© 2017. Published by The Company of Biologists Ltd | Development (2017) 144, 187-200 doi:10.1242/dev.122804

The Company of Biologists

DEVELOPMENT

REVIEW

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 are 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.

Division of Basic Sciences, Fred Hutchinson Cancer Research Center, B2-159, 1100 Fairview Ave. N., Seattle, WA 98109, USA.

*Author for correspondence (cmoens@fhcrc.org)

C.B.M., 0000-0002-1099-0728

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 Frizzled (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, Stbm) 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).

Development (2017) 144, 187-200 doi:10.1242/dev.122804

Table 1. Cell movements controlled by the PCP pathway

	Loss-of-function phenotype		PCP protein localization			
Open/duplicated neural tube	Vangl2	Mouse: Ybot-Gonzalez et al., 2007; Fish: Ciruna et al., 2006; Frog: Goto and Keller, 2002; Ossipova et al., 2014	Localization at apical surface of neuroepithelial progenitor cells	Vangl2	Anterior side (GFP-Vangl2)	Fish: Roszko et al., 2015; Davey et al., 2016; Frog: Ossipova et al., 2015b
				Pk1	Anterior side (GFP-Pk)	Fish: Ciruna et al., 2006; Roszko et al. 2015; Frog: Ossipova et al., 2015b
	Fzd3,6	Mouse: Wang et al., 2006a		Fzd3	Posterior side (Fzd3a-GFP)	Fish: Davey et al., 2016
	Dvl1,2,3	Mouse: Etheridge et al., 2008; Hamblet et al 2002; Wang et al., 2006b; Frog: Wallingford and Harland, 2002; Chick: Nishimura et al., 2012		Dvl2	Anterior and posterior sides (α-Dvl2)	Mouse: McGreevy et al., 2015; Chick: Nishimura et al., 2012
	Celsr1	Mouse: Curtin et al., 2003; Chick: Nishimura et al., 2012		Celsr1	Anterior and posterior sides (α-Celsr1)	Chick: Nishimura et al., 2012
	Ptk7	Mouse: Lu et al., 2004; Williams et al., 2014; Frog: Wehner et al., 2011				
	Scrib	Mouse: Murdoch et al., 2003; Fish: Zigman et al., 2011				
Delayed mesodermal convergent extension	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	Localization on converging mesodermal cells	Vangl2	Anterior side (GFP-Vangl2)	Fish: Roszko et al., 2015
	Pk1	Fish: Veeman et al., 2003; Frog: Takeuchi et al., 2003		Pk1	Anterior side (GFP-Pk)	Fish: Yin et al., 2008
	Fzd7,8	Frog: Djiane et al., 2000; Wallingford et al. 2001				
	Dvl (DN)	Fish: Heisenberg et al. 2000; Frog: Sokol, 1996; Tada and Smith 2000; Wallingford et al., 2000; Wallingford and Harland, 2001		Dvl	Posterior side (GFP-Dvl)	Fish: Yin et al., 2008
	Wnt5a, 11	Mouse: Andre et al 2015; Fish: Heisenberg et al., 2000; Kilian et al. 2003; Frog: Wallingford et al., 2001; Moon et al., 1993; Tada and Smith, 2000				
	Ptk7	Mouse: Yen et al., 2009; Fish: Hayes et al., 2013				
Incomplete/absent NC migration	Vangl2	Fish: Matthews et al., 2008b	Asymmetric localization along leading (non-contacting)-trailing (contacting other NC cells) axis.	Fzd7	Trailing (Fzd7-YFP)	Frog: Carmona-Fontaine et al., 2008
	Dvl (DN)	Fish: Matthews et al., 2008b; Frog: De Calisto et al., 2005; Carmona-Fontaine et al., 2008		Dvl	Trailing (Dvl-GFP)	Frog: Carmona-Fontaine et al., 2008
	Ptk7	Frog: Shnitsar and Borchers, 2008				
	Wnt5, 11	Fish: Matthews et al., 2008b; Frog: De Calisto et al., 2005; Carmona-Fontaine et al., 2008; Matthews et al., 2008a		Wnt11	Trailing (Wnt11-YFP)	Frog: Carmona-Fontaine et al., 2008
Disorganized/ unpolarized longitudinal axon guidance	Vangl2	Mouse: Fenstermaker et al., 2010; Shafer et al., 2011	Dynamic localization in the growth cone	Vangl2	Filopodial tips and membrane (GFP-Vangl2)	Mouse: Shafer et al., 2011
	Fzd3	Mouse: Lyuksyutova et al., 2003; Fenstermaker et al., 2010		Fzd3	Filopodia tips and endocytic vesicles (Fzd3-mCherry)	Mouse: Shafer et al., 2011; Onishi et al., 2013

Continued

Development (2017) 144, 187-200 doi:10.1242/dev.122804

Table 1. Continued

Loss-of-function phenotype			PCP protein localization			
	Celsr3	Mouse: Fenstermaker et al., 2010; Shafer et al., 2011				
	Wnt4, 5a	Mouse: Lyuksyutova et al., 2003; Fenstermaker et al., 2010				
Failure of facial motor neuron migration	Vangl2	Mouse: Vivancos et al., 2009; Glasco et al., 2012; Fish: Jessen et al., 2002; Bingham et al., 2002	Localization in facial motor neurons	Vangl2	Filopodia tips and membrane (GFP-Vangl2)	Fish: Davey et al., 2016
	Pk1	Fish: Mapp et al., 2010, 2011; Rohrschneider et al. 2007; Mouse: Yang et al., 2014				
	Fzd3	Mouse: Vivancos et al., 2009; Qu et al., 2010; Fish: Wada et al., 2006				
	Dvl (DN)	Fish: Davey et al., 2016				
	Celsr1,2,3	Mouse: Qu et al., 2010; Fish: Wada et al., 2006				
	Scrib	Mouse: Vivancos et al., 2009; Fish: Wada et al., 2005				
Failure of lymphatic endothelial valve formation	Vangl2	Mouse: Tatin et al., 2013	Dynamic localization in valve-forming cells	Vangl2	Filopodia tips, homotypic contacts (α-Vangl2 and GFP-Vangl2)	Mouse: Tatin et al., 2013
	Celsr1	Mouse: Tatin et al., 2013			Filopodia tips, homotypic contacts (α-Celsr1 and Celsr1-GFP)	Mouse: Tatin et al., 2013
Reduced breast cancer cell migration/ metastasis	VANGL1	Human: Luga et al., 2012; Anastas et al., 2012; MacMillan et al., 2014	Localization during Wnt-induced migration	VANGL1	Non-protrusive surfaces (Flag-VANGL1)	Human: Luga et al., 2012
	PK1	Human: Luga et al., 2012; Zhang et al., 2016		PK1	Non-protrusive surfaces (α-PK1)	Human: Luga et al., 2012; Zhang et al., 2016
	DVL1,2,3	Human: Luga et al., 2012		DVL	Protrusive surfaces (α-DVL1, α-DVL3)	Human: Luga et al., 2012
	FZD6	Human: Luga et al., 2012		FZD6,7	Protrusive surfaces (α-FZD6, α-FZD7)	
	WNT5A,11	Human: Luga et al., 2012				
	SCRIB	Human: Anastas et al., 2012		SCRIB	Lamellipodia	Human: Anastas et al., 2012

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). Altering the expression of Vang or Fz homologs mosaically in the

