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Tissue polarity and PCP protein function: *C. elegans* as an emerging model

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Abstract

Polarity is the basis for the generation of cell diversity, as well as the organization, morphogenesis, and functioning of tissues. Studies in *Caenorhabditis elegans* have provided much insight into PAR-protein mediated polarity; however, the molecules and mechanisms critical for cell polarization within the plane of epithelia have been identified in other systems. Tissue polarity in *C. elegans* is organized by Wnt-signaling with some resemblance to the Wnt/planar cell polarity (PCP) pathway, but lacking core PCP protein functions. Nonetheless, recent studies revealed that conserved PCP proteins regulate directed cell migratory events in *C. elegans,* such as convergent extension movements and neurite formation and guidance. Here, we discuss the latest insights and use of *C. elegans* as a PCP model.

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C. elegans, Wnt signaling, Tissue polarity, Asymmetric cell division, Spindle positioning, Planar cell polarity, PCP proteins.

Introduction

The establishment of cell and tissue polarity, characterized by the asymmetric distribution of cellular components and structures, is critical for the development and functioning of tissues and organisms. Early insight into the mechanisms of polarity establishment came from the genetic identification of *par* (partitioning-defective) mutants in the nematode *Caenorhabditis elegans*. The evolutionarily conserved PAR proteins have since been found to act as universal regulators of apical-basal epithelial polarity (reviewed in: [1,2]). Epithelial cells are also often polarized collectively within the tissue plane, perpendicular to the apical-basal axis. The molecular mechanisms that underlie this planar cell polarity (PCP) have largely been discovered in *Drosophila* and involve a noncanonical Wnt signaling cascade, known as the core PCP, or noncanonical Wnt/PCP, pathway. This core PCP pathway is conserved in vertebrates and controls a broader range of processes that include gastrulation movements and cell migration events [3]. While most core PCP genes are conserved in *C. elegans*, the epithelial tissue polarity in the worm is organized by a noncanonical Wnt pathway that deviates considerably from noncanonical Wnt/PCP signaling (Figure 1). In contrast, a series of recent studies implicate *C. elegans* core PCP proteins in cell migratory events that resemble PCP controlled processes in *Drosophila* and vertebrates.

In this minireview, we discuss how cell polarity is coordinated with the anterior-posterior body axis in the worm. This appears to occur on an individual cell basis and contrasts with the cell–cell contact-dependent polarization by core PCP proteins in more complex animals. Next, we summarize the latest insights in anterior-posterior oriented cell and neurite migratory processes that involve PCP proteins in the worm. Finally, we speculate about the areas where genetic dissection of PCP mechanisms in *C. elegans* can be most effective.

PCP basics

In the fly, the cells of various body parts and appendages are polarized in the proximal-distal direction. This polarity is easily recognized by the orientation of actin-based hairs, formed at the posterior of individual epithelial cells of the wing, notum, and legs [4]. Two main PCP pathways organize proximal-distal polarity in various tissues. One involves the atypical cadherins Fat and Dachsous, which form heterotypic interactions between cells in a graded pattern along the tissue axis [5]. The second is the above-mentioned core PCP/noncanonical Wnt/PCP pathway. Proteins of this pathway form two separate complexes that localize asymmetrically to apical junctions. Frizzled (Fz) serpentine receptors accumulate at the distal cell junctions, together with intracellular Dishevelled (Dsh/Dvl) and Diego proteins (Figure 1a). The Van Gogh/Strabismus (Vang)

