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► **To cite this version:**

De-Li Shi. Wnt/planar cell polarity signaling controls morphogenetic movements of gastrulation and neural tube closure. Cellular and Molecular Life Sciences, 2022, 79 (12), pp.586. 10.1007/s00018-022-04620-8 . hal-03850603

HAL Id: hal-03850603

<https://cnrs.hal.science/hal-03850603>

Submitted on 14 Nov 2022

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Wnt/planar cell polarity signaling controls morphogenetic movements of gastrulation and neural tube closure

De-Li Shi^{1,2*}

¹Institute of Medical Research, Affiliated Hospital of Guangdong Medical University, Zhanjiang, China

²Laboratory of Developmental Biology, CNRS-UMR7622, Institut de Biologie Paris-Seine (IBPS), Sorbonne University, Paris, France

*Correspondence: de-li.shi@upmc.fr; ORCID: 0000-0002-6104-9137

Running title: Wnt/PCP signaling in gastrulation and neurulation

Abstract

Gastrulation and neurulation are successive morphogenetic processes that play key roles in shaping the basic embryonic body plan. Importantly, they operate through common cellular and molecular mechanisms to set up the three spatially organized germ layers and to close the neural tube. During gastrulation and neurulation, convergent extension movements driven by cell intercalation and oriented cell division generate major forces to narrow the germ layers along the mediolateral axis and elongate the embryo in the anteroposterior direction. Apical constriction also makes an important contribution to promote the formation of the blastopore and the bending of the neural plate. Planar cell polarity proteins are major regulators of asymmetric cell behaviors and critically involved in a wide variety of developmental processes, from gastrulation and neurulation to organogenesis. Mutations of planar cell polarity genes can lead to general defects in the morphogenesis of different organs and the co-existence of distinct congenital diseases, such as spina bifida, hearing deficits, kidney diseases, and limb elongation defects. This review outlines our current understanding of non-canonical Wnt signaling, commonly known as Wnt/planar cell polarity signaling, in regulating morphogenetic movements of gastrulation and neural tube closure during development and disease. It also attempts to identify unanswered questions that deserve further investigations.

Key words: Wnt, PCP, Frizzled, Dishevelled, Vangl1, Vangl2, Prickle, Celsr1, morphogenesis, NTDs, neural tube defects

Introduction

Morphogenetic movements of gastrulation set up the three spatially organized germ layers (ectoderm, mesoderm and endoderm). Neurulation occurs directly after gastrulation to transform the neural plate into a neural tube, the precursor of the central nervous system. Therefore, gastrulation and neurulation are fundamental developmental events that play critical roles in shaping the basic embryonic body plan. Moreover, they operate through common as well as conserved cellular and molecular mechanisms. Convergent extension (CE) movements are mostly driven by cell intercalations that promote extensive exchange of places between neighboring cells [1,2,3,4,5]. While mediolateral intercalation occurs in a coplanar manner, radial intercalation takes place between adjacent planes. During these processes, lateral cells move toward the dorsal midline to narrow the germ layers and promote neural tube closure, while dorsal midline cells extend along the anteroposterior (AP) axis to lengthen the embryo [6]. Other asymmetric cell behaviors, such as oriented cell division and apical constriction, also make an important contribution to epithelial morphogenesis during gastrulation and neurulation [7,8,9].

The phenomenon of planar cell polarity (PCP) is widely conserved among metazoan and is critically involved in coordinating cellular orientation within the plane of an epithelium or a tissue. In vertebrates, as in *Drosophila*, PCP is mostly regulated by a set of evolutionarily conserved proteins including Frizzled (Fzd), Dishevelled (Dvl), Celsr (cadherin EGF LAG seven-pass G-type receptor), Vangl, Prickle, and Ankrd6 (ankyrin repeat domain 6). These “core” PCP proteins form two separate complexes that localize to opposite cell borders [10,11,12]. They transduce Wnt/PCP signaling to regulate cytoskeletal rearrangements through distinct downstream effectors [11, 13]. Several vertebrate Wnt ligands, such as Wnt5a, Wnt5b, and Wnt11, are also important for activating Wnt/PCP signaling to instruct cellular polarization, although they are generally not considered as “core” PCP proteins [14]. Besides the “core” PCP pathway, there are also other conserved protein complexes that function as important PCP regulators. For example, the Fat and Dchs (dachshous cadherin-related) heteromeric protocadherins form ligand-receptor pairs and represent the second PCP pathway [15]; the Scrib

(Scrib1 or Scribble1) polarity system, consisting of Scrib, Dlg (Discs-large) and Lgl (Lethal-giant larvae) proteins in a complex, mostly contributes to establish baso-lateral cell identity [16].

The Wnt/PCP pathway functions in a wide variety of morphogenetic processes and instructs cells with positional cues for directed movements [17]. Particularly, Wnt/PCP signaling is required for cell intercalations during gastrulation, neurulation, and asymmetric organogenesis [6, 18,19,20]. Although Wnt/PCP signaling mostly contributes to mediolateral cell intercalation [21], there is increasing evidence that several “core” PCP proteins also mediate radial cell intercalation and contribute to the establishment of apico-basal polarity [22,23,24]. Dysfunctions of PCP regulators (herein referred to as proteins involved in Wnt/PCP signaling) are closely linked to the congenital disorder of neural tube defects (NTDs), such as spina bifida and anencephaly [25, 26]. This review aims to provide an outline of past achievements and recent advances in deciphering the implication of Wnt/PCP signaling during gastrulation and neural tube closure, by focusing on molecular and cellular mechanisms underlying the regulatory functions of PCP-related genes in development and disease. As an outcome, it attempts to identify challenges in understanding the functional interaction between different polarity pathways as well as the interplay between cell polarity and cell fate specification during morphogenetic movements.

