Wnt signaling establishes anteroposterior neuronal polarity and requires retromer in C. elegans

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Secreted Wnt proteins influence neural connectivity by regulating axon guidance, dendritic morphogenesis and synapse formation. We report a new role for Wnt and Frizzled proteins in establishing the anteroposterior polarity of the mechanosensory neurons ALM and PLM in C. elegans. Disruption of Wnt signaling leads to a complete inversion of ALM and PLM polarity: the anterior process adopts the length, branching pattern and synaptic properties of the wild-type posterior process, and vice versa. Different but overlapping sets of Wnt proteins regulate neuronal polarity in different body regions. Wnts act directly on PLM via the Frizzled receptor LIN-17. In addition, we show that they are needed for axon branching and anteriorly directed axon growth. We also find that the retromer, a conserved protein complex that mediates transcytosis and endosome-to-Golgi protein trafficking, plays a key role in Wnt signaling. Deletion mutations of retromer subunits cause ALM and PLM polarity, and other Wnt-related defects. We show that retromer protein VPS-35 is required in Wnt-expressing cells and propose that retromer activity is needed to generate a fully active Wnt signal.

KEY WORDS: Wnt, Frizzled, Neuronal polarity, Axon guidance, Retromer

INTRODUCTION

The connectivity of the nervous system is established by migrations of cells and growth cones along the dorsoventral and anteroposterior (AP) body axes. Studies in nematodes, flies and vertebrates led to the discovery of several families of conserved signaling molecules that guide these migrations (Yu and Bargmann, 2001). Netrins and Slits direct axon growth and cell migrations along the dorsoventral axis (Brose and Tessier-Lavigne, 2000; Wadsworth, 2002). First identified for their role in embryonic patterning, Wnts have recently been found to regulate migrations of growth cones and cells along the AP body axis (Charron and Tessier-Lavigne, 2005; Fradkin et al., 2005).

Wnts are secreted glycoproteins that control a variety of developmental processes, including asymmetric cell division, cell fate determination and tissue polarity (Logan and Nusse, 2004). Wnts are palmitoylated (Willert et al., 2003). Lipid modification is required for targeting to lipid rafts, packaging for secretion and association with lipoprotein particles, which is essential for long-range diffusion and action (Zhai et al., 2004; Panakova et al., 2005). Wnts can interact with different cell surface receptors, notably seven-transmembrane Frizzleds and Ryk/Derailed tyrosine kinases. Wnt binding to Frizzled can activate canonical β-catenin pathways, which cause changes in gene transcription, and various non-canonical pathways that lead to cytoskeletal rearrangements.

In flies and vertebrates, Wnts act as repellents and attractants for axon growth. Wnt signal via Ryk/Derailed to repel anterior commissural axons in Drosophila and corticospinal tract axons along the AP body axis in mouse (Yoshikawa et al., 2003; Liu et al., 2005). Conversely, Wnts attract commissural axons after midline crossing in mouse (Lyuksyutova et al., 2003). Attraction is mediated by Frizzled 3 and promotes anteriorly directed growth. Ryk and Frizzled can also function as Wnt co-receptors to induce neurite outgrowth (Lu et al., 2004).

The Wnt EGL-20 controls long-range migrations of the neuroblasts QL and QR and their descendants along the AP body axis in C. elegans (Whangbo and Kenyon, 1999). EGL-20 specifies cell fate and posterior migration of QL and its descendants by upregulating expression of the HOX gene mab-5 via the β-catenin BAR-1 (Harris et al., 1996; Maloof et al., 1999). By contrast, EGL-20 influences the anteriorly directed migrations of QR and its descendants by a BAR-1- and MAB-5-independent process.

