

# CHAPTER 12

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## Wnt Signaling in *C. elegans*

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### Abstract

The Wnt signaling pathway controls many aspects of *Caenorhabditis elegans* development. A canonical Wnt pathway functions to control cell migrations and determine certain cell fates. Noncanonical Wnt pathways function primarily to control the polarity of specific cells. *C. elegans* shares the involvement of canonical and noncanonical Wnt pathways in various developmental processes with many other animals. However, other aspects of Wnt signaling appear to be unique to *C. elegans*. This chapter will review the major process in *C. elegans* development that involve Wnt signaling, discuss the interactions of Wnt pathway components involved in each process and compare and contrast Wnt signaling pathways in the worm with those found in other species.

### Introduction

Reading this book, or perusing the literature, should leave one with the impression that Wnt signaling controls many aspects of the development of most animals. The same is true of the development of the nematode *Caenorhabditis elegans*. Like most animals, *C. elegans* has developmental processes that are controlled by both canonical and noncanonical Wnt signaling pathways (see Chapter 1). These processes include the control of cell polarity, cell migrations and cell fate determination involved in the generation of multiple organ systems.

During *C. elegans* embryogenesis blastomeres divide to generate cells of all the major organ systems. Cell migration and other morphogenic events occur so that at hatching the worm body plan is essentially complete. Development then proceeds through four larval stages, each separated by a molt during which the animal sheds its cuticle. Postembryonic larval development is characterized by additional cell divisions, cell migrations and inductions, some of which differentiate the two sexes: self-fertile hermaphrodites and males. Approximately 64 hours after fertilization the life cycle is complete and the worm begins to reproduce. All of the cellular and developmental processes can be easily monitored because the animal is transparent; which allowed the determination of the complete cell lineage.<sup>1,2</sup> Although the cell lineage is essentially invariant, cell-cell interactions are involved in many developmental processes, many of which involve Wnt signaling. This chapter will review the major processes in which Wnt signaling is involved and discuss what we know about the components that are used in each signaling event (Tables 1 and 2).

### Catalog of *C. elegans* Wnt Pathway Components

PCR screens for Wnt-1 homologs identified the first *C. elegans* Wnt genes *cwn-1* and *cwn-2*.<sup>3,4</sup> Genetic screens for mutations that affected particular developmental processes identified

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**Table 1. *C. elegans* Wnt pathway parts list**

Component	Number of Orthologs in: <i>Drosophila</i>	Human	<i>C. elegans</i> Orthologs	Function Determined by: Mutation	RNAi
Porcupine	1	0	<i>mom-1</i>	X	X
Wnt	7	19	<i>mom-2</i> <i>lin-44</i> <i>egl-20</i> <i>cwn-1</i> <i>cwn-2</i>	X X X X	
FrzB	0	5	X73B6BL.21		
Frizzled	4	12	<i>mom-5</i> <i>lin-17</i> <i>mig-1</i> <i>CFz2</i>	X X X	X
Dishevelled	1	3	<i>mig-5</i> <i>dsh-1</i> (C34F11.9) <i>dsh-2</i> (C27A2.6)	X X	X
Casein Kinase I	1	1	<i>kin-19</i>		X
GSK-3	1	1	<i>sgg-1</i> C44H4.6		X
Axin	1	2	<i>pry-1</i>	X	
APC	2	2	<i>apr-1</i>	X	X
$\beta$ -catenin	1	2	<i>wrm-1</i> <i>bar-1</i> <i>hmp-2</i>	X X X	X X X
TCF/LEF	1	3	<i>pop-1</i>	X	X
Groucho	1	4	<i>unc-37</i>	X	X
NLK	1	1	<i>lit-1</i>	X	X
TAK1	1	1	<i>mom-4</i>	X	X
?	?	?	<i>mig-14/mom-3</i>	X	

Orthologs of Wnt pathway components not found in *C. elegans*: Cerberus, WIF, Dickkopf, GBP/Frat, Naked. Numbers of *Drosophila*, Human and *C. elegans* orthologs from Ruvkun<sup>10</sup>, the Wnt gene Home Page (<http://www.stanford.edu/~rnusse/wntwindow.html>) or determined by querying WormPD and HumanPSD databases (Proteome, Inc). Orthologs of Wnt pathway components found in *C. elegans*, but for which a Wnt-related function has not been reported: LRP/Arrow, proteoglycans, Phosphatase PP2C, PAR-1, Slimb/ $\beta$ TrCP (*lin-23*). X, indicates whether a component's function was determined by mutation, RNAi, or both.

