NONCANONICAL WNT SIGNALING PATHWAYS IN *C. ELEGANS* CONVERGE ON POP-1/TCF AND CONTROL CELL POLARITY

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1. ABSTRACT

In the nematode *Caenorhabditis elegans,* a canonical Wnt signaling pathway controls a cell migration whereas noncanonical Wnt pathways control the polarities of individual cells. Despite the differences in the identities and interactions among canonical and noncanonical Wnt pathway components, as well as the processes they regulate, almost all *C. elegans* Wnt pathways involve the sole Tcf homolog, POP-1. Intriguingly, POP-1 is asymmetrically distributed between the daughters of an asymmetric cell division, with the anterior sister cell usually having a higher level of nuclear POP-1 than its posterior sister. At some divisions, asymmetric distribution of POP-1 is controlled by noncanonical Wnt signaling, but at others the asymmetry is generated independently. Recent experiments suggest that despite this elaborate anterior-posterior POP-1 asymmetry, the quantity of POP-1 protein may have less to do with the subsequent determination of fate than does the quality of the POP-1 protein in the cell. In this review, we will embark on a quest to understand Quality (1), at least from the standpoint of the effect POP/Tcf quality has on the control of cell polarity in *C. elegans.*

2. INTRODUCTION

Wnt signaling pathways are conserved from mammals to nematodes and function in diverse developmental processes, such as cell proliferation, cell differentiation, cell fate determination, synaptogenesis, cell migration, and cell polarity (reviewed in refs. 2-4). At least three major conserved Wnt signaling pathways are now recognized: Wnt/beta-catenin, Wnt/calcium and Wnt/JNK or planar cell polarity (PCP). In canonical or Wnt/beta-catenin pathways, Wnt signals function to stabilize beta-catenin level in the cell, allowing beta-catenin to translocate to the nucleus and form a complex with Tcf/Lef factors to activate or repress expression of specific genes. Beta-catenin is not involved in the noncanonical Wnt/calcium and PCP pathways, although in some cases noncanonical Wnt signaling interferes with Wnt/beta-catenin signaling (reviewed in ref. 5).

Wnt signaling pathways control several aspects of *C. elegans* development, including cell fate decisions, cell migrations and cell polarity (reviewed in refs. 6, 7). Both canonical and noncanonical Wnt pathways function during *C. elegans* development. A canonical Wnt pathway controls the migrations of the descendants of the QL neuroblast, collectively called the QL.d (reviewed in refs. 6-8). This canonical pathway includes *egl-20/Wnt, mig-5/Dsh, sgg-1/GSK-3, bar-1/beta-catenin, pry-1/Axin* and *pop-1/Tcf* and functions to control the expression of *mab-5/Hox*, which controls the migration of the QL.d (9-14). There are three beta-catenin homologs in *C. elegans:* BAR-1, HMP-2 and WRM-1. Interestingly, it appears that the adhesion and signaling functions that are performed by a single beta-catenin molecule in other species have been distributed among the three *C. elegans* homologs (12, 15). BAR-1 is the only beta-catenin homolog that interacts strongly with the sole Tcf homolog, POP-1, which has been shown to function as a canonical Tcf (12). WRM-1 also participates in signaling, however it interacts weakly with POP-1 (15, 16) and appears to do so in the absence of the POP-1 amino-terminal beta-catenin binding site (15), indicating that the WRM-1-POP-1 interaction is different than the BAR-1-POP-1 interaction. Furthermore, WRM-1

only functions in pathways that also involve LIT-1, a nemo-like kinase that is involved in a mitogen-activated protein kinase (MAPK)-like pathway. Together, this suggests that Wnt signaling pathways in which WRM-1 participates are noncanonical. Although POP-1 also participates in these pathways, it is regulated differently than it is in the canonical pathways. HMP-2 appears to function only in adhesion: it does not interact with POP-1, but does interact with HMR-1/cadherin (12, 15). Based upon the distinct functions for the three *C. elegans* beta-catenin homologs, signaling pathways that involve BAR-1 appear to be canonical, while those involving WRM-1 are noncanonical. A Wnt/calcium pathway has not been described in *C. elegans* and a PCP-like pathway appears to control the polarity of at least one cell that divides asymmetrically (M.W. and M.A.H., unpublished). Interestingly, in *C. elegans,* all Wnt pathways, canonical or noncanonical, appear to converge on POP-1/Tcf.

Some canonical pathway components, including BAR-1, have also been shown to be involved in controlling the fates of the P12 ectoblast and the vulval precursor cells. However, in these cell fate decisions the full pathway has not been shown to be active and Ras signaling is also involved. Thus, it is not clear whether these cell fates are controlled by the interaction of the canonical Wnt pathway and a Ras pathway or whether the pathway is also noncanonical in some respect.

