologue of a Drosophila planar cell polarity gene. Mech Dev 2002; 116:183–186.
97. Veeman MT, Slusarski DC, Kaykas A, et al. Zebrafish prickle, a modulator of noncanonical wnt/fz signaling, regulates gastrulation movements. Curr Biol 2003; 13:680–685.
98. Takeuchi M, Nakabayashi J, Sakaguchi T, et al. The prickle-related gene in vertebrates is essential for gastrulation cell movements. Curr Biol 2003; 13:674–679.
99. Formstone CJ, Mason I. Combinatorial activity of Flamingo proteins directs convergence and extension within the early zebrafish embryo via the planar cell polarity pathway. Dev Biol 2005; 282:320–335.
100. Yamanaka H, Moriguchi T, Masuyama N, et al. JNK functions in the non-canonical Wnt pathway to regulate convergent extension movements in vertebrates. EMBO Rep 2002; 3(1):69–75.
101. Copp AJ, Greene ND, Murdoch JN. The genetic basis of mammalian neurulation. Nat Rev Genet 2003; 4:784–793.
102. Copp AJ, Greene ND, Murdoch JN. Dishevelled: linking convergent extension with neural tube closure. Trends Neurosci 2003; 26:453–455.
103. Gong Y, Mo C, Fraser SE. Planar cell polarity signalling controls cell division orientation during zebrafish gastrulation. Nature 2004; 430:689–693.
104. Axelrod JD. Unipolar membrane association of Dishevelled mediates Frizzled planar cell polarity signaling. Genes Dev 2001; 15:1182–1187.
105. Das G, Jenny A, Klein TJ, et al. Diego interacts with Prickle and Strabismus/Van Gogh to localize planar cell polarity complexes. Development 2004; 131:4467–4476.
106. Strutt D, Johnson R, Cooper K, et al. Asymmetric localization of frizzled and the determination of notch-dependent cell fate in the Drosophila eye. Curr Biol 2002; 12:813–824.
107. Strutt DI. Asymmetric localization of frizzled and the establishment of cell polarity in the Drosophila wing. Molecular Cell 2001; 7:367–375.
108. Wu J, Klein TJ, Mlodzik M. Subcellular localization of frizzled receptors, mediated by their cytoplasmic tails, regulates signaling pathway specificity. PLoS Biol 2004; 2(7):E158.
109. Bellaiche Y, Beaudoin-Massiani O, Stuttem I, et al. The planar cell polarity protein Strabismus promotes Pins anterior localization during asymmetric division of sensory organ precursor cells in Drosophila. Development 2004; 131:469–478.
110. Lu B, Usui T, Uemura T, et al. Flamingo controls the planar polarity of sensory bristles and asymmetric division of sensory organ precursors in Drosophila. Curr Biol 1999; 9:1247–1250.
111. Montcouquiol M, Sans N, Huss D, et al. Asymmetric localization of Vangl2 and Fz3 indicate novel mechanisms for planar cell polarity in mammals. J Neurosci 2006; 26:5265–5275.
112. Amonlirdviman K, Khare NA, Tree DR, et al. Mathematical modeling of planar cell polarity to understand domineering nonautonomy. Science 2005; 307:423–426.

113. Axelrod JD, Miller JR, Shulman JM, et al. Differential recruitment of Dishevelled provides signaling specificity in the planar cell polarity and Wingless signaling pathways. Genes Dev 1998; 12(16):2610–2622.
114. Rothbacher U, Laurent MN, Deardorff MA, et al. Dishevelled phosphorylation, subcellular localization and multimerization regulate its role in early embryogenesis. EMBO J 2000; 19:1010–1022.
115. Boutros M, Mihaly J, Bouwmeester T, et al. Signaling specificity by Frizzled receptors in Drosophila. Science 2000; 288:1825–1828.
116. Katanaev VL, Ponzielli R, Semeriva M, et al. Trimeric G protein-dependent frizzled signaling in Drosophila. Cell 2005; 120:111–122.
117. Hannus M, Feiguin F, Heisenberg CP, et al. Planar cell polarization requires Widerborst, a B' regulatory subunit of protein phosphatase 2A. Development 2002; 129:3493–3503.
118. Djiane A, Yogev S, Mlodzik M. The apical determinants aPKC and dPatj regulate Frizzled-dependent planar cell polarity in the Drosophila eye. Cell 2005; 121:621–631.