Planar cell polarity protein complexes

Vertebrate Wnt signaling pathways include three branches that trigger distinct biological outcomes. In canonical Wnt or Wnt/ β -catenin signaling, Wnt ligands bind to Fzd receptors and LRP5/6 (low density lipoprotein receptor-related protein 5/6) co-receptors, leading to phosphorylation of Dvl and inhibition of the β -catenin destruction complex consisting of GSK3 β /Axin/APC. Stabilized β -catenin associates with T-cell factor in the nucleus to activate transcription of target genes and regulate cell fate specification. Non-canonical Wnt pathways include Wnt/PCP and Wnt/Ca²⁺ branches that signal independently of β -catenin. Wnt/PCP signaling is activated by binding of non-canonical Wnt ligands to the Fzd-Ror1/2 (receptor tyrosine kinase-like orphan receptor 1/2) complex or the Fzd-Ryk (receptor tyrosine kinase) complex. As will be discussed later, there are also other co-receptors that can interact with non-canonical Wnt ligands. The Wnt/PCP pathway functions to regulate cytoskeletal organization

through two classes of downstream effectors: the well-characterized 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), and the less-studied ciliogenesis and planar polarity effector (CPLANE) proteins including Intu, Fuz and Wdpcp [13]. Wnt/Ca²⁺ signaling triggers phospholipase C activity through heteromeric G-proteins to induce intracellular calcium flux and calcium-dependent responses. Dvl family proteins are important scaffolds that relay signals of all three Wnt branches. They can modulate the activation of Wnt/ β -catenin and Wnt/PCP pathways through conformational changes mediated by intramolecular interaction between the PDZ domain and the extreme C-terminus PDZ-binding motif [27, 28].

The six “core” PCP proteins display the characteristic feature of forming two separate complexes in planar polarized epithelia. Fzd, Dvl and Ankrd6 localize to the posterior side of the cell, while Vangl and Prickle reside at the anterior side; Celsr1 atypical cadherins are distributed on both sides and form homodimers between adjacent cells to propagate polarity information (Figure 1). Recent studies show that Celsr1 can also function in *cis*-interactions to organize Fzd6 and Vangl2 into asymmetric junctional complexes [29]. The asymmetric localization of PCP protein complexes is a hallmark of planar polarization in the tissue. Fat and Dchs heteromeric protocadherins are also localized to opposite sides of adjacent cells to mediate cell-cell interaction, and their activities are positively or negatively regulated by the Golgi resident transmembrane kinase Fj (Four-jointed), respectively [30]. The Scrib polarity system not only regulates baso-lateral polarity but also modulates the localization of “core” PCP proteins, thus contributing to Wnt/PCP signaling [16]. Therefore, through multiple biochemical, functional and genetic interactions, the “core” PCP pathway, the Fat/Dchs polarity module and the Scrib polarity complex play essential roles in regulating gastrulation cell movements and neural tube closure. The following sections will detail their implications in these key developmental processes by emphasizing the consequences of their mutations or dysfunctions on the occurrence of NTDs.

PCP proteins in morphogenetic movements of gastrulation

Wnt/PCP signaling does not appear to regulate dorsal closure during gastrulation in *Drosophila* [11]. However, its requirement for CE movements during gastrulation has been well

documented in vertebrates, particularly using *Xenopus* and zebrafish as models [6, 31,32,33]. The formation of the primitive streak in amniotes and mammals also involves CE movements and requires a functional Wnt/PCP pathway [34, 35]. Mediolateral cell intercalations are major forces that drive CE movements to narrow and elongate tissues in the early embryo, while radial cell intercalations result in thinning of multilayered epithelia during large-scale morphogenesis (Figure 2A,B). Apical constriction that changes tissue curvature and folds epithelial surface also contributes to gastrulation, particularly blastopore formation and mesendoderm invagination. In addition, asymmetric cell division that leads to differential partitions of signaling proteins can contribute to cell lineage specification (Figure 2C). PCP proteins regulate these asymmetric cell behaviors for proper morphogenesis to establish the embryonic axes. Thus, the coordinated actions of embryonic patterning and gastrulation cell movements orchestrate the formation and the spatial organization of the three germ layers, thereby setting up the basic embryonic body plan [36].

Non-canonical Wnt ligands in gastrulation

Zebrafish mutants for *wnt11f2*, previously known as *wnt11* or *silberblick* (*slb*), show abnormal extension of the axial tissue in a cell non-autonomous manner [37]. In these embryos, there is a slowed migration of hypoblast cells within the forming germ ring due to the defective orientation of cellular protrusions along the movement directions [38]. Analysis by in vivo live imaging also reveals a disrupted animal-vegetal polarity of cell intercalations and cell divisions in the dorsal tissues [8]. Wnt11f2 regulates these polarized cell behaviors by modulating cellular cohesion through Rab5c-mediated E-cadherin endocytosis [39]. It also induces the asymmetric accumulation of Fzd7 receptor and the recruitment of Dvl at the plasma membrane of adjacent cells, leading to locally an increase of Celsr2-mediated cell contact persistence [40]. In *Xenopus*, inhibition of Wnt11 function using a dominant negative mutant blocks gastrulation by specifically disrupting Wnt/PCP signaling [41]. Interestingly, *Xenopus* Wnt11 regulates CE movements by promoting the formation of adhesion-modulating complexes consisting of Fzd7 and paraxial protocadherin (PAPC) or Fzd7 and C-cadherin, which function in parallel to prevent C-cadherin clustering and reduce cell adhesion [42]. These observations suggest that Wnt11-mediated

signaling induces cell shape changes and intercalation behaviors at least partially through regulation of cell adhesion.

It is worth mentioning that *wnt11f2* mutants also show craniofacial defects and cyclopia at larval stages due to defective formation of midline structures in the gastrula. Indeed, *wnt11f2* belongs to the “midline group” genes whose mutations impair the extension of axial tissues and remotely affect the development of head cartilages [43]. Similarly, mutations of other components in the Wnt/PCP pathway that disrupt the proper positioning of the prechordal mesoderm at the end of gastrulation can also cause craniofacial phenotypes [44, 45].