WRM-1 and POP-1 function in noncanonical Wnt signaling that is almost exclusively involved in controlling the orientations, or cell polarities, of several cells that divide asymmetrically during *C. elegans* development; specifically the EMS blastomere, the T cells in the tail and the Z1 and Z4 cells in the developing gonad. In each of these asymmetric cell divisions (except for the Z1 and Z4 cells), as well as many others, the nuclear levels of POP-1 are asymmetric with one sister cell, usually the anterior cell, having a higher level of nuclear POP-1 than it's sister. Furthermore, it appears that the function of the WRM-1/LIT-1 noncanonical pathway is to lower the nuclear level of POP-1 in one sister cell. The mechanisms responsible for this regulation, as well as the consequences of cells having different POP-1 nuclear levels, are being intensely investigated. However, while much has been learned, many questions remain. The control of POP-1 nuclear level appears to be fundamental to the control of cell polarity by noncanonical Wnt signaling in *C. elegans,* however modification of POP-1 also seems to be important. This review will focus on the progress that has been made in understanding POP-1/Tcf regulation and role in the control of cell polarity and discuss the remaining questions.

3. NONCANONICAL WNT SIGNALS CONTROL THE POLARITY OF THE EMS BLASTOMERE

At the four-cell stage of *C. elegans* embryogenesis, the EMS blastomere divides asymmetrically to produce an anterior MS cell, that subsequently divides to generate mesodermal precursors, and a posterior E cell, that subsequently divides to generate all the endoderm. Blastomere isolation and reconstitution

experiments demonstrated that the P2 blastomere polarizes the EMS blastomere by inducing the nucleus and centrosomes to rotate 90°, reorienting the spindle along the anterior-posterior axis, as well as inducing the E cell fate at the point of contact between P2 and EMS (17, 18). Forward and reverse genetic approaches have identified many genes that when mutated or inactivated by RNAi affect EMS polarity and endodermal cell fate specification. These include several Wnt pathway components: *mom-2/Wnt, mom-1/Porc, mom-5/Fz, wrm-1/beta-catenin, sgg-1/GSK3, apr-1/APC* and *pop-1/Tcf* (19-21). Two of the three *C. elegans* Dishevelled homologs, *mig-5* and *dsh-2,* are enriched in oocytes (22) and appear to be involved in endoderm induction (23). In addition, MAPK pathway components *lit-1/NLK* and *mom-4/TAK1*, which encodes a MAP kinase kinase kinase protein similar to mammalian TAK1, were also identified (16, 24). The molecular identity of an additional gene, *mom-3,* has not yet been determined.

Mutations in each of the above genes, except *pop-1,* leads to the loss of endoderm, whereas mutation of *pop-1* leads to the loss of mesoderm. Furthermore, upstream components *mom-1, mom-2, mom-5, mig-5/dsh-2* and *sgg-1* are involved in both EMS spindle orientation and endoderm specification, whereas downstream components *apr-1, wrm-1,* and *pop-1* affect only endoderm specification. This suggests that the spindle orientation and endoderm specification pathways branch at *sgg-1* (21, 23). These and other considerations suggest that the spindle orientation pathway may interact directly with the cytoskeleton (25).

3.1. Wnt/MAPK signaling represses of POP-1 function

The Wnt pathway that specifies endoderm involves WRM-1 and is noncanonical. The involvement of LIT-1/NLK and MOM-4/TAK1 also make this Wnt pathway unusual. Both the Wnt and MAPK pathways converge on POP-1/Tcf. In the absence of a signal from P2, POP-1/Tcf represses E cell fate. Thus, endoderm induction is achieved by inhibition of a repressor. POP-1 represses endoderm fate in the MS cell by recruiting a complex that contains the histone deacetylase (HDAC), HDA-1 and UNC-37/Groucho (26). This appears to occur by repressing the transcription of the endoderm-specific GATA-like transcription factor genes, *end-1* and *end-3,* in the MS cell. Two other redundant, zygotically expressed GATA factors, MED-1 and MED-2 bind to the *end-1* and *end-3* promoters and are required for *end* gene expression in the E cell (27). Even in the unstimulated MS cell, MED-1, and presumably MED-2, bind to the *end* gene promoters, but so does POP-1, which somehow inhibits *end* gene expression by the MEDs (28). Interestingly, recent data indicate that the *C. elegans* p300 histone acetyltransferase homolog CBP-1 interacts with POP-1 and acetylates it (29). Furthermore, acetylation of POP-1 is required for POP-1 function in MS, as a *pop-1* construct in which the three acetylated lysine residues (K185, K187 and K188) were changed to either alanine (GFP::POP-1MutAAA) or arginine (GFP::POP-1MutRRR) failed to rescue a *pop-1* mutant, while the control GFP::POP-1 construct was able to rescue (29). Thus, both HDA-1/HDAC and CPB-1/p300

are required in the unsignalled MS cell to block *end* gene expression and repress E cell fate. Interestingly, CBP-1 function is also required for *end-1* expression and endoderm fate (26). Thus, CBP-1 function is required both in MS to repress endodermal fate and in E to promote endoderm fate. One possibility is that the targets of CBP-1 acetylation in MS and E are different. For example, CBP-1 may acetylate POP-1 in MS in order to repress *end* gene expression and to acetylate histones in E to allow *end* gene activation.