C. elegans has five Wnts [CWN-1, CWN-2, EGL-20, LIN-44 and MOM-2 (Shackleford et al., 1993; Herman et al., 1995; Thorpe et al., 1997; Whangbo and Kenyon, 1999)], four Frizzleds [CFZ-2, LIN-17, MIG-1 and MOM-5 (Sawa et al., 1996; Rocheleau et al., 1997; Zinovyeva and Forrester, 2003; Pan et al., 2006)] and a single Ryk, LIN-18 (Inoue et al., 2004). Wnt signaling establishes the polarity of asymmetric cell divisions, determines cell fates and guides cell migrations (Korswagen, 2002; Herman, 2002). In the four-cell stage embryo, a MOM-2/Wnt signal from the blastomere P2 polarizes the endoderm precursor ESM via the Frizzled MOM-5 (Rocheleau et al., 1997; Thorpe et al., 1997; Schlesinger et al., 1999). Polarization of ESM orients its mitotic spindle and specifies endodermal fate in one daughter. Endodermal fate determination requires gene transcription, while mitotic spindle rotation does not (Schlesinger et al., 1999).

In C. elegans, the six mechanosensory neurons ALM, PLM, AVM and PVM extend processes with characteristic morphologies and functions and mediate detection of light touch to different regions of the body. We find that Wnts control the development and organization of the mechanosensory neuron processes along the AP body axis using two completely different mechanisms. For AVM and PVM, the Wnts CWN-1 and EGL-20 act redundantly to promote anteriorly directed process growth; inactivation of both causes many AVM and PVM processes to extend posteriorly rather than anteriorly. By contrast, Wnts influence ALM and PLM development by controlling their overall polarity along the AP body axis. The Wnts CWN-1, CWN-2 and EGL-20 function redundantly to polarize ALM, while LIN-44, CWN-1 and EGL-20 act partially redundantly via the Frizzled LIN-17 to polarize PLM.
We also discovered that the retromer is needed for several Wnt signaling processes, including determination of ALM and PLM polarity. The conserved retromer complex mediates endosome-to-Golgi membrane protein trafficking, such as recycling of hydrolase transporter Vps10p from a prevacuolar endosome to Golgi in yeast (Seaman et al., 1997; Seaman, 2005). Mammalian retromer also regulates transcytosis of the polymeric immunoglobulin receptor (Verges et al., 2004). The retromer is composed of two subcomplexes: one contains VPS26, VPS29 and VPS35 and performs cargo selection; the other consists of the VPS5 and VPS17 dimer in yeast and the VPS5-related proteins SNX1 and, perhaps, SNX2 in mammals, and has a structural role (Haft et al., 2000; Reddy and Seaman, 2001).

C. elegans has VPS-26, VPS-29, VPS-35 and SNX-1/VPS-5 homologs, suggesting that retromer function in intracellular trafficking is similarly conserved. Interestingly, we find that deletion mutations of retromer subunits cause Wnt-related phenotypes, suggesting that retromer mutations disrupt Wnt signaling. We show that retromer protein VPS-35 acts in Wnt-expressing cells and propose that retromer function is needed to generate a competent Wnt signal.

MATERIALS AND METHODS

Genetics

Worms were raised at 20°C and cultured as described (Brenner, 1974). The following strains were used.

LG I: mig-1(e1787), lin-17(n677), lin-44(n1792), mec-4::gfp(zd155); LGII: cwn-1(ok9546), rrf-3(pk1426), mec-7::gfp(muls32), mab-5::gfp(nuls16), mgl-2::gfp(zd545), mlln1[dpy-10(e128)]mls14; LGIII: unc-32(e189), vps-29(tm1320); LGIV: ere-1(mg366), vps-26(tm1523), glp-20(n585), cwn-2(ok895), tph-1::gfp(zd313); LGV: cfc-2(ok1201), rde-1(ne219) mec-7::snb-1-gfp(jsIs37); LGX: lin-18(e620).