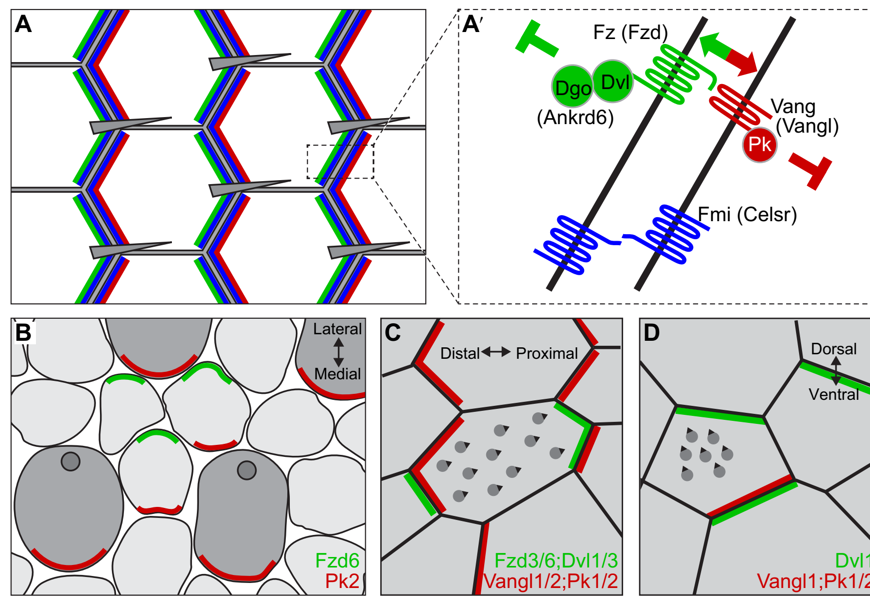


Fig. 1. Planar polarization of epithelial cells. (A,A′) A field of cells in the fly wing is depicted, with distal to the right. Each cells harbors a distal trichome (gray) and polar localization of PCP components, as detailed in the inset (A′). Arrows indicate intercellular recruitment and stabilization, 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 epithelium 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 β-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 c-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 adaptor protein Gipc1 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 Smurf1 and Smurf2, 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 medial and lateral 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* germband 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

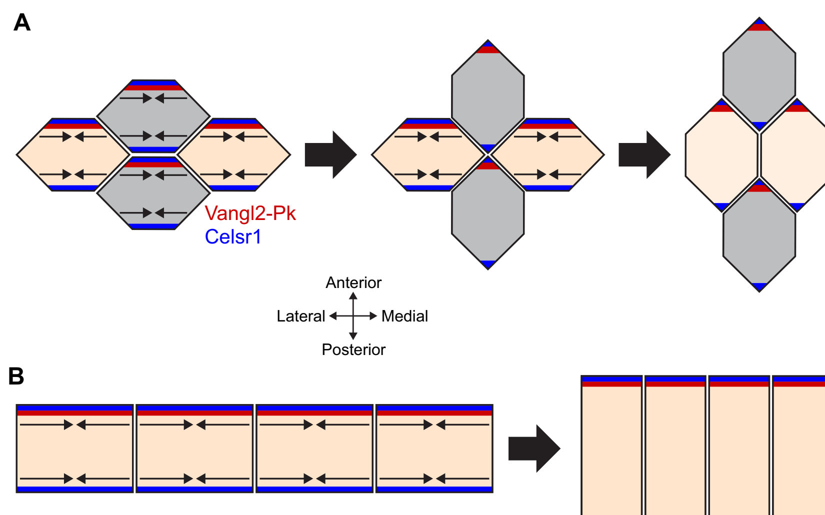


Fig. 2. Asymmetric PCP protein localization drives neuroepithelial CE movements. Schematics of polarized cell behaviors in the neural plate. Views are of the apical surface where PCP proteins localize at apical junctions. (A) An intercalation event separates the two gray cells and creates a new junction 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.

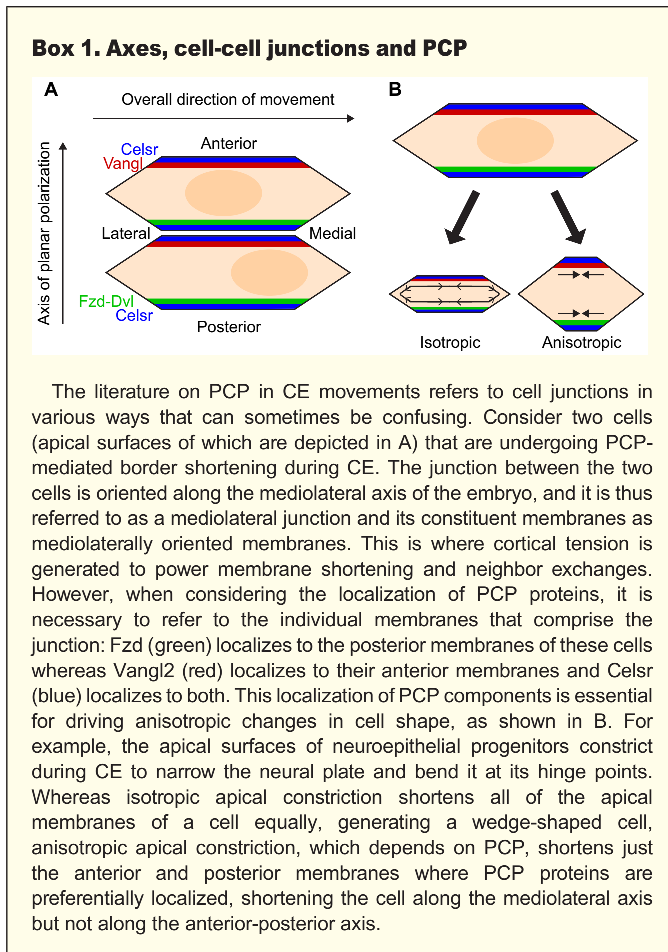
junctions in the neural plate serves to narrow and bend it (Box 1), such that it transforms from a wide, flat epithelium into an elongated tube. When PCP signaling is disrupted, the apical surfaces of neuroepithelial progenitors are distended due to the relaxation of apical actomyosin contractility (Nishimura 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 cranioarachischisis – 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 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 (Devenport 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.

Development (2017) 144, 187-200 doi:10.1242/dev.122804

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).

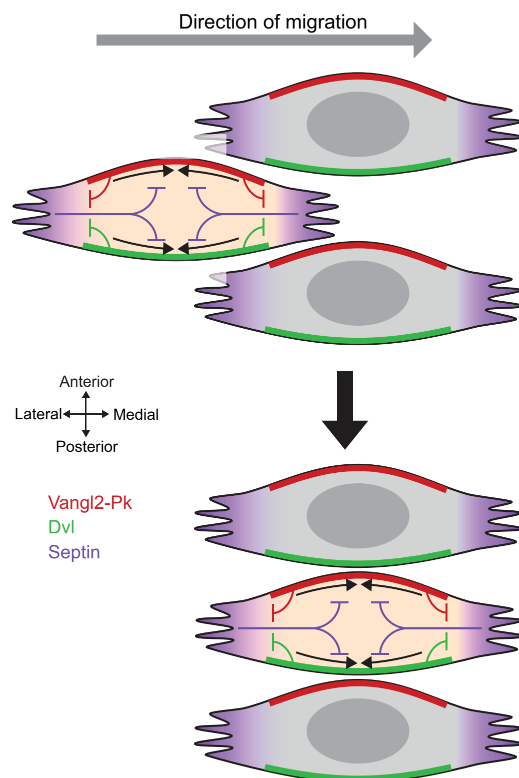


Fig. 3. Asymmetric PCP protein localization drives mesodermal CE movements. Schematic of three mesodermal cells undergoing a medial intercalation event during CE. The vertical dashed line represents the notochord boundary. Vangl and Pk (red) localize to anterior membranes whereas Dvl (green) localizes to posterior membranes. PCP components limit the cortical compartmentalizing protein Septin7 (purple) to the mediolateral ends of cells, which in turn limits actomyosin contractility (black arrows) to the anterior and posterior (mediolaterally oriented) cortex.

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 Septin 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

Development (2017) 144, 187-200 doi:10.1242/dev.122804

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 towards 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 (Devenport 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 Fz-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*$^{lp/lp}$ mutants and in conditional *Vangl2*$^{lp/flox}$*; Wnt1-Cre* 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) (Theveneau et al., 2010), and long-range filopodial contacts between non-neighboring NC cells can also influence migratory behaviors (Teddy and Kulesa, 2004). Thus, 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

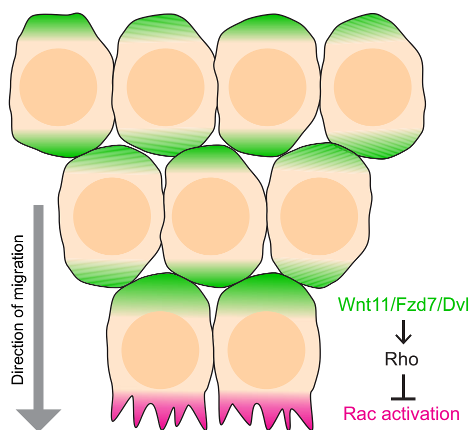


Fig. 4. PCP signaling during neural crest migration. Schematic of a sheet of neural crest (NC) cells with the leading cells at the bottom. The recruitment of Fz-Dvl (green) to sites of cell-cell contact activates Rho to promote actomyosin contractility for forward movement and inhibits Rac1 (red), thus limiting activation of membrane protrusive activity to the leading edge of the migratory front.