Figure 1

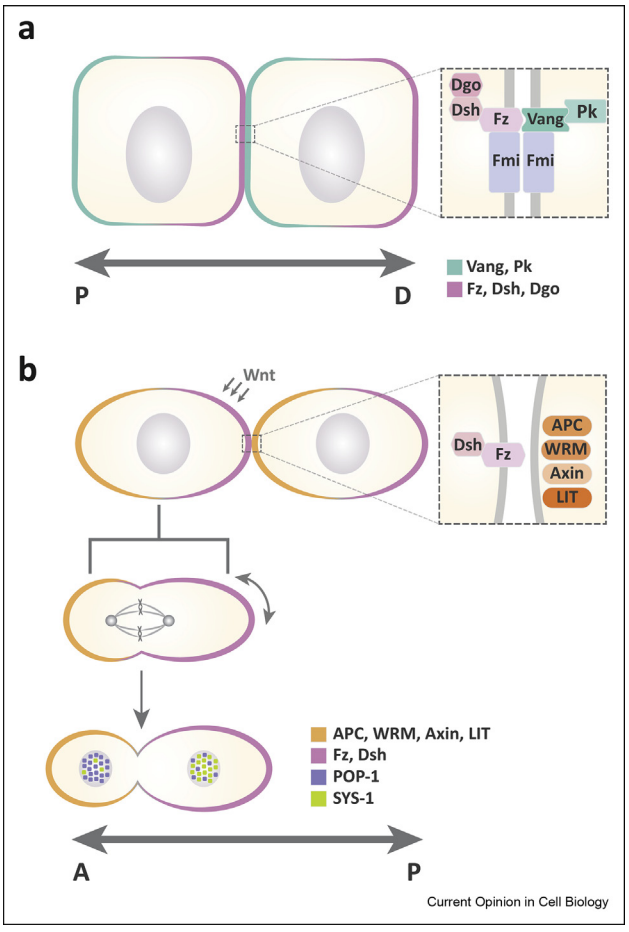


Illustration of the localization of core PCP proteins versus Wnt/β-catenin asymmetry pathway components. (a) The Van Gogh (Vang) and Prickle (Pk) proteins of the Wnt/PCP pathway form a complex and localize to the proximal cell junction, while a complex of Frizzled (Fz), Dishevelled (Dsh) and Diego (Dgo) proteins accumulates at the distal junction. Flamingo (Fmi) cadherins interact with both complexes and mediate the interaction between neighboring cells. **(b)** Asymmetric localization of Wnt/β-catenin asymmetry pathway components in *C. elegans*. Fz and Dsh proteins become enriched at the posterior cell cortex, while APR-1^{APC}, WRM-1$^{\beta\text{-catenin}}$, PRY-1^{Axin}, and LIT-1^{NLK} accumulate in the anterior. Wnt signaling independently instructs cell fate and spindle orientation during division. Wnt signaling induces LIT-1 Nemo mediated POP-1 phosphorylation, which induces nuclear export of POP-1. The level of nuclear POP-1^{TCF} in relation to SYS-1$^{\beta\text{-catenin}}$ determines the daughter cell fate, while the upstream pathway components determine spindle alignment (see text for further information and references).

transmembrane protein localizes to the proximal membrane, in association with cytoplasmic Prickle, and interacts with Fz in the neighboring cell. The atypical cadherin Flamingo (Fmi) forms homotypic interactions between neighboring cells and supports Fz—Vang interaction (Figure 1a). The asymmetric localization of Fz and Vang is enhanced by both stabilizing interactions between the proteins on neighboring cells and antagonistic intracellular interactions between the Vang-Prickle and Fz-Dsh-Diego complexes. This way, the asymmetry cues provided by Wnt ligands, Fat/Dachsous gradients, or mechanical forces can be converted into a uniform polarized orientation of cells within an epithelium (for extensive reviews: [3,6,7]).

Orienting asymmetric cell divisions in *C. elegans*

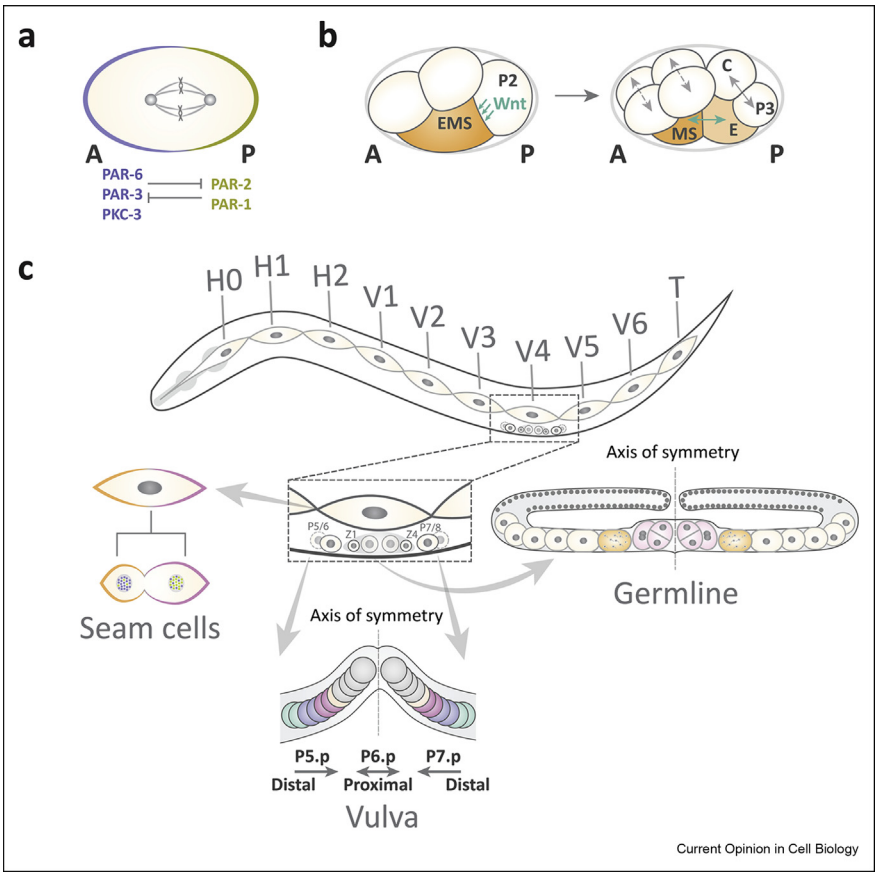
As the anatomy of the worm is simple, tissue polarity is mostly needed to position cells and orient cell divisions along the A-P body axis, for the gonad and vulva with mirror-image symmetry (see below; Figure 2). In large part, these processes are combined through A-P oriented asymmetric cell divisions that produce distinct daughter cells. A divergent canonical Wnt signaling pathway, named Wnt/β-catenin asymmetry pathway, is responsible for differential fate induction and overlaps with noncanonical Wnt-signaling that orients cell division. Dependent on the specific cells and developmental stages, these Wnt signaling pathways use different combinations of *C. elegans* Wnt ligands (CWN-1, CWN-2, EGL-20, LIN-44, MOM-2), Frizzled receptors (FZR-2, LIN-17, MIG-1, MOM-5), and Dishevelled proteins (DSH-1, DSH-2, MIG-5) (reviewed in Ref. [8]).

