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The long and the short of Wnt signaling in *C. elegans*

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The simplicity of *C. elegans* makes it an outstanding system to study the role of Wnt signaling in development. Many asymmetric cell divisions in *C. elegans* require the Wnt/ β -catenin asymmetry pathway. Recent studies confirm that SYS-1 is a structurally and functionally divergent β -catenin, and implicate lipids and retrograde trafficking in maintenance of WRM-1/ β -catenin asymmetry. Wnts also regulate short-range events such as spindle rotation and gastrulation, and a PCP-like pathway regulates asymmetric divisions. Long-range, cell non-autonomous Wnt signals regulate vulval induction. Both short-range and long-range Wnt signals are regulated by recycling of MIG-14/Wntless via the retromer complex. These studies indicate that *C. elegans* continues to be useful for identifying new, conserved mechanisms underlying Wnt signaling in metazoans.

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Wnt signaling is a fundamental, conserved process during metazoan development. The bewildering array of responses of cells in embryos to Wnt signals makes it a daunting task to identify specific Wnt-dependent signaling inputs that influence differentiation. The simplicity of the *C. elegans* embryo and larva makes *C. elegans* an attractive model system for investigating the role of Wnt signaling in a variety of cellular contexts. Here we review recent progress in characterizing several different Wnt-dependent signaling pathways in the *C. elegans* embryo and larva. We have tried to avoid overlap with other recent reviews in what follows. For other recent reviews, please consult [1–3].

Expanding the canon: AXL-1 and the canonical Wnt pathway

As in *Drosophila* and vertebrates, some Wnt-dependent events in *C. elegans* use the canonical Wnt signaling

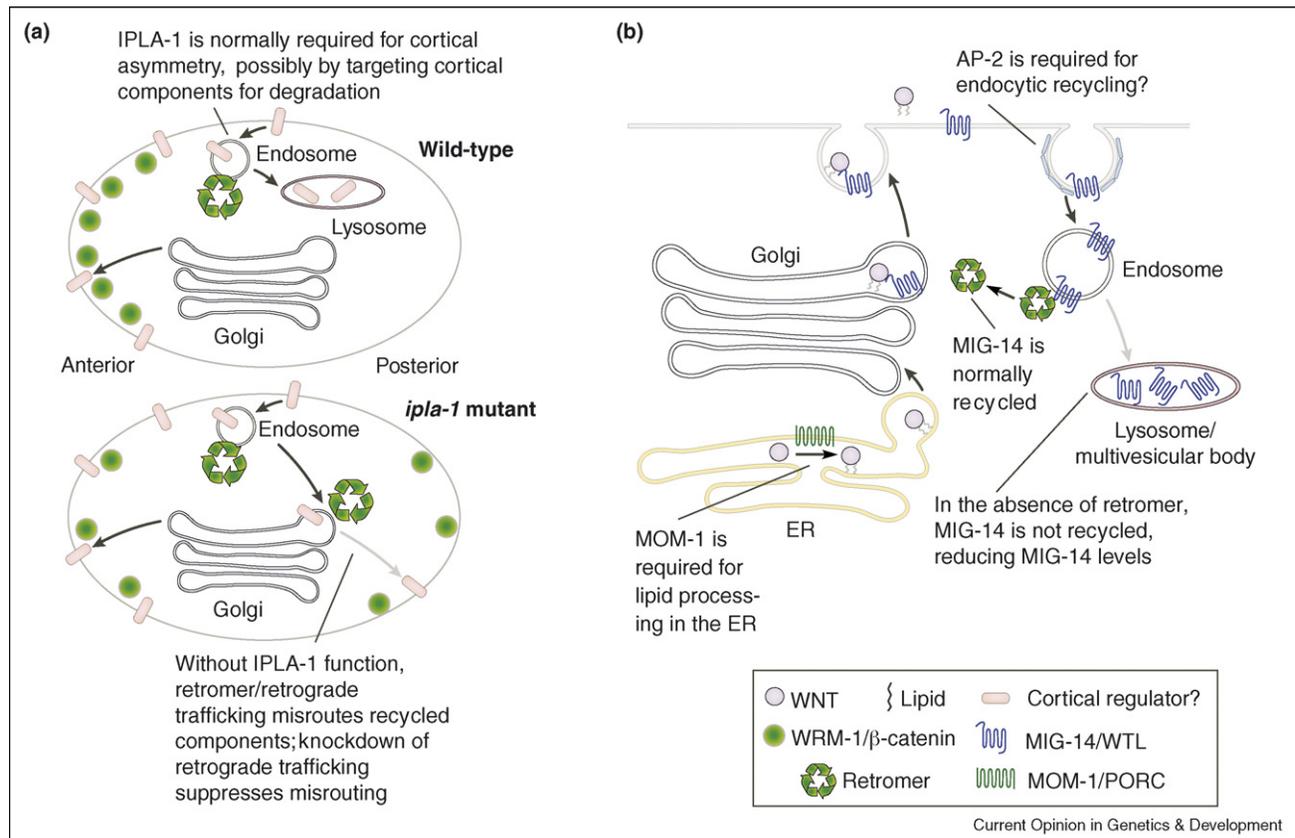
pathway, including migration of the Q neuroblasts and establishment of the fate of vulval precursor cells (VPCs). As in other canonical Wnt signaling pathways, *pry-1/Axin* functions as a negative regulator of the pathway [4]. Oosterveen *et al.* [5] have identified another, divergent Axin-like protein, AXL-1, which like PRY-1, physically interacts with BAR-1/ β -catenin, MIG-5/Dsh and DSH-2/Dsh. Moreover, *axl-1* mutants enhance the *pry-1* null phenotype. *axl-1* mutants display ectopic branching of the excretory cell, which is not enhanced by *pry-1* loss of function, but can be rescued by excretory cell-specific expression of *pry-1*. These results indicate that while partially redundant, *axl-1* and *pry-1* may have functionally distinct roles.

