Planar cell polarity in moving cells: think globally, act locally
Crystal F. Davey and Cecilia B. Moens*

ABSTRACT

The planar cell polarity (PCP) pathway is best known for its role in polarizing epithelial cells within the plane of a tissue but it also plays a role in a range of cell migration events during development. The mechanism by which the PCP pathway polarizes stationary epithelial cells is well characterized, but how PCP signaling functions to regulate more dynamic cell behaviors during directed cell migration is much less understood. Here, we review recent discoveries regarding the localization of PCP proteins in migrating cells and their impact on the cell biology of collective and individual cell migratory behaviors.

KEY WORDS: Cell migration, Planar cell polarity, Planar polarity

Introduction

‘Think Globally, Act Locally’, the catchphrase of grass-roots environmentalism, encapsulates the hopeful idea that small, voluntary changes made at home by individuals can collectively have positive global impacts. If this idea has a representation in the biological world, it is in the context of the planar cell polarity (PCP) pathway, the fundamental function of which is the communication of polarity information between adjacent cells in the plane of a tissue, with outcomes that are far-ranging and diverse. Indeed, the PCP pathway regulates a variety of processes during development, from the coordinated orientation of cells and cell divisions across an epithelium, and the orientation of multicellular epithelial structures such as the mammalian hair follicle or the fly eye, to the directional movements of motile cells across developing vertebrate embryos. All of these processes require a core group of membrane-associated proteins that regulate each other’s localization and the organization of the cytoskeleton. Here, we review the role of PCP in moving cells, focusing on examples of cell movements in which multiple PCP core components have been functionally implicated and on recent studies in which the in vivo localization of those components have provided mechanistic insights (Table 1). We discuss the role of PCP in two general types of directed cell movement. First, the coordinated movements of coherent cells comprising tissues, considering specifically convergence and extension (CE) movements of the neural plate and mesoderm, and the migration of the neural crest. Second, the individual migrations of cells through tissues, considering specifically the longitudinal guidance of commissural axons in the spinal cord and the longitudinal migration of facial motor neurons in the brainstem. We emphasize several principles common to PCP processes: (1) the cell-autonomous and non-cell-autonomous requirement for PCP core components; (2) the mutually exclusive localization and antagonistic functions of PCP core components within cells; and (3) the shared use of downstream regulators of the actin cytoskeleton for diverse migratory outcomes. We also discuss how PCP proteins, deployed to homotypic cell contacts in collectively moving cells versus cellular protrusions in individually migrating cells, can have divergent effects on cell motility.

The basics of the PCP pathway

PCP describes the collective polarization of cells in the plane of a tissue. It is a common feature of many tissues but is most evident in cells that are organized into epithelial sheets. The principles of the PCP pathway were first identified in the fly using a combination of loss- and gain-of-function approaches in genetic mosaics combined with immunohistochemistry, which revealed the polarized asymmetric distribution of core PCP proteins (Goodrich and Strutt, 2011). In vertebrates, mutations in the homologs of fly PCP genes result in phenotypes that are consistent with a conserved role in epithelial planar polarization. The localization of PCP proteins, best visualized by the mosaic expression of fluorescent fusion proteins, is also polarized in vertebrate epithelia. Unlike in flies, however, PCP proteins in vertebrate embryos have crucial roles in the polarized movements of epithelial cells, mesenchymal cells, neurons and their processes, as we discuss in detail below.

Lessons from Drosophila

Two main pathways function in the establishment of planar polarity in the fly: the ‘core’ PCP pathway and the Fat/Dachsous (Fat/Ds) pathway (for a review, see Matis and Axelrod, 2013). A third pathway also operates in the embryonic ectoderm to regulate PCP during germband extension (Bertet et al., 2004; Blankenship et al., 2006; Vichas and Zallen, 2011). Here, and henceforth, we consider exclusively the core PCP pathway, as the role of the Fat/Ds pathway in directional cell movements is less well established.

The conceptual framework for understanding PCP-dependent processes was first elucidated in the fly wing, where planar polarity is easily discerned by the localization of a single actin-rich hair, the trichome, at the distal side of the apical surface of each epithelial cell (Fig. 1A). Screens for mutants in which trichome orientation is disrupted identified six core components, which localize to adherens junctions (AJs) in distinct proximal and distal domains in the wing epithelium. The transmembrane protein Frazzled (Fz) recruits the cytosolic proteins Dishevelled (Dvl; Dsh) and Diego (Dgo) and localizes to distal cell junctions (Fig. 1, green complex), and the transmembrane protein Van Gogh (Vang; also known as Strabismus, Sbrm) recruits the cytosolic protein Prickle (Pk) and localizes proximally (Fig. 1, red complex). The atypical cadherin Flamingo (Fmi; Starry Night) is localized to both proximal and distal membranes (Fig. 1, blue). The molecular asymmetry of these protein complexes precedes the morphological asymmetry of the trichome, and loss of any of these core proteins leads to loss of both molecular and morphological asymmetries (for a detailed review, see Peng and Axelrod, 2012).
Table 1. Cell movements controlled by the PCP pathway

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<tr>
<th>Loss-of-function phenotype</th>
<th>PCP protein localization</th>
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<tr>
<td><strong>Open/duplicated neural tube</strong></td>
<td><strong>Vangl2</strong> Mouse: Ybot-Gonzalez et al., 2007; Fish: Ciruna et al., 2006; Frog: Goto and Keller, 2002; Ossipova et al., 2014</td>
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<td><strong>Fzd3,6</strong> Mouse: Wang et al., 2006a</td>
<td>Localization at apical surface of neuroepithelial progenitor cells <strong>Vangl2</strong> Anterior side (GFP-Vangl2) Mouse: Kibar et al., 2001; Murdoch et al., 2001; Fish: Jessen et al., 2002; Yin et al., 2008; Frog: Darken et al., 2002; Goto and Keller 2002</td>
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<td><strong>Dvl1,2,3</strong> Mouse: Etheridge et al., 2008; Hamblet et al 2002; Wang et al., 2008b; Frog: Wallingford and Harland, 2002; Chick: Nishimura et al., 2012</td>
<td><strong>Dvl2</strong> Anterior side (Fzd3a-GFP) Mouse: McGreevy et al., 2015; Chick: Nishimura et al., 2012</td>
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<td><strong>Celsr1</strong> Mouse: Curtin et al., 2003; Chick: Nishimura et al., 2012</td>
<td>Localization on converging mesodermal cells <strong>Fzd7</strong> Trailing (Fzd7-YFP) Frog: Carmona-Fontaine et al., 2008</td>
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<td><strong>Ptk7</strong> Mouse: Lu et al., 2004; Williams et al., 2011; Frog: Ossipova et al., 2015b</td>
<td>Dynamic localization in the growth cone <strong>Vangl2</strong> Filopodial tips and membrane (GFP-Vangl2) Mouse: Shafer et al., 2011</td>
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Prior to becoming polarized in the plane of the epithelium, PCP proteins are recruited symmetrically to the apical membrane. The symmetry-breaking cues that initiate the polarized localization of the two PCP complexes in *Drosophila* have been a major focus of recent research, which has demonstrated preferential trafficking of Fz and Dvl-containing vesicles towards the plus-ends of apical microtubules, polarization of which depends on Fat/Ds signaling (for a review on this topic, see Galic and Matis, 2015; Yang and Mlodzik, 2015). Once asymmetry is initiated, PCP is amplified and maintained by intracellular antagonistic interactions that destabilize the core components (Fig. 1A′, green and red hammers) and positive intercellular interactions that transmit molecular asymmetries across the epithelium (Fig. 1A′, green and red arrows) (Peng and Axelrod, 2012). Within cells, Dgo binds Dvl to stabilize the Dvl-Fz interaction and membrane accumulation of Fz, whereas Pk antagonizes this interaction. Between cells, Fz and Vang recruit one another to apposing membranes in an Fmi-dependent manner. As a result, in genetic mosaics, not only do Vang or Fz mutant cells have altered trichome orientation, but trichome orientation is also disrupted in neighboring wild-type cells: in other words, Vang and Fz have both cell-autonomous and non-cell-autonomous functions.