Wnt5a and Wnt5b display both specific and redundant roles during CE movements in different species. In zebrafish, Wnt5a can interact with the CD146 receptor, also known as melanoma cell adhesion molecule, to regulate cell motility during gastrulation [46], while Wnt5b signals through Ryk and focal adhesion kinase (Fak) to induce cellular polarization [47, 48]. In addition, Wnt5b functions specifically in posterior regions of the zebrafish gastrula to regulate cell elongation and CE movements, while it shows redundant roles with Wnt11f2 in more anterior regions [49]. In *Xenopus*, Wnt5a specifically interacts with Ror2 to regulate PAPC expression and CE movements through activation of JNK signaling, suggesting that it can also elicit transcriptional responses [50]. Importantly, Wnt5a and Wnt11 can provide directional cues to establish cellular polarization. In ectodermal cells of *Xenopus* midgastrula, they instruct PCP by orienting the Vangl2-Prickle3 complex to anterior cell borders, away from their source [51]. In the avian embryo, Wnt5a and Wnt5b may function redundantly with Wnt11 to regulate cell migration through the primitive streak [52]. In mice, Wnt5a and Wnt11 are also required for the migration of axial and paraxial mesodermal precursor cells to promote notochord formation through regulation of epithelial-mesenchymal transition [53].

“Core” PCP proteins in asymmetric cellular behaviors during gastrulation

Extensive analyses have provided important insights into the functions of “core” PCP proteins in morphogenetic movements of gastrulation. Pioneer studies in *Xenopus* demonstrate that disrupting the functions of Fzd7 or Dvl impairs CE movements but not embryonic patterning [54,55,56]. More recent studies by mutational analyses indicate that zebrafish maternal-zygotic

Dvl2 and Dvl3a cooperatively regulate CE movements independently of Wnt/ β -catenin signaling, suggesting an important contribution of maternal PCP proteins to morphogenetic movements that occur after zygotic genome activation [45]. The membrane recruitment of Dvl by Fzd receptors is important for its activity in Wnt/PCP signaling [28, 57]. In zebrafish, Celsr1 is required for the formation of Fzd-Dvl complex, at least by regulating membrane recruitment of Dvl [58]. Prickle1 regulates Wnt/PCP signaling during gastrulation cell movements through interaction with Dvl and activation of JNK signaling [59,60,61]. It shows asymmetric localization in cells undergoing CE movements and is enriched at the anterior edge, while Dvl is accumulated at the posterior side [62]. This segregation of PCP proteins helps to define distinct anterior and posterior cell properties and provides bias for cell intercalations. Accordingly, inappropriate functioning of Prickle1 causes CE defects, likely by disrupting Fzd-dependent membrane recruitment of Dvl [59]. Intriguingly, recent studies suggest that diffusely distributed cytoplasmic Prickle1 counteracts the activity of Dvl2 to increase F-actin content and cortical tension in *Xenopus* prechordal mesoderm cells, thereby generating mechanical forces to promote cell migration and rearrangements during radial cell intercalations [22].

As Prickle1, zebrafish Vangl2 (also known as Strabismus or Trilobite) displays stage-dependent membrane accumulation and enrichment in highly elongated cells undergoing mediolateral intercalation [63]. Both gain and loss of Vangl2 function disrupts Wnt/PCP signaling and affects mediolateral cell polarity necessary for effective intercalation and directed migration [64,65,66]. In the *Xenopus* gastrula, Vangl2 is also enriched at the constricted apical surfaces of blastopore bottle cells. This asymmetric localization plays a role in blastopore formation and closure by promoting the apical accumulation of Rab11 and its associated motor protein Myosin V, suggesting that Vangl2 can also regulate apico-basal polarity during morphogenetic movements [67].

In zebrafish, Ankrd6 (previously known as Diversin) is required for gastrulation cell movements through interaction with Dvl and activation of JNK [68, 69]. However, biochemical and functional analyses suggest that Ankrd6 represents a molecular switch to stimulate Wnt/PCP signaling but suppress Wnt/ β -catenin pathway in a dose-dependent manner [69]. Therefore,

Ankrd6 may also function in embryonic patterning, and how this impacts on cell behavior changes needs further investigation.

In amniotes, mediolateral cell intercalation has been shown to define the primitive streak before gastrulation [70]. This event occurs in a subdomain of the epiblast where the primitive streak will form during gastrulation and requires the function of several “core” PCP proteins including Dvl, Celsr1, Vangl2 and Prickle1 [70]. Therefore, Wnt/PCP signaling promotes primitive streak formation before gastrulation. Interestingly, as discussed above, this pathway remains active during gastrulation to regulate cell exit from the primitive streak [52].

Co-receptors of the Wnt/PCP pathway in convergent extension movements

Glypican 4 (Knypek in zebrafish), a member of the heparan sulfate proteoglycans (HSPGs) family, functions as a co-receptor for a variety of growth factors and regulates CE movements in zebrafish and *Xenopus* by promoting Wnt/PCP signaling [44, 71]. Both functional and biochemical analyses suggest that it interacts with Wnt5a and Wnt11 during gastrulation [71]. Mutations that disrupt the function of Knypek prevent mediolateral alignment and elongation of ectodermal and mesodermal cells in the paraxial region, leading to CE defects and shortened AP axis [44]. Ror2 and Ryk are also implicated in the regulation of cell polarity and CE movements during gastrulation in vertebrate embryos by functioning as co-receptors for Wnt5a or Wnt11 [72,73,74,75].

Protein tyrosine kinase 7 (PTK7) is an evolutionarily conserved transmembrane receptor that functions as a vertebrate-specific PCP regulator in many developmental processes [76]. In zebrafish, Ptk7 regulates Wnt/PCP signaling by potentiating the activity of Wnt5 and Wnt11; maternal-zygotic *ptk7* mutants show impaired mediolateral intercalation [77]. However, it is not entirely clear how Ptk7 signals in the Wnt/PCP pathway to polarize cell behaviors because CE defects caused by its loss of function can be rescued by expression of a membrane-tethered extracellular domain of the protein [77]. Knockout of Ptk7 in mice also leads to defective mediolateral and radial intercalations, but the identity of Wnt ligands that interact with Ptk7 in these processes remains unclear [78]. Studies in *Xenopus* suggest that Ptk7 functions in Wnt/PCP signaling during morphogenetic movements of gastrulation at least through JNK-

mediated transcriptional response. In this context, Ptk7 interacts with Wnt5a and cooperates with Ror2 to modulate PAPC expression [79].

