Published in final edited form as:

Curr Top Dev Biol. 2010 ; 93: 189–227. doi:10.1016/B978-0-12-385044-7.00007-2.

Planar Cell Polarity Signaling in the *Drosophila* Eye

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Abstract

Planar cell polarity (PCP) signaling regulates the establishment of polarity within the plane of an epithelium and allows cells to obtain directional information. Its results are as diverse as the determination of cell fates, the generation of asymmetric but highly aligned structures (e.g., stereocilia in the human ear or hairs on a fly wing), or the directional migration of cells during convergent extension during vertebrate gastrulation. Aberrant PCP establishment can lead to human birth defects or kidney disease. PCP signaling is governed by the noncanonical Wnt or Fz/PCP pathway. Traditionally, PCP establishment has been best studied in *Drosophila*, mainly due to the versatility of the fly as a genetic model system.

In *Drosophila*, PCP is essential for the orientation of wing and abdominal hairs, the orientation of the division axis of sensory organ precursors, and the polarization of ommatidia in the eye, the latter requiring a highly coordinated movement of groups of photoreceptor cells during the process of ommatidial rotation. Here, I review our current understanding of PCP signaling in the *Drosophila* eye and allude to parallels in vertebrates.

1. Introduction

Epithelia are polarized along the apical–basal axis in order to allow for directional transport of proteins within a cell or secretion of factors into lumina (Dow and Humbert, 2007; Wodarz and Nathke, 2007). In addition, most epithelia are also polarized within the epithelial plane. The latter polarization is called epithelial planar cell polarity (PCP) or tissue polarity. Planar polarization provides a cell not only with positional information but also directional (vectorial) information and correct PCP is a prerequisite for the formation of many organs.

Planar polarization can be very obvious: feathers of birds are nicely aligned; similarly, the fur of animals or hair on human skin is oriented in parallel. Less obvious examples of PCP are found in the organ of Corti in the mammalian inner ear, in which sensory neurons align their ciliary structures in a stereotypic fashion from cell to cell, a prerequisite for a proper response to sound (reviewed in Dabdoub *et al.*, 2005; Jenny and Mlodzik, 2006; Simons and Mlodzik, 2008; Vladar *et al.*, 2009). However, the consequences of PCP not only manifest in

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The effect of PCP signaling on cilia has very recently been shown to be crucial for the establishment of left-right asymmetry in mice and zebrafish (Borovina *et al.* 2010; Song *et al.* 2010). Furthermore, the Golgi resident kinase Fj was shown to phosphorylate the extracellular domains of Ft and Ds, thereby changing the affinity for each other (Brittle *et al.* 2010; Simon *et al.*, 2010).

aligned cytoskeleton-derived structures but can also include cell fate decisions (as in the *Drosophila* eye) or the directed migration of single or groups of cells. To date, the most clinically relevant process shown to be affected by PCP signaling is convergent extension during vertebrate gastrulation and neurulation, during which mesenchymal cells migrate with respect to one another and toward the embryonic midline, where they intercalate, leading to a narrowing and elongation of the body axis (Keller *et al.*, 2000; Yin *et al.*, 2009). Aberrant convergent-extension results in neural folds that are spaced too far apart to correctly close and thus neural tube closure defects, one of the most frequent human birth defects (1–2 infants per 1000; Copp *et al.*, 2003; Doudney and Stanier, 2005). Indeed, mutations in the *vangl1* gene, one of the key players of PCP signaling discussed below, have been identified in spina bifida patients (Kibar *et al.*, 2007, 2009; Reynolds *et al.*, 2010). In addition, defects related to PCP signaling were shown to be responsible for certain cystic kidney diseases in humans and mice due to cilia-related malfunctions (Nephronophthysis and Bardet–Biedl syndrome; Ross *et al.*, 2005; Simons and Mlodzik, 2008; Simons *et al.*, 2005) or aberrant cell division axis orientation and cell migration (Karner *et al.*, 2009; McNeill, 2009; Saburi *et al.*, 2008).

PCP, however, is best studied in *Drosophila melanogaster*, mainly due to the ease of the fly as a model system. In *Drosophila*, PCP is externally visible in the alignment of sensory bristles and hairs (trichomes) on the thorax and abdomen, as well as the wing, where each cell produces a single hair pointing toward the distal tip (Adler, 2005; Lawrence *et al.*, 2007). Arguably the most beautifully polarized structure of *Drosophila* is the eye, with its roughly 800 precisely aligned facets. Facets or ommatidia are the building blocks of insect eyes and are meticulously oriented with respect to each other and to the general axes of the eye (Fig. 7.1). In this review, I will discuss the establishment of PCP in the *Drosophila* eye, which not only involves cytoskeletal rearrangements but also transcriptional responses, cell fate decisions, and directional movement (rotation) of groups of cells.

Most PCP phenomena studied to date, in *Drosophila* and vertebrates, are dependent on the noncanonical Wnt, a.k.a. Frizzled (Fz)–PCP pathway discussed in detail below. However, it is important to note that other mechanisms of planar polarization exist. For instance, Myosin II and Par3 (*bazooka* in flies) are subcellularly polarized during cell intercalation in *Drosophila* gastrulation independent of the Fz–PCP pathway and the interested reader is referred to reviews for further information (Lecuit, 2005; Lecuit and Lenne, 2007; Wirtz-Peitz and Zallen, 2009; Zallen and Blankenship, 2008).

2. PCP in the *Drosophila* Eye

2.1. *Drosophila* eye development

The adult *Drosophila* eye consists of some 800 ommatidia that develop during larval and pupal stages (reviewed in Singh *et al.*, 2005; Wolff and Ready, 1993). Each ommatidium is made up of 20 cells, including eight photoreceptors (PR or R-cells; Fig. 7.1), four lens-secreting cone cells, and bristle and pigment cells. The photoreceptors of each ommatidium—most easily identified by their rhabdomeres, the light-sensitive organelles (inset in Fig. 7.1B)—are organized in a trapezoid made up of the six “outer” (R1–6) and two “inner” photoreceptors (R7/8; R8 is underneath R7 and is not seen in the same plane as R7 in adult

eye sections). The trapezoid of each facet is precisely aligned with those of its neighbors and the overall anteroposterior and dorsoventral axes of the eye. Furthermore, the ommatidia of the dorsal and ventral (black and red arrows, respectively, in Fig. 7.1) eye hemispheres are mirror images of each other and correspond to two chiral forms. This elaborate arrangement is the result of PCP signaling during the 3rd instar larval stage and is essential for proper image formation after wiring of the photoreceptor axons to the underlying lamina, medulla, and brain lobes (see also Chapter 8). Due to the curvature of the eye, individual photoreceptors of an ommatidium point to different areas in space. In order to assemble a correct picture, corresponding photoreceptors of neighboring ommatidia that “see” the same point in space are wired together to represent a single point on the retinotopic map in the brain (Borst, 2009; Clandinin and Zipursky, 2002). In PCP mutants, ommatidia mostly form properly but their chirality and orientation are randomized and visual information thus cannot be properly processed (Fig. 7.1C, D).

The *Drosophila* eye develops from the eye imaginal disk which is initially specified by the *eyeless/Pax6* gene and its associated gene regulatory network (e.g., *eyes absent*, *dachsous*, *sine-oculis*, etc.; Desplan, 1997; Pappu and Mardon, 2004; Treisman and Heberlein, 1998; recently reviewed in this series: Cagan, 2009; see also Chapter 1). During early larval development, *wingless (wg)* expression is induced at the dorsal tip of the eye disk by the GATA factor *pannier* (Cavodeassi *et al.*, 1999; Heberlein *et al.*, 1998). Canonical Wg signaling (see below) then induces the homeobox genes of the Iroquois complex thereby specifying dorsal cell fates in the eye disk. Ultimately, this leads to the activation of Notch (N) along the midline (equator) of the eye separating dorsal from ventral and controlling overall growth of the eye disk.

During the early 3rd larval instar, *wg* is expressed at both poles of the eye disk and forms an activity gradient that is lowest at the equator (Singh *et al.*, 2005; Wehrli and Tomlinson, 1998). At this stage, canonical Wg signaling causes long range, mirror image polarization of the dorsal and ventral eye hemispheres. Clones, patches of mutant cells surrounded by wild-type tissue, of Wg pathway components such as the coreceptor *Lrp6/arrow* lead to the induction of ectopic equators and thus polarity reversals of whole fields of ommatidia (Singh *et al.*, 2005; Wehrli and Tomlinson, 1998). In addition, Wg emanating from the poles is required for proper separation of the eye field from head cuticle structures by maintaining *ey* expression anterior to the morphogenetic furrow (MF; Baonza and Freeman, 2002; Lee and Treisman, 2001; Royet and Finkelstein, 1997).

2.2. Establishment of ommatidial polarity

The MF is an indentation in the eye disk epithelium that functionally corresponds to the site of a switch from cell proliferation to differentiation. Like a wave of differentiation, it sweeps from posterior to anterior across the eye imaginal disk, leaving in its wake rows of differentiating photoreceptor clusters. Roughly every 2 h, a new row of PR clusters is specified (Campos-Ortega and Hofbauer, 1977; Tomlinson and Ready, 1987b). The eye imaginal disk thus represents at any moment a “natural time-course” of differentiation.

R-cell recruitment from the pool of undifferentiated cells occurs in a highly stereotypic fashion (reviewed in Baker, 2001; Roignant and Treisman, 2009). The first photoreceptor

specified is R8 in a process strongly dependent on Notch signaling, followed by EGFR-dependent, pairwise, recruitment of R2/5 and R3/4. Together with R8, the R2/5 and R3/4 equivalence pairs form the five-cell precluster (Figs. 7.1 and 7.2A; note that preclusters can transiently contain one or two “mystery cells”; Tomlinson and Ready, 1987b). The precluster emerging from the MF is initially arc shaped with R3/4 at the outermost positions (Wolff and Ready, 1991). Upon maturation, the precluster tightens up and R3/4 come into contact (Fig. 7.2A). The precluster is subsequently joined by the R1/6 pair and R7. Accessory cells are recruited last (for more information about cell fate decisions see Chapter 6). Thus, ommatidia are generated by recruitment of cells from pools of undifferentiated, clonally (almost) unrelated cells, a fact that has proven enormously helpful to analyze the mechanism of PCP signaling.

