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Running title: Asymmetry in organ morphogenesis

Abstract

The phenomenon of planar cell polarity is critically required for a myriad of morphogenetic processes in metazoan and is accurately controlled by several conserved modules. Six "core" proteins, including Frizzled, Flamingo (Celsr), Van Gogh (Vangl), Dishevelled, Prickle, and Diego (Ankrd6), are major components of the Wnt/planar cell polarity pathway. The Fat/Dchs protocadherins and the Scrib polarity complex also function to instruct cellular polarization. In vertebrates, all these pathways are essential for tissue and organ morphogenesis, such as neural tube closure, left-right symmetry breaking, heart and gut morphogenesis, lung and kidney branching, stereociliary bundle orientation, and proximal-distal limb elongation. Mutations in planar polarity genes are closely linked to various congenital diseases. Striking advances have been made in deciphering their contribution to the establishment of spatially oriented pattern in developing organs and the maintenance of tissue homeostasis. The challenge remains to clarify the complex interplay of different polarity pathways in organogenesis and the link of cell polarity to cell fate specification. Interdisciplinary approaches are also important to understand the roles of mechanical forces in coupling cellular polarization and differentiation. This review outlines current advances on planar polarity regulators in asymmetric organ formation, with the aim to identify questions that deserve further investigation.

Key words: planar cell polarity, Wnt/PCP signaling, left-right asymmetry, heart and gut morphogenesis, lung and kidney branching, inner ear hair cell orientation, limb outgrowth

Introduction

Planar cell polarity (PCP), initially studied in the retina and the wing of insects *Oncopeltus fasciatus* and *Drosophila melanogaster* (Lawrence and Shelton, 1975; Gubb and García-Bellido, 1982), refers to coordinated cellular orientation within the plane of an epithelium or a tissue. This fascinating process exerts wide functions in organizing the asymmetric cellular choreography and the spatially oriented pattern during tissue and organ morphogenesis, contributing to, for example, the architectural beauty of the *Drosophila* compound eye and the mammalian cochlea. The phenomenon of PCP is mostly regulated by a set of evolutionarily conserved proteins, including Frizzled receptors, Flamingo (Celsr1-3 in vertebrates), Dishevelled (Dvl1-3 in vertebrates), Diego (Ankrd6 in vertebrates), Van Gogh (Vangl1-2 in vertebrates), and Prickle (Prickle1-3 in vertebrates). These “core” PCP proteins transduce Wnt/PCP signaling to regulate cytoskeletal rearrangements and polarized cell behaviors in a variety of morphogenetic processes (Wallingford, 2012; Yang and Mlodzik, 2015; Henderson et al., 2018). Increasing evidence suggests that several vertebrate Wnts, such as Wnt5a and Wnt11, are also important components of the Wnt/PCP pathway, although they are not considered as “core” PCP proteins.

There are also other conserved protein complexes that function as important PCP regulators in *Drosophila* and vertebrates. The heteromeric protocadherins Fat4 and Dchs1 (dachsous cadherin-related 1) represent the second PCP pathway (Fat/Dchs module) that is regulated by the Golgi resident transmembrane kinase Four-jointed or Fj (Blair and McNeill, 2018). The Scrib (Scrb1 or Scribble1) polarity complex, originally identified as a regulator of apico-basal cell polarity, consists of Scrib, Dlg (Discs-large) and Lgl (Lethal-giant larvae) proteins (Milgrom-Hoffman and Humbert, 2018). These pathways exert broad activity in cellular signaling and cytoskeletal organization. They function in concert with or independently of the “core” Wnt/PCP pathway to regulate cell polarity. Two additional systems, the Fat2/Lar (leukocyte antigen-related receptor tyrosine phosphatase) and Toll-8/Cirl (adhesion G protein-coupled receptor) pathways, have been recently shown to instruct some *Drosophila* PCP processes, but their functions in vertebrates merit future investigation (Lavalou and Lecuit, 2022).

The "core" PCP pathway, the Fat/Dchs polarity module and the Scrib complex are critically involved in tissue and organ morphogenesis, from the emergence of organ primordia to terminal organogenesis and tissue homeostasis. In vertebrates, the functions of PCP proteins (herein collectively referred to as regulators of PCP-dependent cell behaviors) have been well documented in many morphogenetic processes, such as neural tube closure, embryonic left-right symmetry breaking, heart and gut morphogenesis, lung and kidney branching, orientation of inner ear hair cells, and proximal-distal limb bud elongation (Yang and Mlodzik, 2015; Henderson et al., 2018). Mutations of PCP genes impair organ development and are closely linked to various congenital anomalies, including neural tube defects (Wang et al., 2019), laterality disorders (Grimes and Burdine, 2017), hearing deficits (May-Simera and Kelley, 2012), lung and kidney diseases (Vladar and Königshoff, 2020; Torban and Sokol, 2021), as well as limb abnormalities (Gao and Yang, 2013). Importantly, PCP proteins regulate common cellular processes underlying the outgrowth and elongation of tissue primordia, such as convergent extension (CE) movements driven by oriented cell division and intercalation (Wallingford, 2012). Therefore, their dysfunctions can lead to multiple phenotypes, highlighting a general importance in organogenesis. There are many excellent reviews focusing on PCP functions in specific aspects of morphogenesis, but a more comprehensive analysis of PCP regulators during development of different organs that display PCP-dependent cellular organization is beneficial for understanding their complex interplay in the establishment of cell polarity. This review attempts to present past achievements and latest advances of PCP protein functions in asymmetric organogenesis, with the aim to identify challenges in deciphering the extraordinary process of asymmetry formation.

Wnt signaling pathways and planar cell polarity protein complexes

Vertebrate Wnt pathways can be divided into three branches based on the activation of different biological readouts (Fig. 1A). Wnt/ß-catenin or canonical Wnt signaling induces target gene transcription and cell fate specification by stabilizing ß-catenin through inhibition of its destruction complex. Non-canonical Wnt signaling includes Wnt/PCP and Wnt/Ca^{2+} branches that function independently of ß-catenin. Wnt/PCP signaling controls cell polarity by regulating cytoskeletal rearrangements and/or transcriptional responses. It functions through several wellcharacterized planar polarity effector (PPE) proteins, such as Daam1 (Dishevelled-associated activator of morphogenesis 1), Rho family of small GTPases and Jun N-terminal kinase (JNK), but also via a few less well-studied ciliogenesis and planar polarity effector (CPLANE) proteins including Intu, Fuz and Wdpcp (Adler and Wallingford, 2017). Wnt/Ca^{2+} signaling triggers intracellular calcium flux to induce actin polymerization and NFAT (nuclear factor of activated T-cells)-mediated target gene transcription. Dvl family proteins function in all Wnt pathway branches through distinct domains including DIX, PDZ and DEP (Shi, 2020). Their conserved C-terminus PDZ domain-binding motif can modulate Wnt/ß-catenin and Wnt/PCP signaling through interaction with the PDZ domain (Lee et al., 2015).

The “core” PCP proteins form two separate complexes that are distributed on opposite cell borders within the tissue plane (Fig. 1B,C). Frizzled (Fzd), Dvl and Ankrd6 reside on one side of the cell, while Vangl and Prickle localize to the opposite side. Celsr is present on both sides and forms homodimers between adjacent cells to propagate polarity information across cells. This asymmetric distribution of “core” PCP proteins is a hallmark of planar polarization and reflects the coordinated cellular orientation during tissue or organ morphogenesis. Vertebrate Wnt5a signaling gradient can provide global cues to instruct the asymmetric localization of “core” PCP proteins in several well-documented PCP-dependent processes, such as the proximal-distal development of limb bud and the anteroposterior (AP) polarization of node cells (Gao et al., 2011; Minegishi et al., 2017). Similarly, Fat and Dchs heteromeric protocadherins are localized to opposite sides of adjacent cells (Fig. 1B), and they may function either as a ligand or as a receptor for the other to mediate cell interaction (Blair and McNeill, 2018). Through a multitude of biochemical, functional and genetic interactions, PCP proteins regulate non-canonical Wnt signaling and make an important contribution to the spatiotemporal organization of cellular activities in a variety of tissues and organs (Table 1). The following sections will detail their implications in asymmetric organ morphogenesis during development and disease.

Early embryonic left-right asymmetry

Left-right asymmetry, either external or internal, is a common feature in animals. Although vertebrate embryos are seemingly symmetrical, they already display asymmetric gene expression at early stages of development. This is critical for the asymmetric formation of internal organ primordia and their subsequent morphogenesis to generate left-right differences in sizes, shapes and anatomical locations. Transient structures formed during early development, such as the posterior gastrocoel roof plate (GRP) in *Xenopus*, the Kupffer's vesicle (KV) in zebrafish, the Hensen's node in chick and the node in mice, constitute the left-right organizer (LRO) involved in breaking the bilateral symmetry across the mediolateral plane (Fig. 2A,B). They initiate left-right asymmetry by providing instructive signals mostly through cilia-mediated directional fluid flow (Axelrod, 2020; Little and Norris, 2021). Wnt/PCP signaling acts in the LRO to coordinate the orientation of individual cells and multicellular structures with respect to the embryonic axes. Subsequently, signals generated in a manner that is both dependent and independent on motile cilia create a gradient of Nodal protein to activate left-sided expression of the Nodal-Lefty-Pitx2 network (Grimes and Burdine, 2017; Axelrod, 2020). Thus, differential gene activation and repression establish left-right embryonic polarity that will influence the asymmetric morphogenesis of organ primordia.