Expanding the non-canon: new insights into Wnt/ β -catenin asymmetry

Many asymmetric divisions during *C. elegans* development, including those of EMS, T blast cells and Z1/Z4 (somatic gonadal precursors) use a second Wnt signaling pathway, the Wnt/ β -catenin asymmetry pathway (reviewed in [1,2,6,7]). Like canonical Wnt signaling, β -catenin function is required; however, unlike canonical signaling, a key role of β -catenin is to regulate asymmetric localization of the Tcf transcription factor POP-1, a process termed ‘POP-1 asymmetry’ [8]. In the posterior (distal) daughter cell Wnt signaling activates WRM-1/ β -catenin and LIT-1/Nemo-like kinase. WRM-1 and LIT-1 show an asymmetric nuclear localization that is reciprocal to POP-1 [9,10], consistent with their role in targeting POP-1 for nuclear export [11].

Although the mechanisms by which such asymmetries arise is still largely unknown (see [2] for extensive discussion), it must involve cortical asymmetries, which give rise to eventual nuclear asymmetries. Very recent work by Kanamori *et al.* [12*] shows that loss of function of the intracellular phospholipase A₁, IPLA-1, results in cell autonomous defects in production of seam cells from T blast cells, spindle orientation defects and loss of cortical WRM-1 asymmetry. An *ipla-1* suppressor screen identified *mon-2*, an ARF GEF-like protein and *tbc-3*, a Rab GAP, whose yeast homologues encode proteins involved in endosome-to-Golgi retrograde vesicle trafficking. In addition, knocking down the *C. elegans* homologues of the retromer complex, which is involved in retrograde transport of intracellular sorting receptor complexes in yeast and mammalian cells [13], suppresses *ipla-1* mutants. These results suggest a model in which WRM-1 asymmetry is regulated by turnover of membrane components, whose steady-state levels are influenced by IPLA-1 and recycling endosomes (Figure 1A). Given the importance