Around the stage of PCP signaling, the five-cell precluster is symmetric with respect to the R3/4 cells, with the R3 precursor closer to the equator and the R4 precursor abutting R3 on the polar side (red and blue in Fig. 7.1A, respectively). The immature five-cell preclusters start to rotate in opposing directions on the dorsal (clockwise) and ventral (counterclockwise) sides of the equator once they are about four to five rows away from the MF (Tomlinson and Ready, 1987a; Wolff and Ready, 1991; reviewed in Jenny and Mlodzik, 2006; Mlodzik, 2005; Seifert and Mlodzik, 2007). After a first fast phase of rotation by 45°, ommatidial rotation slows down. Around rows 8/9 after the MF, undifferentiated cells divide once more (“second mitotic wave”) and R1/6/7 are recruited from these newly divided cells (Ready *et al.*, 1976; Wolff and Ready, 1991). The ommatidia then rotate more slowly until they complete a full 90° turn with respect to their initial orientation when they were near the MF (Figs. 7.1 and 7.2). The precise organization, as well as timing and extent of ommatidial rotation has been followed by histochemical staining and more recently by examining apical markers (such as E-Cadherin; Figs. 7.2 and 7.5) or markers that reveal specific R-cell fates.

A central step in PCP signaling is the cell fate specification of the R3 versus the R4 cells, the precursors of which initially are equivalent. This cell fate choice sets the basis for the opposite direction of rotation of photoreceptor clusters on the dorsal and ventral sides of the equator, and for the two chiral forms of ommatidia in the adult eye with their asymmetric position of R3 at the polar-anterior, and R4 posterior-equatorial position (Fig. 7.1). Indeed, this asymmetry can already be detected by molecular markers such as pipsqueak-GFP (enriched in R3) or *mδ-lacZ* (expressed in R4) earlier during the rotation process (Cooper and Bray, 1999; Weber *et al.*, 2008). Even though mutations in PCP genes can uncouple the rotation direction from the chirality choice, the R3/R4 decision probably determines the direction of rotation. Evidence for this comes from the finding that in *pk^{spiny-leg1}* mutants, an allele of the core PCP gene *pk* (see below), the chirality is fully randomized, but ommatidia rotate in the direction appropriate for their acquired chirality (Gubb *et al.*, 1999; Jenny *et al.*, 2003).

3. The Core PCP Pathway and Its Effectors

Most components known to be involved in PCP establishment were initially identified in genetic screens in *Drosophila* and thus were genetically characterized before their molecular interplay was analyzed. Generally, PCP relevant factors fall into three categories (Fig. 7.3;

Sopko and McNeill, 2009; Strutt and Strutt, 2009; Vladar *et al.*, 2009). First, the so-called Fat and Dachshous module appears responsible for a more global polarization of eye, wing, and abdominal tissues. Second, the core PCP genes such as *frizzled* (*fz*) comprise another module required for the establishment of polarity in all tissues. They regulate the third category of genes, the tissue-specific effectors. These “secondary polarity genes” allow for the appropriate tissue-specific responses such as the growth of an actin hair at the distal end of a wing cell (e.g., *inturned* or *multiple wing hairs*) or correct ommatidial rotation (e.g., the *nemo* gene coding for a kinase that is specifically required only for rotation; see also Table 7.1).

3.1. The core PCP module

PCP signaling is controlled by the noncanonical Wnt pathway, which shares some of its core components with the canonical Wnt/Wg pathway. During canonical signaling, a Wnt ligand activates a Fz/Lrp receptor/coreceptor complex leading, via the adapter protein Dishevelled (Dsh), to a stabilization and nuclear translocation of the transcription cofactor β -Catenin. Generally, canonical Wnt signaling controls cell proliferation, differentiation, and embryonic axis formation (reviewed in Clevers, 2006; Sokol, 1999). Both branches of Wnt signaling share Wnt ligands (in vertebrates), Fz, and Dsh, but are otherwise distinct (Jenny and Mlodzik, 2006; Mlodzik, 2002; Veeman *et al.*, 2003).

In addition to *fz* and *dsh*, *strabismus* (*stbm*, a.k.a. *van-gogh*, *vang*), *flamingo* (*fmi*, a.k.a. *starry-night*, *stan*), *prickle* (*pk*, also known as *prickle-spiny legs*), and *diego* (*dgo*) are members of the core PCP genes (Chae *et al.*, 1999; Feiguin *et al.*, 2001; Gubb and García-Bellido, 1982; Gubb *et al.*, 1999; Krasnow and Adler, 1994; Krasnow *et al.*, 1995; Taylor *et al.*, 1998; Usui *et al.*, 1999; Vinson *et al.*, 1989; Wolff and Rubin, 1998; Zheng *et al.*, 1995). All of the core genes affect R3/4 cell fate specification and ommatidial rotation (as well as PCP in other tissues) and have orthologs in vertebrate species that affect convergent extension or inner ear development (Table 7.1; reviewed in Jenny and Mlodzik, 2006; Simons and Mlodzik, 2008; Vladar *et al.*, 2009).

Key to PCP signaling is the G-protein coupled receptor-related, seven-pass transmembrane receptor Fz, which was among the first PCP factors identified (Fig. 7.3; Krasnow and Adler, 1994; Vinson *et al.*, 1989; Wang *et al.*, 1994). Work in the wing showed that Fz is required cell autonomously and can also have nonautonomous instructive roles in PCP signaling. Initial data was consistent with the wing hairs pointing along a decreasing gradient of *fz* activity due to either a diffusible signal or a relay-like signal propagation (Adler, 2005; Axelrod, 2009b; Wu and Mlodzik, 2009). Analogously, Zheng and Carthew showed that *fz* not only affects chirality and ommatidial rotation within mutant tissue but also can influence PCP in about one row of neighboring wild-type ommatidia located on the polar (but not equatorial) side of *fz* mutant clones (Zheng *et al.*, 1995). Fully mutant *fz* eyes show a random chirality over the whole eye (see also Fig. 7.1C). However, the phenotype of *fz* clones is stronger on the polar side than on the equatorial side, consistent with an activity gradient that declines toward the poles of the eye (Zheng *et al.*, 1995) though no ligand (see below) or other mechanistic cause of a graded activity is known to date. In addition, overexpression of Fz is sufficient to induce PCP phenotypes throughout the eye (Strutt *et al.*,

1997). The results of the *fz* clonal analysis are therefore consistent with a model in which Fz is constitutively active, with its activity dampened by a repressive activity declining from the poles toward the equator. Again, although antagonists are known, to date, neither such activity nor evidence for a relay mechanism in the eye has been described.

Downstream of Fz, the signal is transduced to Dishevelled (Dsh), an adapter protein consisting of a DIX, a PDZ, and a DEP domain, followed by a less well-conserved C-terminal region. Dsh is able to interact with a variety of proteins (Wallingford and Habas, 2005) including several components of the core PCP pathway. For example, its PDZ domain binds to Fz and there is evidence for an interaction of the DEP/C-terminal region with the intracellular loops of Fz. These interactions mediate recruitment of Dsh by Fz to the plasma membrane (Axelrod *et al.*, 1998; Wong *et al.*, 2003; Wu *et al.*, 2008).

Genetically downstream of *dsh* are Rho family GTPases such as Rho and Rac (Fanto *et al.*, 2000; Strutt *et al.*, 1997), with the caveat that due to genetic redundancies only *rhoA* mutations were shown to have PCP defects such as chirality inversions and rotation defects (Hakeda-Suzuki *et al.*, 2002; Munoz-Descalzo *et al.*, 2007; Strutt *et al.*, 1997). Reduction of Rho suppressed the overexpression phenotype of Fz or Dsh at the time of PCP signaling (Boutros *et al.*, 1998; Strutt *et al.*, 1997). Furthermore, constitutively active forms of RhoA and Rac cause ommatidial rotation defects and overexpression of Rac can partially suppress the PCP-specific allele *dsh¹* (Fanto *et al.*, 2000). A direct molecular link between Dsh and Rho GTPases in flies remains elusive.

Fz has characteristics of a G-protein coupled receptor (Gilman, 1987) and the G-protein *GαO* has been reported to act just downstream of Fz in canonical Wnt signaling (Katanaev *et al.*, 2005). Although its effects on PCP signaling in the eye have not been analyzed, *GαO* loss causes PCP defects in the wing. However, the analysis of *GαO* function in PCP signaling showed that it is not simply downstream of Fz as in canonical Wnt signaling, but its relationship to the core PCP factors is more complex. For example, the nonautonomous effects of *GαO* are opposite of those due to loss of *fz* (Katanaev *et al.*, 2005).

As mentioned, a key step in PCP signaling is the determination of the R3/4 cell fates in the eye. The requirement of a gene in a particular cell can be determined by correlating mutant phenotypes of ommatidia with the genotype of single cells in ommatidia consisting of homozygous mutant and wild-type cells. Such a genetic mosaic analysis showed that *fz* is required in the R3 precursor: ommatidia with a wild-type R4 precursor and a genotypically mutant *fz* R3 precursor adopted the wrong chirality in >95% of all cases examined (Zheng *et al.*, 1995). Therefore, a Fz-signaling difference between R3/4 is instructive for cell fate (Fig. 7.4). Similar to Fz, Dsh and Diego (Dgo)—an Ankyrin containing protein—act in R3 (Feiguin *et al.*, 2001; Jenny *et al.*, 2005). On the other hand, the four-pass transmembrane protein Strabismus (Figs. 7.3 and 7.4) and the cytoplasmic protein Prickle were demonstrated to be required in R4 for the R3/4 cell fate decision (Jenny *et al.*, 2003; Wolff and Rubin, 1998; Zheng *et al.*, 1995). In general, factors that have positive effects on Fz–PCP signaling are required in R3, while “antagonists” are required in R4. Interestingly and in contrast to *fz* in the eye and wing and *stbm* in the wing, clones of cells mutant for *stbm* in the eye do not affect wild-type ommatidia and thus *stbm* does not act non-autonomously in

the eye. Nevertheless, mosaic ommatidia with a wild-type R3 precursor but a *stbm* mutant R4 precursor will adopt the wrong chirality, indicating that Stbm can antagonize and override a Fz signal (Wolff and Rubin, 1998).