In the E cell, the Wnt and MAPK pathways components function to repress POP-1 function. An unusual aspect of the Wnt pathway involved in endoderm induction is that APR-1/APC and SGG-1/GSK3 act positively in Wnt signal transduction to activate WRM-1, leading to the negative regulation of POP-1. This is very different from canonical Wnt pathways where APC and GSK3 function as part of a complex involved in the degradation of beta-catenin in the absence of Wnt signals and are inhibited in the presence of Wnt signals. WRM-1 and LIT-1 can interact and together phosphorylate POP-1 (16), leading to the inhibition of POP-1 function.

3.2. Wnt/MAPK signaling causes nuclear-cytoplasmic redistribution of POP-1

Recent studies have begun to illuminate how POP-1 function is inhibited (28, 29). The nuclear level of POP-1 is higher in the anterior MS cell than it is in the posterior E cell. However, the overall level of POP-1 in the MS and E cells is similar. Wnt/MAPK signaling appears to cause a nuclear-cytoplasmic redistribution of POP-1 in the E cell. The difference in POP-1 nuclear levels is apparent immediately after the division of EMS. This lead Maduro *et al.* to suggest that the lowered nuclear POP-1 level is due to the inefficient import of POP-1 into the reforming E cell nucleus (28), although other mechanisms are possible. An internal 124 amino acid region of POP-1, that does not include the beta-catenin binding site in the amino-terminus of POP-1 nor the HMG box DNA-binding domain, is required for asymmetric nuclear accumulation (28). Acetylation of POP-1 also influences nuclear-cytoplasmic partitioning. Specifically, acetylation promotes nuclear localization, possibly by increasing nuclear import and blocking nuclear export. However, phosphorylation of POP-1 by WRM-1 and LIT-1 does not affect POP-1 acetylation state, but does cause POP-1 to accumulate in the cytoplasm. This is supported by the observation that both GFP::MutAAA or GFP::MutRRR are predominantly cytoplasmic, yet retain anterior-posterior asymmetric nuclear levels in MS and E (29). Thus, while acetylation is involved in nuclear-cytoplasmic distribution of POP-1, it does not appear to be involved in the anterior-posterior asymmetry. This suggests that phosphorylation of POP-1 is independent of acetylation and can override the promotion of nuclear localization by acetylation. One consequence of Wnt/MAPK signaling, then, appears to be phosphorylation of POP-1, resulting its redistribution from the nucleus to the cytoplasm.

3.3. Quality, not quantity of POP-1 is important

Despite the elaborate regulation of nuclear POP-1 level in the E cell, three experiments suggest that the absolute amount of nuclear POP-1 is not the most important consequence of Wnt/MAPK signaling on E cell fate. First, overexpression of a GFP::POP-1 construct in EMS and E had no effect on endoderm induction, although there was an estimated ten-fold increase in the amount of functional POP-1 in the E cell (28). Second, nuclear POP-1 levels in MS and E are equal in the *lit-1(t1534)* mutant, but endoderm is still properly specified. This demonstrates that POP-1 asymmetric nuclear distribution can be uncoupled from endoderm specification, suggesting that there is a qualitative difference in POP-1 in signaled versus nonsignalled cells. Finally, although some nuclear POP-1 remains in the E cell, it does not bind to the *end* gene promoters, which allows for *end* gene activation by MED-1 and MED-2 (28). Something is different about POP-1 in the E cell nucleus after Wnt/MAPK signaling that prevents it from binding to the *end* gene promoters. This qualitative difference may be reflected in the intranuclear localization of POP-1 in nonsignaled cells. In MS, POP-1 has been observed to localize to puncta within the nucleus; whereas in the nucleus E, it is present in a low, but uniform pattern (28). Maduro *et al.* suggest the puncta observed in the MS nucleus may reflect POP-1's function as a repressor. Whether or not this turns out to be the case, it seems clear that a qualitative difference in POP-1 function, apart from the consequence that Wnt/MAPK signaling has on level of nuclear POP-1, does exist. The function of this qualitative difference and the role of POP-1 asymmetric nuclear distribution remains a puzzle, however.