We performed direct visual and behavioral enrichment screens to isolate mutations affecting ALM, PLM, AVM and PVM development. We treated mec-4::gfp animals, which express GFP in these neurons (Clark and Chiu, 2003), with the mutant ethylmethyl sulfonate (EMS), transferred F1 animals to separate plates and examined their progeny using a fluorescence dissecting stereomicroscope. We screened 4224 haploid transgenic and isolated 23 mutants. Four mutants were recovered in a behavioral enrichment screen of roughly 1000 haploid genomes that entailed picking individual F2 uncoordinated or egg-laying defective animals and scoring their progeny for neuronal defects. We mapped each mutation by two-factor crosses using visible markers or single nucleotide polymorphisms between N2 and CB4856, and performed complementation tests between new mutations and relevant reference alleles. These mutations caused various cell migration and axonal patterning defects and defined 20 genes in total: unc-40(zd170), zd185, zd186, zd187), epi-1(cgl79), zd180, zd184), pag-3(zd160, zd175), unc-53(zd177, zd188), egl-44(zd172), lin-17(zd173), lin-32(zd174), mec-7(zd182), mec-12(zd181), mig-1(zd176), mua-6(zd167), unc-6(zd161), unc-44(zd189), unc-51(zd171), vps-35(zd163) and five genes represented by a single allele (zd168III, zd169I, zd178, zd183I, zd194III).

vps-35 sequence analysis and rescue

zd165 maps to the cluster of LGII. Coding and splice junction regions of several candidate genes were amplified from wild-type and zd165 genomic DNA using flanking primers and PCR. DNA sequences of amplified products or cloned fragments were determined; zd163 contains a 148 bp deletion within F59G1.3 (vps-35) and N2 sequence was identical to that reported by the C. elegans Sequencing Consortium. EST clones yk1358e03 and yk1664g05 were sequenced to ascertain vps-35 gene structure.

We transformed vps-35 animals by co-injecting test DNA (10-50 μg/ml) and gcy-36::dsredT4 marker DNA, pSK223 (20-30 μg/ml), which labels URX, AQR and PQR, into mec-4::gfp; zd163/mln1[dpy-10(e128)]mls14. Transformed F1 and F2 animals were identified by DsRed expression.
the posterior process was long and branched like the wild-type anterior process (Fig. 1B). The long posterior process became entangled in the tail or circumnavigated back towards the anterior. The complete inversion of axonal structures suggests that the overall AP polarity of PLM is in fact reversed. In about half of the defective PLMs, the anterior and posterior processes were both short and roughly equal in length, indicating that PLM polarity was symmetric.

Table 1. ALM and PLM polarity in Wnt, Frizzled, Ryk and retromer mutants

<table>
<thead>
<tr>
<th>Genotype</th>
<th>ALM</th>
<th>PLM</th>
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<tbody>
<tr>
<td></td>
<td>Wild type</td>
<td>Reversed</td>
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<tr>
<td>egl-20</td>
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</tr>
<tr>
<td>mom-2(zygotic rnaI)</td>
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</tr>
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<td>lin-17; egl-20</td>
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<td>0</td>
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<td>Retromer</td>
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<td>vps-35</td>
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Polarity was scored in young adults using *mec-4::gfp* to visualize ALM and PLM. Alleles are listed in the Materials and methods. n=100-200 ALMs and PLMs.
Synaptic vesicles, which can be detected using a mec-7::snb-1-gfp reporter, are selectively enriched in the long anterior PLM process in wild type (Nonet, 1999) (Fig. 2A). In lin-17 mutants, we found that SNB-1-GFP-labeled synaptic vesicles accumulated instead in the long posterior process (Fig. 2B). Synaptic vesicles were enriched in both processes when they were symmetric in length (Fig. 2C). Together, these results confirm that lin-17 mutations reverse the intrinsic AP polarity of PLM: the anterior process exhibits the morphological and synaptic characteristics of the wild-type posterior process and vice versa.

lin-17 mutants have cell fate defects (Sawa et al., 1996). To address whether the PLM polarity defect results from a cell fate transformation, we examined expression of three PLM fate markers: mec-4::gfp, mec-7::gfp and mgl-2::gfp. All three were expressed appropriately in PLM, indicating that PLM cell fate was specified correctly in lin-17 mutants. However, lin-17 mutations often caused ALN, the sister of PLM, to adopt a PLM-like fate based on the ectopic expression of mec-4::gfp and mgl-2::gfp and elevated expression of mec-7::gfp (Fig. 1B). When ALN expressed PLM cell fate markers, ALN also had a reversed polarity like PLM; untransformed ALNs had a wild-type polarity.