many other genes encoding *C. elegans* Wnt signaling components. The first gene found in this way was *lin-44*, which was identified by mutations that affected the orientation of certain asymmetrically dividing cells in the tail to the body axis.<sup>5,6</sup> The identification of other components involved in embryonic endoderm induction by both genetic and sequence-based methods soon followed.<sup>7,8</sup>

The completion of the *C. elegans* genome sequence has allowed the complete cataloging of the *C. elegans* Wnt pathway component orthologs.<sup>9,10</sup> Those that have been identified are listed in Table 1. Briefly, *C. elegans* has five Wnts, compared to seven in *Drosophila* and 19 in Humans; four Frizzleds (Fz), as does *Drosophila*, but compared to 12 in humans; and three Dishevelleds (Dsh) compared to one in *Drosophila* and three also in Humans. Comparisons for

Table 2. Summary of *C. elegans* Wnt controlled processes and the components involved

Canonical Component	QL-d Migration	Endoderm Induction	Spindle Orientation	P12 Cell Fate	VPC Fate	T Cell Polarity	Gonad Polarity	V5 Polarity	Ray Formation
<b>Porcupine</b>									
<b>Wnt</b>	EGL-20 <sup>25</sup>	MOM-1 <sup>7,8</sup>	MOM-1 <sup>40</sup>	LIN-44 <sup>5,5</sup>	MOM-1 <sup>†</sup>	LIN-44 <sup>5,6</sup>	MOM-1 <sup>77</sup>	EGL-20 <sup>78</sup>	EGL-20 <sup>90</sup>
<b>Frizzled</b>	LIN-17 <sup>25</sup>	MOM-2 <sup>7,8</sup>	MOM-2 <sup>40</sup>	LIN-17 <sup>5,5</sup>		LIN-17 <sup>5,7,6</sup>	none <sup>77</sup>	LIN-17 <sup>78</sup>	LIN-17 <sup>90</sup>
	MIG-1*	MOM-5 <sup>7,8</sup>	MOM-5 <sup>40</sup>				LIN-17 <sup>5,7,7</sup>		
<b>Disheveled</b>	MIG-5 <sup>24</sup>	MIG-5 <sup>42</sup>		MIG-5 <sup>24</sup>	MIG-5 <sup>24</sup>				
<b>GSK-3</b>	SGG-1 <sup>11</sup>	DSH-2							
<b>Casein Kinase I</b>		SGG-1 <sup>40</sup>	SGG-1 <sup>40</sup>						
<b>Axin</b>	PRY-1 <sup>40,11</sup>	KIN-19 <sup>41</sup>		PRY-1 <sup>†</sup>	PRY-1 <sup>72</sup>	no**		PRY-1 <sup>78</sup>	
<b>APC</b>		APR-1 <sup>7</sup>			APR-1 <sup>72,73</sup>				
<b>β-catenin</b>	BAR-1 <sup>26</sup>	WRM-1 <sup>7</sup>		BAR-1 <sup>28</sup>	BAR-1 <sup>71</sup>	none <sup>27</sup>	WRM-1 <sup>77</sup>		BAR-1 <sup>90</sup>
<b>TCF/LEF</b>	POP-1 <sup>17,27</sup>	POP-1 <sup>35</sup>			POP-1 <sup>35,72</sup>	POP-1 <sup>27</sup>	POP-1 <sup>77</sup>		
<b>target gene</b>	<i>mab-5</i> <sup>22</sup>	<i>end-1</i> <sup>44</sup>		<i>egl-5</i> <sup>5</sup>	<i>lin-39</i> <sup>1,72</sup>	<i>tlp-1</i> <sup>81</sup>			<i>mab-5</i> <sup>90</sup>
<b>Noncanonical Component</b>									
<b>NLK</b>		LIT-1 <sup>19,36,37</sup>		no <sup>†</sup>	LIT-1 <sup>19</sup>	LIT-1 <sup>77</sup>			
<b>TAK1</b>		MOM-4 <sup>8,37</sup>					no <sup>77</sup>		
<b>MIG-14/MOM-3</b>	yes <sup>25</sup>	yes <sup>8</sup>	yes <sup>40</sup>	yes <sup>28</sup>	yes <sup>28</sup>	no**		yes <sup>78</sup>	

Genes and the proteins involved in the major processes that are controlled by, or involve, Wnt signaling are listed. References that provide evidence for the involvement of each component are indicated. \*S. Clark, personal communication, \*\*M.H. unpublished, †D. Eisenmann, personal communication. If a protein is named, it has been shown to be involved in the process listed in that column. "no" indicates that a function for a particular component was assayed, but found not to be involved. In the case of MIG-14/MOM-3, "yes" indicates that it is involved in the process listed in that column. A blank indicates that the involvement of the component in the process listed has not been assayed. Modified from <http://www.stanford.edu/~russe/pathways/wormsignal.htm>, original by D. Eisenmann.