4. A PARALLEL PATHWAY INVOLVING SRC-1 IS ALSO INVOLVED IN EMS POLARITY

New players have recently entered the picture. Mutations in the Src Kinase homolog *src-1* caused defects in EMS spindle orientation reminiscent of Wnt pathway mutants (23). In addition, *src-1* mutants also caused defects in the germline precursor cells similar to those caused by *mes-1* mutations. MES-1 is a putative transmembrane protein that has a structure of a receptor tyrosine kinase, although it is not predicted to be an active kinase (23, 30). Although *src-1* and *mes-1* mutants do not display endoderm defects on their own, the similarity of the EMS spindle defects led Bei and colleagues to determine whether they might interact genetically with mutations in Wnt pathway components. Double mutant combinations between *src-1* or *mes-1* and *mom-1/Porc, mom-2/Wnt, mom-5/Fz, sgg-/GSK3,* or *mom-3* displayed synergistic EMS spindle orientation and endoderm defects. In addition, the *src-1* or *mes-1* triple mutant combination with *dsh-2/Dsh* and *mig-5/Dsh* also displayed synergistic EMS spindle orientation and endoderm specification defects. This indicates that SRC-1 and MES-1 function in parallel with the noncanonical Wnt pathway to control both EMS spindle orientation and endoderm specification (23). These data were supported by observations that double mutants between Wnt pathway components did not display any genetic interactions. In addition, the nuclear levels of POP-1 were equal in MS and E in *src-1; mom-2* and *mes-1; mom-2* double mutants; indicating that these pathways converge on POP-1. A long-standing curiosity about the involvement of the Wnt pathway in these processes was
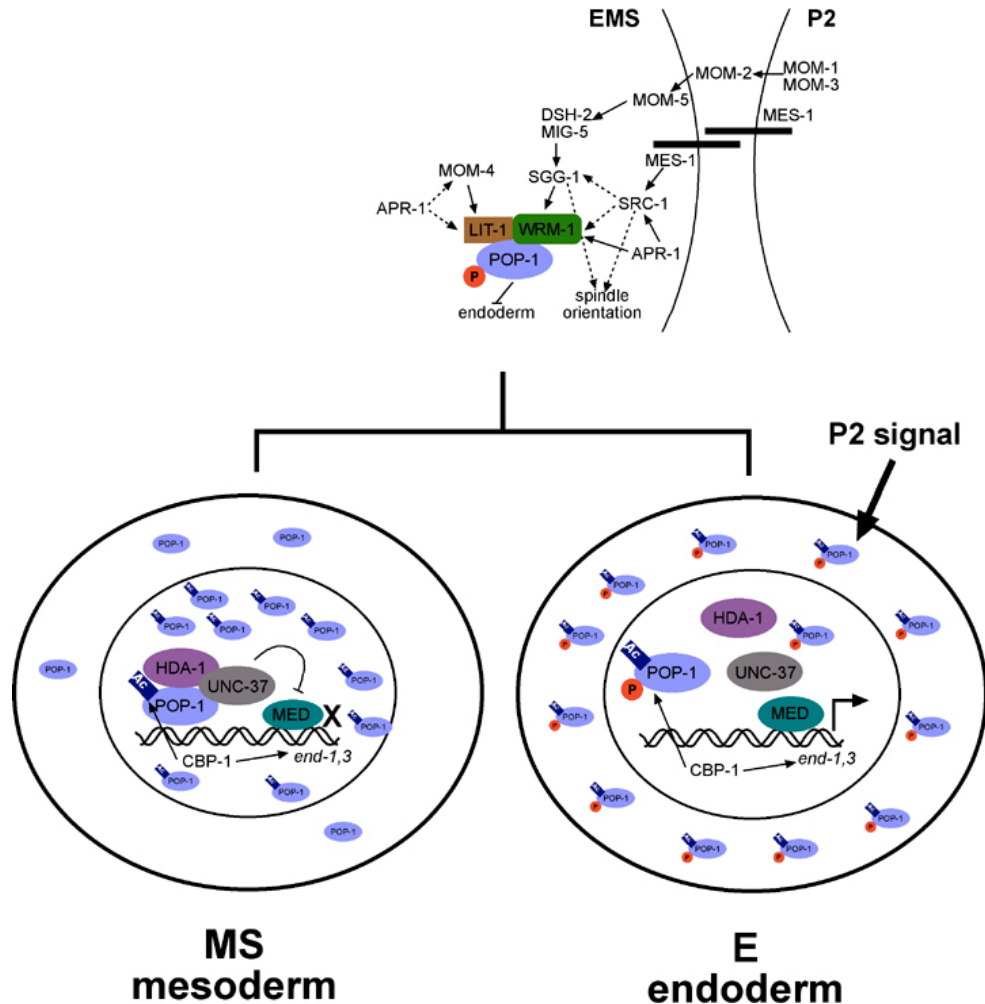
Figure 1. Model of control of polarity in the EMS divisions. Modified from refs. 22 and 27. Model for P2-to-EMS signaling (upper) and subsequent asymmetric division of EMS (lower). Red circles indicate phosphorylation of POP-1, presumably by WRM-1 and LIT-1. Blue rectangles indicate acetylation of POP-1, presumably by CBP-1. CBP-1 may also acetylate histones to modify chromatin structure allowing for *end* gene expression in the E cell. Acetylation promotes nuclear retention of POP-1, thus non-acetylated POP-1 may be found in the cytoplasm of MS. Phosphorylation of POP-1 overrides the acetylation of POP-1 causing redistribution of POP-1 to the cytoplasm in E, thus cytoplasmic POP-1 in E may be both acetylated and phosphorylated. See text for additional details.

that the upstream Wnt pathway mutants showed only a partial loss of P2-to-EMS signaling. Thus, the existence of a parallel pathway, in addition to the LIT-1/MAPK pathway, that also functions to control EMS spindle orientation and endoderm specification, makes sense. This was demonstrated by showing that a *src-1* mutant also genetically interacts with *mom-4* and with *apr-1* mutants. *apr-1* interacts genetically with both the Wnt pathway (19, 23) and *lit-1* (16). *mom-4* also interacts genetically with the Wnt pathway (23, 31). Thus evidence exists for three pathways as well as APR-1; which appears to have some function that is independent of all three pathways. It is not clear exactly where these pathways intersect, although for endoderm specification, it must be upstream of POP-1. There may also be multiple points of interaction among the pathways, leading to the idea that it is more of a network, than separate interacting pathways (23) (Figure 1).