Flamingo (Fmi, also known as Starry-night or Stan) is another component of the core PCP machinery. *fmi* encodes an atypical cadherin with seven transmembrane domains capable of promoting homotypic cell adhesion (Casal *et al.*, 2006; Chae *et al.*, 1999; Usui *et al.*, 1999). Fmi's function in cell adhesion has not been explored, but its loss causes typical PCP defects in the eye, wing, and on the abdomen and thorax (Chae *et al.*, 1999; Das *et al.*, 2002; Usui *et al.*, 1999). In the wing, Fmi antagonizes Fz–PCP signaling (Usui *et al.*, 1999) but its role in the eye is more complicated. Fmi is required in R3 and in R4, consistent with its ability to form homotypic interactions (Fig. 7.4; Das *et al.*, 2002). Initially, Fmi is required for proper membrane localization of Dsh, which could also explain its requirement in R3 (see also below).

Recently, protein–protein interactions between core PCP components were analyzed in more detail in both vertebrates and *Drosophila*. Interestingly, the extracellular domain of Fz can bind to the extracellular surface of Stbm (Wu and Mlodzik, 2008) and Fz coimmunoprecipitated with Fmi (Chen *et al.*, 2008; Montcouquiol *et al.*, 2006). Similarly, mouse orthologs of Stbm and Fmi interacted in coimmunoprecipitation experiments (Devenport and Fuchs, 2008) and these interactions can explain some of the nonautonomous effects in PCP signaling in the wing and abdomen (see Axelrod, 2009a,b; Lawrence *et al.*, 2008; Strutt and Strutt, 2009; Wu and Mlodzik, 2009). In the eye, interactions between Fz, Stbm, and Fmi may allow communication between the R3/4 precursors of an ommatidium, explaining why R3 precursors lacking *stbm* or R4 precursors lacking *fz* always become R3 and R4 cells, respectively, in R3/4 mosaics.

Physical interactions between Dsh and Pk (Tree *et al.*, 2002), Dgo (Jenny *et al.*, 2005), and Stbm (Park and Moon, 2002) were also described, as well as interactions of Pk, Dgo, and Stbm (Bastock *et al.*, 2003; Das *et al.*, 2004; Jenny *et al.*, 2003). The interaction of Pk with Stbm is required for Pk's localization at the plasma membrane, which is lost in *stbm* mutant clones (Bastock *et al.*, 2003; Jenny *et al.*, 2003). Supporting the importance of a Pk/Stbm interaction are experiments in *Xenopus* animal cap assays showing that ectopic expression of Stbm can recruit Pk to the membrane. Furthermore, such membrane recruitment during gastrulation and neurulation in zebrafish requires the *stbm* orthologs *vangl1* and *vangl2* (Ciruna *et al.*, 2006; Jenny *et al.*, 2003). The functional consequences of a direct interaction between Dsh and Stbm are currently unknown.

It was shown that Pk can prevent Dsh recruitment to the cell membrane in U2OS cells in culture, suggesting that sequestration of Dsh from the Fz–Dsh axis could explain Pk's ability to antagonize PCP signaling (Tree *et al.*, 2002). Interestingly, along with Pk and Stbm, Dgo (and its vertebrate orthologs) also interacts with Dsh (Jenny *et al.*, 2005; Moeller *et al.*, 2006; Simons *et al.*, 2005). In *Drosophila*, the basic-PDZ region of Dsh can bind both the C-terminal region of Dgo and Pk (Jenny *et al.*, 2005). Furthermore, an interaction between the Dsh DEP domain and the central PET/Lim region of Pk was also reported (Tree *et al.*, 2002). Importantly, Dgo and Pk compete for Dsh binding *in vitro*. The *in vitro* competition together

with genetic interaction assays, as well as co-overexpression experiments in the wing, suggest a model in which the antagonistic effects of Pk and Dgo on Fz–PCP signaling are explained by direct competition for Dsh binding (Jenny *et al.*, 2005). It is, however, unknown mechanistically why Pk binding to Dsh has repressive effects or why Dgo has enhancing effects. Potentially, either protein could recruit (or prevent the recruitment of) additional factors such as kinases or phosphatases, or could directly alter the stability of higher order PCP signaling complexes. Except for the membrane recruitment of Pk by Stbm (above), the function of the mutual interactions between Stbm, Dgo, and Pk is not well understood and it is unknown whether these interactions are mutually exclusive. *In vivo* analysis of the functional relevance of such interactions is complicated by the lack of a robust functional assay, as apical membrane recruitment during PCP signaling in the eye appears to be partially redundant (Das *et al.*, 2004).

3.1.1. Asymmetric core protein localization—PCP signaling can initiate the formation of asymmetric structures or be required for directional migration of cells (e.g., during vertebrate gastrulation). The core PCP proteins themselves become asymmetrically localized in a variety of situations investigated. For example, in wing cells, after initially localizing uniformly around the apical circumference, proteins required positively for PCP signaling such as Fz, Dsh, and Dgo become (transiently) enriched at the distal edge of the cells (Axelrod, 2001; Das *et al.*, 2004; Strutt, 2001). In contrast, Stbm and Pk become concentrated on the proximal side of the cells, where they abut Fz on the distal side of the more proximal cell. Similarly, in the eye, core PCP proteins are enriched in a double horseshoe-like pattern at the level of apical junctions in R3/4 shortly prior to and during early rotation (e.g., row 5). They are mostly excluded from contact between R3/4 and R2/5 (Fig. 7.2B and schematic in Fig. 7.2C; Das *et al.*, 2002, 2004; Jenny *et al.*, 2003; Rawls and Wolff, 2003; Strutt *et al.*, 2002). Around row 9, they concentrate in a single horseshoe around R4 (Fig. 7.2B, C). Clonal expression of functional GFP fusion proteins showed that Stbm, Dgo, and Fz are asymmetrically localized with respect to the cell border between R3/4 at the later stage: Fz and Dgo are concentrated on the R3 side of the R3/4 border, while Stbm localizes to the equatorial border of R4 (schematics in Fig. 7.5A; Das *et al.*, 2004; Strutt *et al.*, 2002). Thus in the eye and wing, PCP proteins sort to opposite sides within a cell and each group abuts the other across a cell membrane. It is unknown, however, if the asymmetric localization of the core PCP proteins is functionally significant, instructive, indicative of active signaling, or just a “readout” of acquired polarity, because mutant scenarios have been described in which cells can be polarized without apparent asymmetric PCP proteins (e.g., Lawrence *et al.*, 2004, reviewed in Strutt and Strutt, 2009).

Nevertheless, asymmetric localization of PCP proteins is conserved in a variety of PCP signaling scenarios in different organisms. For example, in the organ of Corti of the mammalian inner ear, Vangl2, a Stbm paralog, and Dsh–GFP localize to opposite sides of the sensory neurons (although Fz3 in this case colocalizes with Vangl2; Montcouquiol *et al.*, 2006; Wang *et al.*, 2005). Analogous opposite localizations for Pk2 and Fz6 have been described for the vestibular system (Deans *et al.*, 2007) and for Dsh and Pk in migrating cells during convergent extension in zebrafish (Ciruna *et al.*, 2006; Yin *et al.*, 2008). Although there are variations between the different cell types, the strong conservation of

asymmetric PCP protein localization in the different organisms suggests some function, whether causative or purely as a feedback reinforcement of a distinct initial asymmetric cue. Furthermore, a lack of visible, macroscopic protein asymmetry does not exclude asymmetric protein activities and could explain the cell polarizations observed without apparent asymmetric PCP protein localization (Lawrence *et al.*, 2004).

3.2. Effectors of PCP signaling in the eye

Effectors of the core PCP genes, also called secondary PCP genes, are generally tissue specific. For example, genes such as *multiple wing hairs* or *inturned* only affect PCP signaling in the wing (Adler, 2005). In the eye, several groups of genes were placed downstream of the core genes. For example, a JNK-MAPKinase module signals to the transcription factors Jun and Fos, and their target genes contribute to R3 specification (Fig. 7.3; Table 7.1; Weber *et al.*, 2000, 2008). In addition, Fz signaling regulates a Delta (Dl)–Notch (N) signal that reinforces the R3/4 cell fate decision (Fig. 7.4, Table 7.1; Cooper and Bray, 1999; Fanto and Mlodzik, 1999; Tomlinson and Struhl, 1999). Other genes, such as Nemo (Nmo; Choi and Benzer, 1994), the founding member of the Nemo-like kinase (NLK) family distantly related to MAP Kinases, only affect ommatidial rotation and are not required for PCP signaling in other tissues. Yet other genes such as *misshapen* (*msn*) or *rho kinase* (*drok*) have functions in the eye and wing (Paricio *et al.*, 1999; Winter *et al.*, 2001) and are considered effector genes here, because it is unclear whether their mode of action differs between the eye and wing.