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 migration 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 *Fz3*, *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 serotinergic 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.,

Development (2017) 144, 187-200 doi:10.1242/dev.122804

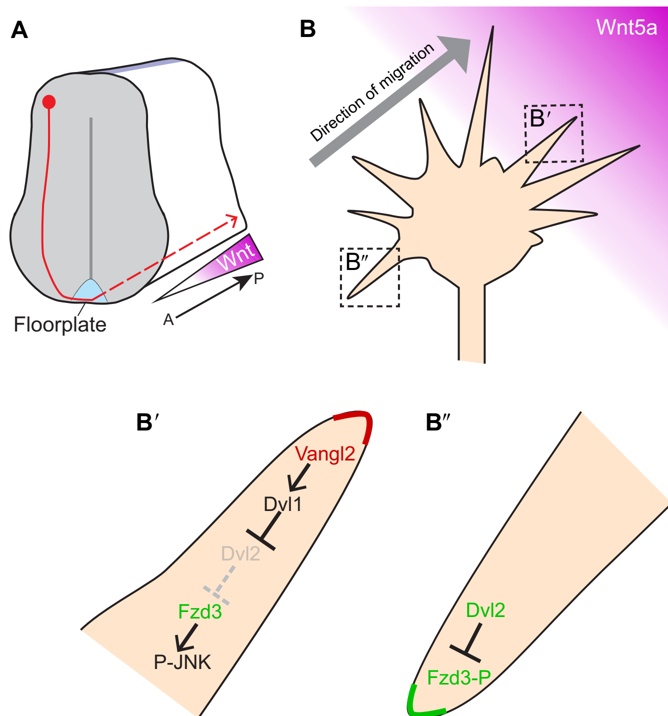


Fig. 5. PCP signaling steers commissural growth cones. (A) Schematic cross-section of the spinal cord. Commissural neurons (red) in the dorsal neural tube project axons ventrally that then cross the midline floorplate. After crossing, they turn and project anteriorly alongside the floorplate (dashed red line), up a Wnt expression gradient (magenta). A, anterior; P, posterior. (B-B″) A post-crossing commissural axon growth cone in a Wnt gradient (magenta). (B′) In filopodia pointing towards the Wnt source, Vangl2 and Dvl1 antagonize Dvl2-mediated Fzd3 phosphorylation, leading to Fzd3 internalization and trafficking down the filopodium. Internalized Fzd3 has higher signaling activity, leading to JNK activation (P-JNK) and growth cone extension. (B″) In filopodia pointing away from the Wnt source, Fzd3 is hyperphosphorylated (Fzd3-P) in a Dvl2-dependent manner, which keeps it at the plasma membrane and thereby prevents signaling through JNK.

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., 2006). 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.

2003; Shafer et al., 2011). High resolution live imaging of growth cones after surface labeling of Fzd3 has revealed that in the presence of Wnt5a, Fzd3 is internalized at the tips of growing growth cone filopodia and trafficked towards the cell body (Onishi et al., 2013). Fzd3 internalization occurs preferentially on the side of the growth cone closer to the Wnt source, suggesting that the directional turning of growth cones *in vivo* is instructed by a Wnt gradient that activates PCP signaling via Fzd3 internalization. Vangl2 also localizes to the tips of growing filopodia, and Vangl2 knockdown eliminates Wnt5a/Fzd3-dependent axon outgrowth, as does knockdown of the endocytic recycling GTPase Arf6 (Onishi et al., 2013; Shafer et al., 2011). These findings, together with cell culture experiments showing that Fzd3 endocytosis promotes PCP signaling (see Box 2), support a model for PCP-dependent axon guidance in which Vangl2 and Dvl2 promote Arf6-mediated Fzd3 endocytosis at filopodial tips, which activates growth cone turning via JNK activation (Fig. 5). Exactly how PCP impacts the cytoskeleton to cause turning is not clear, as it changes neither the number nor the lifetime of growth cone filopodia (Onishi et al., 2013).

Thus, in the dynamic context of axon guidance, the canonical spatial and functional relationships of PCP proteins appear to break down: Fzd and Vangl colocalize at growing filopodial tips, and Vangl promotes Fzd activity in the same cell, rather than antagonizing it. Further unusual relationships have been described in this context: whereas Dvl2 promotes Fzd3 internalization and signaling, Dvl1 has the opposite effect and, by inducing Fzd3 phosphorylation, causes it to remain at the membrane in an inactive form (Onishi et al., 2013). However, it should be noted that these functions were described in isolated growth cones cultured in the absence of the cell-cell and cell-matrix contacts that would normally guide them. As we discuss below, more classical interactions between PCP proteins have been observed *in vivo* in the guidance of migrating neurons.

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 *Scrib* 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).

Development (2017) 144, 187-200 doi:10.1242/dev.122804

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 FBMNs and static planar-polarized epithelial cells) are of different types.

How do PCP proteins function during FBMN migration? High-resolution live imaging of FBMNs 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 FBMNs, 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 Nhsl1/Nhsl1b, 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).

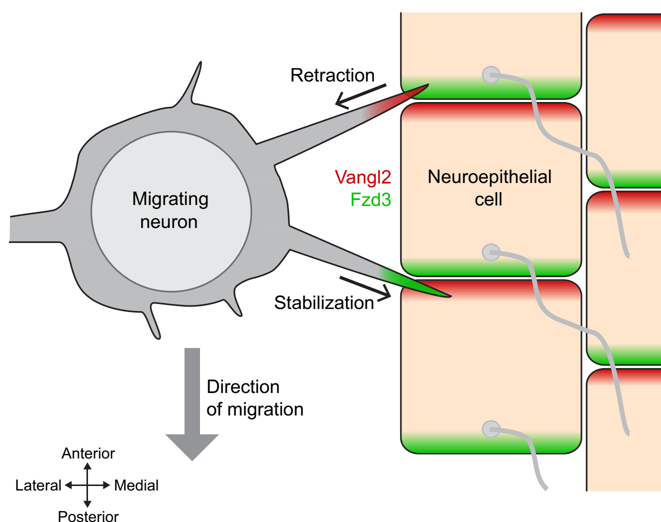
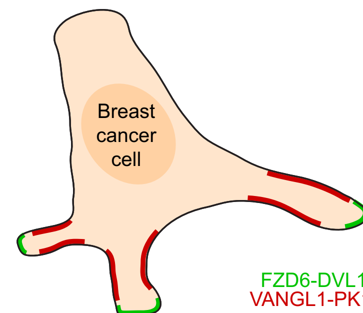


Fig. 6. PCP-mediated control of directional neuron migration. Schematic of a facial branchiomotor neuron (FBMN) migrating posteriorly through the planar-polarized hindbrain neuroepithelium. Vangl2 and Fzd3 localize to anterior and posterior apical sides of the neuroepithelial and floorplate cells, respectively. 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 FBMNs and Fzd3 in the environment destabilize them.

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

Box 3. PCP in cancer cells



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 Dvl 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 to how PCP functions in static epithelia – are providing key insights into the crucial role played by PCP signaling in both developmental and disease contexts.

Acknowledgements
The authors wish to thank the members of the Moens lab, as well as the reviewers for their thoughtful input into the writing of this review.

Competing interests
The authors declare no competing or financial interests.

Funding
Our work in the field of planar polarity in neuronal migration is supported by a grant from the National Institutes of Health (NIH) (to C.B.M.). C.F.D. was supported by a UW Developmental Biology Training Grant from the NIH. Deposited in PMC for release after 12 months.