Other polarity regulators in gastrulation cell movements

Studies in zebrafish have identified additional polarity regulators that function in morphogenetic movements during gastrulation. Maternal-zygotic Dchs1b may be involved in prechordal mesodermal migration and CE movements by regulating actin or microtubule organization in a Fat-independent manner [80]. Maternal but not zygotic Scrib1 shows strong genetic interaction with Vangl2 and the two proteins may form a functional complex in CE movements [81]. Moreover, Scrib1 also interacts with LPP, a zyxin-related actin cytoskeleton protein, to promote the dorsal convergence of paraxial cells [82]. The PDZ domain protein Mcc (mutated in colorectal cancer) directly interacts with the cytoplasmic tail of Vangl2 and can function as an effector of Wnt5a/Ror2/Vangl2 signaling to activate RhoA and JNK in CE movements [83]. Recent research shows that the E3 ubiquitin ligase Mindbomb1 (Mib1) controls the endocytosis of Ryk in gastrulating cells, and therefore, zebrafish *mib1* null mutants display defective PCP-dependent CE movements [84].

Wnt/PCP-regulated cytoskeletal rearrangements and adhesive changes during gastrulation

Wnt/PCP-dependent cellular polarization is mediated by dynamic rearrangements of the actin and microtubule cytoskeleton. Key regulators of the cytoskeleton architecture including Rho and Rac are activated by Fzd receptors through Dvl and the PPE protein Daam1, a formin homology protein with actin-binding activity, thereby promoting asymmetric cellular activities [85, 86]. Moreover, Wnt/PCP signaling mediated by Dvl and Knypek also controls the proper location of the microtubule-organizing center in both ectodermal and mesodermal cells undergoing CE movements [87, 88]. This influences the polarity of the microtubule cytoskeleton, which is required for the asymmetric distribution of PCP proteins [88]. There is also evidence that a spatiotemporal clustering of Prickle2 is correlated with alternative actomyosin oscillation across cell membranes of neighboring cells, which may modulate submembranous accumulations of F-actin and facilitate CE movements [89]. These observations suggest that some PCP proteins can

function to set up the anisotropy of many cellular properties. Therefore, either gain or loss of their activity can disrupt asymmetric cellular behaviors and leads to CE defects [87].

Polarized cellular protrusions also require Wnt/PCP-regulated organization of extracellular matrix (ECM). In *Xenopus*, inhibition of Wnt/PCP signaling disrupts fibronectin assembly in the blastocoel roof by reducing its mechanical tension [90]. Fzd7, Vangl2 and Prickle1 function in mediolateral intercalation of mesodermal cells by regulating fibronectin secretion and assembly at their outer surfaces that contact the overlying ectoderm and the underlying endoderm [91]. In zebrafish, “core” PCP proteins are also differentially involved in ECM organization [92]. Loss of Fzd7 leads to increased fibronectin assembly in the gastrula, while loss of Vangl2 or Prickle1 decreases fibronectin protein levels, thus preventing polarized membrane protrusion and directed migration [93,94,95]. Therefore, Wnt/PCP-dependent ECM organization and cell-substrate adhesion are important to promote polarized cell behaviors for asymmetric movements [96].

PCP regulatory genes in neural tube closure and neural tube defects

The neural plate emerges at the end of gastrulation as the neuroectodermal tissue above the dorsal mesoderm lengthens into columnar cells (Figure 3). The neural tube can be formed through two different processes, called primary and secondary neurulation, which involve fusion of the neural folds or epithelialization from a solid cord of neural tube progenitor cells at the tail bud region, respectively [25]. During primary neurulation in mammals, birds and *Xenopus*, neural folds arise bilaterally at the boundaries between the neural plate and the surrounding epidermis. They elevate and converge toward the midline for fusion to form the neural tube by a “zippering” process either initiating at different closure sites along the AP axis or occurring simultaneously at all axial levels. In zebrafish and other teleost fish, the neural tube is formed through a process similar to secondary neurulation, without the formation of neural folds. As gastrulation, neurulation also involves cell intercalation and apical constriction, which generate major forces driving the AP elongation and bending of the neural plate. Disruption of these polarized cell behaviors prevents neural tube closure and are associated with varying forms of NTDs (Table 1), which are the most common congenital malformations affecting the central nervous systems. The implication of PCP genes in NTDs has been extensively reviewed recently [26]. By integrating

data obtained from complementary animal models with advances on the study of congenital disorders, this work focuses on the analysis of molecular and cellular mechanisms underlying the regulatory roles of PCP-related genes in neural tube closure during development and disease. It not only discusses Wnt/PCP signaling in primary and secondary neurulation but also highlights recent advances in the study of somatic mutations of “core” PCP genes associated with NTDs.

Asymmetric localization of “core” PCP proteins in the neuroectoderm

The subcellular localization of several “core” PCP proteins displays asymmetric bias in the neural epithelium. In *Xenopus*, Vangl2 is enriched at anterior cell boundaries, which is dependent on its interaction with Prickle and Par3 proteins [97, 98]. Wnt5a gradients can provide instructive cues to promote the anterior localization of Vangl2 by inducing its phosphorylation [98]. A recent study further suggests that Fzd3, which is specifically expressed in the neuroectoderm [99], promotes the anterior accumulation of Vangl2-Prickle3 complexes by phosphorylating Vangl2 on specific threonine residues [100]. In addition, the Vangl2-Prickle3 complex is also enriched near the outermost surface of deep neural plate cells that undergo radial intercalations during neurulation [23]. These observations indicate that Vangl2 and Prickle3 can regulate both mediolateral and radial cell intercalations in the neuroepithelium. Importantly, the asymmetric enrichment of PCP proteins is closely correlated with actomyosin-driven contractile behaviors of cell-cell junctions [101]. Moreover, Myosin II can exert a positive feedback on the asymmetric localization of Vangl2 protein, suggesting that mechanical forces contribute to establish PCP in the neural plate [98]. Accordingly, diffusion of locally expressed Wnt11 in the posterior neuroectoderm acts with anisotropic tension imposed by unidirectional tissue stretch to orient PCP through regulation of cell shape changes [102]. Therefore, interactions between “core” PCP proteins and actomyosin-mediated contractility regulate polarized cellular behaviors necessary for driving neural tube closure, such as mediolateral cell intercalation that elongates the neural plate along the AP axis and apical constriction that causes bending of the neural plate [19].