Multiple Wnts control PLM polarity via the Frizzled LIN-17

The PLM polarity defect of lin-17/fz mutants points to Wnts as possible neuronal polarity cues. Indeed, we found that Wnt polarity was often reversed or symmetric in lin-44/wnt mutants (Table 1, Fig. 1C). PLM polarity was unaffected in mom-2/wnt(zygotic rnai) animals and the wnt mutants cwn-1, cwn-2 and egl-20. However, the PLM defect of lin-44; egl-20 double and lin-44; cwn-1; egl-20 triple mutants was greatly enhanced relative to lin-44 single mutants (Table 1, Fig. 1D). These results indicate that LIN-44 is needed to establish PLM polarity and that CWN-1 and EGL-20 also contribute.

Both lin-44/wnt and lin-17/fz mutations caused a highly penetrant PLM polarity defect. To determine whether they act in the same pathway, we examined double mutants and found that the PLM defect was similar to that of either single mutant (Table 1). In addition, the PLM defect of lin-17; cwn-1 and lin-17; egl-20 double mutants was comparable with lin-17 single mutants. These data are consistent with all three Wnts, LIN-44, CWN-1 and EGL-20, acting in the Frizzled LIN-17 pathway.

Wnts might act directly or indirectly on PLM. We found that the PLM defect of lin-17 mutants was rescued by expressing LIN-17A or LIN-17B isoforms in ALM and PLM during embryogenesis using the mec-4 promoter (Fig. 2D). Because the ALM and PLM cell bodies are far apart, we conclude that the Frizzled LIN-17 acts autonomously and postmitotically in PLM and that LIN-44, CWN-1 and EGL-20 act directly on PLM via LIN-17.

Ectopic LIN-44 expression can polarize PLM

LIN-44, the primary Wnt needed for PLM polarity, is expressed during embryonic and postembryonic development in four tail epidermal cells located just posterior to PLM (Herman et al., 1995), whereas EGL-20 is expressed in epidermal and muscle cells located around PLM (Wang et al., 2000) (Fig. 1A). Endogenous EGL-20 only partially compensated for a loss of LIN-44, suggesting that PLM polarization might require Wnt expression from a posteriorly localized source or might need a high overall level of Wnt expression from non-directional sources. We found that expressing LIN-44 in egl-20-expressing epidermal and muscle cells rescued the lin-44 PLM polarity defect (2/2 lines), arguing that a non-localized but high level of Wnt expression from cells anterior and ventral to PLM is sufficient to establish polarity and that LIN-44 acts as a permissive rather than instructive polarity cue.

Three Wnts act redundantly to establish ALM AP polarity

Our finding that Wnts and a Frizzled control PLM polarity led us to explore whether they regulate the polarity of ALM, the anterior homologue of PLM. ALM polarity was unchanged in mom-2/wnt(zygotic rnai) animals, in the wnt mutants lin-44, egl-20 and cwn-2, in frizzled mutants mig-1, lin-17 and cfz-2, and in ryk mutant lin-18 (Table 1). Interestingly, cwn-1; egl-20 and cwn-1; cwn-2 double mutants had a significant ALM polarity defect and cwn-1 single mutants had a slight defect (Table 1, Fig. 3B). Because egl-20...
Wnt signaling establishes neuronal polarity

and cwn-2 are tightly linked (roughly 250 kb apart), we used RNAi to reduce both gene activities simultaneously and observed egl-20 and cwn-2 phenotypes but not an ALM polarity defect. Thus, the Wnts CWN-1, EGL-20 and CWN-2 act redundantly to control ALM AP polarity. In addition, based on the appropriate expression of mec-4::gfp, Wnt mutations did not alter ALM cell fate.