### The PCP pathway in vertebrate epithelia

Orthologs of all six core PCP pathway components are present in vertebrate genomes and are required for the planar polarization of epithelial cells in the vertebrate skin, trachea, central nervous system and sensory structures (Hale and Strutt, 2015). In many of these cells, planar polarity is evident in the microtubule-based primary cilium whose asymmetric position is underlain by the asymmetric localization of core PCP components themselves (Borovina et al., 2010; Hashimoto et al., 2010; Nonaka et al., 2005; Okada et al., 2005). In the irregular epithelia of vertebrate embryos, PCP protein localization is most effectively detected when fluorescent PCP fusion proteins are mosaically expressed, allowing the membranes of single expressing cells to be distinguished from those of non-expressing neighbors. With few exceptions, the relationship between core PCP components in vertebrate epithelial cells is the same as in flies, with Fzd and Dvl localizing together, opposite Vangl and Prickle (hereafter Pk) (Fig. 1B-D) (Butler and Wallingford, 2015; Chu and Sokol, 2016; Ciruna et al., 2006; Davey et al., 2016; Deans et al., 2007; Devenport et al., 2011; Hashimoto et al., 2010; Vladar et al., 2012; Yin et al., 2008).

### Table 1. Continued

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<th>Loss-of-function phenotype</th>
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<td><strong>Failure of facial motor neuron migration</strong></td>
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<td>Vangl2 Mouse: Vivancos et al., 2009; Glassco et al., 2012; Fish: Jessen et al., 2002; Bingham et al., 2002</td>
<td>Localization in facial motor neurons Vangl2 Filopodia tips and membrane (GFP-Vangl2) Fish: Davey et al., 2016</td>
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<td>Pk1 Fish: Mapp et al., 2010, 2011; Rohrschneider et al. 2007; Mouse: Yang et al., 2014</td>
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<td>Fzd3 Mouse: Vivancos et al., 2009; Qu et al., 2010; Fish: Wada et al., 2006</td>
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<td>Dvl (DN) Fish: Davey et al., 2016</td>
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<td>Celsr1,2,3 Mouse: Qu et al., 2010; Fish: Wada et al., 2006</td>
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<td>Scrib Mouse: Vivancos et al., 2009; Fish: Wada et al., 2005</td>
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<td><strong>Failure of lymphatic endothelial valve formation</strong></td>
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<td>Vangl2 Mouse: Tatin et al., 2013</td>
<td>Dynamic localization in valve-forming cells Vangl2 Filopodia tips, homotypic contacts (α-Vangl2 and GFP-Vangl2) Fish: Davey et al., 2016</td>
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<td>Celsr1 Mouse: Tatin et al., 2013</td>
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<td><strong>Reduced breast cancer cell migration/ metastasis</strong></td>
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<td>VANGL1 Human: Luga et al., 2012; Anastas et al., 2012; MacMillan et al., 2014</td>
<td>Localization during Wnt-induced migration VANGL1 Non-protrusive surfaces (Flag-VANGL1) Human: Luga et al., 2012</td>
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<td>PK1 Human: Luga et al., 2012; Zhang et al., 2016</td>
<td>PK1 Non-protrusive surfaces (α-PK1) Human: Luga et al., 2012</td>
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<td>DVL1,2,3 Human: Luga et al., 2012</td>
<td>DVL Protrusive surfaces (α-DVL1, α-DVL3) Human: Luga et al., 2012</td>
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<td>FZD6 Human: Luga et al., 2012</td>
<td>FZD6,7 Protrusive surfaces (α-FZD6, α-FZD7)</td>
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<td>WNT5A,11 Human: Luga et al., 2012</td>
<td>SCRIB Lamellipodia Human: Anastas et al., 2012</td>
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Fig. 1. Planar polarization of epithelial cells. (A,A′) A field of cells in the fly wing is depicted, with distal to the right. Each cell harbors a distal trichome (gray) and polar localization of PCP components, as detailed in the inset (A). Arrows indicate intercellular recruitment and stabilization, hammers indicate intracellular destabilizing interactions with the opposite complex. Vertebrate nomenclature, if different from the fly, is parenthesized. (B-D) Schematized versions of original data showing vertebrate epithelial cells with planar polarized localization of Endogenous or mosaically expressed PCP fusion proteins. (B) Apical surface of the medial region of mammalian vestibular sensory epithelium (Deans et al., 2007). Hair cells (large dark gray cells, with cilia) and support cells (smaller light gray cells) are planar polarized, with Fzd3/6-GFP (green) localizing to the lateral surfaces of support cells whereas endogenous Pk2 (red) localizes to the medial surfaces of hair cells. Pk2-GFP also localizes medially when expressed in support cells. (C) Apical surface of mouse tracheal epithelial cells (Vladar et al., 2012). In both multiciliated (dark gray spots) and surrounding cells, endogenous Vangl1, Pk2 and GFP-Pk1 (red) localize to the distal surface and GFP-Fzd3/6 and GFP-Dvl1/3 localize to the proximal surface. (D) Xenopus epidermal cells (Butler and Wallingford, 2015). In both multiciliated and surrounding cells, Dvl1-GFP localizes to the dorsal side whereas RFP-Pk1/2 and RFP-Vangl1 localize to the ventral side of the same cells. In C,D, ciliary basal bodies are represented as circles, and black arrowheads represent their basal feet, which are rotationally polarized in response to PCP signaling.