The Wnt/β-catenin asymmetry pathway instructs A-P polarity and asymmetric cell division as early as the four-cell stage of embryogenesis [9,10]. At this time, maternal MOM-2^{Wnt} is expressed in the posterior germline precursor cell (P2) (Figure 2b). The neighboring EMS precursor cell receives the Wnt signal through the MOM-5 Frizzled receptor and responds to the signal by aligning the cell division with the A-P body axis and inducing the endoderm (E) fate in the posterior daughter cell. Similar Wnt pathways control many of the subsequent embryonic and larval divisions, including asymmetric divisions of the stem-cell-like epithelial seam cells. Various Wnt/β-catenin pathway components become asymmetrically localized during each of these divisions, with the nuclear level of the transcription factor POP-1 TCF/LEF largely determining the daughter cell fate (Figure 1b). POP-1^{TCF} can either act as a transcriptional repressor, in complex with a Groucho-related corepressor, or as a transcriptional activator together with SYS-1$^{\beta\text{-catenin}}$ (reviewed in Refs. [8,11]).

Noncanonical Wnt-signaling in cell division orientation

Noncanonical Wnt signaling governs the orientation of cell division and overlaps in part with the Wnt/β-catenin asymmetry pathway that controls cell fate [12,13]. In the EMS blastomere, the noncanonical Wnt pathway includes MOM-5^{Fz}, DSH-2^{Dsh}, and MIG-5^{Dsh}, GSK-3, and KIN-19 (casein kinase I). These proteins orient the position of the spindle during mitosis, which, in turn, determines the axis and plane of the cell division

Figure 2



Asymmetric cell divisions align with the anterior-posterior axis. (a) Initial anterior-posterior polarity is established by the self-organization of PAR proteins into two mutually exclusive cortical domains. **(b)** At the four-cell stage, Wnt signaling promotes spindle alignment with the anterior-posterior body axis in the EMS blastomere. **(c)** Combinations of Wnt signaling pathways control cell fate and spindle orientation during asymmetric cell divisions in *C. elegans* larval development. The divisions of epithelial seam cells (left), vulva precursor cells V5-7.p (middle), and somatic gonad precursors Z1 and Z4 (right) are illustrated. The gonad and vulva develop with mirror-image symmetry. See text for further information and references.

[12–14]. How Wnt signaling controls spindle positioning remains poorly understood. The mechanism is thought to involve the regulation of cortical pulling forces that position the spindle. These forces arise from dynein motors in association with the LIN-5/NuMA adaptor, which interacts with shrinking microtubules at the cell cortex [15]. LIN-5^{NuMA} is usually attached to the membrane through a Gα.GDP–GPR-1/2 protein complex (Gα.GDP–Pins/LGN in *Drosophila* and vertebrates). Interestingly, in *Drosophila*, several alternative NuMA localizing proteins have been found to promote spindle alignment with the planar polarity of the epithelia (reviewed in this issue: [16]). These mechanisms have not been observed in *C. elegans*, and recent imaging of fluorescently tagged endogenous proteins did not reveal dynein-dynactin or LIN-5 asymmetries in the EMS blastomere [17].

