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Planar polarity genes and inhibition of supernumerary neurites

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Planar cell polarity (PCP) genes have recently emerged as important players in sculpting neuronal connections. The bipolar VC neurons display stereotypical differences in axon extension along the anterior-posterior (AP) body axis: VC1–3 and VC6 polarize along the AP axis while VC4 and VC5 polarize along the orthogonal left-right (LR) axis generated by the developing vulva. *vang-1* and *prkl-1,* the worm orthologs of Van Gogh and Prickle, are required to restrict the polarity of neurite emergence to a specific tissue axis. *vang-1* and *prkl-1* loss results in ectopic VC4 and VC5 neurites extending inappropriately along the AP axis. Conversely, *prkl-1* overexpression in VC neurons suppresses neurite formation. These findings suggest that a PCP-like pathway acts to silence or antagonize neuronal responses to polarity cues that would otherwise be permissive for neurite growth.

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The first overt sign of polarization in a newly born (post-migratory) neuron is the formation and directed outgrowth of an axon from a more or less featureless cell soma. In *C. elegans*, the ability to directly visualize nascent neurite formation and extension in vivo has led to several recent insights into the mechanisms that govern this aspect of early neuronal polarization. A key finding is that extracellular cues, such as netrins, slits and wnts, which impart directionality to migrating growth cones also specify the site of initial neurite emergence by polarizing guidance receptors and downstream effector molecules along the axis of extension.[1,2,3] However, mechanisms that may act to inhibit non-specific neurite protrusion during neuritogenesis are less well understood. We have recently shown that a PCP-like pathway that includes *vang-1* (Van Gogh/Strabismus), *prkl-1* (Prickle) and *dsh-1* (Dishevelled) acts to block inappropriate neurite formation in VC4 and VC5 motor neurons.[4]

An Emerging Role for PCP Components in Axon Guidance

The Frizzled/PCP pathway was first identified in Drosophila as a key regulator of cell polarity and organization in the plane of the epithelium in eyes, wings, and abdomen.[5] Genetic studies defined a core group of conserved PCP molecules that include the transmembrane proteins Frizzled, Van Gogh and Flamingo and the cytoplasmic proteins Prickle and Dishevelled. In vertebrates, PCP signaling has been implicated in epithelial surface polarity and directed cell migrations during gastrulation and neurulation.[5] In *C. elegans*, PCP-like pathways are known to regulate asymmetric cell division, vulval precursor cell polarity and cell intercalations during intestinal morphogenesis.[6,7,8]

Over the last several years, the involvement of non-canonical Wnt/Frizzled pathways in axon pathfinding kindled speculation that some of these pathways would also include PCP-like mechanisms.[9] This notion was supported by evidence that loss of PCP genes such as *Frizzled3* and *Celsr3/Flamingo* caused similar axon tract formation defects in mouse.[10,11] It has since become apparent that several distinct non-canonical Wnt pathways act in axon guidance, many of which share components that are common to all Wnt/Frizzled pathways including classic PCP pathways. However, only recently have deficits in genes that are specific to PCP signal transduction such as *Van Gogh/Vangl2*

and *Prickle* have been shown to display axon tract defects in mice, flies and worms.[4,12,13,14,15,16]

A *vang-1-prkl-1* Pathway Blocks Inappropriate Neurite Formation in VC4 and VC5 Neurons

The VC neurons are a set of six peripheral motor neurons (VC1–6) that innervate vulval muscles to control egg-laying. Born postembryonically during L1, they undergo neuritogenesis in late L3 and reach final synaptic targets in late L4.[17] The VCs are an advantageous neuronal polarity model as they are bipolar neurons with stereotypical differences in the orientation of process extension along the AP body axis. VC1–3 and VC6 orient process extension along the AP body axis, whereas the processes of VC4 and VC5 (VC4/5) extend along the orthogonal LR axis generated by the developing vulva, an intermediate target tissue during vulval muscle innervation. Time lapse studies indicate that while all VC neurons initially project processes bidirectionally along the AP axis in L3, the vulval proximal VC4/5 neurons rapidly reorient unidirectionally toward the vulval axis of symmetry and eventually bifurcate laterally to grow bidirectionally along the LR axis of the L4-stage vulva.[4] The directional cues that orient VC process growth along the AP axis or toward vulval cells are not known. However, in animals in which the vulva has been physically or genetically ablated, VC4/5 processes, like those of VC1–3 and VC6, extend along the AP axis,[18] suggesting that bidirectional AP growth constitutes the default polarity in the absence of vulval-derived cues.