Dvl, Vangl and Prickle are important for cilia-dependent asymmetric fluid flow. In node cells, Vangl1, Vangl2 and Prickle2 are localized to the anterior side, while Dvl2 and Dvl3 are enriched at the posterior side (Antic et al. 2010; Hashimoto et al., 2010; Grimes and Burdine, 2017). Wnt5a and Wnt5b, which are expressed posteriorly relative to the node, form a diffusible gradient and provide instructive signals to induce the asymmetric localization of Vangl1 and Prickle2, thus polarizing node cells along the AP axis (Minegishi et al., 2017). The asymmetric localization of "core" PCP proteins then restricts the posterior positioning of ciliary basal bodies at the dome-shaped apical surfaces of node cells and promotes the posterior tilting of cilia by coordinating the asymmetric distribution of microtubules and actomyosin networks (Sai et al., 2022). Subsequently, cilia-driven leftward fluid flow across the node initiates left-right asymmetry. Knockout of Dvl2 and Dvl3 impairs the posterior location of ciliary basal bodies (Hashimoto et al., 2010). Loss of Vangl1 and Vang2 disrupts the posterior orientation of primary motile cilia in the LRO, resulting in defective expression of Nodal and Pitx2 on the left side (Borovina et al., 2010; Antic et al., 2010; Song et al., 2010). Consistent with their association into a complex, Prickle1 and Prickle2 interact with Vangl2 to regulate its anterior localization in node cells (Minegishi et al., 2017), while Prickle3 shows reciprocal interactions with Vangl2 for the anterior localization in GRP cells to promote growth and posterior positioning of motile cilia (Chu et al., 2016). Downstream of “core” PCP proteins, JNK activity is required for modulating ciliogenesis and cilia length in the zebrafish KV (Derrick et al., 2022). Altogether, these observations reveal an important implication of Wnt/PCP signaling in the orientation of motile cilia within the LRO. Thus, the AP polarity generated by the asymmetric distribution of PCP proteins can be translated into left-right asymmetry through the directional fluid flow.

The unconventional Myosin1D (Myo1D) also functions to shape the cilia-driven directional fluid flow in the LRO, although unlikely being a component of the Wnt/PCP pathway. It interacts with Vangl2 to initiate left-right axis formation in zebrafish and *Xenopus* embryos (Juan et al., 2018; Tingler et al., 2018). In the zebrafish KV, Myo1D antagonizes the activity of Vangl2 to restrict the localization of posteriorly pointing cilia at the anterior region and anteriorly pointing cilia at the posterior region, thereby establishing a circular geometry of fluid flow (Juan et al., 2018). Thus, the cilia-dependent function of Myo1D acts in concert with Wnt/PCP signaling to break left-right symmetry.

Asymmetric cardiac morphogenesis

The rightward looping of the heart primordium contributes to determine the relative position of cardiac chambers and is the first event of asymmetric organogenesis (Desgrange et al., 2018). It is initiated by the transformation of the cardiac tube into a loop (Fig. 2C). Abnormal left-right patterning is intimately associated with congenital heart diseases, such as X-linked heterotaxy caused by mutations of the ZIC3 transcription factor. Recent evidence suggests that Zic3 regulates the expression of PCP genes and is required for Dvl phosphorylation during node morphogenesis. It also genetically interacts with PCP genes to establish the left-right heart asymmetry (Bellchambers and Ware, 2021). Therefore, there is a possibility that dysregulation of PCP proteins may represent an underlying mechanism of ZIC3 mutations in congenital disorders associated with heterotaxy.

Wnt/PCP signaling critically regulates heart morphogenesis after looping, particularly directional cell movements from the second heart field (SHF) during outflow tract (OFT) development (Henderson et al., 2006). Wnt5a, Wnt5b and Wnt11 are important for modulating extracellular matrix composition, cytoskeletal rearrangements, actomyosin contractility, and cell adhesion during SHF deployment and heart tube remodeling (Zhou et al., 2007; Sinha et al., 2015; Merks et al., 2018). Loss of Wnt5a in mice leads to OFT malformations due to impaired cell deployment in the SHF (Sinha et al., 2015). Moreover, Wnt5a functions through Daam1 in OFT morphogenesis by promoting the AP elongation of the SHF (Li et al., 2019).

Dvl family members collectively contribute to OFT morphogenesis. Loss of Dvl1 and Dvl2 impairs SHF deployment and OFT lengthening by disrupting actin polymerization and filopodia formation (Sinha et al., 2012). Homozygous *Looptail* mice, which carry a single nucleotide mutation in the *Vangl2* gene that is predicted to produce a malfunctional Vangl2 protein, show defects in the polarization of myocardial cells likely due to reduced activity of RhoA and ROCK1 (Rho-associated kinase 1). As a consequence, myocardializing cells fail to extend cellular protrusions into the OFT cushion, resulting in abnormal muscularization of the proximal outlet septum (Phillips et al., 2005). Conditional knockout of Vangl2 indicates that it is solely required within the SHF to promote lengthening of the OFT by regulating cellular polarity in the distal outflow wall (Ramsbottom et al., 2014). Similarly, loss of Prickle1 in mice leads to shortened OFT due to absence of polarized cell orientation in the SHF (Gibbs et al., 2016). Conditional knockout of Daam1 in the myocardium prevents cardiomyocytes from extending protrusions into the OFT (Ajima et al., 2015). Disruption of the CPLANE protein Wdpcp in mice also prevents polarized migration of cardiomyocytes to invade the OFT cushion and causes OFT septation defects (Cui et al., 2013). Thus, Wnt/PCP signaling coordinates directional cell migration in the SHF to promote heart morphogenesis.

PCP proteins are also involved in other aspects of heart development. In mice, Fzd4 is required for arterial and arteriolar formation. It cooperates with Dvl3 to regulate vascular cell proliferation and migration through microtubule stabilization and cellular polarization (Descamps et al., 2012). Fzd2 and Fzd7 are redundantly required for CE movements to promote closure of the ventricular septum (Yu et al., 2012). Vangl2 interacts with RhoA and ROCK in the formation of the coronary vasculature through a non-autonomous manner, likely by regulating deposition of fibronectin in the subepicardial space for the migration of epicardially-derived cells (Phillips et al., 2008). Combined loss of Daam1 and Daam2 in mice causes severe cardiac abnormalities including ventricular noncompaction, ventricular septal defects, irregular sarcomere assembly, and impaired myocardial maturation (Li et al., 2011; Ajima et al., 2015). However, it is unclear whether these defects are all direct consequences of disrupted Wnt/PCP signaling.

Gut looping and elongation

The left-right asymmetry of the developing gut arises through an important morphogenetic process known as gut looping. This event has been shown to rely on physical forces generated by differential growth rates between the gut and the dorsal mesentery that connects the gut to the body wall (Savin et al., 2011). The earliest left-right asymmetric gut morphology is manifested by the leftward curvature of the stomach (Fig. 2D). In the chick embryo, Nodal-induced expression of Pitx2 maintains left-side identity of the early gut tube (Huycke and Tabin, 2018; Grzymkowski et al., 2020). Wnt/PCP signaling acts downstream of Pitx2 to coordinate asymmetric processes of the digestive system, including gut looping, intestine elongation, villification of the intestinal epithelium, stem cell lineage segregation, and maintenance of epithelial homeostasis.

Several PCP proteins display asymmetric expression in the chicken dorsal mesentery, which exhibits polarized cellular behaviors with extensive membrane contacts between adjacent mesenchymal cells on the left side. Fzd4 and Daam2 show Pitx2-dependent left-sided expression, and Daam2 is required for cell adhesion necessary for mesenchymal condensation in the dorsal mesentery (Welsh et al., 2013). Although Wnt5a does not display asymmetric expression, its activity on the right side is antagonized by the right-sided expression of secreted Frizzled-related proteins (sFRPs). Therefore, the asymmetric activation of Wnt5a-Fzd4-Daam2 pathway triggers leftward tilt of the midgut (Welsh et al., 2013).

Intestine elongation critically requires functional PCP signaling. In mice, Wnt5a is expressed in the gut mesenchyme. Constitutive knockout of Wnt5a disrupts midgut elongation by preventing post-mitotic daughter cells from re-intercalating into the epithelial layer (Cervantes et al., 2009) as well as from extending filopodia for basal return of their nuclei during interkinetic nuclear migration (Wang et al., 2018). Ror2 and Ryk differentially regulate midgut elongation through dynamic spatiotemporal interactions with Wnt5a. Mesenchyme-derived Ror2 functions before the early phase of midgut elongation, while both epithelial and mesenchymal Ryk contributes to midgut elongation throughout the early phase (Wang et al., 2020). Vangl2 regulates several polarized cell behaviors in the gut epithelium to couple epithelial morphogenesis with gut tube elongation. In mice, it functions with Wnt5a for oriented cell division along the rostrocaudal axis to increase fore-stomach length (Matsuyama et al., 2009). In *Xenopus*, Vangl2 is enriched at the apical and anterior surfaces of radially elongated gut endoderm cells; it coordinates microtubule organization, cell adhesion and endodermal cell shape changes to promote gut elongation and lumen formation (Dush and Nascone-Yoder, 2019). At late stages of intestine elongation, the Fat4/Dchs1 module acts in parallel with Vangl2 to drive mesenchymal cell clustering necessary for epithelial folding during villus formation, while Wnt5a functions upstream of Fat4 and acts as a chemoattractant to guide directional migration of mesenchymal cells (Rao-Bhatia et al., 2020).