other components are listed in Table 1. Interestingly, *C. elegans* has three  $\beta$ -catenins compared to only one in *Drosophila* and two in humans. *C. elegans* orthologs were not found for several Wnt pathway components identified in other animals. Like *Drosophila*, *C. elegans* does not appear to employ Wnt inhibitors such as Cerberus, WIF, Dickkopf or sFRPs (R. Nusse, World Wide Wnt Window: <http://www.stanford.edu/~rnusse/pathways/pathwleg.html>). However, an apparent FrzB homolog, Y73B6BL.21, is present in the *C. elegans* genome; although its function has not yet been determined. The *C. elegans* genome also does not appear to contain an LRP/Arrow ortholog that functions in Wnt signaling, suggesting Wnts in *C. elegans* bind to Frizzled receptors without the aid of a co-receptor. In addition, orthologs of GBP/Frat and Naked have not been identified in *C. elegans*. However it should be noted that standard BLAST analyses did not identify an Axin homolog, although one exists.<sup>11</sup> Further, although *apr-1/APC*, *pry-1/Axin*, *bar-1/ $\beta$ -catenin* and *pop-1/TCF* are functional orthologs of Wnt pathway components, they are only distantly related to their *Drosophila* and vertebrate counterparts. This suggests that more sophisticated database searches may identify additional *C. elegans* orthologs of Wnt pathway components. A Wnt related function for some *C. elegans* orthologs of Wnt pathway components has not been reported. For example, a role for proteoglycans in *C. elegans* Wnt signaling has not been reported. In addition, *C. elegans* homologs of Phosphatase PP2C which binds Dishevelled and can dephosphorylate Axin in other animals<sup>12</sup> exist, but have not been reported to play a role in Wnt signaling. PAR-1 acts as a positive regulator of Wnt signaling in several animals<sup>13</sup> and functions in establishing the anteroposterior polarity of the *C. elegans* zygote,<sup>14</sup> but its function in *C. elegans* Wnt signaling events has not been established. *lin-23*, the *C. elegans* ortholog of Slimb/ $\beta$ TrCP which functions in the degradation of  $\beta$ -catenin in other animals,<sup>15</sup> is a negative regulator of cell division that functions throughout *C. elegans* development<sup>16</sup> but appears to have no role in Wnt signaling. Thus the *C. elegans* genome contains many, but not all, of the Wnt signaling components found in other animals. Finally, several novel Wnt signaling components have been identified by genetic analyses in *C. elegans*.

### The Three *C. elegans* $\beta$ -Catenins Are Involved in Distinct Processes

Curiously, *C. elegans* has three  $\beta$ -catenin homologs whereas a single protein in *Drosophila* performs both cell adhesion and Wnt signaling functions of  $\beta$ -catenin, and vertebrates have two proteins,  $\beta$ -catenin and plakoglobin, which have partially overlapping roles. Study of the interactions of the three *C. elegans*  $\beta$ -catenin homologs with POP-1/TCF revealed that the *C. elegans*  $\beta$ -catenins have divergent functions.<sup>17</sup> First, it was established that POP-1 binds to a consensus TCF target sequence, indicating that POP-1 can behave like a canonical TCF. In addition, in mammalian tissue culture cells, POP-1 can complex with the *Drosophila*  $\beta$ -catenin, Armadillo, and activate transcription of a reporter gene containing several upstream TCF binding sites. BAR-1 is the only  $\beta$ -catenin homolog that interacts strongly with POP-1. When expressed together, BAR-1 and POP-1 form a complex that can activate transcription of a TCF-reporter gene. An amino-terminally deleted version of POP-1 that removed the putative  $\beta$ -catenin interaction domain,  $\Delta$ N-POP-1, did not interact with BAR-1,<sup>17,18</sup> did not activate a TCF-reporter gene and behaved as a dominant negative protein in phenotypic assays. Thus like other TCF proteins, POP-1 interacts with a  $\beta$ -catenin via an amino-terminal domain.