frog skin or in the sensory epithelum of the chick ear disrupts the polarity of adjacent wild-type cells, suggesting that the molecular interactions between core PCP components that amplify and maintain their asymmetric localization are conserved between fly and vertebrate epithelia (Mitchell et al., 2009; Sienknecht et al., 2011). As we discuss later, this paired cell-autonomous and non-cell-autonomous function is a deeply conserved characteristic of the pathway even in migrating cells. It should also be noted that the core PCP pathway is often referred to as the non-canonical Wnt/PCP pathway, because it involves Fz receptors and Dvl homologs in a j-catenin-independent signaling pathway. Although the role of Wnts in Drosophila planar polarity is debated (Chen et al., 2008; Wu et al., 2013), Wnt ligands are essential as permissive cues for many vertebrate PCP processes including some PCP-dependent cell movements (Heisenberg et al., 2000; Qian et al., 2007), although recent work has identified an instructive role in the establishment of planar polarity in the ectoderm (Chu and Sokol, 2016).

The transduction and maintenance of PCP signaling

How does the PCP pathway influence epithelial cell polarity? PCP signaling has no known transcriptional output; its direct impacts are on the cytoskeleton. In the fly wing, the PCP effectors Fuzzy, Inturned and Fritz colocalize with Vang-Pk at the proximal side of the cell, where they in turn localize a fourth effector, Multiple Wing Hairs (Mwh), which is an atypical formin domain-containing protein that inhibits actin filament elongation, thereby preventing trichome formation at the proximal side of the cell (Collier and Gubb, 1997; Collier et al., 2005; Lu et al., 2015; Park et al., 1996). By contrast, PCP effectors that function at the distal side of the cell, downstream of Fz-Dvl to promote actin assembly and trichome formation, have been more difficult to identify, probably because of pleiotropic phenotypes. However, wing hair abnormalities are observed in mutants harboring defective actin regulators, including the small GTPase RhoA (Rho1), its effector Rho-associated kinase (Rock; Rok) and its target Myosin II (Spaghetti Squash), which drives actomyosin contractility, and the formin Diaphanous, which promotes linear actin filament assembly (Franke et al., 2010; Lu and Adler, 2015; Winter et al., 2001; Yan et al., 2009). In the fly eye, where the PCP pathway functions to orient ommatidia (the units of the compound fly eye), the e-Jun N-terminal kinase (JNK) pathway is activated downstream of Fz-Dvl to phosphorylate key actin regulators (Boutros et al., 1998; Weber et al., 2000). In vertebrates, the PCP pathway recruits the same downstream actin regulators during cell migration, as we discuss below.

Mutations in a number of other vertebrate genes result in PCP phenotypes, implicating these genes either in the establishment of polarized protein domains or in the reception and transduction of PCP signals uniquely in vertebrates. With respect to the establishment of PCP protein localization, Sec24b, a component of the CopII coat protein complex, trafficks Vangl2 from the endoplasmic reticulum (Merte et al., 2010; Wansleeben et al., 2010), and the GTP-binding protein Arfrp1 transports Vangl2 from the trans Golgi network (Guo et al., 2013). Additionally, the adapter protein Gip1 interacts with Vangl2 to promote its endocytic internalization and trafficking away from inappropriate membranes (Giese et al., 2012). PCP protein localization is also maintained intracellularly by localized protein degradation mediated by the E3 ubiquitin ligases Smurfl and Smurfl2, which are recruited to sites of activated Dvl2 where they target Pk1 for degradation (Narimatsu et al., 2009). A number of other proteins have been implicated in the
transduction of PCP signaling. For example, glypican 4 (Knypek), a heparin sulfate proteoglycan, promotes PCP signaling through interactions with Wnt11 (Ohkawara et al., 2003; Topczewski et al., 2001). In mouse limb bud chondrocytes, the transmembrane receptor Ror2 induces phosphorylation of Vangl2 in response to Wnt5a, increasing its proximal localization (Gao et al., 2011). Ptk7, a non-catalytic receptor tyrosine kinase, is also required for planar polarization in a range of vertebrate tissues; however, its mechanism is controversial (Glasco et al., 2012; Lu et al., 2004; Shnitsar and Borchers, 2008; Yen et al., 2009). Finally, scribble (Scrib) mutants have dramatic PCP phenotypes in several vertebrate tissues. Scrib is an apicobasal polarity determinant that physically and genetically interacts with Vangl, but how this interaction influences PCP signaling is still not understood (Courbard et al., 2009; Glasco et al., 2012; Kallay et al., 2006; Montcouquiol et al., 2003; Wada et al., 2005).

**The PCP pathway in collective cell movements**

Collective cell migration refers to the process by which a group of cells move in concert as sheets, cellular streams or clusters by remodeling without losing cell-cell contacts. Collective migrations are characterized by multicellular polarity and ‘supracellular’ organization of the actin cytoskeleton, which enables forces to be generated that are greater than the sum of the forces that could be generated by individual cells (Friedl and Gilmour, 2009). Although PCP has not been strongly implicated in classic in vivo collective migration, in which a group of cells cooperate to translocate relative to their surroundings, a number of studies have shown that PCP is crucial for coordinated cell polarity and force generation in CE movements of the neural plate and mesoderm during vertebrate gastrulation and for communicating joint navigation decisions during neural crest migration. Although the cell types involved in these movements are varied – epithelial in the neural plate and mesenchymal in the mesoderm and neural crest – recent mechanistic studies have revealed commonalities in how PCP is used in these various cell types to generate movement.

**PCP during CE movements**

CE is a process by which the cells comprising a tissue are reorganized to narrow and extend the tissue; in the early embryo, CE movements serve to narrow the embryo along its mediolateral axis and elongate it along its anterior-posterior (AP) axis. CE can be accomplished by different cellular mechanisms in different tissue types or even in different regions of the same tissue. In mesenchymal cells of the mesoderm during gastrulation, lamellipodia become polarized to mediolateral membranes and make stable contacts specifically with mediolateral neighbors to exert traction that results in cellular elongation and alignment with the mediolateral axis (Shih and Keller, 1992; Wilson and Keller, 1991). In the lateral mesoderm of fish and the tailbud of mice, cells move medially by directed migration (Jessen et al., 2002; Yen et al., 2009), whereas in the more densely packed paraxial mesoderm CE progresses via cell intercalations of two types: polarized radial intercalations, in which AP neighbors are separated when a cell from a different layer moves between them; and medial intercalations, in which AP neighbors are separated when a more lateral cell in the same tissue moves between them (Yen et al., 2009; Yin et al., 2008). In the more coherent epithelial cells of the forming neural tube, CE occurs through polarized cell junction remodeling events that drive mediolateral neighbor exchanges similar to those that occur during Drosophila germ band extension, although polarized protrusive activity is also present (Bertet et al., 2004; Blankenship et al., 2006; Nishimura et al., 2012; Williams et al., 2014).