“Core” PCP proteins in neural tube development and disease

Functional studies using animal models and analyses of genetic mutations in humans have firmly demonstrated the contribution of PCP proteins to neural tube closure. In *Xenopus*,

Dvl2 regulates CE movements in the mesoderm and neural plate to generate parallel forces for elongating the AP axis [103]. Perturbation of its function prevents fusion of nascent neural folds and closure of the neural tube [104]. In mice, all the three *Dvl* genes (*Dvl1*, *Dvl2* and *Dvl3*) are involved in neural tube closure, but *Dvl2* appears to play a predominant role [105,106,107]. Since different *Dvl* genes exhibit partial redundant functions and cooperatively regulate Wnt/PCP signaling in CE movements, PCP-dependent neural tube closure is extremely sensitive to Dvl dosage [108, 109]. Consistent with their importance for neurulation, missense mutations of different *DVL* genes have been identified in patients with NTDs or Dandy-Walker malformation characterized by abnormal cerebellar development [110, 111]. However, these missense variants likely produce complex effects, by differentially disrupting the activity of all three Wnt signaling branches.

Vangl2 paralogs are critically involved in neural tube closure [112]. In *Xenopus*, an optimal level of Vangl2 is crucial for proper cellular polarization underlying normal cell intercalation. Therefore, either an increase or a decrease of Vangl2 activity disrupts CE movements of the neuroepithelium and inhibits neural tube closure [113]. This suggests that Wnt/PCP signaling should be tightly regulated at local levels to confirm the tissue-level constraints. In zebrafish, the neural tube is essentially formed through cavitation of the neural keel, a process similar to secondary neurulation. Vangl2-mediated Wnt/PCP signaling regulates neural tube formation by promoting polarization of neural progenitors along the AP axis and re-intercalation of mitotic daughter cells into the neuroepithelium, thereby coupling cell division and neurulation [9]. In mice, missense or null mutations of Vangl2 impair CE movements of the neuroepithelium and produce NTDs, with severity depending on the reduction of Vangl2 activity [114,115,116,117]. It is likely that Vangl2 regulates Wnt/PCP signaling in a dosage-sensitive manner. Thus, heterozygosity of the *Looptail* mutation, which results in a single amino acid substitution that is predicted to produce a malfunctional Vangl2 protein, can exacerbate NTDs in mice carrying heterozygous or homozygous mutations of other polarity genes, often resulting in craniorachischisis [105, 107, 118,119,120,121,122]. There is a possibility that the malfunctional Vangl2 protein aggravates PCP defects by interfering with the proper localization of other “core” PCP components. In

humans, heterozygous missense mutations of *VANGL1*, which disrupt the interaction of *VANGL1* and *DVL* in Wnt/PCP signaling, are linked to varying degrees of spina bifida [123]. Depending on the variants, heterozygous missense mutations of *VANGL2* result in open spinal bifida and closed spinal NTDs or cause anencephaly and are lethal to the fetus [124, 125]. These observations reveal a critical role for VANGL-mediated Wnt/PCP signaling in neural tube closure and strongly implicate VANGL as a risk factor and the genetic causation of human spinal NTDs. Indeed, neural tube closure is particularly vulnerable to loss of *Vangl2* activity. Recent research using mouse embryos indicates that an absence of *Vangl2* function in a minority (16%) of neuroepithelial cells can non-autonomously prevent neural fold elevation by inhibiting apical constriction of neighboring cells, suggesting that tissue mosaicism generated by post-zygotic mutations of some PCP genes may lead to severe failure of morphogenesis [126]. Consistently, somatic mutations of *FZD6*, *VANGL1* and *CELSR1* have been associated with human NTDs [127], suggesting that disruption of Wnt/PCP signaling during different stages of embryonic development can contribute to defective morphogenesis of the neural tube.

Fzd1 and *Fzd2* genetically interact with *Vangl2* in Wnt/PCP signaling to regulate various tissue fusion processes, including neural tube closure at different locations along the neural axis [122]. Combined knockout of *Fzd3* and *Fzd6* in mice causes craniorachischisis with almost 100% penetrance [128]. Heterozygous missense or frameshift mutations of the human *FZD6* gene have been shown to cause different forms of NTDs [129]. Homozygous *Celsr1* mutant mice fail to initiate neural tube closure, resulting in craniorachischisis [130], while heterozygous missense variants of *CELSR1* may represent the underlying pathogenic mechanism of craniorachischisis as well as other forms of NTDs in humans [131,132,133]. In chick embryos, *Celsr1* in floor plate cells recruits *Fzd* receptors and *Dvl* to regulate actomyosin-dependent mediolateral contraction of adherens junctions, promoting bending of the neural plate by causing simultaneous midline convergence and apical constriction [134]. *Prickle1* is required for junctional neurulation in the chick spinal cord, which is a process involving the fusion and connection of the primary neural tube with the posterior neural tube formed by secondary neurulation from the caudal cell mass [135]. Heterozygous missense mutations of *PRICKLE1* in humans have been reported to cause

varying degrees of spina bifida [136]. Although it is unclear how Ankrd6 regulates neural tube closure, rare missense mutations that disrupt its activity in the balance of Wnt/PCP and Wnt/ β -catenin signaling have been identified in human NTDs [137]. The CPLANE protein Fuz, a vertebrate ortholog of *Drosophila* Fuzzy, is required for neural tube closure in *Xenopus* and mice likely by regulating ciliogenesis in the neural plate [138,139,140]. Consistently, mutations of FUZ affecting the formation of primary cilia and ciliary length have been associated with both open and closed NTDs in humans, which are spinal defects exposed at birth or covered by the skin, respectively [141].

Other PCP-related genes in neural tube morphogenesis

Fat1 and Fat4 protocadherins show genetic interaction in cranial neural tube closure, and loss of their functions in mice causes exencephaly [142, 143]. They may function as *cis*-heterodimers to modulate cytoskeletal organization and apical constriction [142]. However, it is unclear whether they regulate polarized cell behaviors and cell-cell interaction by forming ligand-receptor pairs with Dchs protocadherins.