Figure 1



Retrograde trafficking affects Wnt signaling via multiple mechanisms in *C. elegans*. **(A)** WRM-1/ β -catenin asymmetry is disrupted by loss of IPLA-1 function. In this hypothetical model, IPLA-1 is presumed to modify a lipid component that results in steady-state reduction in components at the cortex required for anterior localization of WRM-1. In *ipla-1* mutants, misrouting of these components appears to require retrograde vesicle trafficking, since it is disrupted by knockdown of components of the retromer and retrograde trafficking machinery. **(B)** The retromer complex and AP-2 regulate MIG-14/Wntless trafficking, which indirectly regulates Wnt secretion. MOM-1/Porcupine is required for an early step in Wnt ligand processing, presumably in the endoplasmic reticulum. Later steps in MIG-14 trafficking are affected by AP-2 and retromer. AP-2 knockdown presumably prevents endocytic reuptake of MIG-14, preventing its entry into recycling endosomes; the return of MIG-14 to the Golgi is regulated by retromer components. Their removal results in significant reduction, but not complete abrogation, of MIG-14 function, thereby reducing the amount of Wnt secretion. (A) is adapted from [12*]; (B) is adapted from [40].

of recycling endosomes to Wntless trafficking (see below), these findings are intriguing.

Like other components of the Wnt/ β -catenin asymmetry pathway, *sys-1* is required for specification of distal cell fates in the somatic gonad, but it is not required for POP-1 asymmetry [14]. *sys-1* encodes a protein with weak consensus armadillo repeats, and can bind to the β -catenin binding domain of POP-1 [15]. Several genetic experiments indicate that SYS-1 functions as a limiting coactivator for POP-1 [15,16*, 17*] (reviewed in [2]). SYS-1 has very recently been crystallized and shows a high degree of similarity to β -catenin at the tertiary level, confirming that it is indeed a divergent β -catenin [18*]. *sys-1* function is required throughout development for Wnt/ β -catenin asymmetry, including during endoderm formation [16*], and deletion alleles of *sys-1*, *pop-1* and *wrm-1* show similar embryonic arrest phenotypes (R King, T Walston and J

Hardin, unpublished observations), suggesting that SYS-1 has widespread roles in anterior–posterior fate specification in the *C. elegans* embryo.

Expanding the non-canon: PCP-like signaling?

Evidence for other Wnt-dependent signaling pathways identified in vertebrates, such as the Wnt/ Ca^{2+} [19] and planar cell polarity (PCP [20,21]) pathways is more equivocal. Disruption of calcium signaling, either through loss of function of *plc-1*/phospholipase C- ϵ or *itr-1*/inositol trisphosphate receptor, leads to defects during cell rearrangement in the embryonic epidermis [22,23], but how or when calcium signaling functions in regulating intercalation is unclear.

Perhaps even more enigmatic is whether *C. elegans* possesses a true PCP pathway. Asymmetric division

of the male specific B cell requires *lin-44/Wnt*, *lin-17/frizzled* [24]. While POP-1 shows asymmetric nuclear localization in the B cell daughters, this asymmetry does not require WRM-1 or LIT-1, but instead requires homologues of Wnt/planar cell polarity components. LIN-17/FZ and MIG-5/Dsh both show asymmetric cortical localization reminiscent of Fz and Dsh in other situations involving PCP signaling [25]. Asymmetric division of the B cell requires signaling mediated through the DEP but not the DIX domain of MIG-5/Dsh, as well homologues of the PCP components Vang, Prickle, RhoA, Rock and Daam1, further implying that a PCP-like pathway functions to regulate asymmetric division in the B cell [24]. The PHA neuronal lineage, which requires *dsh-2/Dsh* [26], has similar genetic requirements (K Hinwing, S Lee, L Nykilchuk and N Hawkins, unpublished). Do such experiments demonstrate a *bona fide* PCP pathway in *C. elegans*? Not necessarily, since putative PCP components are not essential [24,25] (R King, T Walston and J Hardin, unpublished). On the contrary, *C. elegans* may be a system poised to identify other, previously unidentified PCP-like effectors.