Components of the PCP pathway have been implicated in each of these cell behaviors that underlie CE movements. Indeed, whereas the characteristic phenotypes of fly PCP mutants arise due to polarity defects in static epithelial cells, the most prominent phenotypes of vertebrate PCP mutants – a short, wide body axis and open neural tube – are due to the failure of CE movements that narrow and extend the mesoderm and neural plate early in development. Fzd, Dvl, Vangl, Pk, Celsr and the PCP effector Fritz have been implicated in both neuroepithelial and mesodermal CE movements in a range of vertebrates, as have the PCP-associated factors Scrib and Ptk7 (as detailed in Table 1). This shared requirement has recently uncovered commonalities in the mechanisms of neuroepithelial and mesodermal CE.

**Neuroepithelial CE movements**

Neuroepithelial progenitor cells in the neural plate are highly dynamic, undergoing apical constriction, medial intercalations and neighbor exchanges (Fig. 2) that lead to the alignment of cells along the mediolateral axis to form supracellular contractile cables (Nishimura et al., 2012; Suzuki et al., 2012; Williams et al., 2014). Anisotropic tension generated along mediolaterally oriented

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**Fig. 2. Asymmetric PCP protein localization drives neuroepithelial CE movements.** Schematics of polarized cell behaviors in the neural plate. Views are of the apical surface where PCP proteins localize at apical junctions. (A) An intercalation event separates the two gray cells and creates a new junction between the two beige cells by virtue of anisotropic contractility (black arrows) that shortens mediolateral junctions. (B) The narrowing and lengthening of a string of mediolaterally aligned cells due to the contractility of supracellular actin cables. In both examples, Celsr (blue) localizes to anterior and posterior membranes and Vangl2-Pk (red) localizes asymmetrically to anterior membranes. Asymmetry of Fzd or Dvl has not been demonstrated at these stages in the neural plate.
junctons in the neural plate serves to narrow and bend it (Box 1), such that it transforms from a wide, flat epithelium into an elongated tube. When PCP signaling is disrupted, the apical surfaces of neuroepithelial progenitors are distended due to the relaxation of apical actomyosin contractility (Nishimura et al., 2012; Ossipova et al., 2014; Yen et al., 2009). This results in a characteristic open neural tube extending from the midbrain to the tail – known clinically as cranioarachnoiditis – due to failure of the neural tube to narrow, bend and fuse dorsally (Table 1) (Curtin et al., 2003; Etheridge et al., 2008; Goto and Keller, 2002; Hamblet et al., 2002; Lu et al., 2004; Murdoch et al., 2003; Nishimura et al., 2012; Wang et al., 2006b; Ybot-Gonzalez et al., 2007).

What role does the PCP pathway play in this process? Neuroepithelial progenitors have been shown to exhibit planar polarized distribution of PCP proteins during CE, with an axis of planar polarity that is orthogonal to the overall direction of convergence. Pk and Vangl localize together at the level of AJs on anterior membranes in the frog neural plate (Ossipova et al., 2015b). In the fish, in which maturation of the apical surface of neuroepithelial progenitors is delayed (Araya et al., 2016), Pk and Vangl2 localize asymmetrically to the anterior side of immature neuroepithelial progenitors (Ciruna et al., 2006; Roszko et al., 2015) and are subsequently recruited to anterior AJs (Davey et al., 2016). The Flamingo homolog Celsr1 localizes to both anterior and posterior AJs, a localization that is particularly apparent when cells become aligned along the mediolateral axis of the chick neural plate (Nishimura et al., 2012) (Fig. 2). Dvl2 also exhibits a planar polarized distribution along mediolaterally oriented membranes (Fig. 2), being strongly recruited there by Celsr1 (McGreevy et al., 2015; Nishimura et al., 2012). Dvl2 asymmetry to posterior versus anterior membranes, if present in the neural plate, was not detectable in these studies.

This localization of PCP components to AP cell membranes puts them in ideal positions to regulate anisotropic membrane shortening by the polarized activation of actomyosin contractility. PCP has been linked to actomyosin contractility in the neural plate in multiple ways. Shroom3 is an apically enriched actin-binding protein that recruits Rho-associated kinase (Rock) to activate myosin II, driving apical constriction and neural tube closure (Nishimura and Takeichi, 2008). A recent report demonstrated that, in the mouse neural plate, Shroom3 and Rock become polarized to mediolaterally aligned membranes by Dvl2 (McGreevy et al., 2015). Daam1, which is a formin homology protein that directs nucleation and elongation of actin filaments, also functions as an adaptor linking Dvl to RhoA in multiple PCP processes (Habas et al., 2001). In the chick neural plate, for example, Dvl2 at mediolaterally oriented junctions binds Daam1, driving both actin assembly and RhoA/Rock-dependent activation of myosin II and anisotropic contractility (Nishimura et al., 2012). The model for neuroepithelial CE that emerges from these studies (Fig. 2) is similar to the model for germband extension in the Drosophila embryonic epithelium, where apical enrichment of actomyosin contractility at dorsoventrally oriented junctions leads to polarized cell intercalations and shape changes that extend the body axis (Bertet et al., 2004; Blankenship et al., 2006; Walck-Shannon and Hardin, 2014). Although germband extension occurs without the asymmetric polarity information conferred by the PCP pathway, recent work has demonstrated the presence of an analogous system whereby heterotypic interactions between Toll-like receptors across dorsoventrally oriented junctions initiate increased actomyosin contractility (Paré et al., 2014). Nevertheless, it is unclear how the asymmetric distribution of PCP proteins, which distinguish anterior Vangl-Pk membranes from posterior Fzd-Dvl membranes in the neural plate, mediates neuroepithelial CE movements. In the fly wing, asymmetrically localized Vang-Pk and Fz-Dvl complexes have opposite effects on the actin cytoskeleton, with Fz/Dvl promoting actin polymerization and Vang-Pk antagonizing that activity; a similar effect during CE might be expected to lead to tissue deformations that are not observed: boundary shortening is anisotropic but not asymmetric (Box 1). The asymmetric distribution of PCP proteins is clearly important, as overexpression of PCP core components during CE typically leads to phenotypes that resemble those observed in the loss-of-function condition (Jessen et al., 2002). Insights into the role of asymmetrically localized PCP components during neuroepithelial morphogenesis have been provided by studies of zebrafish, in which it was shown that Vangl2 mutant cells in the neural keel are unable to re-intercalate into the neuroepithelium after undergoing mediolaterally oriented divisions (Ciruna et al., 2006). Indeed, dividing epithelial cells round up and temporarily lose their polarity, and PCP signaling is a general mechanism by which interphase cells can inform newly divided neighbors about tissue polarity (Devensport et al., 2011). PCP asymmetry might thus serve more to facilitate tissue integrity – analogous to the stacking system of Lego bricks – than to drive morphogenesis directly. The mammalian neural plate is highly proliferative, and although cell divisions are not mediolaterally oriented (Williams et al., 2014) a failure to re-establish tissue polarity after cell division might indirectly impact intercalations during neuroepithelial CE.