References
Ackley, B. D. (2014). Wnt-signaling and planar cell polarity genes regulate axon guidance along the anteroposterior axis in C. elegans. *Dev. Neurobiol.* **74**, 781-796.
Anastas, J. N., Biechele, T. L., Robitaille, M., Muster, J., Allison, K. H., Angers, S. and Moon, R. T. (2012). A protein complex of SCRIB, NOS1AP and VANGL1 regulates cell polarity and migration, and is associated with breast cancer progression. *Oncogene* **31**, 3696-3708.
Andre, P., Song, H., Kim, W., Kispert, A. and Yang, Y. (2015). Wnt5a and Wnt11 regulate mammalian anterior-posterior axis elongation. *Development* **142**, 1516-1527.
Araya, C., Ward, L. C., Girdler, G. C. and Miranda, M. (2016). Coordinating cell and tissue behavior during zebrafish neural tube morphogenesis. *Dev. Dyn.* **245**, 197-208.
Bertet, C., Sulak, L. and Lecuit, T. (2004). Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. *Nature* **429**, 667-671.
Bingham, S., Higashijima, S.-I., Okamoto, H. and Chandrasekhar, A. (2002). The Zebrafish trilobite gene is essential for tangential migration of branchiomotor neurons. *Dev. Biol.* **242**, 149-160.
Blankenship, J. T., Backovic, S. T., Sanny, J. S. P., Weitz, O. and Zallen, J. A. (2006). Multicellular rosette formation links planar cell polarity to tissue morphogenesis. *Dev. Cell* **11**, 459-470.
Borovina, A., Superina, S., Voskas, D. and Ciruna, B. (2010). Vangl2 directs the posterior tilting and asymmetric localization of motile primary cilia. *Nat. Cell Biol.* **12**, 407-412.
Boutros, M., Paricio, N., Strutt, D. I. and Mlodzik, M. (1998). Dishevelled activates JNK and discriminates between JNK pathways in planar polarity and wingless signaling. *Cell* **94**, 109-118.
Brooks, S. P., Coccia, M., Tang, H. R., Kanuga, N., Machesky, L. M., Bailly, M., Cheetham, M. E. and Hardcastle, A. J. (2010). The Nance-Horan syndrome