The gene encoding cilia and flagella associated protein 126, *Cfap126* (also known as *Flattop* or *Fltp*), is transcriptionally activated during Wnt/PCP acquisition in ciliated cells, thus representing a target of Wnt/PCP signaling (Gegg et al., 2014). Cfap126 can function as a PPE, and its transient expression in Lgr5$^+$ intestinal stem cells regulates lineage priming and cell cycle exit at the base of the crypt, contributing to the differentiation of Paneth and enteroendocrine cells (Böttcher et al., 2021). In the adult, Wnt5a is expressed in FoxL1$^+$ telocytes at the crypt-villus junction regions and is required for homeostatic renewal of the intestinal epithelium (Shoshkes-Carmel et al., 2018). These data highlight an essential role of Wnt/PCP signaling in intestinal stem cell lineage segregation and directly link cell polarity to cell fate specification.

Lung branching morphogenesis

The highly elaborated and polarized architecture of the respiratory system emerges from reciprocal interactions between lung epithelium and mesenchyme (Fig. 3A). Lung branching morphogenesis involves repetitive formation of new buds, epithelial sprouting, and tube elongation to generate an arborized airway network. This process is also dependent on several aforementioned asymmetric cell behaviors. PCP proteins regulate different aspects of lung morphogenesis, and their dysfunctions are linked to lung diseases, such as emphysema, a chronic obstructive pulmonary disease, also known as COPD (Vladar and Königshoff, 2020).

In the mouse embryo, Wnt5a is mainly expressed in the mesenchyme of branch points and functions through Ror2 to synchronize radial polarization of mesenchymal cells for tube elongation along the AP axis (Kishimoto et al., 2018). It also activates the Ror2-Vangl2 cascade in both lung epithelium and mesenchyme to coordinate alveolar formation through cytoskeleton-mediated cell shape changes of alveolar type I cells and migration of myofibroblasts. Moreover, Wnt5a and Vangl2 show reduced expression in lung tissues of human patients with bronchopulmonary dysplasia and emphysema, suggesting a possible implication of these genes in lung diseases (Li et al., 2020; Zhang et al., 2020). Fzd2 regulates epithelial cell shape changes to promote branch point formation through RhoA-induced apical localization of phosphorylated myosin light chain 2 (Kadzik et al., 2014). Celsr1 and Vangl2 are enriched at the basal/luminal surface of branching lung epithelial buds. They regulate cytoskeletal remodeling likely via ROCK to maintain epithelial architecture, thus their mutations lead to a reduced number of epithelial branches (Yates et al., 2010b). Tracheal and alveolar epithelial cells from heterozygous *Looptail* mice show highly disrupted actomyosin networks and abnormal focal adhesions, which are correlated with decreased RhoA activity and reduced YAP signaling, suggesting that Vangl2 may function as a regulator of mechanotransduction (Cheong et al., 2020). Scrib is required for lumen morphogenesis. It maintains tight junction integrity and epithelial cohesion through interaction with Wnt/PCP signaling by modulating the localization of Celsr1 and Vangl2 in lung epithelium (Yates et al., 2013).

Airway motile cilia present distinct orientations and highly coordinated movements, which are important for mucociliary clearance. During ciliogenesis, asymmetrically localized PCP complexes first polarize epithelial cells along the proximal (oral) to distal axis, and then provide polarity cues to orient ciliary basal bodies in the proximal direction (Vladar et al., 2016). In airway epithelia, Fzd3, Fzd6, Dvl1 and Dvl3 are enriched at the proximal side of multiciliated cells;

Vangl1, Vangl2 and Prickle2 are distributed at the distal side (Vladar et al., 2012, 2016); Dvl2 and Ankrd6 are localized to the base of cilia (Vladar et al., 2012). Loss of PCP protein expression or localization leads to mis-orientations of airway cilia and chronic inflammatory lung diseases (Vladar et al., 2012, 2016), suggesting an essential role of PCP signaling in maintaining lung homeostasis during fetal and post-natal development. Indeed, conditional knockout of Vangl1 or Prickle2 in mice impairs tracheal epithelial regeneration (Vladar et al., 2016); reducing the activity of Vangl2 causes tissue damage as observed in emphysema and impairs adult lung function (Poobalasingam et al., 2017). Knockout of Cfap126 mostly reduces the diameter of terminal lung bronchioles, leading to constricted distal airways in the post-natal lung (Gegg et al., 2014).

Wnt/PCP signaling also coordinates the development of pulmonary airway and vasculature. Wnt5a interacts with Ror2 to direct avian pulmonary vasculogenesis by regulating VEGF signaling through fibronectin expression in the mesenchyme (Loscertales et al., 2008). Pericytes are perivascular cells that promote vessel maturation. Loss of Wnt5a and Fzd7 impairs endothelium-pericyte interactions during pulmonary angiogenesis by disrupting the motility and polarity of pericytes, which may contribute to pulmonary arterial hypertension (Yuan et al., 2019). Since PCP proteins are critically involved in lung development, homeostasis and disease, it will be important to further decipher the mechanisms by which they orchestrate stage-specific cellular activities, ranging from epithelial-mesenchymal interactions in branching morphogenesis to the maturation of multiciliated cells and the development of respiratory vasculature.

Kidney development

The morphogenesis of the early urinary tract involves reciprocal interactions between the Wolffian duct-derived ureteric bud and its adjacent metanephric mesenchyme. Strikingly, the ureteric bud undergoes reiterative branching morphogenesis with distal elements engaged in directed cell migration, oriented cell division, and cell intercalation. These PCP-dependent cellular behaviors play important roles in ureteric bud branching, tubular elongation, and tubule diameter establishment (Carroll and Yu, 2012). Different polarity pathways are critically implicated in kidney development and potentially linked to various genetic disorders, such as congenital malformations of the kidney and urinary tract (CAKUT) and glomerular diseases (Torban and Sokol, 2021).

It is well documented that Wnt ligands are essential in kidney morphogenesis. Pioneer studies have demonstrated a requirement of Wnt4 for mesenchymal to epithelial transformation that results in epithelialization of the ureteric bud (Stark et al., 1994; Kispert et al., 1998). Wnt5a-Ror2 signaling regulates epithelial tubular formation from the ureteric bud. Loss of Wnt5a or Ror2 in mice impairs the positioning of metanephric mesenchyme and causes its aberrant interaction with the Wolffian duct, resulting in duplicated ureters and kidneys (Nishita et al., 2014; Yun et al., 2014). Wnt9b acts through Rho/JNK signaling to regulate CE movements and maintain polarized cell divisions in the epithelium during early stages of kidney morphogenesis, thus inhibition of Wnt9b-mediated signaling affects epithelial polarity and causes increased tubule diameter (Karner et al., 2009). Wnt11 derived from the ureteric branch tip promotes the stable attachment of nephron progenitors to the epithelial tip by regulating their polarity and motile behaviors, thereby maintaining nephrogenic niche integrity (O'Brien et al., 2018).

Several “core” PCP proteins clearly display asymmetric localization in developing renal tubule epithelia along the proximal-distal axis. In both the collecting duct and proximal tubules, Vangl1 and Vangl2 are accumulated at the proximal side, while Fzd3 and Fzd6 are distributed at the distal side, suggesting that they are involved in the polarization of renal tubules (Kunimoto et al., 2017). Functional analyses indicate that other “core” PCP proteins are also important for renal tubule morphogenesis. Mice deficient for Fzd4 and Fzd8 show renal hypoplasia, with delayed growth, branching and proliferation of the ureteric epithelium (Ye et al., 2011). However, these phenotypes may also result from disrupted Wnt/ß-catenin signaling. Perturbation of Dvl function in *Xenopus* disrupts rosette-based CE movements driven by mediolaterally oriented cell intercalations, resulting in shorter and wider kidney tubules (Lienkamp et al., 2012). Vangl2 regulates morphogenetic processes in the ureteric bud and metanephric mesenchyme. Homozygous *Looptail* mice show defective ureteric branching and glomerular maturation but with normal ureteric bud formation, consistent with impaired CE movements (Yates et al., 2010a). Prickle1 is required for tubule morphogenesis by regulating the distribution of actin cytoskeleton in the ureteric bud; its loss of function impairs cell arrangements in the collecting duct and renal tubules, resulting in abnormal tubule shape (Liu et al., 2014). Celsr1 genetically interacts with Vangl2 to regulate rostrocaudal patterning of renal tubules and maturation of glomeruli; it also promotes ureteric tree growth during early stages of kidney development but prevents tubule overgrowth at late stages (Brzóska et al., 2016). Consistent with these functions, mutations of *CELSR1* are associated with renal disorders, including unilateral renal agenesis, hydronephrosis, and hydroureter (Brzóska et al., 2016). Therefore, it will be important to understand the molecular and cellular mechanisms underlying the stage-specific functions of Celsr1 in kidney development.