Box 1. Axes, cell-cell junctions and PCP

The literature on PCP in CE movements refers to cell junctions in various ways that can sometimes be confusing. Consider two cells (apical surfaces of which are depicted in A) that are undergoing PCP-mediated border shortening during CE. The junction between the two cells is oriented along the mediolateral axis of the embryo, and it is thus referred to as a mediolateral junction and its constituent membranes as mediolaterally oriented membranes. This is where cortical tension is generated to power membrane shortening and neighbor exchanges. However, when considering the localization of PCP proteins, it is necessary to refer to the individual membranes that comprise the junction: Fzd (green) localizes to the posterior membranes of these cells whereas Vangl2 (red) localizes to their anterior membranes and Celsr (blue) localizes to both. This localization of PCP components is essential for driving anisotropic changes in cell shape, as shown in B. For example, the apical surfaces of neuroepithelial progenitors constrict during CE to narrow the neural plate and bend it at its hinge points. Whereas isotropic apical constriction shortens all of the apical membranes of a cell equally, generating a wedge-shaped cell, anisotropic apical constriction, which depends on PCP, shortens just the anterior and posterior membranes where PCP proteins are preferentially localized, shortening the cell along the mediolateral axis but not along the anterior-posterior axis.
Mesodermal CE

PCP signaling is also strongly implicated in CE movements of the mesoderm during gastrulation. Live imaging of mesodermal cells in *Xenopus*, zebrafish and mouse embryos shows that perturbation of PCP signaling disrupts mediolateral polarity and stability of mesodermal cell protrusions, their mediolateral alignment and elongation, and the polarization of both the medial and radial intercalations that normally serve to narrow and extend the AP axis (Table 1) (Darken et al., 2002; Heisenberg et al., 2000; Jessen et al., 2002; Takeuchi et al., 2003; Veeman et al., 2003; Wallingford et al., 2000; Yen et al., 2009; Yin et al., 2008). Although Dvl was initially reported to localize to mediolateral protrusions, where it was thought to promote actin polymerization (Kinoshita et al., 2003), this was subsequently found to be illusory (Panousopoulou et al., 2013). Instead, work in zebrafish has identified a more classically planar polarized distribution of PCP proteins in mediolaterally elongated mesodermal cells during CE (Fig. 3): Pk is detected in dynamic puncta that preferentially localize to anterior membranes, whereas Dvl puncta localize to posterior membranes (Ciruna et al., 2006; Yin et al., 2008). Vangl2 also localizes to anterior membranes and is required for anterior Pk localization (Roszko et al., 2015; Yin et al., 2008). Aside from their failure to elongate and align along the mediolateral axis, Vangl2 mutant mesodermal cells display an aberrant tendency to migrate anteriorly during gastrulation, suggesting that Vangl2 at the anterior membrane normally resists this directional bias (Roszko et al., 2015). This could explain, at least in part, the most striking cell migration defect reported for vangl2 mutant cells: more convoluted trajectories during dorsal convergence compared with those observed in wild-type cells (Jessen et al., 2002).

Recent functional studies in *Xenopus* are consistent with a role for PCP signaling at these non-protrusive, mediolaterally oriented surfaces of mesodermal cells (Shindo and Wallingford, 2014). Increased actomyosin contractility at these junctions shortens and pulls neighboring cells into a classical mediolateral intercalation event. The partitioning of cortical actomyosin contractility to these cell interfaces requires the cortical partitioning protein Septin7, which localizes to mediolateral vertices and restricts actin flow. Disrupting PCP signaling by expression of a dominant-negative form of Dvl (DN-Dvl) or knockdown of the PCP effector Fritz leads to the loss of Septin7 localization and corresponding loss of planar polarized contractility (Kim et al., 2010; Shindo and Wallingford, 2014).

The emerging picture of mesodermal CE (Fig. 3) is, therefore, not dissimilar to the model for neuroepithelial CE (Fig. 2), which was proposed by Nishimura et al. (2012), and suggests that a conserved role for PCP signaling in collective cell behaviors is to promote actomyosin contractility at homotypic cell-cell junctions that lie in a plane that is perpendicular to the direction of planar polarization. However, this is unlikely to be the whole story. In zebrafish and mice, polarized radial intercalations of mesodermal cells (cells moving from a deeper layer into a more superficial layer) also contribute to axial extension and depend on PCP signaling (Yen et al., 2009; Yin et al., 2008), but it is currently unknown how PCP proteins polarize this type of neighbor exchange during mesodermal CE. Recent work in *Xenopus*, in which epidermal cells undergo PCP-dependent radial intercalation, has suggested that Vangl-Pk complexes at the tips of intercalating cells drive invasion into the overlying cell layer (Ossipova et al., 2015a). In this case, the role of Vangl2-Pk is presumably to regulate protrusive activity, which is different from the role of PCP signaling at non-protrusive surfaces during CE. However, the localization of PCP components to dynamic membrane protrusions is characteristic of other types of PCP-dependent cell migration, as we describe below.

PCP in neural crest cell migration

The neural crest (NC) is a multipotent cell population derived from the junction of the neural and non-neural ectoderm. NC cells migrate throughout the embryo in response to well-described chemotropic cues and give rise to numerous neural and non-neural tissues (Mayor and Theveneau, 2013). PCP-dependent homotypic interactions between NC cells are crucial for migration (Carmona-Fontaine et al., 2008). Accordingly, disrupting PCP signaling in *Xenopus* prevents cranial and trunk NC migration (Carmona-Fontaine et al., 2008; De Calisto et al., 2005; Matthews et al., 2008a,b; Shnitsar and Borchers, 2008) (Table 1). In the context of the NC, PCP signaling is specifically implicated in contact-mediated inhibition of cell locomotion: NC cells migrate collectively in cellular streams, with the cells at the front having the highest directional persistence and the most dramatic protrusive activity. Homotypic interactions between NC cells confer this outward directionality by inhibiting protrusive activity at all membranes with the exception of the migratory front (Carmona-Fontaine et al., 2008; Theveneau et al., 2010). NC cells with disrupted PCP signaling thus crawl on top of one another with both leading and trailing cells extending protrusions in all directions (Carmona-Fontaine et al., 2008).