How is a differential VC4/5 polarity achieved? A PCP-like pathway appears to play an important role (**Fig. 1**). In VC4/5 neurons, *vang-1*, *prkl-1* and *dsh-1* act to ensure that neurite outgrowth is exclusively polarized along the vulval axis. Loss-of-function mutants fail to maintain polarized VC4/5 morphology which manifests as a gradual increase in the proportion of neurons with AP-directed ectopic neurites as worms mature from L3 to adulthood. Cell-specific rescue experiments revealed that *vang-1*, *prkl-1* and *dsh-1* act cell-autonomously to maintain VC4/5 polarity. Interestingly, epithelial-specific expression of *vang-1* and *dsh-1* also rescued VC4/5 polarity defects in mutant animals. This is consistent with PCP in flies and vertebrates where some components play both autonomous and non-autonomous roles to transduce polarity signals.[5] The observation that all three genes are expressed in VC neurons and vulval cells during neurite formation and pathfinding suggests that the non-autonomous activity of *vang-1* and *dsh-1* resides in the vulval epithelial cells.

Several observations suggest that *prkl-1* plays a prominent role in mediating neurite growth inhibition. First, *prkl-1* loss and gain-of-function display opposite VC4/5 polarity phenotypes (too many or too few neurites respectively), while loss and gain of *vang-1* and *dsh-1* display similar phenotypes (too many neurites) (**Fig. 1A**). Second, *prkl-1* overexpression restores normal VC4/5 polarity in *vang-1* and *dsh-1* mutants. And third, *prkl-1* overexpression but not that of *vang-1* is sufficient to block neurite emergence or