In concert with the posterior enrichment of DSH-2^{Dsh} in EMS, APR-1 (APC-related) and WRM-1 β-catenin become enriched at the anterior cortex [17,19]. APR-1 stabilizes microtubule plus ends and reduces the anterior pulling forces in the one-cell embryo [20], but is not required for Wnt-induced spindle positioning in EMS [13]. Neither is WRM-1$^{β\text{-catenin}}$; however, its removal from the cell cortex allows correct spindle orientation in EMS, possibly by unmasking a cortical spindle-positioning cue [19]. Complicating the identification of such a cue, an Src-related tyrosine kinase pathway acts redundantly with Wnt signaling to rotate the spindle in EMS [13]. A recent study highlights the potential contribution of actomyosin reorganization in cell division orientation [21]. Physical contacts between cells were found to induce anisotropic actomyosin flows, which likely create a directional torque that orients cell division. Interestingly, contact with a Wnt-producing cell neutralizes this mechanism. However, it remains possible that Wnt-induced actomyosin reorganization induces asymmetries in the LIN-5^{NUMA}-dynein pulling forces that orient the spindle. Several earlier *C. elegans*

studies found actin regulation to affect the asymmetric cell division, and CED-10 Rac to contribute to Wnt-controlled division orientation [22–24].

Importantly, noncanonical Wnt signaling is widely used to control the orientation and plane of cell division, with striking similarities between *C. elegans* and mammals [25,26]. However, a direct molecular connection between epithelial polarity proteins and NuMA-dynein complexes has been identified in only a few situations [16,18,27,28], and it will be important to better define the contribution of the actin cytoskeleton (reviewed in Refs. [16,29]).

A-P polarity through instructive Wnt signaling

Resembling Wnt/PCP signaling, noncanonical Wnt signaling that aligns the spindle orients cell division in a plane orthogonal to the apical-basal polarity axis. Moreover, this signaling generally results in asymmetric posterior enrichment of Frizzled and Dishevelled proteins [17,25,30,31]. However, other core PCP proteins are not needed for cell division orientation in *C. elegans*, and cellular bridge formation through *C. elegans* Van Gogh-like (VANG-1)-Frizzled interactions or Flamingo cadherins have not been described. Instead, A-P polarity orientation in the worm appears fully governed by short and long-range Wnt ligand signaling (reviewed in Ref. [8]). Recent fluorescent tagging of functional endogenous EGL-20^{Wnt} visualized the extracellular spreading and long-range gradient formation of this ligand [32]. However, the specific Wnt ligands are expressed at different locations along the *C. elegans* body axis [8], and an obvious question is as to how cells know which Wnt ligands to respond to and whether Wnt ligands provide permissive or instructive cues.

At least the short-range Wnt signals provide instructive positional cues, as has been best shown for P2-EMS signaling in experiments with combinations of wild-type and mutant blastomeres [25]. Also, the orientation of the V5 and T seam cells is determined by nearby EGL-20 and LIN-44 expression, respectively. Other seam cells are redundantly controlled by multiple Wnt ligands. Upon mutation of all five *C. elegans* Wnt genes, or the *mig-14* Wntless receptor, the A-P seam cell polarity becomes randomized [33,34]. By contrast, lack of three Wnt receptors, LIN-17 and MOM-5 Frizzled together with CAM-1^{Ror}, frequently results in a failure to polarize [34]. This indicates that a receptor-dependent cell-autonomous mechanism establishes A-P polarity, with Wnt ligands coordinating the orientation of this polarity with respect to the body axis. Abnormal expression of CWN-1 in the anterior substantially restored normal V1–V3 cell polarity in triple Wnt mutants [34]. Although this points to a permissive Wnt function, further experiments will be needed to exclude alternative explanations. For instance, *sfrp-1* encodes a secreted Frizzled-related protein that acts as a Wnt inhibitor and is expressed in the anterior [8]. Through such Wnt antagonists, a Wnt-signaling gradient with normal A-P orientation may still be established when Wnt expression is anteriorly displaced.

Does Wnt signaling also instruct the mirror-image symmetry of the gonad and vulva? The somatic gonad and germline are formed during larval development from a four-cell primordium, with two somatic gonad precursors (Z1, Z4) on the outside and two germline precursors (Z2, Z3) in the center (Figure 2c). Z1 and Z4 divide with opposite polarity, which depends on the Wnt/β-catenin asymmetry pathway and a signal from the germline precursors, but critical Wnt ligands have not been identified (reviewed in Ref. [11]). In fact, quintuple Wnt mutants showed normal gonad arm orientations, which has been taken to indicate Wnt-independent polarity establishment in the gonad [34]. However, germ cells express MOM-2^{Wnt}, and the temperature-sensitive *mom-2* allele used in this study might have provided sufficient residual function for polarization of Z1 and Z4.