protein encodes a functional WAVE homology domain (WHD) and is important for co-ordinating actin remodelling and maintaining cell morphology. *Hum. Mol. Genet.* **19**, 2421-2432.
Butler, M. T. and Wallingford, J. B. (2015). Control of vertebrate core planar cell polarity protein localization and dynamics by Prickle 2. *Development* **142**, 3429-3439.
Carmona-Fontaine, C., Matthews, H. K., Kuriyama, S., Moreno, M., Dunn, G. A., Parsons, M., Stern, C. D. and Mayor, R. (2008). Contact inhibition of locomotion in vivo controls neural crest directional migration. *Nature* **456**, 957-961.
Carvajal-Gonzalez, J. M., Balmer, S., Mendoza, M., Dussert, A., Collu, G., Roman, A.-C., Weber, U., Ciruna, B. and Mlodzik, M. (2015). The clathrin adaptor AP-1 complex and Arf1 regulate planar cell polarity in vivo. *Nat. Commun.* **6**, 6751.
Chai, G., Goffinet, A. M. and Tissir, F. (2015). Celsr3 and Fzd3 in axon guidance. *Int. J. Biochem. Cell Biol.* **64**, 11-14.
Chen, W., ten Berge, D., Brown, J., Ahn, S., Hu, L. A., Miller, W. E., Caron, M. G., Barak, L. S., Nusse, R. and Lefkowitz, R. J. (2003). Dishevelled 2 recruits beta-arrestin 2 to mediate Wnt5A-stimulated endocytosis of Frizzled 4. *Science* **301**, 1391-1394.
Chen, W.-S., Antic, D., Matis, M., Logan, C. Y., Povelones, M., Anderson, G. A., Nusse, R. and Axelrod, J. D. (2008). Asymmetric homotypic interactions of the atypical cadherin flamingo mediate intercellular polarity signaling. *Cell* **133**, 1093-1105.
Cho, B., Pierre-Louis, G., Sagner, A., Eaton, S. and Axelrod, J. D. (2015). Clustering and negative feedback by endocytosis in planar cell polarity signaling is modulated by ubiquitinylation of prickle. *PLoS Genet.* **11**, e1005259.
Chu, C.-W. and Sokol, S. Y. (2016). Wnt proteins can direct planar cell polarity in vertebrate ectoderm. *Elife* **5**, e16463.
Ciruna, B., Jenny, A., Lee, D., Mlodzik, M. and Schier, A. F. (2006). Planar cell polarity signalling couples cell division and morphogenesis during neurulation. *Nature* **439**, 220-224.
Classen, A.-K., Anderson, K. I., Marois, E. and Eaton, S. (2005). Hexagonal packing of Drosophila wing epithelial cells by the planar cell polarity pathway. *Dev. Cell* **9**, 805-817.
Collier, S. and Gubb, D. (1997). Drosophila tissue polarity requires the cell-autonomous activity of the fuzzy gene, which encodes a novel transmembrane protein. *Development* **124**, 4029-4037.
Collier, S., Lee, H., Burgess, R. and Adler, P. (2005). The WD40 repeat protein fritz links cytoskeletal planar polarity to frizzled subcellular localization in the Drosophila epidermis. *Genetics* **169**, 2035-2045.
Courbard, J.-R., Djiane, A., Wu, J. and Mlodzik, M. (2009). The apical/basal-polarity determinant Scribble cooperates with the PCP core factor Stbm/Vang and functions as one of its effectors. *Dev. Biol.* **333**, 67-77.
Curtin, J. A., Quint, E., Tsipouri, V., Arkell, R. M., Cattanach, B., Copp, A. J., Henderson, D. J., Spurr, N., Stanier, P., Fisher, E. M. et al. (2003). Mutation of Celsr1 disrupts planar polarity of inner ear hair cells and causes severe neural tube defects in the mouse. *Curr. Biol.* **13**, 1129-1133.
Darken, R. S., Scola, A. M., Rakeman, A. S., Das, G., Mlodzik, M. and Wilson, P. A. (2002). The planar polarity gene strabismus regulates convergent extension movements in Xenopus. *EMBO J.* **21**, 976-985.
Davey, C. F., Mathewson, A. W. and Moens, C. B. (2016). PCP Signaling between migrating neurons and their planar-polarized neuroepithelial environment controls filopodial dynamics and directional migration. *PLoS Genet.* **12**, e1005934.
De Calisto, J., Araya, C., Marchant, L., Riaz, C. F. and Mayor, R. (2005). Essential role of non-canonical Wnt signalling in neural crest migration. *Development* **132**, 2587-2597.
Deans, M. R., Antic, D., Suyama, K., Scott, M. P., Axelrod, J. D. and Goodrich, L. V. (2007). Asymmetric distribution of prickle-like 2 reveals an early underlying polarization of vestibular sensory epithelia in the inner ear. *J. Neurosci.* **27**, 3139-3147.
Devenport, D. and Fuchs, E. (2008). Planar polarization in embryonic epidermis orchestrates global asymmetric morphogenesis of hair follicles. *Nat. Cell Biol.* **10**, 1257-1268.
Devenport, D., Oristian, D., Heller, E. and Fuchs, E. (2011). Mitotic internalization of planar cell polarity proteins preserves tissue polarity. *Nat. Cell Biol.* **13**, 893-902.
Dickson, B. J. (2002). Molecular mechanisms of axon guidance. *Science* **298**, 1959-1964.
Djiane, A., Riou, J., Umbhauer, M., Boucaut, J. and Shi, D. (2000). Role of frizzled 7 in the regulation of convergent extension movements during gastrulation in Xenopus laevis. *Development* **127**, 3091-3100.
Etheridge, S. L., Ray, S., Li, S., Hamblet, N. S., Lijam, N., Tsang, M., Greer, J., Kardos, N., Wang, J., Sussman, D. J. et al. (2008). Murine dishevelled 3 functions in redundant pathways with dishevelled 1 and 2 in normal cardiac outflow tract, cochlea, and neural tube development. *PLoS Genet.* **4**, e1000259.
Fenstermaker, A. G., Prasad, A. A., Bechara, A., Adolfs, Y., Tissir, F., Goffinet, A., Zou, Y. and Pasterkamp, R. J. (2010). Wnt/planar cell polarity signaling controls the anterior-posterior organization of monoaminergic axons in the brainstem. *J. Neurosci.* **30**, 16053-16064.
Franke, J. D., Montague, R. A. and Kiehart, D. P. (2010). Nonmuscle myosin II is required for cell proliferation, cell sheet adhesion and wing hair morphology during wing morphogenesis. *Dev. Biol.* **345**, 117-132.
Friedl, P. and Gilmour, D. (2009). Collective cell migration in morphogenesis, regeneration and cancer. *Nat. Rev. Mol. Cell Biol.* **10**, 445-457.
Galic, M. and Matis, M. (2015). Polarized trafficking provides spatial cues for planar cell polarization within a tissue. *BioEssays* **37**, 678-686.
Gao, B., Song, H., Bishop, K., Elliot, G., Garrett, L., English, M. A., Andre, P., Robinson, J., Sood, R., Minami, Y. et al. (2011). Wnt signaling gradients establish planar cell polarity by inducing Vangl2 phosphorylation through Ror2. *Dev. Cell* **20**, 163-176.
Gault, W. J., Olguin, P., Weber, U. and Mlodzik, M. (2012). Drosophila CK1-gamma, gilgamesh, controls PCP-mediated morphogenesis through regulation of vesicle trafficking. *J. Cell Biol.* **196**, 605-621.
Giese, A. P., Ezan, J., Wang, L., Lasvaux, L., Lembo, F., Mazzocco, C., Richard, E., Reboul, J., Borg, J.-P., Kelley, M. W. et al. (2012). Gipc1 has a dual role in Vangl2 trafficking and hair bundle integrity in the inner ear. *Development* **139**, 3775-3785.
Glasco, D. M., Sittaramane, V., Bryant, W., Fritzsch, B., Sawant, A., Paudyal, A., Stewart, M., Andre, P., Cadete Vilhais-Neto, G., Yang, Y. et al. (2012). The mouse Wnt/PCP protein Vangl2 is necessary for migration of facial branchiomotor neurons, and functions independently of Dishevelled. *Dev. Biol.* **369**, 211-222.
Gombos, R., Migh, E., Antal, O., Mukherjee, A., Jenny, A. and Mihaly, J. (2015). The formin DAAM functions as molecular effector of the planar cell polarity pathway during axonal development in Drosophila. *J. Neurosci.* **35**, 10154-10167.
Goodrich, L. V. and Strutt, D. (2011). Principles of planar polarity in animal development. *Development* **138**, 1877-1892.
Goto, T. and Keller, R. (2002). The planar cell polarity gene strabismus regulates convergence and extension and neural fold closure in Xenopus. *Dev. Biol.* **247**, 165-181.
Gujral, T. S., Chan, M., Peshkin, L., Sorger, P. K., Kirschner, M. W. and MacBeath, G. (2014). A noncanonical Frizzled2 pathway regulates epithelial-mesenchymal transition and metastasis. *Cell* **159**, 844-856.
Guo, Y., Zanetti, G. and Schekman, R. (2013). A novel GTP-binding protein-adaptor protein complex responsible for export of Vangl2 from the trans Golgi network. *Elife* **2**, e00160.
Habas, R., Kato, Y. and He, X. (2001). Wnt/Frizzled activation of Rho regulates vertebrate gastrulation and requires a novel Formin homology protein Daam1. *Cell* **107**, 843-854.
Hale, R. and Strutt, D. (2015). Conservation of planar polarity pathway function across the animal kingdom. *Annu. Rev. Genet.* **49**, 529-551.
Hamblet, N. S., Lijam, N., Ruiz-Lozano, P., Wang, J., Yang, Y., Luo, Z., Mei, L., Chien, K. R., Sussman, D. J. and Wynshaw-Boris, A. (2002). Dishevelled 2 is essential for cardiac outflow tract development, somite segmentation and neural tube closure. *Development* **129**, 5827-5838.
Hashimoto, M., Shinohara, K., Wang, J., Ikeuchi, S., Yoshiba, S., Meno, C., Nonaka, S., Takada, S., Hatta, K., Wynshaw-Boris, A. et al. (2010). Planar polarization of node cells determines the rotational axis of node cilia. *Nat. Cell Biol.* **12**, 170-176.
Hayes, M., Naito, M., Daulat, A., Angers, S. and Ciruna, B. (2013). Ptk7 promotes non-canonical Wnt/PCP-mediated morphogenesis and inhibits Wnt/beta-catenin-dependent cell fate decisions during vertebrate development. *Development* **140**, 1807-1818.
Heisenberg, C.-P., Tada, M., Rauch, G.-J., Saúde, L., Concha, M. L., Geisler, R., Stemple, D. L., Smith, J. C. and Wilson, S. W. (2000). Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* **405**, 76-81.
Hermle, T., Guida, M. C., Beck, S., Helmstädter, S. and Simons, M. (2013). Drosophila ATP6AP2/VhaPRR functions both as a novel planar cell polarity core protein and a regulator of endosomal trafficking. *EMBO J.* **32**, 245-259.
Jaiswal, R., Breitsprecher, D., Collins, A., Corrêa, I. R., Jr, Xu, M.-Q. and Goode, B. L. (2013). The formin Daam1 and fascin directly collaborate to promote filopodia formation. *Curr. Biol.* **23**, 1373-1379.
Jessen, J. R. (2009). Noncanonical Wnt signaling in tumor progression and metastasis. *Zebrafish* **6**, 21-28.
Jessen, J. R., Topczewski, J., Bingham, S., Sepich, D. S., Marlow, F., Chandrasekhar, A. and Solnica-Krezel, L. (2002). Zebrafish trilobite identifies new roles for Strabismus in gastrulation and neuronal movements. *Nat. Cell Biol.* **4**, 610-615.
Kallay, L. M., McNickle, A., Brennwald, P. J., Hubbard, A. L. and Braiterman, L. T. (2006). Scribble associates with two polarity proteins, Lgl2 and Vangl2, via distinct molecular domains. *J. Cell. Biochem.* **99**, 647-664.
Kaucká, M., Petersen, J., Janovská, P., Radaszkiewicz, T., Smyčková, L., Daulat, A. M., Borg, J.-P., Schulte, G. and Bryja, V. (2015). Asymmetry of VANGL2 in migrating lymphocytes as a tool to monitor activity of the mammalian WNT/planar cell polarity pathway. *Cell Commun. Signal.* **13**, 2.
Kibar, Z., Vogan, K. J., Groulx, N., Justice, M. J., Underhill, D. A. and Gros, P. (2001). Ltap, a mammalian homolog of Drosophila Strabismus/Van Gogh, is altered in the mouse neural tube mutant Loop-tail. *Nat. Genet.* **28**, 251-255.

Kilian, B., Mansukoski, H., Barbosa, F. C., Ulrich, F., Tada, M. and Heisenberg, C.-P. (2003). The role of Ppt/Wnt5 in regulating cell shape and movement during zebrafish gastrulation. *Mech. Dev.* **120**, 467-476.

Kim, G.-H., Her, J.-H. and Han, J.-K. (2008). Ryk cooperates with Frizzled 7 to promote Wnt11-mediated endocytosis and is essential for Xenopus laevis convergent extension movements. *J. Cell Biol.* **182**, 1073-1082.

Kim, S. K., Shindo, A., Park, T. J., Oh, E. C., Ghosh, S., Gray, R. S., Lewis, R. A., Johnson, C. A., Attie-Bittach, T., Katsanis, N. et al. (2010). Planar cell polarity acts through septins to control collective cell movement and ciliogenesis. *Science* **329**, 1337-1340.

Kinoshita, N., Iioka, H., Miyakoshi, A. and Ueno, N. (2003). PKC delta is essential for Dishevelled function in a noncanonical Wnt pathway that regulates Xenopus convergent extension movements. *Genes Dev.* **17**, 1663-1676.

Lee, H., Lee, S. J., Kim, G.-H., Yeo, I. and Han, J.-K. (2016). PLD1 regulates Xenopus convergent extension movements by mediating Frizzled7 endocytosis for Wnt/PCP signal activation. *Dev. Biol.* **411**, 38-49.