3.2.1. Misshapen and a JNK module act downstream of Fz/Dsh—MAPKinase modules are usually activated by small GTPases (e.g., Ras during EGF signaling) leading to consecutive phosphorylation of a MAP Kinase Kinase Kinase (MAP3K), a MAP Kinase Kinase (MAPKK), and a MAP Kinase (MAPK), ultimately phosphorylating and activating a transcription factor in the nucleus (reviewed in Pearson *et al.*, 2001). Dominant gain of function interaction tests identified a JNK MAPK cassette acting downstream of the Rho GTPases during PCP signaling in the eye (Fig. 7.3). The PCP phenotype due to overexpression of Fz or Dsh is suppressed by removing one gene dosage of the MAPK *basket* (*bsk*; Strutt *et al.*, 1997) or the MAPKK *hemipterous* (*hep*) or by deficiencies removing those genes (Boutros *et al.*, 1998; Paricio *et al.*, 1999). Furthermore, the PCP phenotype resulting from overexpression of the GTP-bound form of Rac (Rac^{V14}) is dominantly suppressed by removing a copy of *hep* and *bsk*, as well as the transcription factor *jun* (Fanto *et al.*, 2000). Similarly, the PCP-specific allele *dsh^I* can be rescued by overexpressing Hep, Bsk, or Jun at the time of PCP signaling (Boutros *et al.*, 1998). Thus, dominant gain of function genetic interaction experiments indicate that a JNK module acts downstream of RhoA or Rac in PCP signaling in the eye. This is further supported by experiments showing that dominant negative Bsk induces strong PCP defects and that Dsh overexpression can induce Jun phosphorylation in cell culture (Boutros *et al.*, 1998; Weber *et al.*, 2000). Furthermore, clones mutant for the STE20 like kinase Misshapen (Msn; a.k.a. MAP4K) show rotation defects and, more rarely, chirality inversions. Epistasis experiments place *msn* downstream of *rhoA* or *rac*, but upstream of *hep* and *bsk* (Paricio *et al.*, 1999). Analogous genetic interaction tests suggest that the MAP3K dTak can act upstream of Hep and Bsk, but downstream of Msn (Mihaly *et al.*, 2001).

However, the involvement of JNK signaling is controversial (Strutt *et al.*, 2002). To date, it has proven difficult to detect strong loss of function PCP phenotypes for JNK cascade components or their effector Jun. For instance, *hep* or *bsk* mutant tissue in 3rd instar eye imaginal disks show no or only weak PCP defects, which has been attributed to redundancy with other MAPKKs or MAPKs (such as p38). Indeed, deficiencies removing MKK4 or p38 also suppress the PCP phenotype of Dsh overexpression (Paricio *et al.*, 1999). In addition, *jun* clones show weak PCP defects and *jun* could function partially redundantly with *fos*. Jun and Fos are components of the AP1 transcription factor and can either hetero- or homodimerize (Wagner and Eferl, 2005). *fos* is required for tissue survival and progression through mitosis (Ciapponi *et al.*, 2001) and *jun/fos* double mutants are not viable and clones cannot be recovered. Hypomorphic alleles of *fos* (*kaj*) show mild PCP defects in adult eye sections and clonal analysis using molecular markers in eye disks show a requirement for *fos* in the R3 precursor, similar to *fz*, *msn*, and *jun* (Paricio *et al.*, 1999; Weber *et al.*, 2000, 2008; Zheng *et al.*, 1995), consistent with a role downstream of, or in parallel to, *fz* in R3/4 cell fate specification. Also consistent with a role for JNK in PCP signaling in the eye is that loss of *hindsight/pebbled*, which is required to downregulate JNK signaling, causes PCP phenotypes in the eye (Pickup *et al.*, 2002).

Interestingly, a conservation of JNK involvement in convergent extension further supports its involvement in PCP signaling. In *Xenopus*, knockdown of JNK using morpholinos or dominant negative MKK7 affects convergent extension, and JNK knockdown is also sufficient to suppress convergent-extension defects induced by the overexpression of Dsh (Yamanaka *et al.*, 2002). However, new data showed that JNK can also function downstream of Wnt5/Ror and thus can also work in parallel to Fz/Dsh during convergent extension (Schambony and Wedlich, 2007).

Additional evidence suggests an influence of EGFR signaling on R3/4 cell fate choice. In particular, EGFR signaling stimulates its downstream transcription factor Pointed (Pnt) to promote R4 specification, while inhibiting Yan, a transcription repressor that can promote R3 fate (Weber *et al.*, 2008).

3.2.2. Delta and Notch reinforce the R3/4 fate decision—Typically, after loss of core PCP function, ommatidia adopt a chirality stochastically rather than in a directed manner, suggesting a mechanism that can reinforce a weak cellular bias induced by Fz–PCP signaling. Indeed, the Delta (DI)/Notch (N)-signaling pathway has been shown to affect the specification of cell fates downstream of the core PCP module (Cooper and Bray, 1999; Fanto and Mlodzik, 1999; Tomlinson and Struhl, 1999). *mδ-lacZ*, a reporter line in which a short fragment of the N target gene *mδ* controls expression of β -Galactosidase, is expressed strongly in developing R4 cells in 3rd instar eye imaginal disks (Cooper and Bray, 1999). Importantly, *mδ-lacZ* expression is reduced in *fz* and *dsh* mutants. Overexpression of constitutively active N leads to expression of *mδ-lacZ* in both R3 and R4 precursors and gives rise to ommatidia that appear symmetric and contain two R4 cells (as judged by the morphology of adult ommatidia; Fig. 7.1A; Cooper and Bray, 1999). Conversely, temperature-sensitive alleles of *N* or *DI* clones give rise to R3/3 symmetric ommatidia (Cooper and Bray, 1999; Fanto and Mlodzik, 1999; Tomlinson and Struhl, 1999). Clonal analysis showed that DI is required in R3 to promote R4 fate in its neighbor (Table 7.1).

Similarly, reduction of N activity in a mosaic fashion showed that N in R4 specifies R4 cell fate (Tomlinson and Struhl, 1999). Furthermore, N signaling is epistatic to *fz* (Cooper and Bray, 1999; Fanto and Mlodzik, 1999; Tomlinson and Struhl, 1999) and N can repress *Dl* in R4 (Cooper and Bray, 1999).

Several transcriptional targets of Fz–PCP signaling were identified (Fig. 7.4). First of all, *Dl* is expressed at higher levels in R3 at the time of PCP signaling and is upregulated upon overactivation of Fz, initiating the *Dl*/N bias between R3 and R4 (Cooper and Bray, 1999; Fanto and Mlodzik, 1999). The RING-finger E3-ubiquitin ligase *neuralized* (*neur*) is a transcriptional target of the Fz pathway as well. *neur* is required in R3 to promote *Dl* activity (Del Alamo and Mlodzik, 2008), either via the stimulation of *Dl* endocytosis leading to higher N activation (Lai *et al.*, 2001) or by modification of the *Dl*/N binding interaction (reviewed in Le Borgne *et al.*, 2005). Conversely, N represses *neur* in R4 to reiterate the bias (Del Alamo and Mlodzik, 2008). Furthermore, a *Dl* transcriptional reporter is upregulated in *fmi* clones while *Fmi* overexpression in R3/4 leads to a reduction of *Dl* and thus the formation of R3/R3 symmetric ommatidia due to a loss of N activity (Das *et al.*, 2002). In addition, *Fmi* itself is a target of N signaling: if only one of the R3/4 precursors of an ommatidium overexpresses *Fmi*, that cell will become R4 because *Dl* levels in that cell are lower than its partner, resulting in higher *cis*-N activity (thus reinforcing the bias). It is currently unclear how *Fmi* represses *Dl*. However, a repressive effect directly on Fz–PCP signaling would be consistent with data from the wing, where it has been shown that overexpression of *Fmi* antagonizes Fz (tissue-specific expression of *Fmi* reorients wing hairs toward the expression domain, while upon Fz overexpression, hairs point away from the source of Fz; Usui *et al.*, 1999).

Taken together, experimental evidence suggests a model in which Fz controls *Dl* at the transcriptional level in R3, which then nonautonomously activates N in the neighboring R4 precursor to cement the *fz* activity bias between R3 and R4. However, an alternative model was proposed in which Dsh, previously shown to bind to N, directly inhibits N in R3 (Strutt *et al.*, 2002). However, this model is difficult to reconcile with the finding that the N-reporter *mδ-lacZ* is reduced, rather than upregulated, in a *dsh¹* mutant background (Cooper and Bray, 1999).

3.2.3. Ommatidial rotation—As described above, dorsal and ventral ommatidia rotate 90° clockwise and counterclockwise, respectively (Figs. 7.1 and 7.2). The initial rotation of 45° is relatively fast and occurs over ommatidial rows 4–7/9, while a second, slower phase is completed around rows 15–18 (Gaengel and Mlodzik, 2003; Wolff and Ready, 1991).

During rotation, groups of adhering photoreceptors move between stationary, interommatidial cells (IOCs; Fiehler and Wolff, 2007). To date, it is unknown whether this specialized type of cell migration is dependent on protrusive activities of cells such as lamellipodia and/or filopodia (as during convergent extension; Wallingford *et al.*, 2002; Yin *et al.*, 2009), or whether rotation is mainly achieved via restructuring of apical junctions (as in germband extension during *Drosophila* embryogenesis; Bertet *et al.*, 2004; Blankenship *et al.*, 2006). Unfortunately, no *in vivo* imaging of the rotation process has been reported to date, mainly because of the difficulties in culturing eye disks. It is, however, expected that rotation is controlled by cytoskeletal proteins, cell adhesion, and the extracellular matrix. In

addition there are mechanisms regulating the start and stop of the process. Indeed, genes affecting rotation have been described for each of these classes and include *nemo*, *argos*, *drok*, *cadherins*, and *laminin A*, but their interplay with the core genes and each other is not well understood (Choi and Benzer, 1994; Henchcliffe *et al.*, 1993; Mirkovic and Mlodzik, 2006; Winter *et al.*, 2001).