Mutations that impair Scrib function in mice severely affect neural tube closure and cause craniorachischisis by disrupting cell intercalation required for neural plate CE movements and by preventing apical constriction necessary for neural plate bending [119, 144, 145]. Scrib may regulate polarized cell behaviors by influencing the localization and expression of junctional and cytoskeletal proteins [144]. It also directly binds to Vangl2 through its PDZ domains to promote the proper localization of Vangl2 in mouse neuroepithelial cells [146]. Combined heterozygous mutations of Scrib and Vangl2 or Scrib and Celsr1 produce variable phenotypes ranging from craniorachischisis to other forms of NTDs [144, 145, 147, 148]. Further supporting its implication in neural tube closure, there are several studies linking heterozygous missense mutations of *SCRIB* gene with human NTDs [131, 146, 149].

Dishevelled binding antagonist of β -catenin 1 (Dact1), also known as Dapper1, is a cytoplasmic protein that interacts with Dvl2 and Vangl2 to regulate their expression at the post-translational level [150,151,152]. Knockout of Dact1 in mice disrupts Wnt/PCP signaling and causes malformations at the posterior primitive streak [152, 153]. Consistent with its requirement

for CE movements and neural tube closure, several rare heterozygous missense mutations that lead to loss or reduction of DACT1 activity have been identified in human NTDs [154].

SEC24B is a cargo-binding protein in the coat protein complex II (COPII) and is involved in vesicle trafficking. It functions in Wnt/PCP signaling by selectively sorting Vangl2 to COPII during neural tube closure. In mice, mutations of *Sec24b* gene that introduce a premature stop codon disrupt PCP-dependent CE movements and cause craniorachischisis by inhibiting proper endoplasmic reticulum (ER) to Golgi transport of Vangl2 [155, 156]. Heterozygous missense mutations of *SEC24B* that disrupt the subcellular localization of Vangl2 have been associated with open and closed spina bifida in humans [157].

PTK7 displays conserved roles in neural tube morphogenesis. Inhibition of PTK7 function in *Xenopus* impairs CE movements of the neural plate by preventing Dvl membrane localization mediated by Fzd7 and the adaptor protein RACK1 [118, 158]. In mice, PTK shows strong genetic interaction with Vangl2 or Celsr1 and is required for the initiation of neural tube closure [118, 159]. Its loss of function disrupts the polarity of cell motility and intercalation in the neural plate by preventing polarized localization of Myosin IIB, leading to craniorachischisis [160]. Consistent with this importance in neural tube closure, rare heterozygous missense mutations of *PTK7* gene have been associated with various forms of NTDs in human patients [161, 162].

Although LRP6 generally functions as a co-receptor in Wnt/ β -catenin signaling, there is evidence that it regulates neural tube closure through the Wnt/PCP pathway. In mice, both gain and loss of LRP6 activity affect neural tube closure and cause varying degrees of NTDs, which may be attributed to defective Wnt/ β -catenin or Wnt/PCP signaling [163,164,165,166,167,168]. Heterozygous missense mutations in LRP6 that disrupt Wnt/ β -catenin or Wnt/PCP signaling, or both, have been identified in human NTDs [169,170,171]. The mechanism by which gain and loss of LRP6 link Wnt/PCP signaling to NTDs remain elusive. It is possible that the extracellular domain of LRP6 antagonizes Wnt/PCP signaling through sequestration of non-canonical Wnt ligands such as Wnt5a and Wnt11 [172, 173]. Therefore, LRP6 may serve as a molecular switch from Wnt/ β -catenin or Wnt/PCP signaling during neural tube closure in a dosage-dependent manner.

Wnt/PCP signaling in secondary neurulation

It is well established that Wnt/PCP signaling plays crucial roles in primary neurulation, as discussed above. However, there is also evidence that PCP genes are involved in secondary neurulation. In mice, analyses of genetic interactions between multiple heterozygous mutations of PCP genes indicate that some double heterozygous mutants exhibit NTDs that may result from impaired secondary neurulation [148]; conditional knockout of *Vangl2* in the surface ectoderm during neurulation disrupts polarized cell body orientation and results in caudal spina bifida that may be partially due to secondary neurulation defects [174]. Interference with the activity of PPE proteins such as *Cdc42* and *Rac1* disrupts mesenchymal-epithelial transition during secondary neurulation in the chick embryo [175]. As aforementioned, secondary neurulation occurs throughout the neural axis in zebrafish, *Vangl2*-mediated Wnt/PCP signaling is required for neural tube formation by polarizing neural progenitors along the AP axis [9]. Furthermore, it is likely that closed spina bifida occurs as a consequence of defective secondary neurulation [176]. Indeed, mutations of PCP genes can cause varying degrees of NTDs. Specifically, some heterozygous missense mutations in *CELSR1*, *VANGL1* and *VANGL2* genes have been identified in human NTDs with phenotypes reminiscent of abnormalities in secondary neurulation [124, 132, 177, 178]. These observations suggest that there are common molecular mechanisms regulating primary and secondary neurulation. The implication of Wnt/PCP signaling in secondary neurulation may be dependent on the genetic interactions, dosage or activity, and spatiotemporal expression of different PCP genes.

Concluding remarks

Gastrulation and neurulation are highly coordinated morphogenetic processes that are regulated by common molecular and cellular mechanisms. The “core” Wnt/PCP pathway functions in cooperation with or independently of other PCP regulatory genes to control polarized cellular behaviors. Extensive studies using different animal models have greatly advanced our understanding on the implication of these PCP regulators in morphogenetic cell movements that contribute to establish the basic body plan. However, there also raise many intriguing questions that deserve future investigation.

It is well documented that “core” PCP proteins display polarized localization within the cell of various organs undergoing asymmetric morphogenesis [20]. This feature seems to be also conserved in cells undergoing mediolateral and radial intercalations during gastrulation and neurulation. In addition to instructive cues provided by Wnt gradients [51], mechanical signals generated by cell movements also play an important role to redistribute PCP proteins. In *Xenopus*, it has been shown that mechanical strain acts through microtubules to determine the axis of planar polarity and transport PCP proteins in a directed manner [179, 180]. Thus, it will be of interest to decipher the interplay between different polarity modules and mechanotransduction during morphogenetic movements of gastrulation and neurulation.