Wnts control axon growth and branch formation
We analyzed the Wnt mutants further to determine whether Wnts are needed for axon guidance in worms as they are in flies and vertebrates. The mechanosensory neurons AVM and PVM extend a single process that enters the ventral nerve cord (VNC) and turns anteriorly; the AVM process continues into the head and the PVM process stops in the anterior body (White et al., 1976) (Fig. 4A,C,F). Process outgrowth was unaffected in lin-44, cwn-1 and egl-20 wnt mutants and in mom-2(zygotic rna) animals. However, cwn-1; egl-

20 double mutants had striking defects in anteriorly directed process growth (Fig. 4B,F). Processes extended ventrally and entered the VNC correctly, but then they turned anteriorly and stopped short, turned posteriorly, or split and extended in both directions. Thus, CWN-1 and EGL-20 act redundantly to guide the anteriorly directed growth of the AVM and PVM processes.

Wnt mutations also blocked formation of axon branches. The ALM and AVM processes have distal branches that make contact within the nerve ring (Fig. 4C,D). In 2% of cwn-1 (n=50) and 48% of cwn-2 mutants (n=50), the ALM and AVM processes stopped at the nerve ring and/or lacked the nerve ring branch. Furthermore, all cwn-1; cwn-2 double mutants (n=50) had such defects showing that CWN-1 and CWN-2 promote formation of the nerve ring branch and final anterior extension of the ALM and AVM processes (Fig. 4E).

vps-35 mutants have AP polarity defects like Wnt and Frizzled mutants
vps-35 encodes a subunit of the retromer complex, which mediates transcytosis and endosome-to-Golgi protein trafficking. In our screen, we isolated a mutation in vps-35 that surprisingly caused polarity defects like Wnt and Frizzled mutations (Table 1). In vps-35 mutants, polarity of both ALM and PLM was often reversed or symmetric suggesting that Wnt signaling was compromised (Fig. 1E, Fig. 3C). vps-35 mutants were slightly small and uncoordinated and most died during embryogenesis. Animals had a reduced brood size as well as vulval and egg-laying defects. All phenotypes but the reduction in brood size were maternally rescued. Zygotic expression was sufficient, as heterozygous cross progeny from homozygotes appeared wild type.

Genetic mapping, germline rescue, RNAi experiments and sequence analysis firmly established that our mutation, zd163, was a deletion in vps-35 (Fig. 5A). A 5.6 kb vps-35 genomic fragment rescued zd163 and reduction of vps-35 function by RNAi caused ALM and PLM polarity and other zd163 phenotypes. Based on sequence analysis of two full-length cDNAs and genomic DNA, vps-35 has eight exons, a four nucleotide 5′ untranslated region (UTR) and a 497 nucleotide 3′ UTR (Fig. 5B). Analysis of zd163 genomic DNA revealed a 148 bp deletion that removes part of intron 7 and exon 8 and probably eliminates most or all function. vps-35 is predicted to encode an 821 amino acid protein that is a homolog of yeast and mammalian VPS35 (Paravicini et al., 1992; Edgar and Polak, 2000; Zhang et al., 2000).

Retromer mutations disrupt EGL-20 signaling
The retromer is composed of two subcomplexes: one contains VPS-26, VPS-29 and VPS-35; the other contains VPS-5 and VPS-17 in yeast and the VPS-5-related proteins SNX1 and, perhaps, SNX2 in mammals. To determine whether VPS-26 and VPS-29 are needed for polarity, we examined vps-26 and vps-29 deletion mutations (kindly provided by S. Mitani) and found that vps-26 but not vps-29 mutants had both ALM and PLM polarity defects (Table 1).

Because ALM and PLM polarity is regulated in part by EGL-20, we investigated whether retromer mutations caused other egl-20 phenotypes. EGL-20 controls cell fate specification and posterior migration of the neuroblast QL and its descendants, such as PVM, by upregulating expression of HOX gene mab-5 (Harris et al., 1996; Whangbo and Kenyon, 1999). egl-20 mutations block mab-5::gfp expression in QL and its descendants and both mab-5 and egl-20 mutations cause PVM to migrate anteriorly rather than posteriorly. In vps-26, vps-29 and vps-35 mutants (n=50-96), 100% of the PVMs migrated anteriorly (Fig. 3C). In addition, vps-26 and vps-29
mutations eliminated mab-5::gfp expression in QL and its descendants (Fig. 6A,B; data not shown). The shared PVM migration and mab-5::gfp expression phenotypes of retromer and egl-20 mutants strongly suggest that EGL-20 signaling is blocked by retromer mutations.