The vulva arises from divisions of three equipotent precursor cells; usually, P5.p, P6.p, and P7.p distributed along the A-P axis in the ventral epidermis (Figure 2c, Middle). The cell division pattern and cell fates of P5.p and P7.p lineages are mirror images (ABCD and DCBA). This is achieved by the expression of Wnt ligands MOM-2 and LIN-44 in the center of the vulva [35]. The LIN-17^{Fz} and LIN-18^{Ryk} receptors mediate this response, and in their absence, P7.p divisions reverse orientation. Interestingly, this reversed orientation is a response to long-range EGL-20 Wnt signaling from the posterior, to which P7.p descendants respond through CAM-1^{Ror} and VANG-1^{Vangl}. This is a rare example of an epithelial cell division, which orients toward a Wnt source through a *C. elegans* PCP pathway component.

PCP protein functions in *C. elegans* cell rearrangements

As outlined above, noncanonical Wnt/Fz/Dsh signaling aligns cell divisions with the A-P axis of the worm. What about the functions of PCP-related genes that are conserved in *C. elegans*: do *vang-1*^{Vangl}, *prkl-1*^{Prickle}, and *fmi-1*^{Flamingo} work together with Frizzled receptors and Dishevelled proteins in tissue polarity or other PCP-controlled processes? Although not needed for epithelial polarity, VANG-1, PRKL-1, and FMI-1 function in cell migration-related processes that include cell intercalation, convergent extension movements, neurite growth, axon guidance, and neuronal migration. This indicates a clear analogy with PCP-controlled processes in other animals (reviewed in Ref. [3]). In fact,

convergent extension movements during gastrulation in the *Xenopus* and zebrafish embryo were the first observed PCP-driven processes in vertebrates.

Developmental cell intercalation is critical for morphogenesis in the worm; however, the mechanisms used are mostly distinct from convergent extension [36]. Interestingly, the correct organization of the *C. elegans* intestinal tube depends on cell intercalation and *vang-1* [37]. During intestinal morphogenesis, VANG-1 relocalizes to apical junctions and interacts through its C-terminal PDZ-binding site with DLG-1 Discs Large. Careful observations demonstrated that wild-type embryos show some variation in the early steps of intestinal formation [38]. Sometimes, cells become displaced during the initial E-lineage divisions; however, their repositioning invariably results in the formation of a planar organized 8-cell intestinal primordium. *vang-1* mutants fail to reposition such variant cells and possibly miss later cell intercalation events (E16 stage), which eventually leads to intestines with an abnormal organization (Figure 3a). Sets of two intestinal cells normally connect through apical junctions to form a common lumen. In *vang-1* embryos, some of these intestinal rings contain three epithelial cells (Figure 3a) [37,38]. This process remains the only example of VANG-1 critically contributing to the organization of epithelial cells in the worm.

Convergent extension-related movements have only recently been described in *C. elegans*, specifically to direct elongation of the worm ventral nerve cord during embryogenesis [39]. In this process, neuronal precursors from the left and right lateral sides of the embryo move toward the ventral midline and contact each other to initially form rosettes. This is temporally organized from anterior to posterior and, upon resolution of the rosettes, is followed by cellular intercalation (Figure 3b). Together with additional rearrangements, this results in the formation of a single file of motor neurons, which subsequently acquire their proper anterior-posterior positions along the ventral nerve cord. Interestingly, the *vang-1* and *prkl-1* PCP genes contribute in this process, as well as a parallel acting SAX-3 Robo trans-membrane receptor. Mutations in the PCP genes cause anteriorly displaced motor neuron positioning, which is strongly enhanced when combined with *sax-3* mutations. VANG-1^{Vangl} and PRKL-1^{Prickle} act cell autonomously in the neurons, and VANG-1 accumulates with nonmuscle myosin (NMY-2) at contacting membranes and the rosette center (Figure 3b). Given this connection and the cellular phenotype, *C. elegans* VANG-1 and PRKL-1 are likely to control directional actomyosin contractions in this convergent extension process, in clear similarity with convergent extension processes in other animals [3].