Mutations in *nemo* (*nmo*), a distant MAPK relative and Wnt antagonist, arrest rotation at about 45° and *nmo* regulates the speed of rotation (note it is unclear whether available alleles are null mutations; Choi and Benzer, 1994; Fiehler and Wolff, 2008). Not much is known about the mechanism of action of Nmo during PCP signaling, but it could potentially phosphorylate members of the core PCP proteins or components of the cytoskeletal or adhesion machineries to regulate their activities. Mutations in *argos* (*aos*), a diffusible inhibitor of EGFR signaling initially identified as relevant for PCP signaling due to the phenotype of the *aos^{roulette}* (*aos^{rlt}*) allele, lead to strong over- and under-rotation of ommatidia (Brown and Freeman, 2003; Choi and Benzer, 1994; Gaengel and Mlodzik, 2003; Strutt and Strutt, 2003). Initially, since *nmo/aos* double mutants arrest rotation at 45°, it was assumed that Aos controlled the second phase of rotation. More recently, it appears that precise control of EGFR signaling is crucial for the correct extent of rotation. Not only the inhibitor Aos but also weak alleles of *egfr* (a.k.a. *torpedo*, *top*) show rotation defects. Multiple explanations, which are not necessarily mutually exclusive, were proposed to explain the observed phenotypes. First, a higher frequency of mystery cells, an R-like cell normally only briefly associated with ommatidial preclusters, was observed. These mystery cells also remain part of the photoreceptor precluster for longer than normal and affect Fz and Fmi subcellular localization and thus might perturb Fz–PCP activity (Strutt and Strutt, 2003). In addition, a genetic interaction between EGFR signaling components and *E-cadherin* was observed, suggesting a regulation of cell adhesion by EGF signaling (Brown and Freeman, 2003).

The EGF signal is usually mediated via the small GTPase Ras that activates different downstream branches such as Raf/Rolled MAPK, PI3 Kinase, Rgl, or Canoe (Cno)/AF-6 (Prober and Edgar, 2002). Using Ras effector loop mutants that are able to activate only subsets of the different Ras branches, it was shown that EGFR can affect rotation not only via the Raf/Rolled MAPK cascade but also via Canoe/AF-6 and potentially Rgl/Ral or PI3 Kinase (Gaengel and Mlodzik, 2003). Indeed, mutations in the adherens- and tight-junction associated protein Canoe show over- and underrotation defects similar to *aos^{rlt}*, even early during the larval 3rd instar stage. Although not assessed in PCP signaling, *cno* genetically interacts with *scabrous* (*sca*), an endosome-associated protein involved in N signaling (Li *et al.*, 2003; Miyamoto *et al.*, 1995). Ommatidia posterior to *sca* mutant clones overrotate and *sca* thus appears to be required non-cell autonomously for ommatidia to stop at the right position (Chou and Chien, 2002). It remains puzzling to explain the genetic interaction of *cno* and *sca* mechanistically. However, consistent with an involvement of EGFR signaling in rotation, mutations in the phospholipase C γ , *small-wing*, which is involved in the ER retention of the processed EGFR ligand Spitz, show rotation defects (Schlesinger *et al.*, 2004).

3.2.4. Impact of cell adhesion and the cytoskeleton on ommatidial rotation—

Ommatidial rotation can be considered a special type of cell migration, in which groups of tightly adhering cells change their position relative to cells in their circumference. Cells not part of maturing ommatidia (IOCs) are not moving along with the clusters (Fiehler and Wolff, 2007; Wolff and Ready, 1991), implying that members of the PR clusters adhere more strongly to one another than to undifferentiated neighbors and that a force generator must exist to allow movement of cluster cells with respect to IOCs. Cytoskeletal as well as junctional components are thus expected to play key roles downstream of core PCP genes and indeed, members of each group have been identified that affect rotation.

Downstream of RhoA, Rho Kinase (Rock, Drok in flies) regulates acto-myosin contractility (Riento and Ridley, 2003). Rock activates Myosin regulatory light chain (Spaghetti squash, Sqh) and inhibits Myosin phosphatase, which itself inactivates Sqh, ultimately increasing Myosin II activity (Lee and Treisman, 2004; Riento and Ridley, 2003; Winter *et al.*, 2001). *drok* mutant clones show rotation defects (in addition to a severe loss of photoreceptors) and in the wing, *drok* genetically interacts with *fz* and *dsh* consistent with being downstream of the core PCP cassette (Winter *et al.*, 2001). Similarly, hypomorphic alleles of Myosin II heavy chain (*zipper*, *zip*), as well as overexpression of wild-type or dominant negative forms of Zip, cause over- and under-rotation of ommatidia (Fiehler and Wolff, 2007). Furthermore, *zip* and *drok* genetically interact in the eye (although most evident by the suppression of PR defects caused by overexpression of constitutively active Drok, rather than by effects on rotation; Fiehler and Wolff, 2007; Verdier *et al.*, 2006). Interestingly, MyoII is concentrated around (although not restricted to) the perimeter of the cells already recruited into the PR cluster (schematic in Fig. 7.5B), which led Fiehler *et al.* to suggest that it might be the driving force for rotation, similar to the zippering up of the *Drosophila* embryo during dorsal closure (Fiehler and Wolff, 2007; Franke *et al.*, 2005). Alternatively, such a localization pattern could also be indicative of a mechanism compacting the ommatidial cluster, or, since confocal imaging cannot resolve on which side of the cell–cell border Zip is localized, Zip could be enriched in non-PR cells around the rotating cluster. Importantly, the function of Rho kinase and MyoII downstream of the core PCP module is conserved during convergent extension in mouse, *Xenopus*, and zebrafish (Kim and Han, 2005; Marlow *et al.*, 2002; Skoglund *et al.*, 2008; Ybot-Gonzalez *et al.*, 2007).

During cellular movements, cell–cell contacts dynamically change. A major component of adherens junctions are cadherins (Halbleib and Nelson, 2006; Hulpiau and van Roy, 2009). Classical cadherins form homotypic interactions between adjacent cells, bind intracellularly to α/β Catenin complexes, and are responsible for cell adhesion. The *Drosophila* genome codes for an *E-cadherin* (*shotgun*, *shg*) and two *N-cadherin* genes, all of which are involved in the rotation process (in addition to E-Cadherin's requirement for the maintenance of epithelial integrity; Mirkovic and Mlodzik, 2006). Hypomorphic *shg* mutations show a clear underrotation defect visible already in very young ommatidia close to the MF. In contrast, N-Cad double mutant ommatidia rotate too quickly, but stop at the correct angle of 90°, indicating not only that N-Cad slows down the rotation process but also that an N-Cad independent process exists that stops ommatidia (Mirkovic and Mlodzik, 2006). Analysis of *N-Cad/sca* double mutants could elucidate whether Sca function (see above) is part of such a stopping process.

Particularly striking are the complementary expression patterns of E-Cad and N-Cad1: E-Cad is relatively uniformly expressed at lower levels throughout the developing eye imaginal disk, but highly enriched at the borders where R2 touches R3 and R8, and where R5 touches R4 and R8 (Fig. 7.2 and schematic in Fig. 7.5B; Mirkovic and Mlodzik, 2006) but is not enriched at the contacts of R3/4 or where cluster cells touch IOC. In contrast, N-Cad1 is mainly enriched at the shared border of R3/4 (Fig. 7.5B), where core PCP localization is asymmetric. With their complementary expression pattern and qualitatively opposing phenotype, it is tempting to speculate that the cadherins mediate precluster integrity/adhesion as well as balance the speed of ommatidial rotation. As mentioned above, E-Cad genetically interacts not only with EGFR signaling pathway components but also with a subset of the core PCP genes and RhoA (Mirkovic and Mlodzik, 2006). It is, however, unknown how these interactions function on a molecular basis; in particular, it is unknown whether Rho kinase can (differentially) phosphorylate components of adhesion complexes. Alternatively, genetic interactions could also be mediated by the Ras family GTPase Rap1 which also affects rotation, binds to Canoe, and modifies E-cadherin localization (O'Keefe *et al.*, 2009). Furthermore, due to the low level of uniform E-Cad expression, its ability to form homotypic interactions, and the existence of other adhesive proteins, it is expected that the effects of adhesion on rotation are more complex, as they are not fully "ommatidium autonomous" and could be affected by interactions with IOCs.

Indeed, two additional cell adhesion molecules, the IgCAM Echinoid (Ed), capable of homophilic and heterophilic interactions (Islam *et al.*, 2003), as well as its paralog Fred (Friend of Echinoid), have recently been described to be required for proper rotation (Fetting *et al.*, 2009; Ho *et al.*, 2010). While loss of their function causes a wide variation in the rotation angle of individual ommatidia, genetic interactions showed that *ed* and *fred* antagonistically interact with the EGFR signaling components *pointed* and *canoe* (Fetting *et al.*, 2009). Ed is a known inhibitor of EGFR signaling in other contexts (Bai *et al.*, 2001), but enhances rotation defects of *pnt* and *cno* (Fetting *et al.*, 2009). In contrast, *fred* mutations suppress loss of *cno* or *pnt*, again pointing toward a tight interplay between EGFR signaling, cell adhesion, and rotation. Interestingly, Ed appears to be enriched in cells newly recruited into the PR clusters and the neighboring IOCs, but reduced in the rotating preclusters and their contact interfaces with IOCs (Fetting *et al.*, 2009; Ho *et al.*, 2010; Fig. 7.5B). Fred, on the other hand, shows an expression pattern more similar to the core PCP proteins with a double horseshoe around R3/4 at early stages of rotation and a higher level on membranes around R4 cells (with the exception of where R4 abuts R3; Fetting *et al.*, 2009). How the dynamic localization patterns of Ed and Fred mechanistically can be reconciled with their genetic requirements in R1,6,7 and cone cells (Table 7.1; Fetting *et al.*, 2009) remains to be addressed in greater detail. Recently, an indirect mechanism for Ed's effect on rotation has been proposed (Ho *et al.*, 2010). Ed is required for the endocytosis of Fmi in IOCs. In the absence of Ed, Fmi levels strongly increase in non-PR cells and such excessive ectopic Fmi levels can lead to PCP defects, similarly to prolonged Fmi presence in perduring mystery cells in *argos* mutants (see also above Strutt and Strutt, 2003).