VPS-35 is expressed broadly and colocalized with Golgi
To determine the vps-35 expression pattern, we generated a transcriptional GFP reporter. Starting in early embryos, vps-35::gfp expression was detected in most or all cells (Fig. 6C). Expression continued throughout larval and adult stages in most tissues, including epidermis, body wall muscle, intestine, somatic gonad, coelomocytes, pharynx and some neurons. vps-35::gfp expression in tail epidermis overlapped with lin-44/wnt expression throughout development (Fig. 6D). The broad embryonic and postembryonic expression of VPS-35 is consistent with the observed vps-35 developmental defects.

The retromer shuttles between endosomes and Golgi. To ascertain whether VPS-35 associates with Golgi in C. elegans, we compared localization of a full-length VPS-35-mCherry fusion with a Golgi-specific marker, MANS-YFP (Rolls et al., 2002). When expressed in tail epidermis, VPS-35-mCherry and MANS-YFP were detected in a punctate pattern around the nucleus (Fig. 6E-G). A subset of VPS-35-mCherry and MANS-YFP puncta were co-labeled, showing that VPS-35 associates with a subset of Golgi/MANS and non-Golgi/MANS vesicles.

VPS-35 acts in Wnt-signaling cells
VPS-35 might act in Wnt-responding cells or in Wnt-signaling cells. Polarity defects were not rescued when VPS-35 was expressed in Wnt-responding neurons ALM and PLM using the mec-4 promoter (0/2 lines). However, the PLM polarity defect was specifically rescued when VPS-35 was expressed in tail epidermis using the lin-44/wnt promoter (2/2 lines). Furthermore, ALM/PLM polarity and PVM migration defects were rescued when VPS-35 was expressed in EGL-20-expressing epidermal and muscle cells (2/2 lines). Thus, VPS-35 functions non-autonomously and is needed in LIN-44 and EGL-20 signaling cells.

DISCUSSION
Wnt signaling establishes neuronal polarity along AP body axis
Our studies uncover a new role for Wnts and Frizzleds in establishing the intrinsic AP polarity of individual postmitotic neurons in C. elegans. ALM and PLM are bipolar and extend two processes with dramatically different morphologies and synaptic characteristics. A block in Wnt signaling causes an inversion of ALM and PLM polarity: the anterior process adopts the length, branching pattern and synaptic properties of the wild-type posterior process, and vice versa.

Three Wnts act redundantly to control ALM polarity; inactivation of more than one is needed to confer a polarity defect. cwn-1; egl-20 and cwn-1; cwn-2 double mutants had a synthetic ALM polarity defect, indicating that EGL-20 and CWN-2 act in parallel to CWN-
1. CWN-2 and EGL-20 possibly act in the same pathway because reducing both activities at the same time by RNAi did not lead to a polarity defect. A single Wnt, LIN-44, largely governs PLM polarity. Genetic interactions indicate that CWN-1 and EGL-20 possibly act in the same pathway because they were now expressed in ALN as well as PLM postmitotically. lin-17 mutations also cause the blast cells B and T to undergo symmetric cell divisions, producing two daughters with the size and fate of the anterior daughter (Herman, 2002). LIN-44 has been proposed to be a ligand for LIN-17 for B and T development. However, lin-44, cwn-1, cwn-2 and egl-20 mutations alone or in various combinations did not alter ALN cell fate. As such, ALN cell fate regulation by LIN-17 might involve several redundantly acting Wnts or not require Wnts. In addition, these results indicate that PLM polarization and ALN cell fate specification entail independent LIN-17-mediated processes.