PCP in axon guidance and neurite formation

In addition to convergent extension movements, PCP components also participate in subsequent steps of *C. elegans* nervous system development, such as axon guidance, as well as the positioning and directional outgrowth of neurites in response to extracellular cues. This was first observed in *fmi-1* Flamingo/Celsr mutants, which show strong axon guidance defects during ventral nerve cord development [40]. The ventral nerve cord is composed of neuronal cell bodies and two bundles of axons separated by a midline. Combinations of attractive and repellent signals prevent the neurites from crossing the midline, or instruct that they cross only once [41]. Herein, pioneer neurons initially lay out the A-P trajectories and provide a scaffold for the migration of subsequent neurons. Midline avoidance of the pioneering axons depends on both extracellular and intracellular domains of FMI-1, while axons that follow the pioneer scaffold require only the extracellular FMI-1 part, most likely for cell adhesion. A recent study described that mutations that disrupt Wnt ligands, CAM-1^{Ror}, LIN-17^{Fz} or VANG-1^{Vangl} also result in severe midline guidance defects [42]. Genetic experiments support that these proteins function within a single pathway, and act in parallel to UNC-6 Netrin/UNC-40 DCC and SAX-3 Robo receptor signaling. Surprisingly, however, the function of CAM-1^{Ror}, LIN-17^{Fz} or VANG-1^{Vangl} is not required in the affected neurons, but in a neuron at the midline (PVT), which secretes UNC-6^{Netrin} and acts as a guidepost cell to direct axon migration (Figure 3c). Thus, while axon guidance defects have been reported for both *fmi-1* and other PCP mutants, in this situation, FMI-1 does not act in the same cells as the other transmembrane PCP proteins.

Similarly, loss of *fmi-1*, but not *vang-1* or *prkl-1,* results in anterior outgrowth and synapse formation defects in a class of inhibitory motor neurons (GABAergic VD neurons) [43,44]. In this case, the function of *fmi-1* is needed in the neighboring excitatory neurons, while the CDH-4 Fat cadherin is required in the inhibitory GABAergic neurons. In specific motor neurons that innervate egg-laying muscle, FMI-1 appears to antagonize other PCP proteins. Mutation of *vang-1*, *prkl-1,* or *dsh-1* leads to extra and abnormally oriented neurites, suggesting that the encoded proteins, function within a single pathway in these neurons [45] (Figure 3d, Top). This phenotype is suppressed by loss of *fmi-1*, indicating that FMI-1 normally promotes neurite formation, opposite to the other core PCP proteins.

Recent studies of specific mechanosensory (touch receptor) neurons provided further insight in PCP regulation of both neurite outgrowth and branching [46,47]. Wnt ligands act as repellent signals that prevent (ALM