Effectors of Wnt/PCP signaling are implicated in different aspects of renal tubule development. In *Xenopus* and zebrafish pronephros, Daam1 may function through Rho GTPase to regulate cytoskeletal rearrangements for proper tubulogenesis and tubule morphology (Miller et al., 2011). It also colocalizes with E-cadherin at cell-cell contact regions in the *Xenopus* nephric primordium to ensure intercellular adhesion and epithelial tissue organization during CE movements (Krneta-Stankic et al., 2021). In mice, Fuz appears to regulate ureteric bud branching morphogenesis through cilia-dependent and -independent pathways, raising the possibility that it may play a role in ciliogenesis (Wang et al., 2021).

Fat and Dchs heteromeric protocadherins are important for cell interactions in the developing kidney. Loss of Fat4 disrupts polarized cell behaviors and leads to cyst formation (Saburi et al., 2008). Fat1 cooperates with Fat4 in renal tubular elongation (Saburi et al., 2012). Importantly, the interaction between Fat4 and Dchs1 is necessary for ureteric bud branching and tubule diameter establishment. Dchs1 is specifically expressed in the cap (condensing) mesenchyme that coalesces around the ureteric bud, while Fat4 is only present in stromal cells that surround the cap mesenchyme (Fig. 3B). These expression patterns establish a crosstalk whereby Fat4 interacts with Dchs1 to regulate self-renewal and differentiation of ureteric epithelial progenitors through Hippo, Notch or FGF signaling (Das et al., 2013; Bagherie-Lachidan et al., 2015). Fat4/Dchs1-mediated stroma-to-cap signaling may also function to polarize the cap mesenchyme for proper nephrogenesis (Mao et al., 2015). There is also a complex interplay between Fat family protocadherins and other signaling pathways during kidney development. In zebrafish pronephros, Fat1 functions with Scrib in Hippo signaling through regulation of YAP1. Depletion of Fat1 and Scrib causes severe cyst formation, suggesting that Scrib may couple Fat1 with the Hippo pathway to coordinate cellular polarization and growth (Skouloudaki et al., 2009). In mice, Fat4 can act non-autonomously to prevent ectopic ureteric bud formation and kidney duplication by fine-tuning the signaling function of nephric duct-expressed RET, a GNDF receptor that regulates cell rearrangements in ureteric bud morphogenesis (Zhang et al., 2019). Thus, Fat4 plays a critical role in the cap mesenchyme to control the nephron progenitor pool.

Wnt/PCP signaling is also important for proper organization of podocytes, which are highly specialized and polarized glomerular visceral epithelial cells that are essential for maintaining the correct function of the glomerular filtration barrier to prevent proteinuria. Podocyte-specific loss of Vangl2 in mice causes abnormal glomerular maturation in fetal kidneys and increases the susceptibility of glomerular injury in the adults (Rocque et al., 2015). Thus, Vangl2 play an important role to promote glomerular development and protect glomerular injury.

The improved understanding of PCP proteins in kidney morphogenesis helps to clarify the link between Wnt/PCP signaling and chronic kidney failure. Nephron, the functional unit of the kidney, uses numerous ciliated epithelial tubules to reabsorb nutrients and concentrate waste products. Dysfunctions of primary cilia contribute to the pathogenesis of polycystic kidney disease (PKD). As Wnt/PCP signaling, PKD genes encoding proteins mainly localized to primary cilia are also required for oriented cell divisions and CE movements in developing renal tubules, inferring that defective Wnt/PCP signaling may contribute to PKD as well. However, recent studies indicate that mutations of PKD genes disrupt renal tubule morphogenesis independently of Wnt/PCP signaling and that the asymmetric localization of PCP proteins remains intact in adult cystic kidney (Kunimoto et al., 2017). Moreover, collecting duct-specific loss of Vangl1 and Vangl2 in mice impairs oriented cell divisions and CE movements, leading to abnormal tubule diameter in pre-natal renal tubules but not cyst formation in post-natal development (Kunimoto et al., 2017; Derish et al., 2020). Therefore, PCP proteins play an important role in the accurate control of tubule lumen diameter. Nevertheless, it remains to be determined in more detail how they regulate early and late stages of kidney epithelial morphogenesis and how they are linked to other kidney diseases.

Inner ear development and hair cell polarity

The inner ear exhibits marvelous features of PCP-dependent asymmetric morphogenesis, making it an attractive system for studying PCP regulation and function. Mammalian inner ear consists of a snail-shaped cochlea required for hearing and a vestibular system necessary for maintaining balance. Cochlear extension and development of the spiral organ of Corti from a thicker and shorter primordium involve cell intercalations at the luminal surface and directed migration of hair cells toward the apex (Driver et al., 2017). These highly regulated cellular processes promote cochlea growth and contribute to the spatial distribution of sensory cells. Thus, the organ of Corti forms one row of inner hair cells (IHCs) and three rows of outer hair cells (OHC1, OHC2, and OHC3), which are separated by specialized supporting cells. Based on their locations with respect to the center of the cochlear spiral, IHCs are referred to as neural/medial (or proximal), while OHCs are described as abneural/lateral (or distal). Strikingly, both cochlear and vestibular hair cells display uniformly oriented stereociliary bundles on their apical surfaces. Wnt/PCP signaling functions to promote cochlea growth and organize the highly choreographed orientation of stereociliary bundles.

In cochlear and vestibular epithelia of the developing mouse embryo, PCP proteins display striking asymmetric localization in both sensory and supporting cells in a pattern that is tightly linked to hair cell polarity (Fig. 4A). Fzd3, Fzd6 and Dvl1 accumulate at the neural boundary of IHCs and OHCs, opposite to the site of stereociliary bundle formation, while Dvl2 and Dvl3 are apparently enriched at the abneural edge (Najarro et al., 2020). Ankrd6 localizes to the neural edge (Jones et al., 2014). Celsr1 is likely enriched both at the neural side of hair cells and at the abneural side of supporting cells (Duncan et al., 2017). Analysis by stimulated emission depletion microscopy indicates that Vangl2 is present at the abneural side of supporting cells (Giese et al., 2012). Prickle2 is enriched at the neural side of vestibular hair cells before the formation of stereocilia, and Vangl2 maintains this asymmetric distribution (Deans et al., 2007).

Dysfunctions of PCP proteins affect cochlear outgrowth and hair cell polarity. Wnt5a alone is not sufficient for hair cell polarization in the developing cochlea, but multiple Wnt ligands collectively contribute to this process (Najarro et al., 2020). During cochlear extension to form the organ of Corti, Dvl1, Dvl2 and Vangl2 regulate cell intercalation that drives unidirectional elongation of the primordium. Loss of their functions interferes with outgrowth and morphogenesis of the cochlea, resulting in abnormal patterning of the cochlear duct and defective uniform hair bundle orientation (Montcouquiol et al., 2003; Wang et al., 2005). Combined loss of Fzd3 and Fzd6 mostly affects hair bundle orientation in the organ of Corti (Wang et al., 2006). In mice, missense mutations that may reduce the structural integrity of Celsr1 protein disrupt the earliest stages of hair cell polarity in the cochlea and lead to mis-oriented stereociliary bundles between vestibular hair cells associated with defective vestibular behaviors (Curtin et al., 2003). Knockout of Fat4 mildly affects hair bundle orientation by mostly leads to defects in cochlear extension and organization of OHCs, which are exacerbated by reducing the dosage of Fat1 (Saburi et al., 2012).

Different PCP genes show genetic interactions in cochlear extension and stereociliary bundle orientation, likely due to functional redundancy and interdependent asymmetric localization of their protein products. In particular, there is evidence showing Vangl2 interaction with other PCP proteins in auditory hair cells by utilizing the *Looptail* mutant. Inhibiting the secretion of Wnt ligands, including Wnt5a, in a heterozygous *Looptail* background aggravates defects in cochlear extension and sensory hair cell polarization (Najarro et al., 2020). Loss of Fzd1 or Fzd2 causes mis-orientations of hair cells only when Vangl2 activity is also reduced (Yu et al., 2010). Although *Ankrd6* mutant mice show disrupted uniform orientation of hair cells in the utricle, only *Ankrd6* and *Vangl2* compound mutants display defective hair cell polarity and patterning in the cochlea (Jones et al., 2014). Fat4 cooperates with Vangl2 to regulate cochlear elongation and sensory epithelium patterning (Saburi et al., 2012). There are also physical and genetic interactions between Scrib and Vangl2 in the planar polarization of stereociliary bundles (Montcouquiol et al., 2003, 2006). It is possible that the *Looptail* mutation contributes partially to enhance hair cell phenotypes because the resulting malfunctional Vangl2 protein can interfere with the proper localization of other PCP complexes.

During post-natal development, loss of Vangl2 has mild effects on the orientation of auditory stereociliary bundles and on hearing loss, but mostly causes profound changes in the shape and distribution of supporting cells that are necessary for cochlear amplification mediated by OHCs (Copley et al., 2013). Thus, it will be of interest to determine the post-natal requirement of other PCP proteins in maintaining hearing function.