Li, X., Roszko, I., Sepich, D. S., Ni, M., Hamm, H. E., Marlow, F. L. and Solnica-Krezel, L. (2013). Gpr125 modulates Dishevelled distribution and planar cell polarity signaling. *Development* **140**, 3028-3039.

Lu, Q. and Adler, P. N. (2015). The diaphanous gene of Drosophila interacts antagonistically with multiple wing hairs and plays a key role in wing hair morphogenesis. *PLoS ONE* **10**, e0115623.

Lu, X., Borchers, A. G. M., Jolicoeur, C., Rayburn, H., Baker, J. C. and Tessier-Lavigne, M. (2004). PTK7/CCK-4 is a novel regulator of planar cell polarity in vertebrates. *Nature* **430**, 93-98.

Lu, Q., Schafer, D. A. and Adler, P. N. (2015). The Drosophila planar polarity gene multiple wing hairs directly regulates the actin cytoskeleton. *Development* **142**, 2478-2486.

Luga, V., Zhang, L., Viloria-Petit, A. M., Ogunjimi, A. A., Inanlou, M. R., Chiu, E., Buchanan, M., Hosein, A. N., Basik, M. and Wrana, J. L. (2012). Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. *Cell* **151**, 1542-1556.

Lyuksyutova, A. I., Lu, C.-C., Milanesio, N., King, L. A., Guo, N., Wang, Y., Nathans, J., Tessier-Lavigne, M. and Zou, Y. (2003). Anterior-posterior guidance of commissural axons by Wnt-frizzled signaling. *Science* **302**, 1984-1988.

MacMillan, C. D., Leong, H. S., Dales, D. W., Robertson, A. E., Lewis, J. D., Chambers, A. F. and Tuck, A. B. (2014). Stage of breast cancer progression influences cellular response to activation of the WNT/planar cell polarity pathway. *Sci. Rep.* **4**, 6315.

Mapp, O. M., Wanner, S. J., Rohrschneider, M. R. and Prince, V. E. (2010). Prickle1b mediates interpretation of migratory cues during zebrafish facial branchiomotor neuron migration. *Dev. Dyn.* **239**, 1596-1608.

Mapp, O. M., Walsh, G. S., Moens, C. B., Tada, M. and Prince, V. E. (2011). Zebrafish Prickle1b mediates facial branchiomotor neuron migration via a farnesylation-dependent nuclear activity. *Development* **138**, 2121-2132.

Marlow, F., Topczewski, J., Sepich, D. and Solnica-Krezel, L. (2002). Zebrafish Rho kinase 2 acts downstream of Wnt11 to mediate cell polarity and effective convergence and extension movements. *Curr. Biol.* **12**, 876-884.

Matis, M. and Axelrod, J. D. (2013). Regulation of PCP by the Fat signaling pathway. *Genes Dev.* **27**, 2207-2220.

Matis, M., Russler-Germain, D. A., Hu, Q., Tomlin, C. J. and Axelrod, J. D. (2014). Microtubules provide directional information for core PCP function. *Elife* **3**, e02893.

Matthews, H. K., Broders-Bondon, F., Thiery, J. P. and Mayor, R. (2008a). Wnt11r is required for cranial neural crest migration. *Dev. Dyn.* **237**, 3404-3409.

Matthews, H. K., Marchant, L., Carmona-Fontaine, C., Kuriyama, S., Larrain, J., Holt, M. R., Parsons, M. and Mayor, R. (2008b). Directional migration of neural crest cells in vivo is regulated by Syndecan-4/Rac1 and non-canonical Wnt signaling/RhoA. *Development* **135**, 1771-1780.

Mayor, R. and Theveneau, E. (2013). The neural crest. *Development* **140**, 2247-2251.

McGreevy, E. M., Vijayraghavan, D., Davidson, L. A. and Hildebrand, J. D. (2015). Shroom3 functions downstream of planar cell polarity to regulate myosin II distribution and cellular organization during neural tube closure. *Biol. Open* **4**, 186-196.

Merte, J., Jensen, D., Wright, K., Sarsfield, S., Wang, Y., Schekman, R. and Ginty, D. D. (2010). Sec24b selectively sorts Vangl2 to regulate planar cell polarity during neural tube closure. *Nat. Cell Biol.* **12**, 41-46; sup pp. 41-48.

Mitchell, B., Stubbs, J. L., Huisman, F., Taborek, P., Yu, C. and Kintner, C. (2009). The PCP pathway instructs the planar orientation of ciliated cells in the Xenopus larval skin. *Curr. Biol.* **19**, 924-929.

Montcouquiol, M., Rachel, R. A., Lanford, P. J., Copeland, N. G., Jenkins, N. A. and Kelley, M. W. (2003). Identification of Vangl2 and Scrb1 as planar polarity genes in mammals. *Nature* **423**, 173-177.

Moon, R. T., Campbell, R. M., Christian, J. L., McGrew, L. L., Shih, J. and Fraser, S. (1993). Xwnt-5A: a maternal Wnt that affects morphogenetic movements after overexpression in embryos of Xenopus laevis. *Development* **119**, 97-111.

Mottola, G., Classen, A.-K., Gonzalez-Gaitan, M., Eaton, S. and Zerial, M. (2010). A novel function for the Rab5 effector Rabenosyn-5 in planar cell polarity. *Development* **137**, 2353-2364.

Murdoch, J. N., Doudney, K., Paternotte, C., Copp, A. J. and Stanier, P. (2001). Severe neural tube defects in the loop-tail mouse result from mutation of Lpp1, a novel gene involved in floor plate specification. *Hum. Mol. Genet.* **10**, 2593-2601.

Murdoch, J. N., Henderson, D. J., Doudney, K., Gaston-Massuet, C., Phillips, H. M., Paternotte, C., Arkell, R., Stanier, P. and Copp, A. J. (2003). Disruption of scribble (Scrb1) causes severe neural tube defects in the circletail mouse. *Hum. Mol. Genet.* **12**, 87-98.

Narimatsu, M., Bose, R., Pye, M., Zhang, L., Miller, B., Ching, P., Sakuma, R., Luga, V., Roncari, L., Attisano, L. et al. (2009). Regulation of planar cell polarity by Smurf ubiquitin ligases. *Cell* **137**, 295-307.

Nishimura, T. and Takeichi, M. (2008). Shroom3-mediated recruitment of Rho kinases to the apical cell junctions regulates epithelial and neuroepithelial planar remodeling. *Development* **135**, 1493-1502.

Nishimura, T., Honda, H. and Takeichi, M. (2012). Planar cell polarity links axes of spatial dynamics in neural-tube closure. *Cell* **149**, 1084-1097.

Nonaka, S., Yoshiba, S., Watanabe, D., Ikeuchi, S., Goto, T., Marshall, W. F. and Hamada, H. (2005). De novo formation of left-right asymmetry by posterior tilt of nodal cilia. *PLoS Biol.* **3**, e268.

Ohkawara, B., Yamamoto, T. S., Tada, M. and Ueno, N. (2003). Role of glypican 4 in the regulation of convergent extension movements during gastrulation in Xenopus laevis. *Development* **130**, 2129-2138.

Ohkawara, B., Glinka, A. and Niehrs, C. (2011). Rspo3 binds syndecan 4 and induces Wnt/PCP signaling via clathrin-mediated endocytosis to promote morphogenesis. *Dev. Cell* **20**, 303-314.

Okada, Y., Takeda, S., Tanaka, Y., Izpisua Belmonte, J.-C. I. and Hirokawa, N. (2005). Mechanism of nodal flow: a conserved symmetry breaking event in left-right axis determination. *Cell* **121**, 633-644.

Onishi, K., Shafer, B., Lo, C., Tissir, F., Goffinet, A. M. and Zou, Y. (2013). Antagonistic functions of Dishevelleds regulate Frizzled3 endocytosis via filopodia tips in Wnt-mediated growth cone guidance. *J. Neurosci.* **33**, 19071-19085.

Ossipova, O., Kim, K., Lake, B. B., Itoh, K., Ioannou, A. and Sokol, S. Y. (2014). Role of Rab11 in planar cell polarity and apical constriction during vertebrate neural tube closure. *Nat. Commun.* **5**, 3734.