As is the case for collectively moving cells undergoing CE, PCP proteins localize to the non-protrusive cell surfaces of NC cells that are engaged in homotypic interactions. Dvl, Wnt11 and Fzd7, for
example, are recruited to sites of NC cell-cell contact both in vivo and in vitro (Carmona-Fontaine et al., 2008). As a result, cells at the migratory front have a Fzd-Dvl asymmetry (Fig. 4). PCP signaling via Fzd-Dvl at homotypic NC contacts then leads to local activation of RhoA, which promotes retraction at the rear of the cell via Rock2-mediated actomyosin contractility. Additionally, RhoA/Rock activation inhibits the related small GTPase Rac1, such that Rac1 activity becomes polarized toward the leading edge of the cell (Fig. 4) where it drives protrusive activity (Carmona-Fontaine et al., 2008; Matthews et al., 2008b; Ridley, 2015). The localization of Fzd-Dvl to the trailing side of migrating cells is not universal, however: in migrating B lymphocytes and breast cancer cells, Dvl-Fzd localizes to the leading edge of cell protrusions, whereas Vangl-Pk localizes to non-protrusive or trailing sides (Kaucká et al., 2015; Luga et al., 2012; Zhang et al., 2016). This suggests that the recruitment of Fzd and Dvl to sites of homotypic cell-cell contact might instead be the more universal phenomenon, particularly in cells expressing Celsr or related adhesion G protein-coupled receptors (Devendport and Fuchs, 2008; Li et al., 2013; Nishimura et al., 2012). However, the localization of Celsr, Vangl or Pk has not been examined in the context of NC migration, so it is not known whether the usual antagonistic relationship of Fzd-Dvl and Vangl-Pk complexes underlies NC cell polarity.

A role for PCP signaling in NC migration in mammals is less clear. Superficially, NC migration is normal in constitutive Vangl2<sup>b<sub>b</sub>.<sub>b</sub></sup> mutants and in conditional Vangl2<sup>b<sub>b</sub>.<sub>b</sub>, Wnt1<sub>-Cre</sub></sup> mutants in which Vangl2 is deleted in the entire neural crest lineage (Pryor et al., 2014). However, Celsr3 and Fzd3 are required within the NC lineage for normal innervation of the gut by NC-derived enteric neurons, suggesting a more subtle role in NC development (Sasselli et al., 2013). Contact inhibition of locomotion is influenced not only by PCP signaling but also by diffusible signals such as Sdf1 (Cxcl12) (Theneveau et al., 2010), and long-range filopodial contacts between non-neighboring NC cells can also influence migratory behaviors (Teddy and Kulesa, 2004). Thus, it is not surprising that different mechanisms could predominate in different species.

**PCP and individual cell migration**

The PCP-dependent cell movements that we have discussed so far are all collective ones, involving PCP recruitment to dynamically remodeled homotypic cell-cell contacts, activating actomyosin contractility to drive apical constriction, and junctional remodeling and/or forward movement. Because these cell-cell contacts are changing on the scale of hours, it seems likely that in this context PCP complexes form and disassemble at a faster rate than in stable epithelial cells. However, cells in vivo frequently migrate over and between other types of cells, making and breaking heterotypic cell-cell and cell-extracellular matrix contacts on the scale of minutes as they do so. This is particularly true during central nervous system development, when neurons and axon growth cones navigate a complex neuroepithelial environment to reach their synaptic targets. The PCP pathway has been implicated in such directional movement of individual cells and, as we discuss below, the emerging picture is that it regulates actin dynamics within short-lived cellular protrusions. Recent evidence also suggests that the classical antagonistic relationship between Vangl-Pk and Fzd-Dvl complexes, and their effects on actin dynamics, appears to be largely preserved.

**PCP signaling during axon guidance**

A role for PCP in axon guidance has been described in both vertebrates and invertebrates (Tissir and Goffinet, 2013). Loss of either Fzd3 or Celsr3 in the mouse results in loss of the major axon tracts that connect the thalamus and the cortex, and failure of commissural neurons in the spinal cord to project anteriorly following midline crossing (Chai et al., 2015). Because the axon guidance defects observed in some PCP mutants are not detected in others, it has been suggested that core PCP components function independently of one another in some guidance events (Qu et al., 2014). Here, we focus on spinal commissural axon guidance in mammals, where components of both the Fzd and Vangl complexes have been implicated, and in which live imaging of PCP protein localization during guidance decisions has provided mechanistic insights.

Commissural neurons in the dorsal spinal cord project axons ventrally to cross the midline at the floorplate (Fig. 5) in response to well-characterized diffusible floorplate-derived cues (Dickson, 2002). After midline crossing, commissural axons turn to project anteriorly alongside the floorplate in a PCP-dependent manner: in Fzd3, Vangl2 and Celsr3 mouse mutants, post-crossing axons either stall or project randomly both anteriorly and posteriorly along the floorplate (Lyuksyutova et al., 2003; Shafer et al., 2011) (Table 1). This is a cell-autonomous requirement, as conditional knockout of Celsr3 in commissural neurons is sufficient to cause guidance defects (Onishi et al., 2013). Fzd3a, Celsr3 and Vangl2 are also required for the guidance of serotonergic and dopaminergic axons along the floorplate in the mouse brainstem, suggesting a general requirement for PCP in floorplate-guided longitudinal pathfinding (Fenstermaker et al., 2010). More generally, PCP signaling is often required for anterior-posteriorly oriented axon guidance events, including the AP extension of enteric neuron processes in the mouse gut (Sasselli et al., 2013) and the AP guidance of mechanosensory axons in the worm (Ackley, 2014).