119. Simons M, Gloy J, Ganner A, et al. Inversin, the gene product mutated in nephronophthisis type II, functions as a molecular switch between Wnt signaling pathways. Nat Genet 2005; 37:537–543.
120. Ross AJ, May-Simera H, Eichers ER, et al. Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. Nat Genet 2005; 37:1135–1140.
121. Fischer E, Legue E, Doyen A, et al. Defective planar cell polarity in polycystic kidney disease. Nat Genet 2006; 38(1):21–23.
122. Morgan D, Turnpenny L, Goodship J, et al. Inversin, a novel gene in the vertebrate left-right axis pathway, is partially deleted in the inv mouse. Nat Genet 1998; 20:149–156.
123. Mochizuki T, Saijoh Y, Tsuchiya K, et al. Cloning of inv, a gene that controls left/right asymmetry and kidney development. Nature 1998; 395:177–181.
124. Otto EA, Schermer B, Obara T, et al. Mutations in INVS encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. Nat Genet 2003; 34:413–420.
125. Schwarz-Romond T, Asbrand C, Bakkers J, et al. The ankyrin repeat protein Diversin recruits Casein kinase Iepsilon to the beta-catenin degradation complex and acts in both canonical Wnt and Wnt/JNK signaling. Genes Dev 2002; 16(16):2073–2084.
126. Watanabe D, Saijoh Y, Nonaka S, et al. The left-right determinant Inversin is a component of node monocilia and other 9+0 cilia. Development 2003; 130:1725–1734.
127. Germino GG. Linking cilia to Wnts. Nat Genet 2005; 37:455–457.
128. Beales PL. Lifting the lid on Pandora's box: the Bardet-Biedl syndrome. Curr Opin Genet Dev 2005; 15:315–323.
129. Deardorff MA, Tan C, Conrad LJ, et al. Frizzled-8 is expressed in the Spemann organizer and plays a role in early morphogenesis. Development 1998; 125:2687–2700.
130. Djiane A, Riou J, Umbhauer M, et al. Role of frizzled 7 in the regulation of convergent extension movements during gastrulation in Xenopus laevis. Development 2000; 127:3091–3100.
131. Umbhauer M, Djiane A, Goisset C, et al. The C-terminal cytoplasmic Lys-thr-X-X-X-Trp motif in frizzled receptors mediates Wnt/beta-catenin signalling. EMBO J 2000; 19:4944–4954.
132. Wallingford JB, Rowning BA, Vogeli KM, et al. Dishevelled controls cell polarity during Xenopus gastrulation. Nature 2000; 405:81–85.
133. Theisen H, Purcell J, Bennett M, et al. dishevelled is required during wingless signalling to establish both cell polarity and cell identity. Development 1994; 120:347–360.
134. Tada M, Smith JC. Xwnt11 is a target of Xenopus Brachyury: regulation of gastrulation movements via Dishevelled, but not through the canonical Wnt pathway. Development 2000; 127(10):2227–2238.
135. Shimada Y, Usui T, Yanagawa S, et al. Asymmetric colocalization of Flamingo, a seven-pass transmembrane cadherin, and Dishevelled in planar cell polarization. Curr Biol 2001; 11:859–863.
136. Darken RS, Scola AM, Rakeman AS, et al. The planar polarity gene strabismus regulates convergent extension movements in Xenopus. EMBO J 2002; 21(5):976–985.
137. Goto T, Keller R. The planar cell polarity gene strabismus regulates convergence and extension and neural fold closure in Xenopus. Dev Biol 2002; 247:165–181.
138. Park M, Moon RT. The planar cell polarity gene stbm regulates cell behaviour and cell fate in vertebrate embryos. Nat Cell Biol 2002; 4:20–25.
139. Fanto M, Clayton L, Meredith J, et al. The tumor-suppressor and cell adhesion molecule Fat controls planar polarity via physical interactions with Atrophin, a transcriptional co-repressor. Development 2003; 130:763–774.
140. Strutt H, Mundy J, Hofstra K, et al. Cleavage and secretion is not required for Four-jointed function in Drosophila patterning. Development 2004; 131:881–890.
141. Tahinci E, Symes K. Distinct functions of Rho and Rac are required for convergent extension during Xenopus gastrulation. Dev Biol 2003; 259:318–335.
142. Marlow F, Topczewski J, Sepich D, et al. Zebrafish rho kinase 2 acts downstream of wnt11 to mediate cell polarity and effective convergence and extension movements. Curr Biol 2002; 12:876–884.