Intriguingly, PCP proteins can function in parallel to instruct hair cell orientation. Zebrafish lateral line neuromasts also consist of mechanosensory hair cells and support cells. Loss of Vangl2 directly leads to PCP-dependent defects in hair cells. However, loss of Wnt11 or Fzd7 indirectly affects hair cell polarity by disrupting the alignment of support cells, suggesting a contribution of support cells in sensory cell orientation (Navajas Acedo et al., 2019). Interestingly, supporting cells can also dictate hair cell polarization in the mammalian cochlea. The evolutionarily conserved transmembrane receptor protein tyrosine kinase 7 (PTK7) functions as a vertebrate-specific PCP regulator. Disruption of PTK7 mostly affects stereociliary bundle orientation in OHC3 (Lu et al., 2004). Mechanistically, PTK7 acts in parallel with Wnt/PCP signaling to polarize hair cells by promoting junctional localization of myosin II near the apical surfaces of supporting cells (Lee et al., 2012). Thus, PTK7 restricts the abneural positioning of kinocilia by exerting local tension at the neural edge of hair cells.

Cochlear OHCs are innervated by type II spiral ganglion neurons, which project peripheral axons that extend beyond the IHCs and then turn 90° toward the cochlear base. Disruption of Wnt secretion suggests that non-canonical Wnts are necessary to trigger Wnt/PCP signaling during axon guidance (Ghimire and Deans, 2019). Fzd3 and Fzd6 are present at the basolateral boundary between adjacent supporting cells along the trajectory of axonal growth cones; they promote the asymmetric localization of Vangl2 and function redundantly to direct cochlear innervation (Ghimire and Deans, 2019). Consequently, Vangl2 is enriched at intercellular junctions between cochlear supporting cells and is non-autonomously required for axon turning (Ghimire et al., 2018). Although it is unclear how Prickle1 and Prickle2 regulate hair cell polarity, Prickle1 is required for neurite outgrowth of type II spiral ganglion neurons (Yang et al., 2017a). Together, these observations demonstrate an essential role for Wnt/PCP signaling in hair cell innervation.

Generally, dysfunctions of PCP proteins affect the uniform orientation of stereociliary bundles between neighboring hair cells, but individual hair cells still retain polarized stereocilia (Fig. 4B). This is because there are independent but nevertheless interconnected mechanisms coordinating hair cell polarity. Intrinsic or intracellular PCP instructs hair bundle polarization within a hair cell, while tissue-level or intercellular PCP establishes the uniform orientation of hair cells. Indeed, several cytoskeletal regulatory proteins, such as Inscuteable (Insc), Partner of Inscuteable (Pins), Cfap126 and Wdpcp, have been shown to control or restrict positioning of the tubulin-based kinocilium and arrowhead-shaped distribution of actin-rich stereocilia (Ezan et al., 2013; Tarchini et al., 2013; Cui et al., 2013; Gegg et al., 2014). Therefore, they can function to establish intrinsic hair bundle polarity. However, the emergence of asymmetric hair bundle position in the hair cell apical surface is tightly coupled with long-range orientation cues in the tissue. Evidence is accumulating that G protein signaling functions to establish intrinsic asymmetry and interpret intercellular PCP signaling. Accordingly, inhibition of Gαi function leads to randomized positioning of kinocilia and mis-orientations of OHCs (Tarchini et al., 2013). Daple, a guanine nucleotide exchange factor and a Dvl-binding protein, mediates Wnt-stimulated heterotrimeric G protein and phosphoinositide 3-kinase signaling to regulate both microtubule-dependent eccentric kinocilium positioning and asymmetric localization of PCP proteins (Siletti et al., 2017; Landin Malt et al., 2020). Therefore, a complex interplay between intracellular and intercellular PCP signaling coordinates the acquisition of hair cell polarity.

Proximal-distal limb outgrowth and elongation

Vertebrate limb buds arise from the lateral plate mesoderm and its overlying ectoderm at the presumptive forelimb and hindlimb locations. Dynamic formation of cellular protrusion and biased orientation of cell division are major driving forces that elongate the limb bud along its proximal-distal axis (Boehm et al., 2010; Wyngaarden et al., 2010). Wnt/PCP signaling plays an essential role in regulating these directional cell behaviors (Barrow, 2011; Gao and Yang, 2013).

Early studies show that *Wnt5a* is expressed in the apical ectoderm ridge (AER) and distal mesenchyme of the limb bud as a gradient along the proximal-distal axis (Gavin et al., 1990; Dealy et al., 1993). Similarly, Vangl2 protein also displays a proximal-distal gradient of asymmetric distribution (Gao et al., 2011).

Wnt5a plays an important role in establishing cell polarity for limb outgrowth (Fig. 4C). Mice mutant for Wnt5a exhibit reduced individual skeletal elements, with absence of distal phalanges (Yamaguchi et al., 1999). Analysis of cell behaviors by living imaging in mouse embryos at E10.5 demonstrate that Wnt5a deficiency essentially disrupts the orientation of mesenchymal cell movements and divisions toward the overlying ectoderm (Gros et al., 2010). Conversely, implantation of Wnt5a-soaked beads into the lateral plate mesoderm of chick embryos reveals that Wnt5a acts as a chemoattractant in the emerging limb bud to instruct cellular polarization necessary for limb outgrowth (Wyngaarden et al., 2010). Moreover, locally oriented cell shape changes and biased cell division planes are conserved cellular mechanisms in vertebrate limb morphogenesis (Wyngaarden et al., 2010).

Wnt5a promotes the interaction between Ror2 and Vangl2 to establish the asymmetric localization of Vangl2 in limb chondrocytes (Gao et al., 2011). It acts through Ror2 to induce a proximal-distal gradient of Vangl2 phosphorylation in the mesenchyme, setting up a higher Vangl2 activity in most distal cells (Gao et al., 2011). This phosphorylation is mediated by casein kinase 1 and functions to polarize cellular behaviors of limb chondrocytes in a dose-dependent manner (Yang et al., 2017b). Wnt5a and Vangl2 also show genetic interaction in limb skeletal development. Reducing *Wnt5a* dosage in *Looptail* mice not only aggravates defects in distal limb skeletal elements but also causes shortened long bones in the limb, reminiscent of Robinow syndrome and brachydactyly type B (Wang et al., 2011). Therefore, a Wnt5a gradient provides global cues, which are interpreted by Ror2 and Vangl2, to coordinate directional cellular behaviors for proximal-distal limb elongation. Interestingly, Wnt5a can also generate mechanical signals to trigger digit elongation likely through ROCK-mediated actomyosin contractility (Parada et al., 2022). Loss of Wnt5a disrupts anisotropic active stresses and CE movements in developing digits, leading to absence of digit-organizing centers (also known as phalange-forming regions) and digit formation (Parada et al., 2022). Ror2 has been shown to regulate phalange development mediated by the digit-organizing center (Witte et al., 2010). Thus, Wnt5a may interact at least partially with Ror2 to combine mechanical cues and molecular signals in digit emergence.

Other PCP proteins also contribute to limb outgrowth. Ryk regulates Wnt/PCP signaling and limb elongation in part by promoting Vangl2 stability (Andre et al., 2012). Dvl family proteins are required for Wnt5a-induced Vangl2 phosphorylation by facilitating the interaction of casein kinase 1 and Vangl2 (Yang et al., 2017b). Mice deficient for Prickle1 show loss of one phalangeal segment on digits 2-5 in both forelimbs and hindlimbs (Yang et al., 2013; Liu et al., 2014). Thus, biochemical and functional interactions between PCP proteins are essential for proper limb morphogenesis.

Concluding remarks

PCP proteins contribute to the highly choreographed cellular organization in many morphogenetic processes. Extensive research has greatly advanced our understanding on their implications in controlling asymmetric organogenesis and tissue homeostasis. Accumulating evidence also suggest that dysfunctions of PCP proteins are linked to a variety of human diseases. Nevertheless, there are still many intriguing questions that deserve further investigation.

Although Wnt ligands can provide global cues to initiate cell polarity in several PCP-dependent processes, it remains to be determined whether molecular pathways act in concert with other orientation cues to instruct and establish PCP features. Interestingly, a recent research shows that the orientation of tissue stretch cooperates with Wnt signaling gradient to align PCP in *Xenopus* neuroectoderm (Hirano et al., 2022). Since mechanical constraints act as important cues in planar polarization, it will be important to further develop interdisciplinary approaches for understanding the interplay of PCP signaling and mechanotransduction in morphogenesis. Functional gene disruptions combined with in vivo live imaging and manipulation of mechanical state should help to push the field forward.

Another important aspect is to understand how cell polarity can be translated to cell fate specification. These two processes are clearly interconnected in several contexts, such that molecular asymmetry provided by PCP proteins helps to promote cell type-specific differentiation. For example, cell polarity cues are coupled with cell lineage segregation during intestinal stem cell self-renewal and differentiation (Böttcher et al., 2021). Deciphering the interaction between molecular signals and mechanical forces in the coupling of cell fate decision and morphogenetic movements also represents a significant challenge. In this regard, it is noteworthy that Wnt5a signaling functions in a mechanical feedback that links specification and elongation of developing digits (Parada et al., 2022).