How, then, does PCP signaling influence axon guidance? It is known that growth cones make guidance decisions in response to cell-surface and diffusible cues. For example, several Wnts that function in the non-canonical Wnt pathway (Wnt4a, Wnt5a, Wnt7b) are expressed in gradients along the AP axis of the hindbrain and spinal cord, and can drive commissural axon outgrowth when provided remotely to spinal cord explants in vitro, suggesting that Wnts can act as diffusible directional cues in this process (Fenstermaker et al., 2010; Lyuksyutova et al.,...
Box 2. PCP protein trafficking

Although PCP in epithelial cells is characterized by the stable asymmetric localization of Fzd and Vangl complexes to opposite sides of cells, live imaging of fluorescent PCP fusion proteins has demonstrated that these proteins are highly dynamic, even in stably polarized cells. Imaging of the fly wing disc epithelium, for example, has revealed cytoplasmic endosomal vesicles containing Fz and Dvl that are transcytosed along proximodistally oriented apical microtubules (Shimada et al., 2008). Vang-Pk complexes are also internalized (Cho et al., 2015), and both membrane-associated Fz-Dvl and Vang-Pk complexes exhibit a high rate of turnover, particularly in membrane domains where they are not stably localized (Strutt et al., 2011). Furthermore, an array of endosomal proteins has been identified in screens for planar polarity defects in flies (Carvajal-Gonzalez et al., 2015; Classen et al., 2005; Gault et al., 2012; Hermle et al., 2013; Mottola et al., 2010). This polarized membrane trafficking of fly PCP complexes is thought to be part of the mechanism by which PCP protein asymmetry is initially established (Matis et al., 2014) and subsequently amplified (Cho et al., 2015). Endosomal trafficking of PCP proteins is also involved in establishing their asymmetric localization in vertebrate epithelia (Devenport et al., 2011; Giese et al., 2012). However, in moving cells, PCP protein internalization is also directly implicated in downstream signaling to the actin cytoskeleton. In the presence of Wnt, Dvl interacts with clathrin adaptor proteins to drive internalization of activated Fzd (Chen et al., 2003; Onishi et al., 2013; Yu et al., 2007). Inhibition of Fzd internalization prevents Rac activation and JNK phosphorylation – classical readouts of PCP signaling – and prevents growth cone turning, CE movements and endothelial sprouting (Boutros et al., 1998; Kim et al., 2008; Lee et al., 2016; Ohkawara et al., 2011; Sato et al., 2010; Sewduth et al., 2014; Shafer et al., 2011; Yamanaka et al., 2002). Thus, rather than targeting Fzd for degradation or delivery to the ‘correct’ plasma membrane domain, endocytosis of Fzd in moving cells is associated with activated signaling. How internalized Fzd promotes cortical contractility or protrusive activity to generate cell movement is not clearly understood.

PCP signaling in neuronal migration

The PCP pathway is strongly implicated in the longitudinal migration of neurons in the plane of the neuroepithelium. This finding stems primarily from studies of mouse and zebrafish facial branchiomotor neurons (FBMNs), which originate ventrally in hindbrain rhombomere (r)4 and undergo highly stereotypical posterior migration along the floorplate to r6 and r7 (Wanner et al., 2013). Mutations in the genes encoding Vangl2, Pk1, Fz3 and Celsr2 all prevent FBMN migration in zebrafish and mice, as do Scrb and Ptk7 mutations (Bingham et al., 2002; Glasco et al., 2012; Mapp et al., 2011; Qu et al., 2010; Vivancos et al., 2009; Wada et al., 2005, 2006; Yang et al., 2014) (Table 1). The role of Wnt ligands in FBMN migration is uncertain: mice lacking Wnt5a have a mild defect whereas Wnt4a, Wnt5a and Wnt11r mutants in zebrafish all exhibit normal FBMN migration (Vivancos et al., 2009) (our unpublished results). Similarly, the role of Dvl genes in FBMN migration remains uncertain (Davey et al., 2016; Glasco et al., 2012).
As is the case for other cell migration and axon guidance events that involve PCP, signaling is required within the migrating cells themselves (Davey et al., 2016; Jessen et al., 2002; Walsh et al., 2011). However, chimeric analysis in zebrafish has demonstrated an equally important non-cell-autonomous role for Vangl2, Fzd3a, Celsr2 and Scrib, as wild-type FBMNs fail to migrate through a neuroepithelium that is mutant for any of these factors (Davey et al., 2016; Jessen et al., 2002; Sittaramane et al., 2013; Wada et al., 2005, 2006; Walsh et al., 2011). It should also be noted that cells of both the neuroepithelium and the floorplate exhibit planar polarization along their AP axes that depends on the same PCP core components that are required for FBMN migration; these factors exhibit canonical epithelial localization, with Vangl-PK opposite Fzd-Dvl along the axis of polarization (Borovina et al., 2010; Ciruna et al., 2006; Davey et al., 2016; Walsh et al., 2011). Thus, the fundamental characteristic of PCP signaling – that it acts within and between contacting cells to coordinate cell polarization – appears to hold even when the cells involved (migrating FBMs and static planar-polarized epithelial cells) are of different types.

How do PCP proteins function during FBMN migration? High-resolution live imaging of FBMs in PCP mutant embryos has revealed a role in filopodial protrusive activity, with Vangl2 localizing transiently to the tips of FBMN filopodia and signaling retraction events (Davey et al., 2016). Chimeric analysis showed that Fzd3a and Vangl2 regulate FBMN filopodial protrusive activity in opposing ways (Fig. 6). Within FBMs, Fzd3a is required to stabilize filopodia whereas Vangl2 has an antagonistic, destabilizing role. Conversely, in the migratory environment, Fzd3a destabilizes FBMN filopodia whereas Vangl2 has a stabilizing role (Davey et al., 2016). Although transient (on the scale of seconds to minutes), these functional interactions are analogous to the intracellular antagonistic versus intercellular stabilizing functions that Fzd and Vangl complexes exhibit in stably polarized epithelia. Thus, conserved interactions between PCP core components regulate different aspects of actin dynamics, ranging from the formation of stable actin-rich hairs to the extension and retraction of dynamic actin-based protrusions with consequences on directional cell migration. Interestingly, in breast cancer cells in culture, in which PCP signaling in response to signals from stromal cells is required for migration and metastasis (see Box 3), Fzd and Vangl complexes localize in complementary domains, with Fzd-Dvl at the tips of cellular protrusions and Vangl2-PK at their base (Luga et al., 2012). Although the target(s) of PCP signaling in filopodia are unknown, a putative FBMN-specific PCP effector is Nhs1/ Nhs1b, a WAVE-homology domain-containing regulator of cellular protrusive activity that localizes to FBMN filopodia and is required cell-autonomously for FBMN migration (Brooks et al., 2010; Walsh et al., 2011).

**Conclusions and future perspectives**

We have discussed here distinct roles for PCP signaling in collectively versus individually migrating cells in vivo, based on live cell imaging studies of normal and mutant embryos and on the localization of PCP fusion proteins. In collectively moving cells, recent evidence favors a conserved role for PCP signaling at homotypic cell contacts perpendicular to the axis of tissue polarity, which promotes cortical actomyosin contractility leading to

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**Fig. 6. PCP-mediated control of directional neuron migration.** Schematic of a facial branchiomotor neuron (FBMN) migrating posteriorly through the planar-polarized hindbrain neuroepithelium. Vangl2 and Fzd3 localize to anterior and posterior apical sides of the neuroepithelial and flooplate cells, respectively. Vangl2 localizes to the tips of retracting FBMN filopodia. Based on chimeric analysis, Fzd3 in the neurons and Vangl2 in the neuroepithelial environment stabilize FBMN filopodia, whereas Vangl2 in FBMs and Fzd3 in the environment destabilize them.