Ossipova, O., Chu, C.-W., Fillatre, J., Brott, B. K., Itoh, K. and Sokol, S. Y. (2015a). The involvement of PCP proteins in radial cell intercalations during Xenopus embryonic development. *Dev. Biol.* **408**, 316-327.

Ossipova, O., Kim, K. and Sokol, S. Y. (2015b). Planar polarization of Vangl2 in the vertebrate neural plate is controlled by Wnt and Myosin II signaling. *Biol. Open* **4**, 722-730.

Panousopoulou, E., Tyson, R. A., Bretschneider, T. and Green, J. B. A. (2013). The distribution of Dishevelled in convergently extending mesoderm. *Dev. Biol.* **382**, 496-503.

Paré, A. C., Vichas, A., Fincher, C. T., Mirman, Z., Farrell, D. L., Mainieri, A. and Zallen, J. A. (2014). A positional Toll receptor code directs convergent extension in Drosophila. *Nature* **515**, 523-527.

Park, W. J., Liu, J., Sharp, E. J. and Adler, P. N. (1996). The Drosophila tissue polarity gene inturned acts cell autonomously and encodes a novel protein. *Development* **122**, 961-969.

Peng, Y. and Axelrod, J. D. (2012). Asymmetric protein localization in planar cell polarity: mechanisms, puzzles, and challenges. *Curr. Top. Dev. Biol.* **101**, 33-53.

Pryor, S. E., Massa, V., Savery, D., Andre, P., Yang, Y., Greene, N. D. E. and Copp, A. J. (2014). Vangl-dependent planar cell polarity signalling is not required for neural crest migration in mammals. *Development* **141**, 3153-3158.

Qian, D., Jones, C., Rzadzinska, A., Mark, S., Zhang, X., Steel, K. P., Dai, X. and Chen, P. (2007). Wnt5a functions in planar cell polarity regulation in mice. *Dev. Biol.* **306**, 121-133.

Qu, Y., Glasco, D. M., Zhou, L., Sawant, A., Ravni, A., Fritzsch, B., Damrau, C., Murdoch, J. N., Evans, S., Pfaff, S. L. et al. (2010). Atypical cadherins Celsr1-3 differentially regulate migration of facial branchiomotor neurons in mice. *J. Neurosci.* **30**, 9392-9401.

Qu, Y., Huang, Y., Feng, J., Alvarez-Bolado, G., Grove, E. A., Yang, Y., Tissir, F., Zhou, L. and Goffinet, A. M. (2014). Genetic evidence that Celsr3 and Celsr2, together with Fzd3, regulate forebrain wiring in a Vangl-independent manner. *Proc. Natl. Acad. Sci. USA* **111**, E2996-E3004.

Ridley, A. J. (2015). Rho GTPase signalling in cell migration. *Curr. Opin. Cell Biol.* **36**, 103-112.

Rohrschneider, M. R., Elsen, G. E. and Prince, V. E. (2007). Zebrafish Hoxb1a regulates multiple downstream genes including prickle1b. *Dev. Biol.* **309**, 358-372.

Roszko, I., Sepich, D. S., Jessen, J. R., Chandrasekhar, A. and Solnica-Krezel, L. (2015). A dynamic intracellular distribution of Vangl2 accompanies cell polarization during zebrafish gastrulation. *Development* **142**, 2508-2520.

Sasselli, V., Boesmans, W., Vanden Berghe, P., Tissir, F., Goffinet, A. M. and Pachnis, V. (2013). Planar cell polarity genes control the connectivity of enteric neurons. *J. Clin. Invest.* **123**, 1763-1772.