The formation of separately localized “core” PCP protein complexes is important to create cellular polarization. What are the exact roles of each complex in the coordination of polarized cellular behaviors? How do they converge to regulate cell polarity? Similarly, it is unclear how and when the “core” PCP pathway and the Fat/Dchs polarity module coordinate to regulate polarized cellular behaviors. In *Drosophila*, it appears that they may be coupled by specific Prickle isoforms through regulation of microtubule plus-end bias (Strutt and Strutt, 2021). Whether this is conserved in vertebrates awaits future investigation. Therefore, future research using both invertebrate and vertebrate PCP systems will certainly bring important breakthrough for some of these issues and provide the link between PCP protein dysfunctions and human diseases.

Conflict of interest

The authors declare no competing interests.

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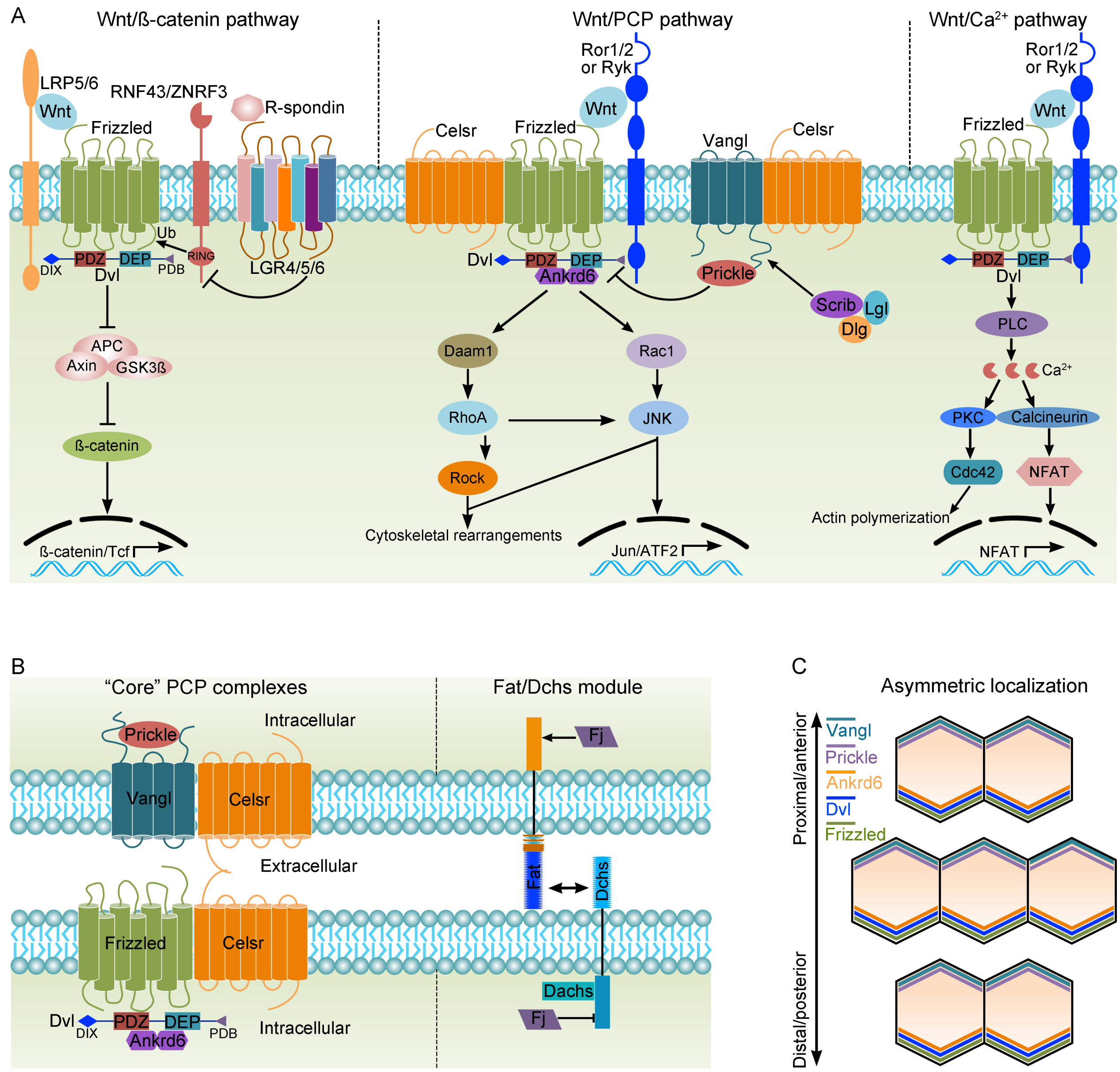

Fig. 1. Wnt signaling branches and asymmetric localization of PCP protein complexes. **A**: Wnt/ß-catenin signaling is activated by ligands binding to Fzd receptors and LRP5/6 (low density lipoprotein receptor-related protein 5/6) co-receptors. The E3 RING ubiquitin ligases RNF43/ZNRF3 mediate the ubiquitination (Ub) of Fzd receptors for lysosomal degradation. This activity is inhibited by R-spondins binding to LGR4/5/6 (leucine-rich repeat containing G-protein coupled receptors). The Wnt/PCP pathway is induced by interaction of Wnts with the Fzd/Ror1/2 (receptor tyrosine kinase-like orphan receptor 1/2) complex or the Fzd/Ryk (receptor tyrosine kinase) complex. The activation of downstream effectors leads to cytoskeletal rearrangements and/or transcription of ATF2 (activating transcription factor-2) target genes. The Scrib complex

can function to regulate Vangl asymmetric localization. The Wnt/Ca^{2+} branch triggers phospholipase C (PLC) activity through heteromeric G proteins to induce calcium-dependent responses. Dvl proteins mediate all Wnt signaling branches through distinct domains: N-terminal DIX, central PDZ, C-terminal DEP, and extreme C-terminus PDZ domain-binding (PDB) motif. **B**: The "core" PCP proteins function as two separately localized complexes, with Celsr forming homodimers between neighboring cells. Fat and Dchs form heterodimers between adjacent cells, acting as a ligand-receptor pair. Dachs is a Dchs-interacting unconventional myosin that functions as a key effector of Fat/Dchs signaling. **C**: Asymmetric subcellular localization of "core" PCP proteins along the proximal (anterior) and distal (posterior) axis in *Drosophila* epithelia.

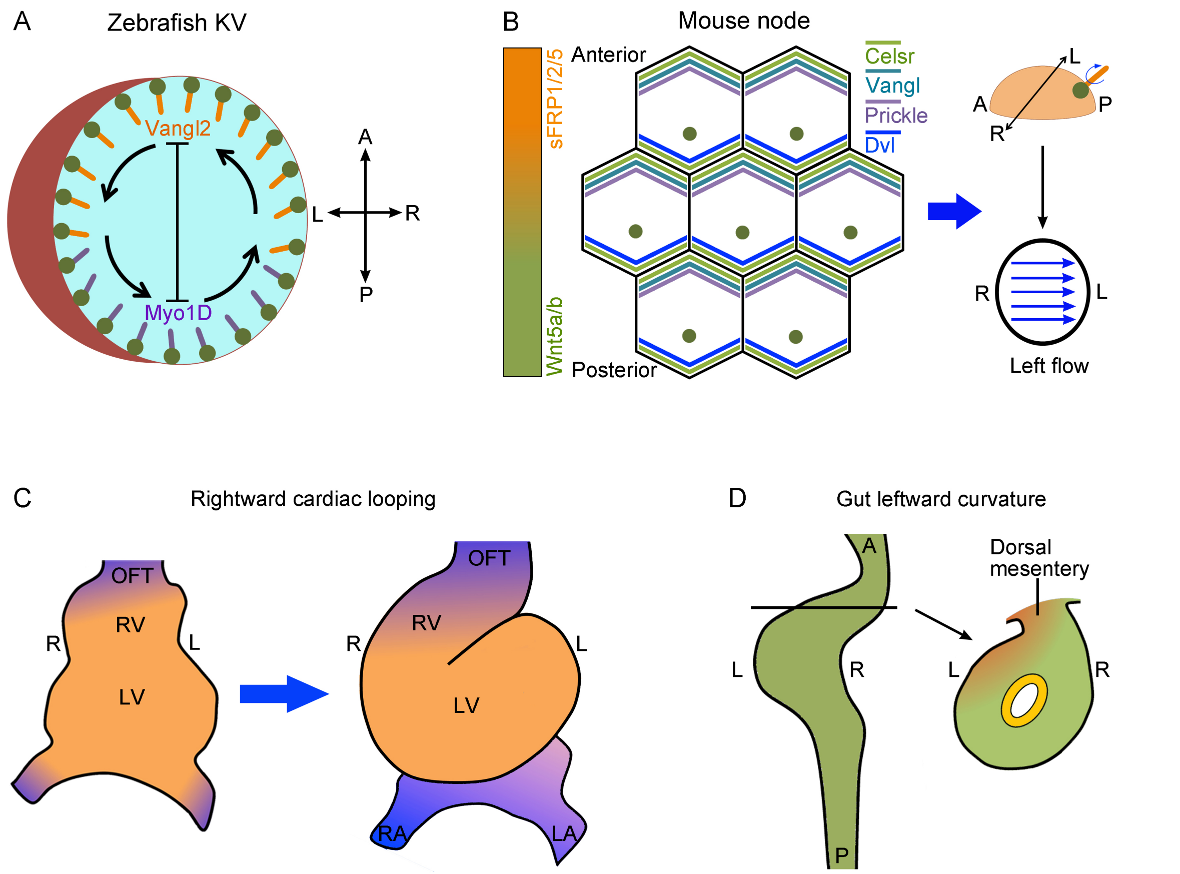

Fig. 2. Left-right organizers and asymmetric organogenesis. **A**: In the zebrafish KV, Vangl2 and Myo1D coordinate leftward fluid flow (arrows) by regulating the localization of anteriorly (orange) and posteriorly (purple) pointing cilia. **B**: In the mouse node, Wnt5a/b gradients along the AP axis promote the asymmetric localization of PCP proteins. The posterior positioning of ciliary basal bodies (green dots) at the dome-shaped apical surfaces of node cells and the posterior tilting of