4. Upstream PCP Components

The least understood and potentially most interesting, as well as controversial, questions in the PCP field currently are how the global polarization of the eye field, wing, and abdomen relate to the polarization of the individual structural units. Are there secreted factors such as Wnts involved? How do the atypical cadherins Fat and Dachsous act, and how and where are they connected to the PCP pathway?

4.1. Involvement of a Wnt in PCP signaling?

Wnts (Wingless, Wg in flies) are a family of secreted proteins functioning as classical ligands for Fz receptors during canonical Wnt signaling and could be responsible for a global activation of the Fz–PCP pathway (van Amerongen and Nusse, 2009). Considerable data is available that Wnts are involved in PCP signaling in vertebrates. Wnt7a induced PCP signaling regulates the number and expansion of satellite stem cells after muscle injuries in mice (Le Grand *et al.*, 2009). In *Xenopus* and zebrafish, Wnt5a and Wnt11 are both required for convergent-extension movements (Heisenberg *et al.*, 2000; Lele *et al.*, 2001; Moon *et al.*, 1993; Tada and Smith, 2000), and double mutants of Wnt5a and Wnt11 with concomitant morpholino-mediated knockdown of Wnt4 leads to neurulation defects in zebrafish similar to loss of *vangl1/2*, the paralogs of *stbm/vang* (Ciruna *et al.*, 2006). Similarly, Wnt5 knockout mice show convergent-extension defects in the organ of Corti in the inner ear, a typical vertebrate PCP-related effect (Qian *et al.*, 2007). Because injection of Wnt11 mRNA into the oocyte can rescue convergent-extension defects of zebrafish *wnt11* mutants, the directionality of Wnt input in PCP signaling has been questioned. It has, however, not been assessed whether ubiquitous Wnt11 mRNA leads to ubiquitous Wnt11 protein or, even more importantly, activity. Support for directional input of Wnts on PCP signaling comes from the finding that Wnt11 provides a directional cue for the orientation and elongation of muscle fibers during early chicken development (Gros *et al.*, 2009). Muscle fiber orientation and elongation are also perturbed by manipulating core PCP components and the Wnt11 effects were thus attributed to noncanonical Wnt/Fz–PCP signaling.

In contrast to vertebrates, although not assessed in the eye, no data currently supports the involvement of *wg* or any of the other six *Drosophila* Wnts in PCP signaling (Chen *et al.*, 2008; Lawrence *et al.*, 2002). In particular, compound mutant clones of the five *wnts* expressed in the wing during PCP signaling cause no PCP defects (Chen *et al.*, 2008). These results thus show that simple redundancy does not account for the failure to detect Wnt involvement in PCP signaling in *Drosophila*. To date, the existence of a Fz ligand required for PCP signaling remains elusive, and the absence of a Wnt requirement is the biggest difference between PCP establishment in flies and vertebrates. However, Wg can affect ommatidial orientation more globally, due to its involvement in the establishment of the equator of the eye (see above). Loss of canonical Wnt signaling, as occurs in clones of the obligate canonical coreceptor *arrow* (Lrp5/6 ortholog) or *dsh*, leads to the formation of ectopic equators on the equatorial side of such clones relative to the endogenous equator (Strutt and Strutt, 2002; Wehrli and Tomlinson, 1998), as characterized by the induction of an equatorial marker (i.e., the enhancer trap line *eqI*; Wehrli and Tomlinson, 1998), arguing that this mechanism is distinct from PCP signaling.

4.2. The Fat/Ds system

fz clones in the eye, wing, and abdomen not only have cell autonomous effects but also affect neighboring wild-type tissue (Lawrence *et al.*, 2004; Vinson and Adler, 1987; Zheng *et al.*, 1995). Multiple molecular mechanisms have been invoked in order to explain noncell autonomy involving either diffusible factors (“factor X”; Lawrence *et al.*, 2002; Struhl *et al.*, 1997; Wehrli and Tomlinson, 1998) or relay systems and direct intercellular protein–protein interactions (Amonlirdviman *et al.*, 2005; Chen *et al.*, 2008; Tree *et al.*, 2002; Wu and Mlodzik, 2008). Most experiments addressing cell nonautonomous functions of PCP components were performed in the wing and abdomen, and models have been reviewed recently (Axelrod, 2009a; Lawrence *et al.*, 2007, 2008; Strutt and Strutt, 2009; Wu and Mlodzik, 2009). This review concentrates on experimental data obtained from the eye. Basically, analogous mechanisms involving intermediary, diffusible factors, or locally relayed activities can be envisaged to explain nonautonomous effects in the eye. To date, nonautonomy in the eye has been neglected and no data exists that favors a particular model.

In addition to Fz, the atypical cadherins Fat (Ft) and Dachshous (Ds), as well as the Golgi resident, type II transmembrane protein Four-jointed (Fj) cause nonautonomous PCP defects in the eye (Fanto *et al.*, 2003; Rawls *et al.*, 2002; Strutt and Strutt, 2002; Yang *et al.*, 2002; Zeidler *et al.*, 1999b) and are implicated in setting up a global orientation gradient in the eye and wing (Ma *et al.*, 2003; Matakatsu and Blair, 2004; Strutt and Strutt, 2002). While fully mutant eyes for *ds* and *ft* show random chirality inversions, *fj*[−] mutants show only very rare PCP defects. Surprisingly, mutant clones of all three genes induce very strong PCP defects with strikingly distinct nonautonomous defects (Rawls *et al.*, 2002; Yang *et al.*, 2002; Zeidler *et al.*, 1999b). *fj* and *ft* clones show chirality inversions on the polar side of the mutant tissue, while *ds* clones are affected only on the equatorial side. Thus, wild-type tissue next to mutant tissue can correct PCP defects on the equatorial side of *ft* and *fj* clones and on the polar side of *ds* clones (Fanto *et al.*, 2003; Rawls *et al.*, 2002; Strutt and Strutt, 2002; Yang *et al.*, 2002; Zeidler *et al.*, 1999b). In addition, wild-type tissue on the equatorial side of *ds* and the polar side of *ft* and *fj* clones can revert the chirality, demonstrating an effect of mutant tissue on wild-type tissue (Fanto *et al.*, 2003; Strutt and Strutt, 2002; Yang *et al.*, 2002; Zeidler *et al.*, 1999b), although one report explicitly did not find the latter type of defects (Rawls *et al.*, 2002).

Initially, it was shown that *ft* and *fj* are required in the R3 cell, while *ds* was required in R4 (Fanto *et al.*, 2003; Yang *et al.*, 2002), and it was proposed that these factors directly act on the R3/4 cell fate specification, because Ft and Fj can promote R3, and Ds can promote R4 fates in R3/4 mosaic analysis (Table 7.1; Fanto *et al.*, 2003; Yang *et al.*, 2002). However, the validity of this clonal analysis was questioned because of a nonrandom incorporation of photoreceptor precursors into the ommatidial preclusters (Rawls *et al.*, 2002). The main reason for concern over the strict requirement of Ft, Fj, and Ds, however, comes from the fact that the induced clonal phenotypes can induce mirror symmetry lines similar (although mechanistically not necessarily equivalent) to ectopic equators, and the chirality choice of a single ommatidium is thus not “ommatidia autonomous,” but can be influenced by its neighbors (Fanto *et al.*, 2003; Rawls *et al.*, 2002; Strutt and Strutt, 2002; Zeidler *et al.*, 1999b). Nevertheless, since the effects of *ft* and *ds* in R3/4 mosaic ommatidia for promoting

R3 and R4 fates, respectively, are >80–90% penetrant, *ft* and *ds* probably can have an instructive role for the R3/4 cell fate (Fanto *et al.*, 2003; Yang *et al.*, 2002). This interpretation is also supported by the finding that the instructive role of *ft* requires *fz*: *ft* loses its ability to promote R3 in R3/4 mosaics in a homozygous *fz* mutant background (Yang *et al.*, 2002), indicating that the initial bias is not simply due to the geometry of the eye disk and the manner cells are recruited into the ommatidial preclusters.

Ft protein is expressed uniformly in the eye disk but elevated around the MF. Ds, in contrast is expressed more strongly at the poles than at the equator close to the MF, while Fj (as assessed by a *fj-lacZ* transcriptional reporter) is expressed in an equatorial to polar gradient (Yang *et al.*, 2002). It is currently thought that Fj antagonizes Ds, which in turn can antagonize Ft activity (Fig. 7.3B) resulting in a Ft activity gradient (Sopko and McNeill, 2009; Strutt, 2009). In addition, *fj* transcription is repressed by Ft signaling and by Fj itself, while Ds can activate *fj* transcription (Fig. 7.3B; Yang *et al.*, 2002). Ft and Ds form heterotypic interactions (Ma *et al.*, 2003), which could explain their antagonism in the R3/4 fate decision. Furthermore, Fj is an extracellular kinase that can phosphorylate Ft as well as Ds on their transit through the Golgi (Ishikawa *et al.*, 2008). How such a phosphorylation could modify Ft and Ds activity and/or interaction, and whether their extracellular phosphorylation is essential, remains to be demonstrated.

A direct effect on R3/4 cell fate choice is not sufficient to explain the nonautonomous phenotypes of loss of *fj*, *ft*, and *ds* in clones and it has thus been proposed that the Ft/Ds system additionally modulates the activity of a factor X (Fanto *et al.*, 2003). Consistently, Atrophin (Atro), a transcription repressor that binds to the intracellular C-tail of and acts downstream of Ft shows PCP phenotypes similar to Ft. Importantly, these phenotypes include autonomous and nonautonomous effects. This supports the existence of a relay molecule regulated by the Ft/Atro axis (Fanto *et al.*, 2003). Again, such a factor X would need to be identified and does not necessarily have to be secreted, but could also be a factor that alters a cell-relay mechanism.