It becomes increasingly evident that Wnt/PCP signaling also couples embryonic polarity and patterning. The coordinated action of Wnt/PCP and Wnt/ β -catenin signaling regulates ectodermal cell fate during *Xenopus* development. Wnt/PCP signaling controls the apico-basal cell polarity by directing the asymmetric localization of the canonical Wnt co-receptor LRP6, leading to elevated Wnt/ β -catenin signaling in the deep layer of ectoderm cells and differentiation of multiciliated cells [181]. Moreover, the asymmetric localization of PCP proteins can contribute to cell differentiation by promoting radial intercalation. The Vangl2-Prickle3 complex, for example, is enriched at the apical side of multiciliated cell progenitors, which undergo intercalation and move to the external surface during neurulation [98]. Wnt/PCP signaling also interacts with other patterning signals in morphogenetic cell movements. In zebrafish, it cooperates with Nodal signaling to regulate embryonic axis extension [182, 183]. Therefore, it will be of interest to elucidate how PCP proteins coordinate cell polarity with gene expression and how cell polarization is coupled with cell fate specification. However, since Wnt/PCP signaling can also induce transcriptional changes, specific readouts need to be applied for examination of cell behavior changes. Live imaging of cell behaviors and PCP protein dynamics should contribute to mechanistic analyses of PCP functions in morphogenesis [101, 184].

Since PCP proteins use similar cellular mechanisms, such as oriented cell division and cell intercalation, to regulate tissue and organ morphogenesis, it would be not surprising that their dysfunctions can cause general anomalies in multiple organs that display these PCP-dependent

cell movements. As such, mutations of PCP genes may need to be taken into consideration when there is a co-existence of NTDs and other congenital disorders. Biochemical and functional interactions between different polarity pathways in NTDs also need further investigations. Deciphering the detailed functions of PCP proteins and their interplay in regulating cell behaviors during development and disease should help to define therapeutic approaches targeting Wnt/PCP signaling.

Declarations**Ethics approval and consent to participate**

Not applicable

Consent for publication

Not applicable

Availability of data and material

Not applicable

Competing interests

The authors declare no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (grant number 32070813), the French Muscular Dystrophy Association (AFM-Téléthon grant number 23545), and by the annual supports from the Centre National de la Recherche Scientifique (CNRS) and the Sorbonne University.

Authors' contributions

DLS conceived the project, performed literature analyses, prepared figures, and wrote, reviewed and edited the manuscript.

Acknowledgements

I would like to thank members of the laboratories for discussion and supports during the preparation of this work.

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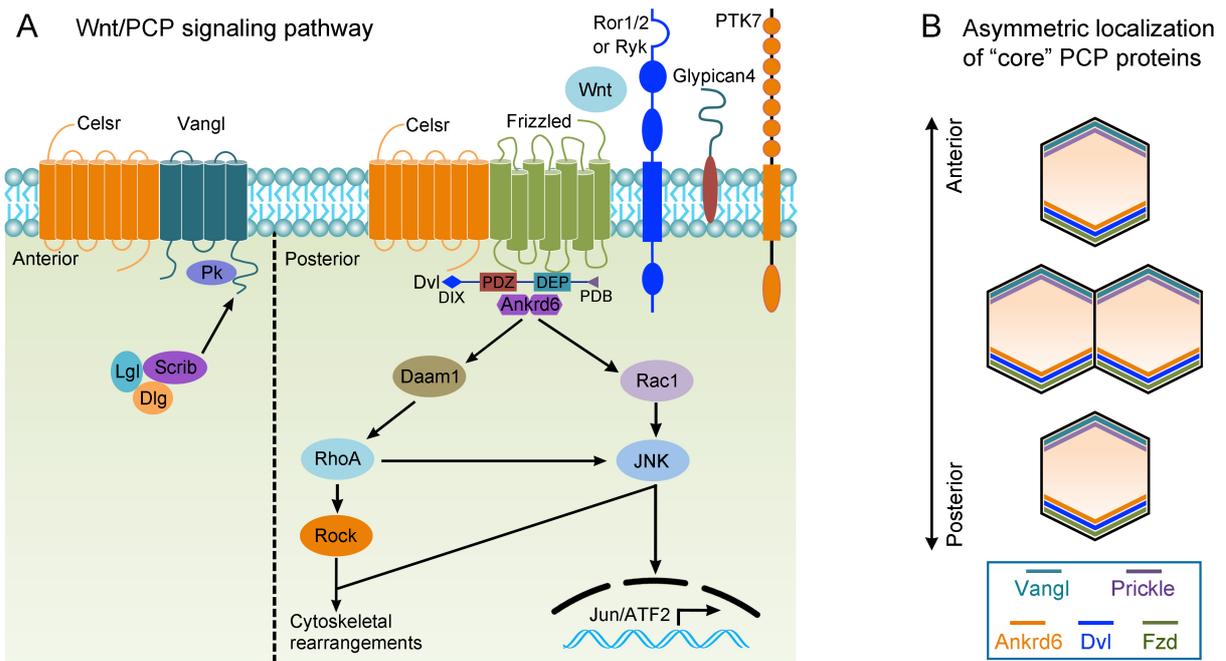


Figure 1. Wnt/PCP signaling pathway and asymmetric distribution of “core” PCP protein complexes. (A) Wnt/PCP signaling is induced by non-canonical Wnt ligands binding to Fzd receptors and co-receptors such as Ror2, Ryk, glypican4/Knypek, and PTK7. Dvl-activated downstream effector proteins relay the signal to induce cytoskeletal rearrangements and/or transcription responses. The “core” PCP proteins form two separate complexes localized to opposite cell borders. The C-terminal cytoplasmic region of Vangl interacts with Prickle (Pk) and Scrib, which may contribute to the anterior localization of the Vangl-Prickle complex within a cell. Celsr protocadherins form homodimers between adjacent cells to propagate polarity information. Dvl family proteins function as important scaffolds in different Wnt signaling pathways. The N-terminal DIX (blue diamond) is involved in Wnt/ β -catenin signaling, while the central PDZ, the C-terminal DEP domains, and the extreme C-terminus PDZ domain-binding motif (PDB, purple triangle) regulate Wnt/PCP signaling. (B) Diagram shows the asymmetric subcellular localization of “core” PCP proteins within the epithelial plane.