5. WNT-DEPENDENT AND INDEPENDENT CONTROLS ON POP-1 ASYMMETRIC DISTRIBUTION IN OTHER CELLS

POP-1 is asymmetrically distributed to anterior-posterior sister cells at many other divisions during *C. elegans* development (13, 32). What controls POP-1 asymmetry at these divisions? Park and Priess have recently addressed this question by examining the controls over POP-1 asymmetry within the AB cell lineage (33). The two-cell *C. elegans* embryo consists of an anterior blastomere, AB, and a posterior blastomere, P1. AB then divides along a skewed anterior-posterior plane to generate AB.a and AB.p (the AB^2 stage). Next, these cells divide transversely along a left-right plane to generate AB.al, AB.ar, AB.pl and AB.pr (the AB^4 stage). Each of these, and subsequent, AB descendants divide along an anterior-

posterior plane (the AB^8, AB^{16}, AB^{32}, etc. stages). Within the AB lineage, POP-1 is not asymmetrically distributed until the third division (the AB^8 stage) (32). To determine what prevents POP-1 asymmetry during the first two AB divisions and what establishes it at the third, Park and Priess examined POP-1 levels in embryos and cultured embryonic cells. They first used RNAi to inhibit the function of the G-alpha proteins encoded by *goa-1* and *gpa-16* to randomize the EMS division plane (34). They observed that if EMS divided along a transverse (left-right) plane, POP-1 was not asymmetric in the daughter cell nuclei, whereas if EMS divided along the normal anterior-posterior plane, POP-1 was asymmetric. Normally, the AB^2 cells divide transversely, with both cells contacting P2, and there is no POP-1 asymmetry. In similar *goa-1(RNAi); gpa-16(RNAi)* embryos, POP-1 was asymmetric if the AB^2 cells divided along an anterior-posterior axis. Furthermore, the POP-1 asymmetry in these embryos was dependant upon MOM-2/Wnt. Isolated AB^2 cells also divided transversely. If an isolated P2 cell is placed perpendicular to the division axis, which is the normal configuration, POP-1 is not asymmetric in the daughter cell nuclei, as expected. However, if an isolated P2 cell is placed in line with the division axis, POP-1 is asymmetric in the daughter cell nuclei, with the distal daughter having a higher nuclear level of POP-1. Thus, AB^2 cells can respond to polarity signals from P2 (which depend upon MOM-2), but the transverse division plane places both daughters in contact with P2, precluding asymmetric distribution of POP-1 in the daughter cell nuclei. As a result, POP-1 is not asymmetric in the AB^4 cells.

When the AB^4 cells divide to generate the AB^8 cells, POP-1 asymmetry is observed. How is POP-1 asymmetry established at this division? Isolated C, P3 (both P1 descendants), E and to some extent MS cells, are capable of inducing POP-1 asymmetry at the division of the AB^2, AB^4 and AB^8 cells. Furthermore, C and P2 require MOM-2 to induce POP-1 asymmetry, whereas MS does not. Among the multiple cells that are capable of inducing POP-1 asymmetry at the division of the AB^4 cells, blastomere recombination experiments established that P2, E and C have precedence over MS. This suggests that C, P3 and E function together to orient POP-1 asymmetry at the division of the AB^4 cells. In addition, the observation that the AB^8 cells display POP-1 asymmetry in *mom-2* mutants (24, 32) can be explained by a Wnt-independent polarity signal from MS.

After division of the AB^8 cells, the AB and EMS descendants can generate POP-1 asymmetry independent of MOM-2/Wnt signals. This was demonstrated by sequential isolation of embryonic AB daughter cells in culture. POP-1 was not asymmetric at the AB^4 or AB^8 stages, but became asymmetric at the AB^{16} stage. The acquisition of POP-1 asymmetry in these experiments was independent of MOM-2, yet still required MOM-5/Fz. This is reminiscent of planar cell polarity pathways in *Drosophila*, which are dependant upon Frizzled, but for which no Wnt signal has been identified (reviewed in ref. 35). Interestingly, when adjacent AB^{16} cells divided, POP-1 asymmetry was mirror symmetric. Instead of nuclear POP-1 levels being

high/low, high/low, they were low/high, high/low. This is in contrast to what occurs in intact embryos, where the POP-1 asymmetry is always anterior-posterior asymmetric high/low. This suggests the existence of two signaling pathways that generate POP-1 asymmetry, one that is Wnt-dependant and another that is not. However, it is not clear whether all the high/low POP-1 asymmetries that are generated during development (and there are many), do so in response to a Wnt signal, or whether in an intact animal, other cellular behaviors dictate the high/low POP-1 asymmetry independent of Wnt signals.