One of the most intriguing, complicated, and controversial questions is how Ft/Ds signaling is mechanistically related to the core PCP genes (Fig. 7.3B). Based on experiments in the abdomen, in which a *fz*[−] cell can be repolarized by the Ft system, it was proposed that the two systems act in parallel (Casal *et al.*, 2006), although it is not clear where the two systems would converge (recently reviewed in Axelrod, 2009a; Lawrence *et al.*, 2007, 2008; Strutt and Strutt, 2009; Wu and Mlodzik, 2009). Similarly, *ds* and *fz* can function additively under certain conditions in the eye (Strutt and Strutt, 2002). Based on experiments in the wing, it was proposed that Ft/Ds provide a global signal inducing local refinement by the core Fz system (Ma *et al.*, 2003). The dependence on *fz* of the Ft instructive effect in R3/4 mosaics discussed above (Yang *et al.*, 2002) suggests that, at least to a certain extent, the Ft module might have upstream functions. Consistent with this, the asymmetric localization of core PCP proteins in the wing follows the “induced global polarity” upon Ft/Ds manipulation in the wing (Ma *et al.*, 2003; Strutt and Strutt, 2002). Importantly, forced asymmetric expression of Ft and, in particular, reverse asymmetric expression of Ds in an equatorial to polar gradient in a *fj* mutant background can almost fully invert the chirality in the whole eye, demonstrating that graded *ds* expression can polarize the eye field (Simon, 2004).

Surprisingly, *ds* and *ff* gradients in the wing are dispensable (Matakatsu and Blair, 2006; Simon, 2004). Furthermore, ubiquitous expression of Fat lacking the extracellular domain is sufficient to rescue the PCP defects of Fat mutants in the wing, suggesting that if a graded Ft activity is required, an intracellular system must exist to control it (Matakatsu and Blair, 2006).

Puzzling questions remain. First, comparison of a potential Fz activity gradient in the eye (equatorial to polar) and in the wing (proximal to distal) to a hypothetical Ft activity gradient shows that the latter would be reverted with respect to Fz in the wing (distal to proximal vs. equatorial to polar in the eye; Matakatsu and Blair, 2004, 2006; Simon, 2004). The situation is even more complicated on the abdomen, where the relative gradient orientation inverts within each segment (Casal *et al.*, 2006; reviewed in Strutt, 2009). In spite of these apparently contradictory findings, it is worth noting that Ft plays a role in PCP signaling not only in the fly, but knockouts of Fat4, the mouse *ft* paralog, also show PCP-related phenotypes in the inner ear and kidney, clearly revealing a conserved role in PCP signaling (Saburi *et al.*, 2008).

Second, in elegant experiments using heat-shock time-course rescue, it was shown that the nonautonomous PCP signaling phase and the autonomous requirement of Fz can be temporally separated with the nonautonomous phase preceding the autonomous one in the wing as well as in the eye (Strutt and Strutt, 2002). Intriguingly, it was concluded that the nonautonomous, *ds*-like phase of PCP signaling in the eye occurs ahead of the MF prior to the recruitment of photoreceptors into ommatidial clusters (Fig. 7.1). R3/4 mosaic analysis demonstrated that *fz* and *stbm* mutant cells push the wild-type partner cell into R3 and R4 fate, respectively, with >95% efficiency, which clearly requires communication between the future R3/4 cells of the same cluster. If R3/4 cells are globally prespecified even before they are photoreceptors, and even longer before they are in contact with each other (ommatidial row 3; Fig. 7.2; Wolff and Ready, 1991), a mechanism would have to be predicted that is able to revert the previously imposed R3/4 fates later.

Third, in the wing and abdomen, not only *fz* but also *stbm*/*vang* clones show domineering nonautonomy (Lee and Adler, 2002; Taylor *et al.*, 1998) and feedback models explaining nonautonomy consider both of these transmembrane molecules crucial (e.g., Amonlirdviman *et al.*, 2005; Chen *et al.*, 2008; Lawrence *et al.*, 2002; Wu and Mlodzik, 2008). However, in the eye, no nonautonomy has been observed for *stbm* clones (Wolff and Rubin, 1998) and a more comprehensive analysis of the effects and mechanistic causes of nonautonomy in the eye is thus required in the future.

4.3. JAK/Stat signaling

The JAK (Janus kinase)/STAT (signal transducer and activator of transcription) signaling pathway was initially discovered in mammals for its role mediating cytokine signaling (Hou *et al.*, 2002; Rawlings *et al.*, 2004; Shuai and Liu, 2003). In *Drosophila*, Unpaired (Upd) signals to the receptor Domeless, which activates the JAK Hopscotch (Hop), leading to phosphorylation and nuclear translocation of the transcription factor Stat. Interestingly, loss of *upd*, *hop*, and to a lesser extent *stat92e* leads to polarity inversions similar to loss of *ft* (Luo *et al.*, 1999; Zeidler *et al.*, 1999a). In particular, mutant clones of *hop* and *stat92e* lead

to inversion of ommatidial chirality on the polar side of clones and show a nonautonomous behavior similar to *ft* clones (Zeidler *et al.*, 1999a). However, in contrast to loss of canonical Wnt signaling which causes induction of true ectopic equators based on the induction of the equatorial marker *eq1* (see above; Wehrli and Tomlinson, 1998), *hop* clones do not change the expression of equatorial markers (Zeidler *et al.*, 1999a). The phenotype reminiscent of ectopic equators seen in *hop* or *stat92e* clones is instead due to effects on PCP signaling. These findings have led to the suggestion that JAK/STAT signaling gradients are another means to regulate the elusive “factor X” controlling ommatidial orientation (Zeidler *et al.*, 1999a; reviewed in Strutt, 2009). Interestingly, however, the ligand Upd can also act independent of JAK/STAT to influence the equator by changing the expression of *mirror*, a key gene required for the establishment of the endogenous equator (McNeill *et al.*, 1997; Zeidler *et al.*, 1999a). Similar to the Ft system, future studies are needed to decipher the interplay between the JAK/STAT and the core Fz–PCP module during PCP establishment in the eye.

5. Additional Components Affecting PCP Signaling in the Eye

Several additional genes have been identified that also affect PCP signaling, but do not belong the groups of genes described so far. For example, genes required for apical/basal cell polarity can affect the establishment of PCP in the eye. In particular, the apical dPatj (PALS-1 associated tight-junction) protein recruits aPKC to Fz, which leads to a reduction of Fz activity in non-R3/4 cells of the ommatidial precluster (Djiane *et al.*, 2005). Consistent with this function, dPatj is strongly expressed around the apical circumference of R2 and R5 (Fig. 7.5B) and reduced around R3/4. In contrast, Par3/Bazooka is enriched more like a typical core PCP protein and can prevent aPKC from phosphorylating Fz in R3/4 and thus prevents its inhibition in the R3/4 precursor cells (Djiane *et al.*, 2005). Similarly, Scribble, a member of the Scribble/Lethal giant larva (Lgl)/Discs large (Dlg) complex localized to septate junctions (Bilder, 2004), has been shown to interact with Stbm and *scrib* mutants to cause PCP defects (Courbard *et al.*, 2009). Importantly, mouse *Scrb1* (*spin-cycle*) genetically interacts with *vangl2* mutants during PCP establishment in the inner ear (Montcouquiol *et al.*, 2003).

Furthermore, Nhe2, a Na⁺/H⁺ exchanger has been shown to be required for Dsh membrane recruitment, a process considered critical for canonical as well as noncanonical Wnt signaling (Axelrod, 2001; Bilic *et al.*, 2007). *Nhe2* genetically interacts with *fz* and its overexpression can induce PCP defects. It has thus been suggested that a local more-alkaline pH favors an additional, direct membrane binding of Dsh upon recruitment by Fz (Simons *et al.*, 2009). Interestingly, *bedraggled* (*bdg*), another putative membrane transporter also genetically interacts with PCP genes and Bdg overexpression can induce PCP defects in the eye (Rawls *et al.*, 2007), suggesting that the membrane microenvironment is critical for PCP signaling.

In addition, mutations in *rasputin* (*rin*) cause photoreceptor recruitment defects as well as typical PCP defects in the eye (Pazman *et al.*, 2000). Rin is the *Drosophila* ortholog of the Ras-GTPase-activating SH3 domain-binding protein (G3BP). G3BPs contain, amongst others, several SH3 domain-binding sites and RNA-binding motifs and interact with

RasGAP. G3BPs are involved in a variety of functions from cell signaling to RNA metabolism and transcription, but how these functions relate to each other is unknown (reviewed in Irvine *et al.*, 2004). *rin* genetically interacts with Ras and Rho signaling, potentially via the regulation of a RasGAP and RhoGAP. Further experiments are required to more precisely determine the mechanism of *rin* function in PCP signaling. It would be of particular interest to know if the RNA-binding activities of Rin are required for PCP signaling.

6. Conclusions

Much progress has been made in the past years in the understanding of PCP establishment in the *Drosophila* eye. Most importantly, genetics and molecular and genetic interactions have defined requirements for genes in PCP signaling that are conserved from invertebrates to vertebrates. Nevertheless, several key questions remain to be answered in order to understand PCP signaling in the eye and ultimately in human development and disease.

The most obvious problem to solve is defining the precise molecular linkage between the Ft/Ds system and the core Fz PCP module. If these systems act in parallel, where do they converge? If the Ft/Ds system feeds into the Fz system, at what position? Is the intersection or lack thereof the same in every tissue in *Drosophila* and in vertebrates?