cilia generate leftward fluid flow within the node. **C**: Asymmetric heart morphogenesis. Ventral view shows rightward cardiac looping in E8.5-E9.5 mouse embryos. Progenitor cells derived from the SHF (blue) promote heart tube elongation, contributing to the development of OFT, right ventricule (RV) and the atria. RA, right atrium; LA, left atrium. **D**: Leftward curvature of gut tube. Transverse section at the horizontal line shows Wnt/PCP-mediated mesenchymal condensation (orange) on the left side of the dorsal mesentery in the chick embryo.

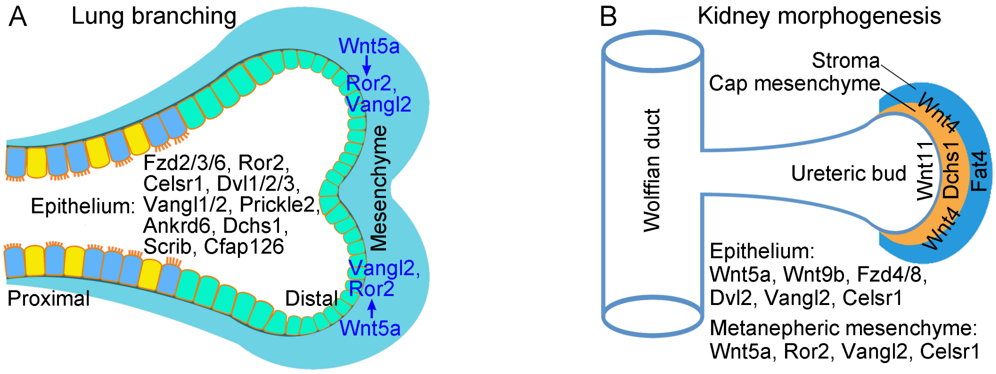

Fig. 3. PCP regulators in lung and kidney morphogenesis. **A**: At the earliest stage of lung branching morphogenesis in E10.5 mouse embryos, mesenchyme-derived Wnt5a activates the Ror2-Vangl2 cascade in the epithelium and mesenchyme to initiate distal lung morphogenesis. PCP proteins display asymmetric localization in bronchial epithelium composed of multiciliated cells and other cell types (yellow) as well as in distal lung epithelium. **B**: Schematic of ureteric bud development in E11 mouse embryos shows the interaction between the Wolffian duct-derived ureteric bud and its adjacent metanephric mesenchyme (stroma and cap mesenchyme). PCP proteins act in the mesenchyme and/or in the ureteric bud to promote ureteric bud branching, tubular elongation, and tubule diameter establishment. The Fat/Dchs polarity module mediates stroma-to-cap mesenchyme signaling in nephrogenesis.

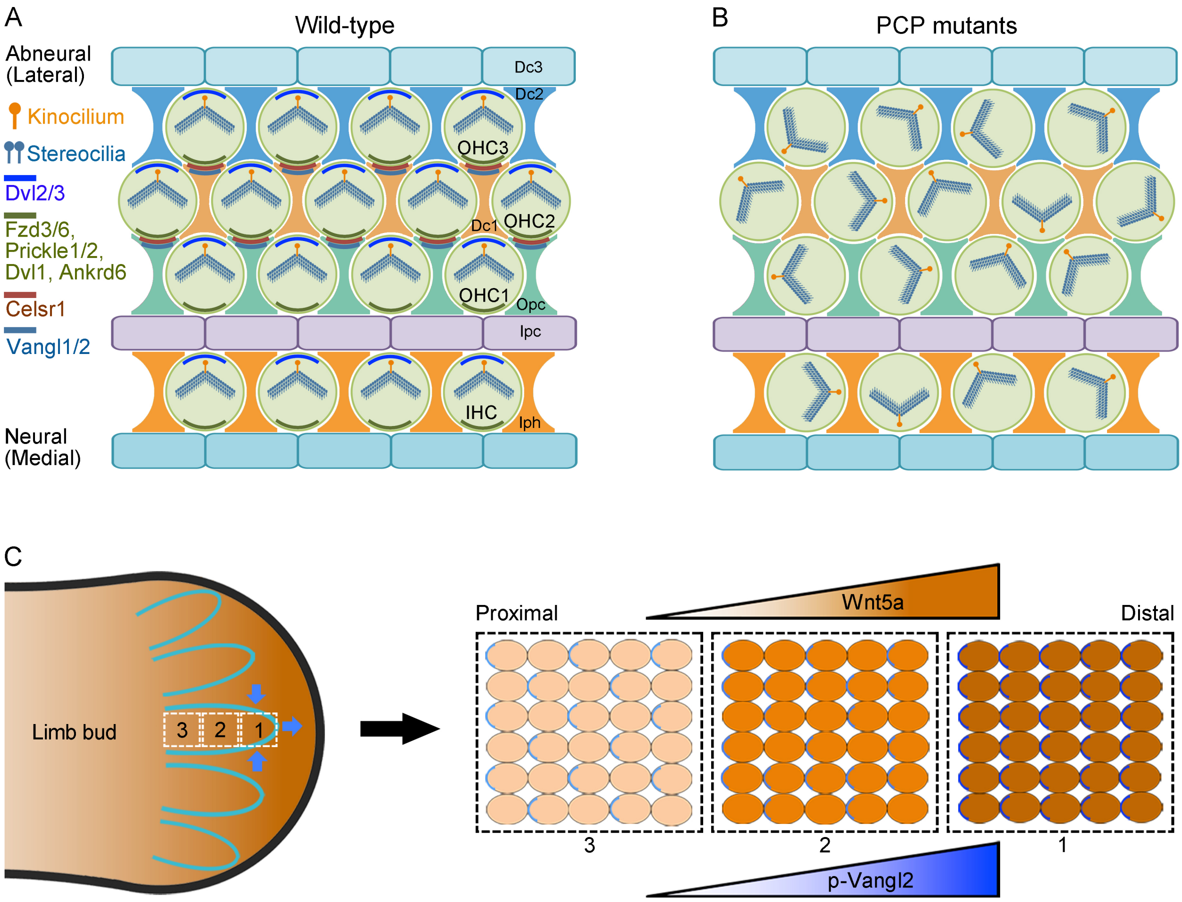

Fig. 4. Wnt/PCP signaling in stereociliary bundle orientation and proximal-distal limb elongation. **A**: In the organ of Corti, hair cells are aligned with supporting cells, including inner phalangeal cells (Iph), inner pillar cells (Ipc), outer pillar cells (Opc), and Deiter cells (Dc1, Dc2, and Dc3). The arrowhead-shaped hair bundles are uniformly oriented toward the abneural (lateral) edge of the sensory epithelium. **B**: PCP mutants display mis-orientations of hair bundles, but each hair cell still retains polarized stereocilia. Severe PCP phenotypes lead to strongly shortened and thickened cochlea with additional rows of IHCs or OHCs. **C**: The graded expression of Wnt5a in the developing limb bud provides global cues to establish a gradient of Vangl2 phosphorylation and localization (blue) along the proximal-distal axis. Vangl2 induces polarized mesenchymal cell behaviors toward the overlying ectoderm (black line), ensuring limb elongation and chondrocyte differentiation. Circles in dashed boxes depict limb chondrocyte condensates expressing Wnt5a and phosphorylated Vangl2. Wnt5a also generates anisotropic active stresses and controls CE movements (blue arrows) to promote the formation of digit-organizing centers for digit specification and elongation.