Localized expression of LIN-44 in four epidermal cells located just posterior of PLM suggested that LIN-44 might act as an instructive polarizing cue (Fig. 7A). However, ectopic expression of LIN-44 in cells anterior and ventral to PLM rescued the lin-44 PLM defect. Thus, a posterior source of LIN-44 is not required for generating proper PLM polarity, supporting the idea that LIN-44 instead acts as a permissive cue. Interestingly, EGL-20 and LIN-44 act as permissive cues in the regulation of the asymmetric cell divisions of V5 and T, respectively (Herman and Horvitz, 1994; Whangbo et al., 2000). To establish polarity, Wnts might act in concert with an instructive polarizing signal or function to stabilize or activate a transient cellular asymmetry.

For planar cell polarity in Drosophila, Frizzleds are localized and activated independently of Wnt signals and provide asymmetric cues for subsequent developmental events. In C. elegans, the Frizzled MOM-5 is enriched at the posterior pole of cells prior to cell division; enrichment is dependent on the Wnt MOM-2 in early but...
not older embryos (Park et al., 2004). For PLM polarity, Frizzleds might be asymmetrically localized in the postmitotic cell but require Wnt activation to induce polarization. A LIN-17-mCherry fusion did not appear asymmetrically localized in undifferentiated PLMs; however, LIN-17 overexpression did cause PLM axonal defects, suggesting that LIN-17 levels can influence PLM development (B.C.P. and S.G.C., unpublished).

**Model for PLM polarity regulation by LIN-44**

Centrosome localization determines polarity of hippocampal neurons (de Anda et al., 2005). The centrosome, Golgi and endosomes are clustered at the pole opposite from the plane of the last mitotic cell division; the first neurite forms at this site and develops into the axon, while subsequent neurites become dendrites. As such, the mitotic division plane of a neuronal precursor determines the default polarity of its postmitotic daughters. After cell division, the daughters would have opposite polarities because their centrosomes are located at opposite poles. The PLM/ALN precursor divides along the AP body axis to produce the anterior daughter PLM and posterior daughter ALN (Fig. 7B). As their centrosomes are at opposite poles, PLM and ALN would be expected to have inverted AP polarities. However, after differentiation, PLM and ALN have the same AP polarity: each forms a long anterior process that makes synapses and a short posterior process that lacks synapses. In Wnt and Frizzled mutants, the polarity of PLM is in fact inverted relative to ALN, as predicted based on the division plane of the PLM/ALN precursor. We propose that Wnt signaling flips the polarity of PLM by causing the centrosome to move to the opposite pole of the postmitotic cell. In the absence of Wnt signaling, the centrosome does not move and PLM polarity is inverted relative to wild type. Wnt signaling via GSK-3 can induce centrosome movement and reorient the mitotic spindle in the *C. elegans* early embryo independently of gene transcription (Rocheleau et al., 1997; Thorpe et al., 1997; Schlesinger et al., 1999). Similarly, the ALM/BDU precursor divides along the AP body axis to produce the anterior daughter ALM and posterior daughter BDU. ALM migrates posteriorly from the head to a specific position in the midbody. The posterior migration and final polarity of ALM is controlled by Wnt signaling. We propose that Wnts reorient the polarity of ALM by inducing centrosome movements and/or cytoskeletal rearrangements after undergoing its posteriorly directed migration.

**Wnt proteins act redundantly to control axon guidance and branching**

Our results also highlight the importance of Wnts as axon guidance and branch-inducing factors. In flies and vertebrates, Wnts can act as attractive and repulsive guidance molecules to direct axon growth along the AP body axis. Similarly, we find that anteriorly directed growth of the AVM and PVM processes is controlled by CWN-1 and EGL-20. When both are eliminated, the processes stop prematurely or become redirected toward the posterior. CWN-1 and EGL-20 are primarily expressed in posterior body regions, suggesting that they might act as repellents for AVM and PVM processes (Whangbo et al., 2000; Pan et al., 2006). Ectopic expression experiments support the idea that EGL-20 is a repellent for AVM and PVM processes (Pan et al., 2006). In mouse, Wnt3 induces branching of sensory neuron axons (Krylova et al., 2002). We find that two Wnts, CWN-1 and CWN-2, govern branching of the ALM and AVM processes; branching is reduced in cwn-2 mutants and is completely eliminated in cwn-1; cwn-2 double mutants. Our findings reveal that regulation of branching by Wnts is conserved.