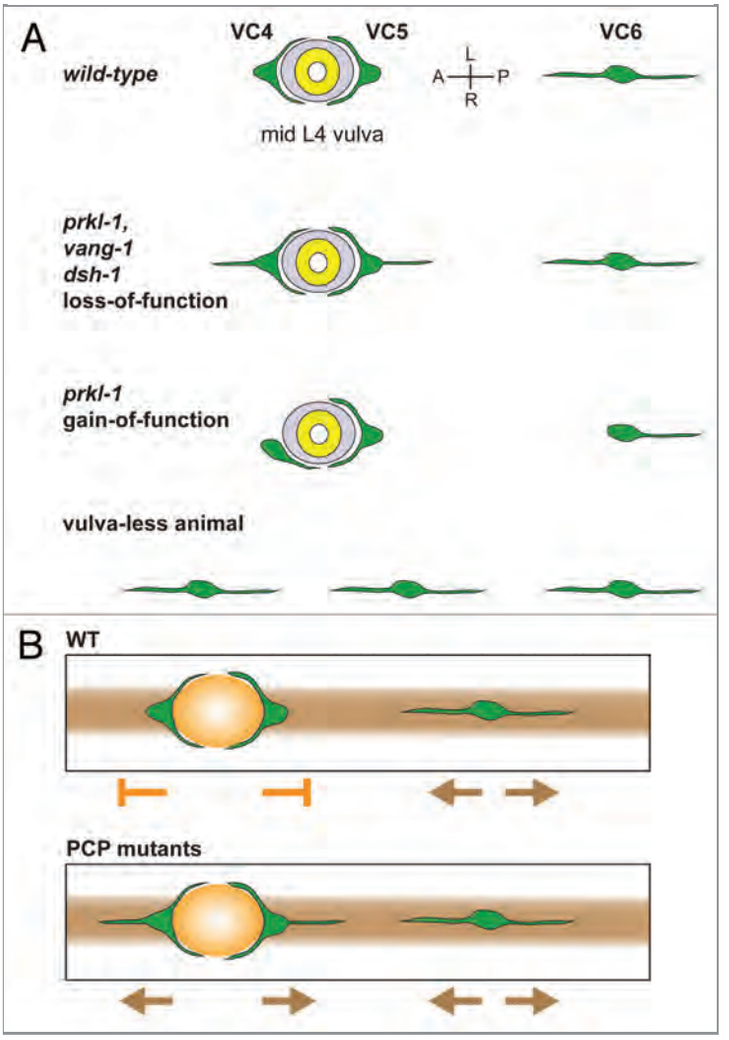

Figure 1. A PCP-like pathway blocks inappropriate neurite formation in VC4 and VC5. (A) VC4 and VC5 neurons polarize neurite extension bidirectionally along the LR axis of the vulval epithelium while vulval distal neurons like VC6 polarize bidirectionally along the AP axis. In PCP mutants such as *vang-1* or *prkl-1*, VC4 and VC5 display ectopic AP-directed neurites. Overexpression of *prkl-1* but not *vang-1* inhibits VC neurite formation in vulva-distal and vulval-proximal VC neurons. In vulval-less animals all VC neurons polarize bidirectionally along the AP axis. (B) A PCP-like pathway in VC4 and VC5 involving cell-cell interactions with vulval guidepost cells (orange) acts to antagonize or silence default AP polarity signals (brown) which would otherwise promote ectopic neurite formation. Polarity cues are depicted as hypothetical permissive corridors or instructive gradients.

extension in VC6 resulting in a bipolar to unipolar-like morphology change. Surprisingly, while *prkl-1* could block VC6 neurite growth in a *vang-1* and *dsh-1*-independent manner, the resulting orientation of VC6 along the AP axis was *vang-1* and *dsh-1*-dependent. In other words, *prkl-1* overexpression preferentially resulted in the loss of the anterior-directed neurite in a *vang-1* or *dsh-1*$^{+/+}$ background and the posterior-directed neurite in a *vang-1* or *dsh-1*$^{-/-}$ background. Collectively, these results are consistent with PRKL-1 acting both upstream and downstream of VANG-1 and DSH-1 and suggest that an output of PCP-like signaling is to orient a neurite inhibitory complex in VC4/5 cell bodies.

Asymmetric PCP Complexes?

Van Gogh and Prickle orthologs are known to physically interact in vitro and found to colocalize in vivo in asymmetrically distributed complexes that correlate with later visible features of morphological polarization.[19,20] Interactions among asymmetric PCP complexes appear to be an important feature of polarity generation in tightly packed cell sheets such as epithelia but whether they are a defining feature of PCP in cells with more transient cell-cell contacts is less clear.[21] Localization of GFP-tagged VANG-1 and PRKL-1 proteins revealed distinct punctate plasma membrane distributions but no apparent asymmetries in VC4/5 somas during neuritogenesis. This finding suggests that polarized distributions may not be an important determinant of VC polarity. Alternatively, polarized protein distributions in VC4/5 may be transient (for example in collapsing growth cones) or involve post-translational modifications and therefore difficult to detect. Notably, in Drosophila mushroom body neurons, where a PCP-like pathway regulates axon branching, *Van Gogh*, *Frizzled*, *Prickle*, and *Dishevelled* were also not found to be differentially distributed.[14] The dynamic and transient aspect of PCP-like signaling during neurite extension is highlighted in commissural growth cones where Vangl2 is preferentially localized to the tips of leading edge filopodia to promote Wnt/Frizzled3-dependent advance.[15]

A Model for PCP-Like Regulation of VC4/5 Polarity

VC4/5 polarity shows several hallmarks of classic PCP-like signaling including autonomous and non-autonomous gene activity, similar loss and gain-of-function phenotypes, and complex genetic interactions; however, the PCP-like mechanism that inhibits VC4/5 neurite formation along the AP axis is currently not known. VC4/5 neurons likely encounter at least two polarity cues during neuritogenesis, a default polarity cue that promotes neurite outgrowth along the AP axis and a vulval-derived one that promotes outgrowth toward and along the LR axis of the developing vulva. The observation that many VC4/5 neurons in PCP mutants show normal early polarization suggests that the initial responses to these cues are PCP-independent. Given that only VC4/5 among the VC neurons are in continuous direct contact with vulval cells during neurite formation and pathfinding, a logical hypothesis is that VC neuron-vulval cell interactions, involving *vang-1* and *dsh-1*, activate a *prkl-1*-dependent PCP effector pathway in the VC4/5 neurons to block or collapse non-specific supernumerary growth cones (**Fig. 1**). In this model, vulval cells are predicted to act as guidepost cells that participate in the reorientation of VC4/5 neurite growth toward the vulva and in a PCP-like pathway to antagonize or silence default polarity signals that would otherwise promote neurite formation or extension along the AP axis. Testing this model will require a better understanding of the extrinsic cues that guide VC neurite growth and intrinsic factors that control PCP-dependent cytoskeletal changes that promote growth cone collapse.

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