6. ROLE OF POP-1 IN THE CONTROL OF T CELL POLARITY

Noncanonical Wnt signaling also controls the polarities of the B and T cells that divide asymmetrically in the tail of developing *C. elegans* larvae. Mutations in *lin-44/Wnt* cause the polarities of the B and T cells to be reversed, while mutations in *lin-17 /Fz* cause a loss of polarity in the same cells (review in refs. 6, 8). This suggests that LIN-17 is the LIN-44 receptor. LIN-44 is expressed in the epidermal cells at the tip of the developing tail, posterior to the T cells (36). The difference in polarity effects of *lin-44* and *lin-17* mutants (reversal versus loss of polarity) is curious and suggests the existence of a second Wnt signal that emanates from a source anterior to the T cells that serves to orient the T cell division in the absence of *lin-44* (37). Such an anterior signal has yet to be found, however. Other Wnt pathway components that have been shown to function in the control of T cell polarity include LIT-1 (16), WRM-1 (H. Takeshita and H. Sawa, personal communication) and POP-1 (13). Interfering with the function of these genes cause a loss of T cell polarity, rather than the reversal of polarity observed in *lin-44* mutants. Effects of this pathway may occur through the action of chromatin remodeling complexes as mutations in *egl-27*, which encodes a protein with similarity to a factor isolated as a component of the NURD complex as well as mutations in *psa-1* and *psa-4,* which encode homologs of the SWI/SNF complex all cause a loss of T cell polarity (38, 39). *tlp-1* encodes a zinc-finger protein required for neural cell fates within the T.p and T.ap cell lineages and may be a target gene, as TLP-1 is localized specifically to T.p and responds to LIN-44/Wnt signals (40). The involvement of WRM-1 and LIT-1 suggests that the noncanonical Wnt pathway controlling T cell polarity may bear some resemblance to that involved in endoderm induction. However, the role POP-1 plays in the T cell divisions suggests that there might be some differences.

As in many other anterior-posterior asymmetric cell divisions, the nuclear level of POP-1 is higher in the anterior T cell daughter, T.a, than it is in the posterior daughter, T.p (13). However, in *lin-44* mutants, the nuclear level of POP is lower in T.a, reflecting the reversal of T cell polarity. Surprisingly, inactivation POP-1 by RNAi, mutation or expression of a dominant negative POP-1 construct that lacks the amino-terminal beta-catenin binding site causes a loss of polarity similar to that observed for *lin-17* mutants; the production of two epidermal cells, the anterior cell fate (13, 41). This is
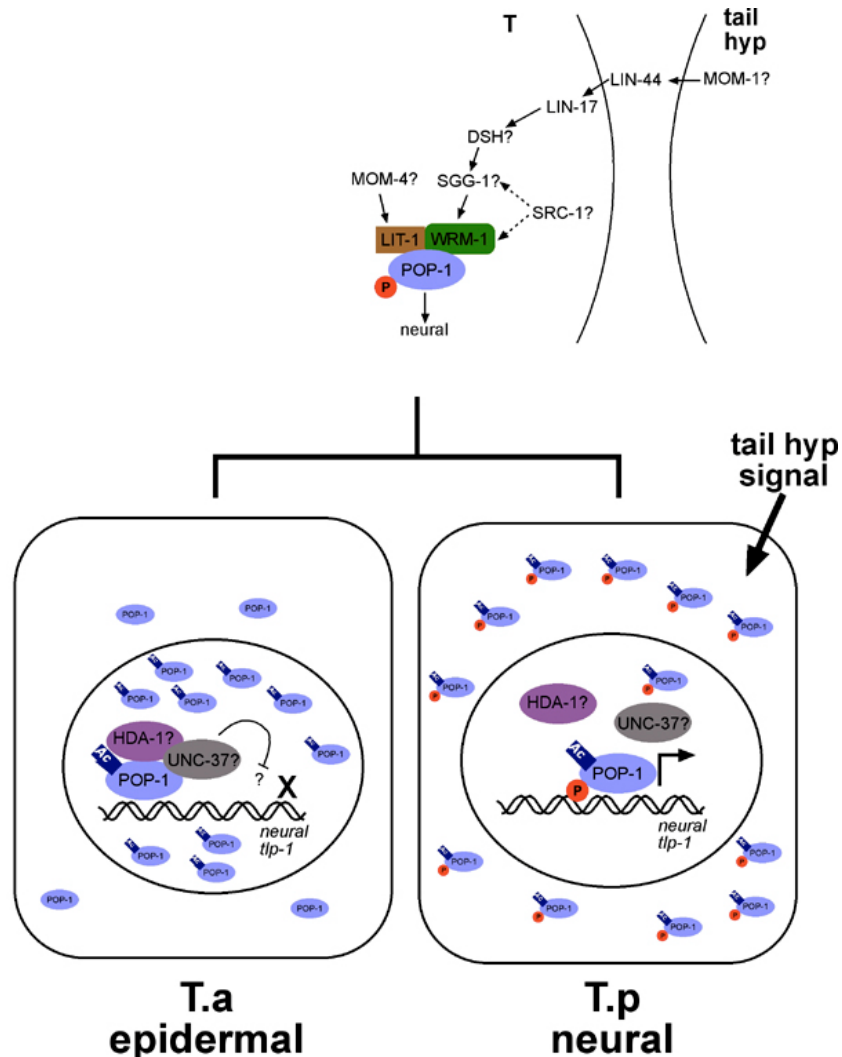
Figure 2. Model for LIN-44 signaling (upper) and control of T cell asymmetric division (lower). Proposed interactions based upon results observed for EMS polarity (see **Figure 1)**. ? indicates that a role for the component has not been reported. MOM-3 and APR-1 do not appear to play a role in T cell polarity (M.A.H., unpublished). See text for additional details.