In summary, Wnts play a global role in organizing multiple aspects of the nervous system along the *C. elegans* AP body axis. They guide long-range migrations and final positioning of several...
neurons and neuroblasts (Whangbo and Kenyon, 1999; Pan et al., 2006). Our results show that Wnts guide anteriorly directed axon growth and establish AP neuronal polarity.

The retromer is needed for Wnt signaling

Retromer plays a key role in transcytosis and endosome-to-Golgi protein trafficking. We discovered that the retromer has an unexpected role in Wnt signaling. Retromer mutants vps-26, vps-29 and vps-35 had PVM migration defects like egl-20 mutants, and vps-26 and vps-29 mutations blocked expression of a MAB-5 transcriptional reporter. These results support the idea that EGL-20 signaling is compromised by retromer mutations. Furthermore, vps-26 and vps-35 mutants had ALM and PLM polarity defects that are consistent with a reduction of CWN-1, EGL-20, LIN-44 and other Wnt activities. vps-35 mutants also had defects in long-range migrations of ALM, AVM and HSN cell bodies (B.C.P and S.G.C., unpublished), which are in part guided by Wnt and Frizzled signaling (Harris et al., 1996).

VPS-35 serves as the core of the retromer complex, binding independently to VPS-26 and VPS-29 to form a high-affinity heterotrimeric subcomplex (Haft et al., 2000; Reddy and Seaman, 2001; Collins et al., 2005). vps-26 and vps-35 deletion mutations confer similar phenotypes, suggesting that both cause a complete loss of retromer function. By contrast, vps-29 deletion mutants have fewer Wnt-related defects, suggesting that some retromer activity is retained. In yeast, the elimination of Vps29p causes Vps35p to become unstable and reduces its affinity for sorting nexins Vps5p and Vps17p (Reddy and Seaman, 2001); however, our results indicate that VPS-29 is less essential for retromer function in Wnt signaling in C. elegans.

VPS-35 functions non-autonomously and acts in Wnt-expressing cells. In particular, we show that VPS-35 activity in lin-44-expressing tail epidermis rescued PLM polarity defects and activity in egl-20-expressing cells rescued ALM/PLM polarity and PVM migration defects. We also find that VPS-35 associates with Golgi and non-Golgi vesicles consistent with the well-described role of retromer in endosome-to-Golgi protein trafficking. We propose that VPS-35, and, by inference, the retromer complex function in signaling cells to generate a fully active Wnt signal.

Even when retromer function was abolished by vps-26- and vps-35-null mutants, retromer phenotypes were often less severe than Wnt-null mutants. For example, the PLM polarity defect of lin-44-null mutants was more severe than vps-26 or vps-35 mutants, suggesting that LIN-44 activity was reduced but not eliminated. Furthermore, vps-26 and vps-35 mutants had ALM polarity defects like Wnts mutants but did not have AVM/PVM process growth defects like cwn-1; egl-20 double mutants or ALM/AVM branching defects like cwn-1; cwn-2 double mutants. By contrast, all retromer mutants had completely penetrant defects in PVM migration as did strong egl-20 mutants.

The partial and selective loss of Wnt activity by retromer mutations suggests that retromer might directly or indirectly affect Wnt expression levels, processing or gradient formation. We did not observe a change in intracellular levels of EGL-20 in vps-35 mutants (B.C.P and S.G.C., unpublished). Wnts undergo post-translational modifications to become fully active at both short and long range. Wnt palmitoylation is needed for targeting to lipid rafts, for packaging for secretion and for association with lipoprotein particles, which is essential for long-range diffusion (Zhai et al., 2004; Panakova et al., 2005). The retromer complex might function in trafficking various Wnt processing enzymes or proteins required for Wnt transport or diffusion, such as lipoproteins.

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