Probably intertwined with the last problem is the need to resolve the mechanistic aspects of the directional nonautonomy in more detail. In particular, are *fz* nonautonomous effects in the wing truly equivalent to those in the eye? As discussed, in contrast to the wing, *stbm/vang* acts cell autonomously in the eye. Furthermore, in the wing, asymmetric PCP protein localization reiterates from cell to cell. In the eye, PCP protein localization has only been studied in PR cells and no asymmetries have been reported that would support a relay system passing across IOC. Along similar lines, the function of the cytoskeleton and cell adhesion components in PRs as well as IOCs need to be better analyzed in order to understand their respective contribution to the rotation process, which in turn will help us to better understand this type of collective cell migration.

The molecular link between Dsh and the genetically downstream Rho family GTPases remains enigmatic. In *Xenopus*, the formin xDAAM was shown to bridge signaling between Dsh and Rho. Furthermore, xDAAM is activated by xDsh, which in turn leads to an activation of Rho by a poorly understood process (Habas *et al.*, 2001; Liu *et al.*, 2008). Genetic analysis of *Drosophila* did not implicate *daam* in PCP signaling in the fly; thus, the molecular link between RhoA/Rac and Dsh remains obscure (Matusek *et al.*, 2006).

Historically, the *Drosophila* eye has provided an excellent model system for studies of PCP establishment and recent genetic evidence demonstrating requirements for *stbm*, *fmi*, *vangl2*, and *celsr1* in mammalian eye development corroborate the relevance of the fly system and are thus very gratifying (Sugiyama *et al.*, 2010).

Acknowledgments

I am grateful to the members of my lab and colleagues in the field for their discussions and support and would like to thank Drs. G. Dollar, F. Marlow, and H. McNeill for their comments on this chapter. Our work is supported by NIH grant 1R01GM088202.

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Figure 7.1.

Establishment of PCP in the *Drosophila* eye. Anterior is to the left, dorsal is up in all panels. (A) Schematic of a 3rd instar eye imaginal disk with the dorsoventral midline (equator) in yellow and the morphogenetic furrow (MF) in gray. Purple bar outlines the approximate region of nonautonomous/*dachsous* signaling phase. Initially, ommatidial preclusters are symmetrical with the precursors for R3 (pale red) and R4 (light blue) next to each other. The cell of the R3/4 pair closer to the equator is specified as R3 (red) upon Fz–PCP signaling. The neighbor becomes R4 (blue). Ommatidia rotate 90° in opposing directions on either half of the eye. The rhabdomeres of adult ommatidia are thus mirror symmetric (chiral) with the rhabdomere of R3 at the polar-anterior tip and the R4 more equatorial and posterior (schematic on the right). Far right: schematic representing symmetric ommatidia of the R3/3 and R4/4 type occurring in certain mutant situations. Colors of the flagged arrows correspond to the ones shown in the sections in B–D. (B–D) Tangential sections through wild-type (B), *fz* (C), and *dsh* (D) mutant adult *Drosophila* eyes. Note the randomized chirality and degree of rotation in the mutants. Schematic below the sections indicates the polarity of ommatidia (see (A) for arrows). Circles represent ommatidia with defects in the

photoreceptor complement. Yellow dots represent the equator. Inset in (B): high magnification of a single ommatidium with numbered photoreceptors. Note that R8 is below R7 and thus cannot be seen. Purple arrow points to pigment granules associated with the rhabdomere of photoreceptors. The presence of these granules is used as a marker during genetic mosaic analysis. See text for details.

Figure 7.2.

Subcellular localization of core PCP genes during PCP signaling. (A) Dorsal area at the apical level of a 3rd instar eye disk stained for Fmi in green (single channel image in A') and E-Cad in red (single channel image in A''). The morphogenetic furrow (MF) is on the anterior (left). Note the progressive rotation of anterior (young) to posterior (more mature) ommatidia. Ommatidial row numbers are indicated at the bottom. (B, C): (B) Higher magnification of the ommatidial clusters indicated with yellow squares in A. In row 5, Fmi, a representative core PCP protein, is enriched in apical membranes in a "double horseshoe" pattern in R3 and R4, but reduced where they are in contact with R2/5. After the initial phase of rotation (about row 9), Fmi enrichment is visible as a single "horseshoe" in R4.

Colors as in (A). (C) Schematic drawing outlining the cluster cells and localization of typical core PCP proteins (as described in B). In the upper panel, PRs are numbered. AC, PC: the anterior and posterior cone cells. Note that based on localization at the cell membrane it is not possible to determine of which cells touching each other actually expresses the protein (see also Fig. 7.5). Images courtesy of K. Gängel and M. Mlodzik.

Figure 7.3.

Schematic of the genes involved in PCP signaling. (A) Core PCP genes, JNK cascade, and cytoskeletal branch. In flies, the link between *Dsh* and the Rho family GTPases is currently unknown. The involvement of MRLC is inferred from wing data. (B) Ft/Ds module. Note that the hierarchical relationship between Ft/Ds and the core PCP pathway is unclear.

Figure 7.4. Schematic summarizing PCP signaling during R/3/4 cell fate specification. Factors in bright colors are genetically required in the respective cell.

Figure 7.5.

Schematic of protein expression patterns during PCP signaling in ommatidial clusters. (A) Subcellular core PCP protein expression. Use of clonal expression of GFP fusion proteins of Fz, Dgo, and Stbm showed that the overall similar single horseshoe pattern (green) is actually due to asymmetric protein localization that is distinct for Fz/Dgo (red) and Stbm (blue). While Fz and Dgo localize to the polar border of R3 (and R4), but are excluded from the equatorial side of R4, Stbm localizes to the equatorial side of R4 (and its polar side). The situation at the R3/4 cell interface is thus equivalent to the proximal/distal border of wing cells, where Stbm/Pk on the proximal side of distal cells abut Fz/Dgo/Dsh on the distal side of the more proximal cell. (B) Schematic summarizing simplified expression patterns of E- and N-cadherin 1, Echinoid, Myosin II (Zip), and dPatj. See text for a more detailed description and references. Note that these proteins are expressed at lower levels in most cells and green color indicates a strong enrichment. In particular, Ed and Myosin II expression is highly simplified. Ed is absent from early preclusters and later enriched at the interface between the ommatidia and IOC. Similarly, Myo II is also expressed within the cytoplasm of cluster cells (as indicated by pale green circles).

Table 7.1

PCP genes involved in eye development

PCP gene	Tissues affected in <i>Drosophila</i>	Function in vertebrate PCP	PR requirement	Molecular features
Core genes				
<i>frizzled (fz)</i>	All adult tissues	M, X, Z	R3	Seven-pass transmembrane receptor; binds Wnt ligands, Dsh; recruits Dsh and Dgo to membrane
<i>dishevelled (dsh)</i>	All adult tissues	M, X, Z	R3	Cytoplasmic protein containing DIX, PDZ, DEP domains, recruited to membrane by Fz, binds Fz, Pk, Sbm, and Dgo
<i>flamingo (fmi)/starry-night (stan)</i>	All adult tissues	Z, M	R3, R4	Cadherin with seven-pass transmembrane receptor features, homophilic cell adhesions
<i>diego (dgo)</i>	Eye, wing, notum in GOF ^a	X, Z	R3	Cytoplasmic Ankyrin repeat protein, recruited to membrane by Fz, binds Dsh, Sbm, and Dgo. Diversin/Inversin in vertebrates.
<i>strabismus (sbm)/van-gogh (Vang)</i>	All adult tissues	H, M, X, Z	R4	Novel four-pass transmembrane protein, binds Pk, Dsh, and Dgo, recruits Pk to membrane
<i>prickle (pk)</i>	All adult tissues	M, X, Z	R4	Cytoplasmic protein with 3 LIM domains and PET domain, recruited to membrane by Sbm, physically interacts with Dsh, Sbm, and Dgo
Fv/Ds module				
<i>ft</i>	All adult tissues	M	R3 (?)	Atypical cadherin
<i>ds</i>	All adult tissues		R4 (?)	Atypical cadherin
<i>fj</i>	All adult tissues		R3 (?)	Golgi resident luminal kinase
Secondary genes				
<i>RhoA/Rac</i>	Eye, wing ^a	X		Small GTPase, acts downstream of Dsh
<i>tho kinase (drok)</i>	Eye, wing	M, X, Z		Ser-Thr kinase
<i>daam</i>	γb	X		Formin, actin polymerizing
<i>misshapen (msn)</i>	Eye (wing)		R3	Ste20 like kinase
<i>jun</i>	Eye	X (JNK)	R3	AP1 transcription factor component
<i>fos (kajak)</i>	Eye		R3	AP1 transcription factor component
<i>pointed (pnt)</i>	Eye		R4	Transcription factor
<i>yan</i>	Eye		R3	Transcriptional repressor
<i>Notch (N)</i>	Eye		R3	Transmembrane protein
<i>Delta (DI)</i>	Eye		R4	Transmembrane protein
<i>neutralized (neur)</i>	Eye		R3	E3 ubiquitin ligase
<i>nemo (nmo)</i>	Eye			Kinase distantly related to MAPKs

PCP gene	Tissues affected in <i>Drosophila</i>	Function in vertebrate PCP	PR requirement	Molecular features
<i>atg</i> (<i>aos</i>)	Eye			EGF (Spitz) binding inhibitor
Cell adhesion				
<i>E-cadherin</i> (<i>shotgun</i>)	Eye	X, Z		Cadherin, cell adhesion
<i>N-cadherin</i>	Eye			Cadherin, cell adhesion
<i>ectinoid</i> (<i>ed</i>)	Eye		R1, R6, R7, CC	Ig CAM
<i>fred</i>	Eye		R1, R6, R7, CC	Ig CAM

H: human; M: mouse; X: *Xenopus*; Z: Zebrafish. Functional homolog unknown. CC: cone cells. See text for references.

^aOther tissues were not tested.

^b*Drosophila* Daam has no PCP LOF phenotype.