contrary to what one might expect if POP-1 functions similarly in the EMS and T cell divisions. Loss of POP-1 function in the EMS division leads to the production of two endodermal cells, the posterior cell fate. If the same were true of the T cell division, *pop-1* mutations should lead to the production of two neural cells, the posterior cell fate, rather than two epidermal cells. This suggests that POP-1 may play a positive role in specifying the neural T.p cell fate, despite the lower POP-1 nuclear levels observed in T.p. Similar to what is proposed for endoderm specification, it appears that it is the quality of POP-1, rather than the quantity of POP-1 that is important for determining neural cell fate and establishing cell polarity (13). If POP-1 is phosphorylated by WRM-1 and LIT-1 in T.p, as it is in E, the mechanism for POP-1 nuclear to cytoplasmic redistribution may be similar, while the functional consequence of that modification on cell fate specification may be different (Figure 2). The current challenge is to determine which mechanisms T cell polarity shares with EMS polarity and which are different.

7. OTHER CELL POLARITIES THAT INVOLVE NONCANONICAL WNT SIGNALING

Noncanonical Wnt signaling also controls the asymmetric cell divisions of the Z1 and Z4 cell that generate the somatic gonad. The gonad primordium lies in the center of the animal, the Z1 and Z4 cells flank the two primordial germ cells Z2 and Z3. The hermaphrodite gonad develops an anterior and a posterior arm, each having a proximal-distal axis (reviewed in (42). The polarities of the Z1 and Z4 divisions are oriented along the proximal-distal axis of the gonad. Mutations in *mom-1/Porc, lin-17/Fz, wrm-1/beta-catenin, lit-1/NLK* and *pop-1/Tcf* cause the loss of polarity of the Z1 and Z4 divisions (41). Thus, this pathway is similar to that involved in the control of EMS and T cell polarities. In fact, it may be a bit more like the T cell pathway in that interfering with each of these genes has the same effect on Z1 and Z4 polarities, instead of *pop-1* having the opposite effect as occurs with EMS polarity. It is also not known whether nuclear POP-1

levels are asymmetric at any of these asymmetric divisions, as POP-1 antibodies do not stain the gonads of L1 animals. Finally, the Wnt involved in specifying Z1 and Z4 polarity is also not known, as mutation or interference with each of the *C. elegans wnt* genes did not cause gonad defects. It is possible that two or more of the *C. elegans* Wnts function redundantly, however.

Polarity of the male specific blast cell, B, is also specified by Wnt signaling. In wild-type males the B cell divides asymmetrically generating a larger anterior daughter, B.a, and a smaller posterior daughter, B.p. In addition to the difference in size, these asymmetric daughter cells have different fates, each producing different numbers of progeny. Mutations in *lin-44* cause both the difference in size and subsequent division pattern to be reversed (43). Mutations in *lin-17* cause a loss of polarity with two anterior cell fates being produced (44). It is not yet clear which other Wnt pathway components function to control B cell polarity. However, recent data suggest that components of the PCP pathway might play a role (M.W. and M.A.H., unpublished).

8. CONLUSIONS AND REMAINING QUESTIONS

8.1. EMS spindle effects

Wnt signals that control asymmetric cell divisions and cell polarity affect the mitotic spindle and subsequent asymmetric cell fate determination. In the EMS blastomere, the spindle must rotate for the asymmetric division to occur. The rotation of the EMS spindle is controlled by Wnt pathway components upstream of SGG-1 that appear to function in parallel with a SRC-like pathway (23). The target of signals that control spindle rotation may be the cytoskeleton, perhaps mediated by Rho-like GTPases (23). This is reminiscent of PCP pathways that control cell polarity in the *Drosophila* wing that also appear to function through regulating Rho-like GTPases (review in ref., 35). Spindle rotation does not appear to be involved in the asymmetric division of the T, Z1 and Z4 cells, thus Wnt effects on the spindle are not absolutely required for asymmetric cell divisions and may be unique to the EMS division. However, displacement of the mitotic spindle must be important for asymmetric divisions that generate daughters of different sizes such as the division of the B cell and certain other asymmetric divisions that occur within the T cell lineage (43).

8.2. POP-1 and asymmetric cell fate determination

POP-1/Tcf is involved in most, if not all, Wnt signaling pathways in *C. elegans*. In particular, the noncanonical Wnt pathways that control cell polarities and asymmetric cell fate determination during *C. elegans* development converge upon POP-1, leading to modifications that affect the function and cellular distribution of POP-1. One important modification is acetylation, which affects the nuclear-cytoplasmic distribution of POP-1 by promoting nuclear retention. Acetylation is also important for POP-1 function, as a mutant POP-1 protein that cannot be acetylated is unable to repress expression of endodermal genes in the mesodermal

precursor cell MS. However, the mechanism by which acetylation affects POP-1 function is not clear. Perhaps POP-1 must be acetylated to interact properly with HDA-1/HDAC and UNC-37/Groucho in order to inhibit MED induced expression of the *end* genes in MS (Figure 1). Other unidentified modifications may also prove to be important for POP-1 function in MS.