Table 1. Functions of PCP regulators in asymmetric organogenesis

Organs	PCP proteins	Functions	References
Left-right organizer	Wnt5a/b	Asymmetric localization of “core” PCP proteins in node cells	Minegishi et al., 2017
	Dvl2/3	Posterior positioning of ciliary basal bodies in node cells	Hashimoto et al., 2010
	Vangl1/2	Posterior tilting of primary motile cilia in node cells; posterior orientation of anteriorly positioned cilia in the zebrafish KV (Vangl2)	Borovina et al., 2010; Antic et al., 2010; Song et al., 2010; Juan et al., 2018
	Prickle1/2	Asymmetric distribution of microtubules and actomyosin networks for posterior positioning of ciliary basal bodies in node cells	Minegishi et al., 2017; Sai et al., 2022
	Prickle3	Basal body organization and cilia growth in *Xenopus* GRP	Chu et al., 2016
	Dchs1/2	Vangl1 localization and basal body positioning in node cells	Sai et al., 2022
	JNK1/2	Ciliogenesis and regulation of cilia length in the zebrafsh KV	Derrick et al., 2022
Heart	Wnt5a/b	SHF deployment and AP elongation of the heart tube; OFT septation; polarization of actomyosin during heart tube remodeling (Wnt5b)	Sinha et al., 2015; Merks et al., 2018; Li et al., 2019
	Wnt11	OFT development and polarization of actomyosin networks during heart tube remodeling	Zhou et al., 2007; Merks et al., 2018
	Fzd4	Microtubule stabilization and cellular polarization during arterial and arteriolar formation	Descamps et al., 2012
	Fzd2/7	Redundantly involved in the closure of the ventricular septum	Yu et al., 2012
	Dvl1/2/3	SHF deployment and OFT lengthening	Sinha et al., 2012
	Vangl2	Polarized migration of myocardializing cells and OFT lengthening; formation of the coronary vasculature	Phillips et al., 2005, 2008; Ramsbottom et al., 2014

	Prickle1	Polarized cell orientations and intercalations for OFT lengthening	Gibbs et al., 2016
	Wdpcp	Polarized migration of cardiomyocytes to invade the OFT cushion	Cui et al., 2013
	Daam1/2	Cytoskeletal organization and cell adhesion for protrusion of cardiomyocytes into OFT	Li et al., 2011; Ajima et al., 2015
Gut	Wnt5a	Leftward tilt and gut elongation; re-intercalation of post-mitotic cells into gut epithelium and post-mitotic filopodial pathfinding in nuclear trafficking; chemoattractant for oriented migration of mesenchymal cells during villus formation; homeostatic renewal of adult intestinal epithelium	Cervantes et al., 2009; Matsuyama et al., 2009; Welsh et al., 2013; Wang et al., 2018; Shoshkes-Carmel et al., 2018; Dush and Nascone-Yoder, 2019; Rao-Bhatia et al., 2020
	Ror2	Midgut elongation (mesenchyme-derived Ror2 before phase I)	Wang et al., 2020
	Ryk	Midgut elongation by promoting post-mitotic filopodial pathfinding	Wang et al., 2020
	Vangl2	Oriented cell divisions to increase fore-stomach length; gut elongation and lumen formation; mesenchymal cell clustering in villus formation	Matsuyama et al., 2009; Dush and Nascone-Yoder, 2019; Rao-Bhatia et al., 2020
	Daam2	Mesenchymal condensation in the dorsal mesentery	Welsh et al., 2013
	Fat4/Dchs1	Mesenchymal cell clustering during villus formation	Rao-Bhatia et al., 2020
	Cfap126	Lineage priming and cell cycle exit at the base of the crypt for differentiation of Paneth and enteroendocrine cells	Böttcher et al., 2021
Lung	Wnt5a	Distal lung morphogenesis and lung maturation; alveologenesis; mesenchymal cell polarization for trachea and esophagus formation	Kishimoto et al., 2018; Li et al., 2020; Zhang et al., 2020
	Fzd2	Epithelial cell shape changes to promote branch point formation	Kadzik et al., 2014
	Fzd7	Pulmonary endothelium-pericyte interactions during pulmonary angiogenesis	Yuan et al., 2019
	Ror2	Pulmonary vasculogenesis; alveologenesis	Loscertales et al., 2008; Zhang et al., 2020
	Celsr1/Vangl2	Maintenance of epithelial architecture; branching morphogenesis;	Yates et al., 2010b; Li et al., 2020; Zhang et al.,

		alveologenesis	2020
	Vangl1/Prickle2	Airway epithelial homeostasis and adult lung function	Vladar et al., 2016; Poobalasingam et al., 2017
	Scrib	Tight junction integrity and epithelial cohesion in lumen morphogenesis	Yates et al., 2013
	Cfap126	Diameter formation in terminal lung bronchioles	Gegg et al., 2014
Kidney	Wnt4	Mesenchymal to epithelial transformation for epithelialization of the ureteric bud	Stark et al., 1994; Kispert et al., 1998
	Wnt5a/Ror2	Metanephric mesenchyme positioning for interaction with the Wolffian duct; epithelial tubular formation from the ureteric bud	Nishita et al., 2014; Yun et al., 2014
	Wnt9b	CE movements and polarized cell divisions for tubular diameter formation	Karner et al., 2009
	Wnt11	Attachment of nephron progenitors to the epithelial tip for nephrogenic niche integrity	O'Brien et al., 2018
	Fzd4/8	Growth, branching and proliferation of the ureteric epithelium	Ye et al., 2011
	Dvl2	Rosette-based CE movements during kidney tubule elongation	Lienkamp et al., 2012
	Vangl1/2	Oriented cell divisions and CE movements in developing renal tubules; ureteric branching and glomerular maturation; organization of podocytes to protect glomerular injury in the adult (Vangl2)	Yates et al., 2010a; Rocque et al., 2015; Kunimoto et al., 2017
	Prickle1	Cell arrangements in the collecting duct and renal tubules	Liu et al., 2014
	Celsr1	Rostrocaudal patterning of renal tubules and maturation of glomeruli; promoting ureteric tree growth at early stages and inhibiting tubule overgrowth at late stages	Brzóska et al., 2016
	Fat1/Scrib	Cooperation with Fat4 in renal tubular elongation (Fat1); activation of Hippo signaling to regulate cell polarization and growth	Saburi et al., 2012; Skouloudaki et al., 2009
	Fat4/Dchs1	Oriented cell divisions for renal tubule elongation; Ureteric bud branching and tubule diameter formation; differentiation of ureteric epithelial	Saburi et al., 2008; Das et al., 2013; Mao et al., 2015; Bagherie-Lachidan et al., 2015; Zhang et

		progenitors; polarization of cap mesenchyme; inhibition of ectopic ureteric bud formation and kidney duplication (Fat4)	al., 2019
	Daam1	Pronephric tubulogenesis; intercellular adhesion and epithelial tissue organization in CE and polarized movements	Miller et al., 2011; Krneta-Stankic et al., 2021
	Fuz	Cilia-dependent and -independent ureteric bud branching	Wang et al., 2021
Inner ear	Wnts	Cochlear extension and hair bundle orientation (interaction with Vangl2)	Najarro et al., 2020
	Dvl1/2/3	Cochlear extension and hair bundle orientation	Montcouquiol et al., 2003; Wang et al., 2005
	Wnt11/Fzd7	Alignment of support cells in zebrafish lateral line neuromasts	Navajas Acedo et al., 2019
	Fzd1/2	Hair bundle orientation (interaction with Vangl2)	Yu et al., 2010
	Fz3/6	Hair bundle orientation in the organ of Corti; cochlear innervation by type II spiral ganglion neurons	Wang et al., 2006; Ghimire and Deans, 2019
	Vangl1/2	Cochlear extension and hair bundle orientation; post-natal organization of supporting cells to promote the function of OHCs (Vangl2); axon turning toward the cochlear base to innervate OHCs (Vangl2)	Montcouquiol et al., 2003; Wang et al., 2005; Copley et al., 2013; Ghimire et al., 2018
	Prickle1	Neurite growth of type II spiral ganglion neurons toward OHCs	Yang et al., 2017a
	Celsr1	Earliest stages of hair cell polarity in the cochlea; hair bundle orientation in the vestibule associated with vestibular behaviors	Curtin et al., 2003; Duncan et al., 2017
	Ankrd6	Hair bundle orientation in the utricle; polarity and patterning of hair cells in the cochlea (interaction with Vangl2)	Jones et al., 2014
	Fat1/4	Cooperation in cochlear extension and patterning of OHCs	Saburi et al., 2012
	PTK7	Hair bundle orientation in OHC3; functioning in supporting cells to exert polarized contractile tension on hair cells	Lu et al., 2004; Lee et al., 2012
	Scrib	Planar polarization of stereociliary bundles (interaction with Vangl2)	Montcouquiol et al., 2003, 2006

	Cfap126	Kinocilium positioning and hair bundle morphogenesis in the cochlea	Gegg et al., 2014
	Wdpcp	Vangl2 asymmetric expression and kinocilium localization	Cui et al., 2013
Limb bud	Wnt5a	Orientation of mesenchymal cell movements and divisions; chemoattractant for limb outgrowth; asymmetric localization and phosphorylation of Vangl2; generation of active stresses for the formation of digit-organizing centers	Yamaguchi et al., 1999; Gros et al., 2010; Wyngaarden et al., 2010; Gao et al., 2011; Parada et al., 2022
	Ror2	Phalange development and elongation through the digit-organizing center; phosphorylation of Vangl2	Witte et al., 2010; Gao et al., 2011
	Dvl1/2/3	Interaction of casein kinase 1 with Vangl2 for Vangl2 phosphorylation	Yang et al., 2017b
	Vang2	Polarization of chondrocyte behaviors; elongation of proximal-distal axis; limb skeletal development	Gao et al., 2011; Wang et al., 2011
	Prickle1	Limb outgrowth; formation of distal skeletal elements; chondrocyte polarity and proximal-distal outgrowth of endochondral elements	Yang et al., 2013; Liu et al., 2014
	Ryk	Regulation of Vangl2 stability; interaction with Vangl2 in limb elongation	Andre et al., 2012