Core PCP proteins are upregulated in a number of cancers and have been found to regulate tumor cell motility (Jessen, 2009). The downregulation of Fzd6, Dvl1, Vangl1, PK1 and Wnt11 reduces protrusive activity and migration of breast cancer cells (BCCs), and PK1 is required to promote BCC metastasis in mice (Luga et al., 2012). Additionally, a link between Fzd2, Dvl1, Vangl1, Scrib and Wnt5a and cancer cell motility has been shown in several cancer cell lines (Anastas et al., 2012; Gujral et al., 2014; MacMillan et al., 2014; Yamamoto et al., 2010). A model for how PCP signaling regulates the migration of individual cancer cells has recently been proposed. Upon Wnt11 stimulation of BCC migration, Fzd6 and Dvl1 become enriched at the leading edge of cell protrusions whereas PK1 and Vangl1 localize to non-protrusive membranes (Luga et al., 2012). This complementary distribution of Fzd-Dvl and Vangl-PK with respect to dynamic cellular protrusions is reminiscent of the complementary patterns of PCP protein localization observed in vivo, and suggests that the mutual intracellular antagonism of the two complexes is preserved in individually migrating cancer cells. PK1 at non-protrusive surfaces inhibits RhoA GTPase activity; RhoA in turn activates focal adhesion maturation and force generation only at PK1-negative (Fzd-Dvl positive) membrane domains (Zhang et al., 2016). Interestingly, although PK1 knockdown and ectopic PK1 have opposite effects on cellular protrusions, migration is inhibited under both conditions. Thus, canonical PCP interactions create a dynamic pro-migratory balance of protrusive and non-protrusive surfaces in cancer cells.
polarized cell intercalations and neighbor exchanges. By contrast, in individually migrating cells and axons that are pathfinding along the axis of tissue polarity, PCP proteins localize dynamically to membrane protrusions where Fzd-Dvl promotes the growth and stabilization of protrusions and consequent directional migration.

A recent study captured both forms of PCP-dependent migration in a single cell type (Tatin et al., 2013), namely lymphatic endothelial cells. Such cells migrate to form lymphatic valves, a process that requires both Celsr1 and Vangl2. During an early phase of migration along the axis of the vessel, both proteins localize to cellular protrusions, but as the endothelial cells re-orient into a plane that is perpendicular to the axis of the endothelium, Celsr1 and Vangl2 are abruptly recruited to sites of homotypic cell contact. We suggest that an emerging theme is that PCP signaling in cellular protrusions is associated with migration along an axis of planar polarity whereas signaling at homotypic cell contacts is characteristic of migration that is predominantly perpendicular to the axis of planar polarity.

The antagonistic intracellular relationship between Fzd-Dvl and Vangl-Pk that leads to their mutually exclusive localization in fly epithelia also appears to be broadly conserved in moving cells. In collectively moving cells undergoing CE, Vangl-Pk complexes localize on the anterior side and Fz-Dvl complexes localize on the posterior side. Although both complexes, together with Celsr, are required for normal CE movements, how their asymmetric localization facilitates these movements remains an important question. In single cells migrating in culture, FZD-DVL and VANGL-PK localization is also mutually exclusive, with FZD-DVL at the leading edge of protrusions and VANGL-PK at adjacent non-protrusive surfaces (Luga et al., 2012). These complementary domains actively balance protrusive and non-protrusive regions of the membrane to enable persistent directional migration; both too much and too little protrusive activity inhibits migration (Zhang et al., 2016). This is consistent with functional analysis of Fzd and Vangl in migrating motor neurons in vivo, where both complexes are required for directional migration but have opposite cell-autonomous effects on filopodial dynamics (Davey et al., 2016).

In growth cones, the relationship between Fzd and Vangl complexes in cellular protrusions is less canonical, with Vangl promoting, rather than antagonizing, Fzd via internalization and JNK activation, with consequences on directional turning but not filopodial dynamics (Onishi et al., 2013; Shafer et al., 2011). Different directional cues in the embryo – a diffusible Wnt ligand for commissural axons versus local polarity cues on nearby neuroepithelial progenitors for migrating facial motor neurons – might determine how PCP signaling is transduced within cellular protrusions to influence migration. Differential recruitment of vertebrate-specific PCP-associated proteins such as Ptk7, Ror2, Knypek and others in specific cell movement contexts might also influence the activities of PCP core components in as-yet undiscovered ways.

Finally, the effectors of PCP that regulate actomyosin contractility in collectively moving cells are frequently the same multifunctional effectors that regulate actin assembly downstream of PCP in individually migrating cells. Indeed, the same effectors function downstream of PCP in fly epithelia. For example, ROCK and JNK activation downstream of Fzd-Dvl is required for CE movements (Marlow et al., 2002; Nishimura et al., 2012; Yamanaka et al., 2002) as well as for commissural axon guidance (Shafer et al., 2011) and FBMN migration (Vivancos et al., 2009). Furthermore, Daam1 interacts with Dvl1 and Rho GTPases to promote actomyosin contractility during neural plate CE (Nishimura et al., 2012) but also has a prominent role in the bundling of linear F-actin filaments in filopodia (Jaiswal et al., 2013). This actin-bundling activity was recently shown to be required for the PCP-dependent growth and guidance of axons in the Drosophila mushroom body (Gombos et al., 2015). What determines how these conserved PCP effectors influence diverse migratory cell behaviors downstream of PCP signaling will be a topic of future research.

In this Review, we have emphasized several principles common to PCP-mediated cell movements during development but there are clearly many open questions. Furthermore, although we have focused exclusively on cell movements that require multiple PCP core components, including at least one from each of the Fzd and Vangl complexes, it should be noted that many other cell movements and axon guidance events have been shown to involve some, but not other, core PCP components. It thus remains to be determined whether any of the principles we have described here hold true in those contexts. We have also focused on cells in which the localization of core PCP components and the live imaging of migratory behaviors in vivo have provided insights into the underlying cell biology. However, by necessity, these studies have used ectopically expressed fluorescent fusion proteins, which could alter PCP protein dynamics, and it remains an important goal to validate these findings by studying endogenous PCP components. Nonetheless, these findings – together with studies relating how PCP functions in static epithelia – are providing key insights into the crucial role played by PCP signaling in both developmental and disease contexts.

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