Short-range signals: spindles, shrinking apices and synapses

Wnt signaling has long been known to regulate spindle rotation of the EMS and ABar blastomeres, a process that does not rely on POP-1/Tcf or new transcription (reviewed in [27]). Recent work by Zhang *et al.* suggests that Wnt signaling may function to localize dynactin in this process [28]. In wild-type embryos dynactin accumulates at the border between P2 and EMS, similar to DSH-2/Dsh and MOM-5/Fz. *mom-5* knockdown results in reduced dynactin accumulation, and dynactin accumulation is abolished in *mom-5*; *src-1* double knockdown embryos, consistent with the synergy between *mom-5* and *src-1* in regulating spindle rotation. Accumulation of dynactin at the P2-EMS border could recruit dynein and function to trap astral microtubules, placing torque on the attached centrosomes.

Wnt signaling also regulates an actomyosin-mediated event during gastrulation in *C. elegans*. MOM-2/Wnt and MOM-5/Fz are well known to be required for differentiation of the endodermal precursors, Ea and Ep, which apically constrict as they ingress. However, Lee *et al.* [29^{*}] noticed that in some *mom-2* and *mom-5* mutant embryos in which endodermal fate appears normal, Ea and Ep fail to ingress. Such defects cannot be explained by a simple delay in the cell cycle, nor by loss of localized PAR or NMY-2/non-muscle myosin heavy chain, which are properly localized in the mutants. However, enrichment of phosphorylated regulatory myosin light chains at the apical surface of Ea and Ep is reduced in *mom-5* mutants, which may lead to reduced apical constriction and gastrulation failure.

A third example of short-range Wnt signaling involves synapse formation. Wnts are known to positively influence synaptic connectivity in vertebrates and *Drosophila* (reviewed in [30]). Using SNB-1/synaptobrevin localization in the DA9 motor axon to monitor synapse formation in the posterior region of *C. elegans* hermaphrodites, Klassen and Shen [31^{**}] show that local Wnt signaling influences the location of presynapses. In *lin-44/Wnt*, *lin-44*; *egl-20/Wnt* double mutants and *lin-17/ Frizzled* mutants, however, DA9 has ectopic presynapses in the tail, suggesting that Wnt signaling normally inhibits synapse formation. Consistent with this model, misexpression of *lin-44* under control of the *egl-20* promoter leads to changes in the location of the synaptic region. How such local control of synaptogenesis is regulated is unclear. DSH-1/Dsh appears to be involved, but loss of function of *pop-1*, *lit-1*, *wrm-1*, *bar-1* or *pry-1* does not phenocopy loss of Wnt function. Further work will be needed in each of these three cases to identify how Wnts control local accumulation of subcellular components in specific regions of a single cell.

Long-range signals: sources and sinks during vulval induction

In addition to these examples of short-range Wnt-mediated signaling, there are several well-known cases of long-range Wnt signaling in *C. elegans*. Neuronal migration along the anterior–posterior axis has been extensively reviewed recently in this journal [3]. Another example involves the vulva. The ‘Pn.p cells’ are born in the L1 larva; five of these cells then fuse with the hyp7 syncytium (the ‘F’ fate) and another cell, P3.p, sometimes fuses with hyp7 in the L2 larva. The remaining (typically six) cells express the Hox gene *lin-39* and become the vulval precursor cells (VPCs), which adopt different fates (1°, 2° or 3°) owing to EGF and Notch signaling (reviewed in [32]). Multiple Wnts and Frizzleds regulate *lin-39* expression via *bar-1/β-catenin*, and hence VPC differentiation [33^{*}].

Two recent studies have extended these findings. First, Myers and Greenwald examined Wnt regulation of the F fate in VPCs and found that CWN-1 and EGL-20 are involved in suppressing fusion of P4.p with hyp7 in L2 larvae. They went on to use tissue-specific promoters to rescue *mig-14/Wntless* function (and hence Wnt secretion) in various cells and found that expression of *mig-14* in neurons, muscle or the epidermis can partially rescue P4.p fusion defects. This suggests that Wnt signaling may provide general competence-promoting functions during VPC specification; reduction of EGF signaling or ablation of the somatic gonadal primordium at the L1 stage synergizes with loss of multiple Wnt signals, suggesting both signals may promote VPC competence.