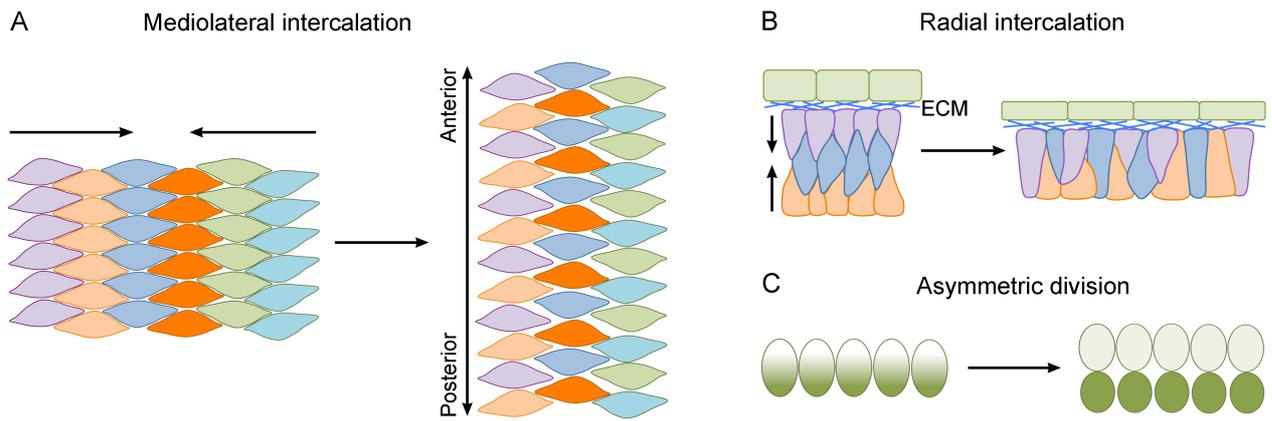


Figure 2. Asymmetric cellular behaviors in morphogenetic movements during gastrulation and neurulation. (A) Mediolateral cell intercalation (“en face” view) drives CE movements to narrow and extend tissues along mediolateral and AP axes, respectively. (B) Radial cell intercalation (sagittal plane) reduces the number of cell layers and thins a multilayered epithelium into sheets or tubes. ECM deposition at the inner (basal) surface of the superficial layer can be also regulated by Wnt/PCP signaling and is involved in radial intercalation of multilayered deep cells. (C) Asymmetric cell division leads to differential partitions of signaling proteins and lineage segregation.

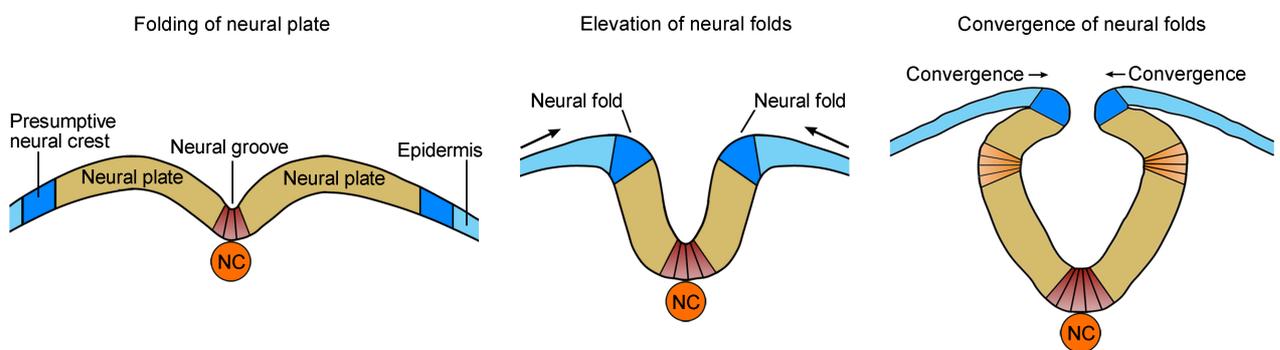


Figure 3. Neural plate formation and neural tube closure. Transverse sections in successive stages of neurulation show bending of the neural plate and the convergence of neural folds with presumptive neural crest approaching at the dorsal midline. Apical constriction generates wedge-shaped cells at the notochord (NC)-anchored medial hinge point (red) corresponding to the floor plate and at the dorsolateral hinge points (orange). These hinge points promote bending of the neural plate and convergence of the neural folds. It remains to be determined how PCP proteins regulate apical constrictions in different hinge points.

Table 1. Mutations of PCP regulatory genes linked to the congenital disorder of human NTDs.

Genes	Mutations*	Phenotypes	References
<i>FZD6</i> **	Heterozygous missense and frameshift	Myelomeningocele, meningocele, caudal regression syndrome	[127, 129]
<i>DVL1-3</i>	Heterozygous missense	Varying forms of NTDs	[110, 111]
<i>VANGL1</i> **	Heterozygous missense	Cranial, open and closed*** spinal dysraphisms	[123, 127, 177]
<i>VANGL2</i>	Heterozygous missense	Anencephaly, open spinal bifida, closed spinal NTDs***	[124, 125]
<i>PRICKLE1</i>	Heterozygous missense	Varying degrees of open spina bifida	[136]
<i>CELSR1</i> **	Heterozygous missense	Craniorachischisis, other forms of NTDs, tight filum terminale***	[127, 131,132,133]
<i>ANKRD6</i>	Heterozygous missense	Lipomyeloschisis, meningocele, scoliosis, and other forms of NTDs.	[137]
<i>SCRIB</i>	Heterozygous missense	Varying forms of open and closed NTDs	[131, 146, 149]
<i>FUZ</i>	Heterozygous missense	Open and closed NTDs	[141]
<i>PTK7</i>	Heterozygous missense	Varying forms of NTDs	[161, 162]
<i>DACT1</i>	Heterozygous missense	Craniorachischisis, closed NTDs, encephalocele	[154]
<i>SEC24B</i>	Heterozygous missense	Open and closed NTDs	[157]
<i>LRP6</i>	Heterozygous missense	Spina bifida	[169,170,171]

*Shown here are most frequently occurred mutations. Other mutations, such as truncations and inframe deletions, have been also identified in NTDs.

**Somatic mutations of these genes have been also associated with NTDs.

***Possible consequences of secondary neurulation defects.