Of particular interest are the mechanisms that lead to the anterior-posterior difference in nuclear POP-1 levels between sisters cells of an asymmetric cell division. This appears to occur by affecting the nuclear-to-cytoplasmic ratios of POP-1 without affecting total cellular POP-1 levels (28), suggesting that degradation of POP-1 is not involved as was previously thought. What the mechanisms might regulate the differences in the nuclear-to-cytoplasmic POP-1 distribution in sister cells? POP-1 acetylation can affect its nuclear-to-cytoplasmic distribution, however there is no evidence for differential POP-1 acetylation between asymmetric sister cells. Wnt/MAPK/Src signaling can, however, override the influence of acetylation and cause a redistribution of POP-1 to the cytoplasm. This appears to occur by modification of POP-1, presumably phosphorylation by WRM-1 and LIT-1, without affecting the acetylation state of POP-1. How does the redistribution of POP-1 from the nucleus to the cytoplasm occur? Some evidence indicates that redistribution only occurs after cell division, suggesting that modified POP-1 is excluded from the reforming nucleus in the signaled cell (28). However, nuclear export may also be involved. In either case, it is of interest to identify the proteins and mechanisms responsible for the exclusion or export of modified POP-1 from the nucleus, as well as to determine the role of SRC-1 signaling. Specifically, it is not yet clear exactly where the Wnt/MAPK and Src pathways intersect and how they interact.

Later in embryonic development, unidentified Wnt-independent mechanisms of POP-1 asymmetric nuclear distribution take over. It appears that MOM-5/Fz may play role, suggesting parallels with the PCP pathway (33). Much later in development, during the first larval stage, the asymmetric distribution of POP-1 nuclear levels again comes under the control of Wnt signals that control the polarity of the T cell divisions. What governs the transition from Wnt-independence to Wnt-dependence? The Wnt pathway that controls T cell polarity bears some resemblance to the EMS polarity pathway in that WRM-1 and LIT-1 are involved. It is not known whether SRC-1 signaling is also involved. Although this seems unlikely as, in contrast to EMS polarity where mutations in the upstream components *mom-2/Wnt* and *mom-5/Fz* cause a only a partial loss of cell polarity, mutations in *lin-44/Wnt* and *lin-17/Fz* cause highly penetrant, cell polarity defects. Thus in the T cell division, there does not seem to be a need for a parallel signaling pathway (Figure 2).

8.3. On the nature of POP-1 Quality

The functional consequence for the asymmetric distribution of nuclear POP-1 is still not clear. For example, it appears that it is not the difference in quantity of POP-1 between the MS and E cells that is important for endoderm induction, but instead the difference in quality of

POP-1 in each cell (28). For the MS cell, POP-1 has a quality that allows it to interact with HDA-1/HDAC and UNC-37/Groucho to inhibit MED induced expression of the *end* genes. This quality may be related to the observation of puncta of POP-1 accumulation in the MS nucleus. POP-1 puncta are also observed in other anterior sisters of asymmetric divisions during embryonic development, but they may not be involved in all anterior asymmetric sisters. For the E cell, POP-1 has a quality that allows it *not* to interact with the MEDs, which allows *end* gene expression and endoderm fate. This quality is likely to be the phosphorylation of POP-1 by WRM-1 and LIT-1. Perhaps phosphorylated POP-1 cannot interact with and inhibit the MEDs , allowing for *end* gene expression. Interestingly, the same quality of POP-1 may also lead to its redistribution to the cytoplasm. However, we will not know for sure until the sites on POP-1 that are phosphorylated are identified and the functional consequence of each phosphorylation determined. For the T cell division, POP-1 quality in the posterior daughter, T.p may have consequences different from that in the E cell. In the EMS cell division, the effect of removing POP-1 function on cell fate is the same as modifying and lowering the nuclear level of POP-1; the posterior endodermal cell fate is produced. In the T cell division, the effect of removing POP-1 function on cell fate is different than modifying and lowering the nuclear level of POP-1: the posterior T.p cell has a low nuclear level of POP-1 and generates neural cells whereas removal of POP-1 function causes the anterior epidermal cell fate to be produced. This suggests that POP-1 may have a quality that allows it to play a positive role in specifying the neural cell fate. Is the quality of POP-1 in E and T.p similar? If so, how can the consequences be different? If not, what are the differences in POP-1 qualities in these two cells and how are they generated? Finally, observations that the difference in POP-1 nuclear quantity plays little functional role in the specification of asymmetric cell fates, yet exists for many asymmetric cell divisions, remains a major mystery to be resolved by further study. Hopefully, workers in this field will have a better go at defining quality than did Phædrus, who found that although "there is such a thing as Quality, but that as soon as you try to define it, something goes haywire. You can't do it." (1).

9. ACKNOWLEDGEMENTS

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