Figure 3

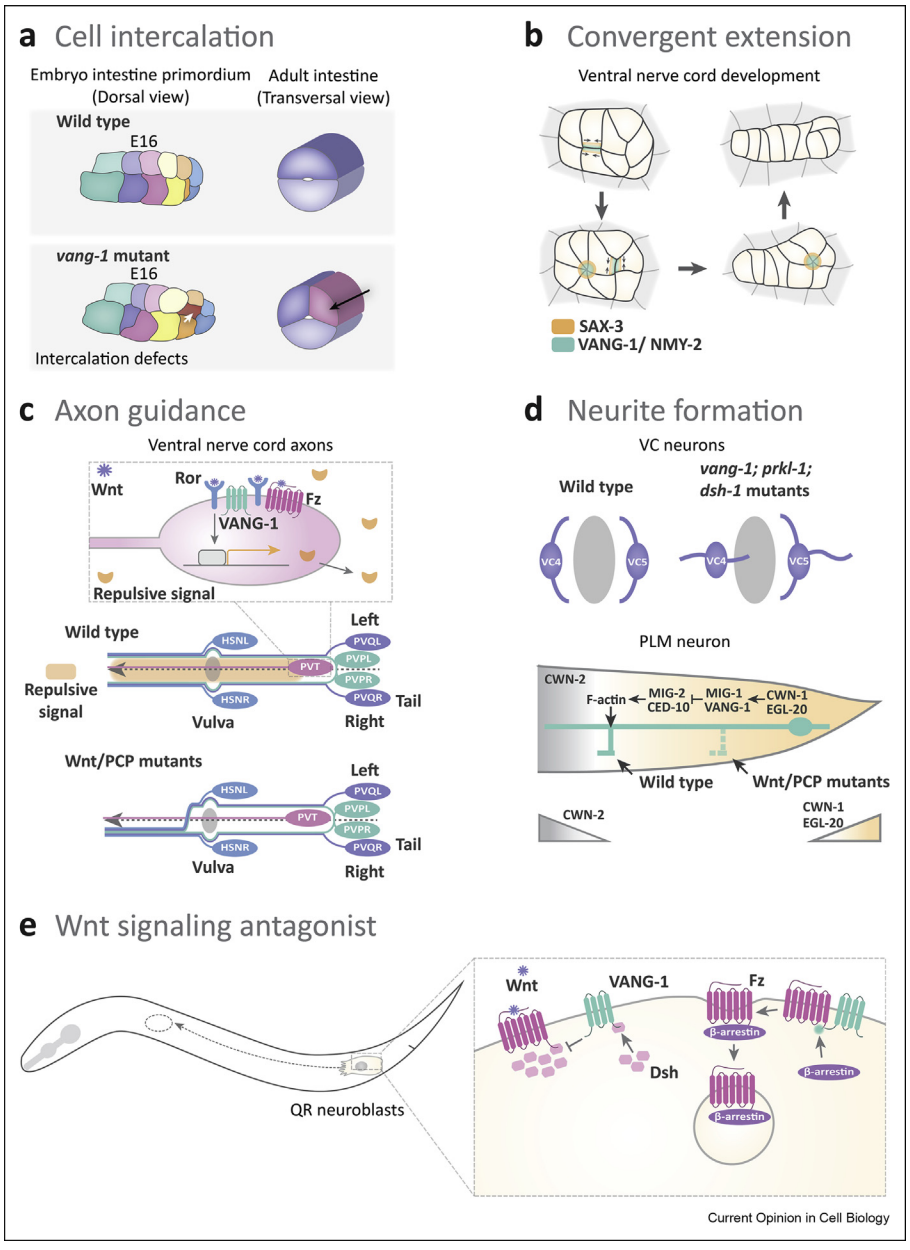


Illustration of core PCP protein-controlled processes in *C. elegans*. *(a)* *vang-1* contributes to intestinal cell intercalation. Illustration of the E16 intestinal primordium. The dorsal side contains two rows of cells (this view; ventral cells are not visible). *vang-1* mutants frequently fail to properly rearrange cells at the E4-E8 stage and show occasional misplacement of ventral cells to the dorsal row (white arrow). Right: illustration of the normal arrangement of intestinal cells (top) and three connected intestinal cells, as observed in *vang-1* mutant larvae (bottom, black arrow). **(b) VANG-1 participates in convergent extension movements of ventral nerve cord precursor cells.** Neuronal precursors migrate to the midline during the formation of the ventral nerve cord and form rosettes. VANG-1 Vangl, NMY-2 non-muscle myosin, and SAX-3 Robo colocalize at contacting membranes and in the rosette center. These proteins are required for proper rosette formation and resolution. Subsequent cell intercalation and additional cell movements result in the formation of a single file of motor neurons. **(c) Axon guidance in the ventral nerve cord.** The neurons PVQ left and right (L/R), and PVP (L/R) are located in the tail and extend axons along the A-P axis toward the nerve ring (anterior, left). The PVT neuron is positioned at the midline (dashed line) and acts as a guidepost cell for PVP and PVQ. Axons from the hermaphrodite specific neurons (HSN), follow the trajectories of the guide neurons. Wnt signals, LIN-17 Frizzled, and the proposed co-receptors CAM-1 Ror and VANG-1 are needed for the repulsive function of the PVT neuron, which prevents the inappropriate crossing of the midline by PVPL and PVQL axons. **(d) Neurite formation of VC4 and VC5 motor neurons** (Top). VC4 and VC5 neurons that innervate egg-laying muscle are normally bipolar but form extra and abnormally oriented neurites in the absence of *vang-1*, *dsh-1*, or *prkl-1*. Correct neurite branching of PLM neurons (Bottom). A discrete gradient along the anterior-posterior axis of Wnt ligands CWN-1, EGL-20 and CWN-2 controls the PLM branching pattern. High CWN-1 and EGL-20 promote clustering of MIG-1 Frizzled, which in turn inhibits the Rac family GTPases, CED-10, and MIG-2 and restrains F-actin assembly to a narrow zone along the anterior-posterior axis. CWN-2 also prevents distal branching. VANG-1 promotes MIG-1 endocytosis, which appears to promote MIG-1 signaling. Mutants of the core PCP genes (*vang-1* and *mig-1*) show mispositioned neurite

touch neuron) or restrict (PLM touch neuron) the outgrowth of posteriorly directed neurites. VANG-1, PRKL-1, and FMI-1 contribute to this repellent process, possibly in a modulatory role, because the phenotypes of the PCP mutants are less severe than those of *lin-44* Wnt and *lin-17* Fz mutants [46]. In addition to outgrowth, the specification of branching of the anterior PLM neurite is also determined by redundant Wnt ligands in association with Frizzled receptors and VANG-1 [47]. In this process, CWN-1^{Wnt} provides an instructive signal, while EGL-20^{Wnt} appears to act permissively through clustering of the MIG-1^{Fz} receptor, which may sensitize signaling. This signaling pathway specifies the branching point by restricting F actin assembly, through inhibition of Rac family GTPases (Figure 3d, Bottom). Interestingly, VANG-1 promotes the endocytosis of MIG-1^{Fz}, which in this situation, appears to stimulate MIG-1^{Fz} signaling [47]. This Wnt-Fz-VANG-1 pathway provides anterior-posterior spatial control over neurite branching, while independent Netrin signaling controls the outgrowth in the perpendicular dorsal-ventral direction.