Sato, A., Yamamoto, H., Sakane, H., Koyama, H. and Kikuchi, A. (2010). Wnt5a regulates distinct signalling pathways by binding to Frizzled2. *EMBO J.* **29**, 41-54.
Sewduth, R. N., Jaspard-Vinassa, B., Peghaire, C., Guillabert, A., Franzl, N., Larrieu-Lahargue, F., Moreau, C., Fruttiger, M., Dufourcq, P., Couffinhal, T. et al. (2014). The ubiquitin ligase PDZRN3 is required for vascular morphogenesis through Wnt/planar cell polarity signalling. *Nat. Commun.* **5**, 4832.
Shafer, B., Onishi, K., Lo, C., Colakoglu, G. and Zou, Y. (2011). Vangl2 promotes Wnt/planar cell polarity-like signaling by antagonizing Dvl1-mediated feedback inhibition in growth cone guidance. *Dev. Cell* **20**, 177-191.
Shih, J. and Keller, R. (1992). Patterns of cell motility in the organizer and dorsal mesoderm of Xenopus laevis. *Development* **116**, 915-930.
Shimada, Y., Yonemura, S., Ohkura, H., Strutt, D. and Uemura, T. (2006). Polarized transport of Frizzled along the planar microtubule arrays in Drosophila wing epithelium. *Dev. Cell* **10**, 209-222.
Shindo, A. and Wallingford, J. B. (2014). PCP and septins compartmentalize cortical actomyosin to direct collective cell movement. *Science* **343**, 649-652.
Shnitsar, I. and Borchers, A. (2008). PTK7 recruits dsh to regulate neural crest migration. *Development* **135**, 4015-4024.
Sienknecht, U. J., Anderson, B. K., Parodi, R. M., Fantetti, K. N. and Fekete, D. M. (2011). Non-cell-autonomous planar cell polarity propagation in the auditory sensory epithelium of vertebrates. *Dev. Biol.* **352**, 27-39.
Sittaramane, V., Pan, X., Glasco, D. M., Huang, P., Gurung, S., Bock, A., Li, S., Wang, H., Kawakami, K., Matise, M. P. et al. (2013). The PCP protein Vangl2 regulates migration of hindbrain motor neurons by acting in floor plate cells, and independently of cilia function. *Dev. Biol.* **382**, 400-412.
Sokol, S. Y. (1996). Analysis of Dishevelled signalling pathways during Xenopus development. *Curr. Biol.* **6**, 1456-1467.
Strutt, H., Warrington, S. J. and Strutt, D. (2011). Dynamics of core planar polarity protein turnover and stable assembly into discrete membrane subdomains. *Dev. Cell* **20**, 511-525.
Suzuki, M., Morita, H. and Ueno, N. (2012). Molecular mechanisms of cell shape changes that contribute to vertebrate neural tube closure. *Dev. Growth Differ.* **54**, 266-276.
Tada, M. and Smith, J. C. (2000). Xwnt11 is a target of Xenopus Brachyury: regulation of gastrulation movements via Dishevelled, but not through the canonical Wnt pathway. *Development* **127**, 2227-2238.
Takeuchi, M., Nakabayashi, J., Sakaguchi, T., Yamamoto, T. S., Takahashi, H., Takeda, H. and Ueno, N. (2003). The prickle-related gene in vertebrates is essential for gastrulation cell movements. *Curr. Biol.* **13**, 674-679.
Tatin, F., Taddei, A., Weston, A., Fuchs, E., Devenport, D., Tissir, F. and Makinen, T. (2013). Planar cell polarity protein Celsr1 regulates endothelial adherens junctions and directed cell rearrangements during valve morphogenesis. *Dev. Cell* **26**, 31-44.
Teddy, J. M. and Kulesa, P. M. (2004). In vivo evidence for short- and long-range cell communication in cranial neural crest cells. *Development* **131**, 6141-6151.
Theveneau, E., Marchant, L., Kuriyama, S., Gull, M., Moepps, B., Parsons, M. and Mayor, R. (2010). Collective chemotaxis requires contact-dependent cell polarity. *Dev. Cell* **19**, 39-53.
Tissir, F. and Goffinet, A. M. (2013). Shaping the nervous system: role of the core planar cell polarity genes. *Nat. Rev. Neurosci.* **14**, 525-535.
Topczewski, J., Sepich, D. S., Myers, D. C., Walker, C., Amores, A., Lele, Z., Hammerschmidt, M., Postlethwait, J. and Solnica-Krezel, L. (2001). The zebrafish glypican knypek controls cell polarity during gastrulation movements of convergent extension. *Dev. Cell* **1**, 251-264.
Veeman, M. T., Slusarski, D. C., Kaykas, A., Louie, S. H. and Moon, R. T. (2003). Zebrafish prickle, a modulator of noncanonical Wnt/Fz signaling, regulates gastrulation movements. *Curr. Biol.* **13**, 680-685.
Vichas, A. and Zallen, J. A. (2011). Translating cell polarity into tissue elongation. *Semin. Cell Dev. Biol.* **22**, 858-864.
Vivancos, V., Chen, P., Spassky, N., Qian, D., Dabdoub, A., Kelley, M., Studer, M. and Guthrie, S. (2009). Wnt activity guides facial branchiomotor neuron migration, and involves the PCP pathway and JNK and ROCK kinases. *Neural Dev.* **4**, 7.
Vladar, E. K., Bayly, R. D., Sangoram, A. M., Scott, M. P. and Axelrod, J. D. (2012). Microtubules enable the planar cell polarity of airway cilia. *Curr. Biol.* **22**, 2203-2212.
Wada, H., Iwasaki, M., Sato, T., Masai, I., Nishiwaki, Y., Tanaka, H., Sato, A., Nojima, Y. and Okamoto, H. (2005). Dual roles of zygotic and maternal Scribble1 in neural migration and convergent extension movements in zebrafish embryos. *Development* **132**, 2273-2285.
Wada, H., Tanaka, H., Nakayama, S., Iwasaki, M. and Okamoto, H. (2006). Frizzled3a and Celsr2 function in the neuroepithelium to regulate migration of facial motor neurons in the developing zebrafish hindbrain. *Development* **133**, 4749-4759.
Walck-Shannon, E. and Hardin, J. (2014). Cell intercalation from top to bottom. *Nat. Rev. Mol. Cell Biol.* **15**, 34-48.
Wallingford, J. B. and Harland, R. M. (2001). Xenopus Dishevelled signaling regulates both neural and mesodermal convergent extension: parallel forces elongating the body axis. *Development* **128**, 2581-2592.
Wallingford, J. B. and Harland, R. M. (2002). Neural tube closure requires Dishevelled-dependent convergent extension of the midline. *Development* **129**, 5815-5825.
Wallingford, J. B., Rowning, B. A., Vogeli, K. M., Rothbächer, U., Fraser, S. E. and Harland, R. M. (2000). Dishevelled controls cell polarity during Xenopus gastrulation. *Nature* **405**, 81-85.
Wallingford, J. B., Vogeli, K. M. and Harland, R. M. (2001). Regulation of convergent extension in Xenopus by Wnt5a and Frizzled-8 is independent of the canonical Wnt pathway. *Int. J. Dev. Biol.* **45**, 225-227.
Walsh, G. S., Grant, P. K., Morgan, J. A. and Moens, C. B. (2011). Planar polarity pathway and Nance-Horan syndrome-like 1b have essential cell-autonomous functions in neuronal migration. *Development* **138**, 3033-3042.
Wang, J., Hamblet, N. S., Mark, S., Dickinson, M. E., Brinkman, B. C., Segil, N., Fraser, S. E., Chen, P., Wallingford, J. B. and Wynshaw-Boris, A. (2006a). Dishevelled genes mediate a conserved mammalian PCP pathway to regulate convergent extension during neurulation. *Development* **133**, 1767-1778.
Wang, Y., Guo, N. and Nathans, J. (2006b). The role of Frizzled3 and Frizzled6 in neural tube closure and in the planar polarity of inner-ear sensory hair cells. *J. Neurosci.* **26**, 2147-2156.
Wanner, S. J., Saeger, I., Guthrie, S. and Prince, V. E. (2013). Facial motor neuron migration advances. *Curr. Opin. Neurobiol.* **23**, 943-950.
Wansleeben, C., Feitsma, H., Montcouquiol, M., Kroon, C., Cuppen, E. and Meijlink, F. (2010). Planar cell polarity defects and defective Vangl2 trafficking in mutants for the COPII gene Sec24b. *Development* **137**, 1067-1073.
Weber, U., Paricio, N. and Mlodzik, M. (2000). Jun mediates Frizzled-induced R3/R4 cell fate distinction and planar polarity determination in the Drosophila eye. *Development* **127**, 3619-3629.
Wehner, P., Shnitsar, I., Urlaub, H. and Borchers, A. (2011). RACK1 is a novel interaction partner of PTK7 that is required for neural tube closure. *Development* **138**, 1321-1327.
Williams, M., Yen, W., Lu, X. and Sutherland, A. (2014). Distinct apical and basolateral mechanisms drive planar cell polarity-dependent convergent extension of the mouse neural plate. *Dev. Cell* **29**, 34-46.
Wilson, P. and Keller, R. (1991). Cell rearrangement during gastrulation of Xenopus: direct observation of cultured explants. *Development* **112**, 289-300.
Winter, C. G., Wang, B., Ballew, A., Royou, A., Karess, R., Axelrod, J. D. and Luo, L. (2001). Drosophila Rho-associated kinase (Drok) links Frizzled-mediated planar cell polarity signaling to the actin cytoskeleton. *Cell* **105**, 81-91.
Wu, J., Roman, A.-C., Carvajal-Gonzalez, J. M. and Mlodzik, M. (2013). Wg and Wnt4 provide long-range directional input to planar cell polarity orientation in Drosophila. *Nat. Cell Biol.* **15**, 1045-1055.
Yamamoto, H., Oue, N., Sato, A., Hasegawa, Y., Yamamoto, H., Matsubara, A., Yasui, W. and Kikuchi, A. (2010). Wnt5a signaling is involved in the aggressiveness of prostate cancer and expression of metalloproteinase. *Oncogene* **29**, 2036-2046.
Yamanaka, H., Moriguchi, T., Masuyama, N., Kusakabe, M., Hanafusa, H., Takada, R., Takada, S. and Nishida, E. (2002). JNK functions in the non-canonical Wnt pathway to regulate convergent extension movements in vertebrates. *EMBO Rep.* **3**, 69-75.
Yan, J., Lu, Q., Fang, X. and Adler, P. N. (2009). Rho1 has multiple functions in Drosophila wing planar polarity. *Dev. Biol.* **333**, 186-199.
Yang, Y. and Mlodzik, M. (2015). Wnt-Frizzled/planar cell polarity signaling: cellular orientation by facing the wind (Wnt). *Annu. Rev. Cell Dev. Biol.* **31**, 623-646.
Yang, T., Bassuk, A. G., Stricker, S. and Fritzsch, B. (2014). Prickle1 is necessary for the caudal migration of murine facial branchiomotor neurons. *Cell Tissue Res.* **357**, 549-561.
Ybot-Gonzalez, P., Savery, D., Gerrelli, D., Signore, M., Mitchell, C. E., Faux, C. H., Greene, N. D. E. and Copp, A. J. (2007). Convergent extension, planar-cell-polarity signalling and initiation of mouse neural tube closure. *Development* **134**, 789-799.
Yen, W. W., Williams, M., Periasamy, A., Conaway, M., Burdsal, C., Keller, R., Lu, X. and Sutherland, A. (2009). PTK7 is essential for polarized cell motility and convergent extension during mouse gastrulation. *Development* **136**, 2039-2048.
Yin, C., Kiskowski, M., Pouille, P.-A., Farge, E. and Solnica-Krezel, L. (2008). Cooperation of polarized cell intercalations drives convergence and extension of presomitic mesoderm during zebrafish gastrulation. *J. Cell Biol.* **180**, 221-232.
Yu, A., Rual, J.-F., Tamai, K., Harada, Y., Vidal, M., He, X. and Kirchhausen, T. (2007). Association of Dishevelled with the clathrin AP-2 adaptor is required for Frizzled endocytosis and planar cell polarity signaling. *Dev. Cell* **12**, 129-141.
Zhang, L., Luga, V., Armitage, S. K., Musiol, M., Won, A., Yip, C. M., Plotnikov, S. V. and Wrana, J. L. (2016). A lateral signalling pathway coordinates shape volatility during cell migration. *Nat. Commun.* **7**, 11714.
Žigman, M., Trinh, L. A., Fraser, S. E. and Moens, C. B. (2011). Zebrafish neural tube morphogenesis requires Scribble-dependent oriented cell divisions. *Curr. Biol.* **21**, 79-86.