The second study focused on the ROR receptor tyrosine kinase, CAM-1, which had been shown previously to

antagonize Wnt responses in neuronal cells through its CRD domain [34,35]. The simplest explanation for these data is that CAM-1 binds and sequesters Wnt ligands. In CAN neurons, genetic data suggest that CAM-1 activity is cell autonomous [36], but CAM-1 could also act cell non-autonomously to sequester Wnts. Green *et al.* examined this possibility in the context of VPC specification [37^{*}]. *cam-1* mutants synergize with *lin-17*/Frizzled and *lin-18*/Ryk mutants, resulting in production of more than the usual 22 vulval cells. This phenotype can be ameliorated by reduction of *egl-20*/Wnt and *cwn-1*/Wnt function, suggesting that CAM-1 acts by inhibiting these Wnts, to which it can bind *in vitro*. Significantly, expression of *cam-1* in neurons or muscle is sufficient to cause loss of 1° VPC induction, similar to loss of *bar-1* and Wnt gene function. Taken together with the results of Myers and Greenwald, these results indicate that Wnt signaling is 'buffered' in the environment surrounding VPCs. The simplicity of the *C. elegans* larva may be an ideal place to dissect out the specific contribution of surrounding tissues in VPC specification in the future.

The long and the short of Wnt signaling: vesicular trafficking and MIG-14/Wntless

Production of both short-range and long-range Wnt signals requires correct processing of Wnts. Wnt processing is a complex subject, and has been reviewed extensively elsewhere (e.g. [38–40]). Very recently two groups working in *C. elegans* [41^{**},42^{**}] along with simultaneous work in *Drosophila* (reviewed in [43]) provided new insights into Wnt secretion. The new work builds on two important recent results. First, two groups had shown that in the context of neuronal migration, the retromer complex regulates Wnt signaling in *C. elegans* [44^{**},45^{**}]. Second, the Basler group identified a novel seven-pass membrane protein, Wntless (WLS), which is required for secretion of Wnts. The *C. elegans wntless* homologue corresponds to mutations originally identified through their role in endoderm induction (*mom-3*) and in neuronal migration (*mig-14*) [46^{**}]. In *wntless* mutants, Wnts accumulate inside the cells that produce them, and secretion of Wnts is ultimately blocked.

Work by the Korswagen and Garriga labs now shows that retromer function is important in recycling MIG-14/WLS and that in the absence of retromer function, MIG-14 is targeted for lysosomal degradation [41^{**},42^{**}]. First, the Garriga group shows that *dpy-23* encodes the *C. elegans* homologue of the μ subunit of the AP-2 clathrin adaptor complex [41^{**}]. They and the Korswagen group both show that loss of DPY-23 in Wnt producing cells results in phenotypes similar to Wnt loss of function. Both groups show that loss of retromer function also disrupts Wnt signaling. Significantly, the subcellular distribution of MIG-14::GFP is disrupted in *dpy-23* and retromer loss of function backgrounds. In the former, MIG-14 accumulates at the plasma membrane, while in the latter MIG-14 accumulates in vesicular structures deeper in the cyto-

plasm, which the Korswagen group shows colocalize with lysosomal markers [42^{**}]. Taken together, these results suggest that DPY-23, working at the plasma membrane, and retromer components, working at the level of recycling endosomes, are both required for recycling of MIG-14 (Figure 1B). MIG-14 presumably plays a modulatory role in this process, after initial lipid modifications to Wnts that occur in the endoplasmic reticulum, which are thought to be mediated by MOM-1/Porcupine (reviewed in [39,40]).

Conclusions

C. elegans continues to be a powerful model system to identify new regulators of Wnt signaling, and how these new regulators function. Along with new insights from other genetic model systems, results from *C. elegans* promise to continue to refine our understanding of how cells respond to multiple signaling inputs, and how they translate such signals into specific, molecular readouts to alter cell fate, motility and subcellular structure.

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