The simultaneous requirement for Frizzled and CAM^{Ror} in neuronal PCP processes, as well as the interaction of CAM-1^{Ror} with DSH-1^{Dsh}, supports the view that CAM-1^{Ror} acts as a Frizzled co-receptor. Notably, Robo receptors participate frequently in Wnt/PCP-controlled processes in the worm. Robo receptors are well known for their repellent functions in combination with Slit ligands; however, both in convergent extension movements and several neurite outgrowth processes, SAX-3 Robo acts independently of Slit [39]. Surprisingly, a recent study identified SAX-3 as a Wnt ligand co-receptor, which directly binds CWN-2^{Wnt}, as well as CAM-1^{Ror} [48]. This complex mediates the polarized membrane recruitment of the Dsh effector in response to Wnt signals.

Neuronal cell migration

Wnt signaling and PCP proteins also act together in the migration of neuronal precursor cells. This has been best documented in the Q neuroblast lineages (Figure 3e). Two Q neuroblasts, QL and QR, are born in late embryogenesis at similar positions on the left and right side of the animal. QL and its descendants migrate posteriorly in response to the activation of a canonical Wnt pathway. In contrast, the descendants of QR migrate toward the anterior dependent on several sequential Wnt/Fz signaling mechanisms [8]. Interestingly, Fat-family cadherins are involved in the initial polarization of QR neuroblasts [49], which is followed by QR migration that depends on EGL-20^{Wnt}-CAM-1^{Ror} signaling, and the final positioning of QR descendants through cell-autonomous functions of VANG-1 and PRKL-1 [50]. In two recent studies, *vang-1* was identified as a general negative regulator of both canonical and noncanonical Wnt signaling in neuronal precursor migration [51,52]. One of the studies showed that VANG-1 associates with MIG-5 Dishevelled, in a *prkl-1* independent manner, and interferes with MIG-5^{Dsh} plasma membrane localization [51]. The other study detected VANG-1 interaction with the MIG-1 and LIN-17 Frizzled receptors and identified receptor endocytosis as the Wnt-antagonizing mechanism [52]. Reporter experiments in mammalian cell culture and some observations of mouse and zebrafish development provide support for an evolutionarily conserved role of Vangl as a negative regulator of canonical Wnt signaling [51,53–56].

Concluding remarks

C. elegans epithelial organization does not depend on core PCP protein-mediated cellular communication. The importance of coordinating polarity between neighboring cells is likely to depend on the tissue size. *C. elegans* may simply not need a cell–cell polarization mechanism, because of its limited size and cell numbers, and absence of trichomes or cilia on epithelial cells. Local Wnt signals and long-range Wnt gradients appear to reliably achieve A-P polarity on an individual cell basis in the worm. This results in posterior enrichment of Frizzled–Dishevelled complexes, as in noncanonical Wnt/PCP signaling. Exposure to a local Wnt signal also orients the mitotic spindle and induces asymmetric cell division in mammalian stem cells [26], which highlights that studying these processes in *C. elegans* may reveal evolutionarily conserved molecular mechanisms.

In contrast to epithelia, the functions of PCP signaling in cell migratory events and anterior-poster neurite growth show strong similarities between *C. elegans* and other animals. The processes involved integrate signals through a wide variety of transmembrane proteins, including cadherins, VANG-1, several classes of Wnt receptors, and receptors for other guidance cues that include Netrins. For creating directional cellular responses, multiple receptors may act combinatorially, either in the migrating cell or noncell autonomously. As such, SAX-3 Robo was found to act as a Wnt co-receptor, and to induce asymmetric clustering of Dishevelled together with CAM-1^{Ror}[48]. Other receptors may act sequentially in the same cells, as recently shown for QR

branching. **(e) VANG-1 antagonizes WNT signaling in neuroblast migration.** The QR neuroblast migrates toward the anterior in response to Wnt signaling. The final positions of QR descendants depend on VANG-1 Vangl. Two different levels of regulation have been proposed to modulate the response to Wnt signaling in the QR lineage. On the one hand, sequestration of MIG-5 Dishevelled (Dsh) by VANG-1 prevents clustering of MIG-5 and association with LIN-17 Frizzled (Fz). In addition, VANG-1 has been shown to promote LIN-17 internalization through β-arrestin2. See text for further information and references.

neuroblasts, in which polarization and protrusion formation precedes directional migration and migration termination [49–51]. Dissecting these evolutionarily conserved and complex processes will require extensive analysis in vivo, with a high temporal resolution at the single-cell level. The reproducible development, transparency, and advanced methods for temporal and spatial control of gene function [57] make *C. elegans* attractive for such studies.

Conflict of interest statement

Nothing declared.

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