Weak interactions between WRM-1/ $\beta$ -catenin and POP-1/TCF in yeast hybrid assays have been reported,<sup>18,19</sup> but stable interactions were not observed in co-immunoprecipitation assays.<sup>17</sup> Furthermore, WRM-1 also interacted weakly with  $\Delta$ N-POP-1, suggesting that WRM-1 interacts with POP-1 in a manner different from BAR-1 and canonical  $\beta$ -catenin-TCF interactions. The third *C. elegans*  $\beta$ -catenin homolog, HMP-2, does not interact with POP-1 in any assay, but does interact with the cadherin homolog HMR-1 and the  $\alpha$ -catenin HMP-1.<sup>17,18</sup>

One interesting conclusion from this work is that the functions of the *C. elegans*  $\beta$ -catenins in adhesion and in signaling are performed by separate proteins. This is a major difference between *C. elegans* and other systems. Of the three *C. elegans*  $\beta$ -catenins, BAR-1 appears to function in signaling pathways as a canonical  $\beta$ -catenin. In addition, of the two  $\beta$ -catenins involved in signaling, BAR-1 is involved in canonical  $\beta$ -catenin signaling whereas WRM-1 is involved in unusual Wnt signaling pathways (see below).

Why might the functions of the  $\beta$ -catenin orthologs be separated among different processes in *C. elegans*? It should be noted that these proteins are quite divergent; the Hydra  $\beta$ -catenin ortholog<sup>20</sup> is more similar to vertebrate  $\beta$ -catenins that are the *C. elegans* orthologs. Perhaps this arrangement is unique to nematodes, although why this might be is unclear. One possibility is that this arrangement separates the adhesion, canonical signaling and non-canonical signaling,  $\beta$ -catenin pools to prevent pathway cross-talk.

## A Canonical Wnt Pathway Controls the Migration of the Descendants of the QL Neuroblast

QL and QR are left/right homologous neuroblasts that migrate and each generate three neurons and two cells that undergo programmed cell death during the first larval stage (L1). The QR cell and its descendants (known as the QR.d) migrate anteriorly, whereas the QL neuroblast and its descendants (known as the QL.d) migrate posteriorly and divide. The posterior QL daughter, QL.p, stops migrating and generates two neurons, and a cell that undergoes programmed cell death. The anterior QL daughter, QL.a, continues a posterior migration and divides to generate a cell that undergoes programmed cell death and a neuron, which continues to migrate posteriorly until it reaches its characteristic position in the tail (Fig. 1).<sup>1</sup> The direction Q cell descendants migrate is controlled by the Hox gene *mab-5*. Cells that express *mab-5* migrate posteriorly, whereas cells that do not, migrate anteriorly. Ectopic expression of *mab-5* in both the QL.d and QR.d descendants caused them to migrate posteriorly, just as the QL.d do in wild-type animals.<sup>21,22</sup> Loss of *mab-5* expression (as in *mab-5* mutants) cause the QL.d and QR.d to migrate anteriorly.<sup>22,23</sup>

A canonical Wnt signaling pathway controls the migration of the QL.d by regulating the expression of *mab-5*.<sup>17,24-27</sup> Mutations in *egl-20/Wnt*, *lin-17/Fz*, *mig-5/Dsh* and *bar-1/ $\beta$ -catenin*, all cause the loss of *mab-5* expression in the QL lineage and anterior migration of the QL.d. The inhibition of *pop-1/TCF* function by RNAi also causes anterior migration of the QL.d, as well as loss of *mab-5* expression in the QL lineage<sup>27</sup> as does the expression of the dominant negative  $\Delta$ N-POP-1 construct (see below).<sup>17</sup> Furthermore, overexpression of *pop-1* also causes anterior migration of the QL.d, antagonizing Wnt signaling in a manner similar to observations of TCFs in other Wnt pathways.<sup>27</sup> In addition, mutations in *pry-1* cause posterior migration of the QR.d and the ectopic expression of *mab-5* in the QR lineage, suggesting that it functions as a negative regulator of the Wnt pathway.<sup>26</sup> Genetic analysis demonstrated that *pry-1* functions downstream of *egl-20/Wnt* and upstream of *bar-1/ $\beta$ -catenin*, *pop-1* and *mab-5*; and it was recently shown that *pry-1* encodes the *C. elegans* Axin homolog.<sup>11,26</sup> Overexpression of *pry-1* and *sgg-1*, the *C. elegans* GSK-3 ortholog, caused anterior migration of the QL.d, indicating that they function negatively in the pathway. Finally, PRY-1 interacts with BAR-1, SGG-1, APR-1/APC and MIG-5/Dsh in a canonical manner in a yeast two-hybrid assay.<sup>11</sup> Although a role for APR-1 in QL.d migration has not been established, it appears to function negatively in vulval development (see below). Taken together, current evidence indicates that the Wnt pathway that controls QL.d migration and *mab-5* expression is a canonical Wnt pathway (Table 2, Fig. 9). In addition to these components, *mig-14* mutants also cause anterior migrations of the QL.d as well as a loss of *mab-5* expression, suggesting that MIG-14 functions in the canonical Wnt pathway.<sup>25,28</sup> The molecular identity of MIG-14 has not yet been deter-

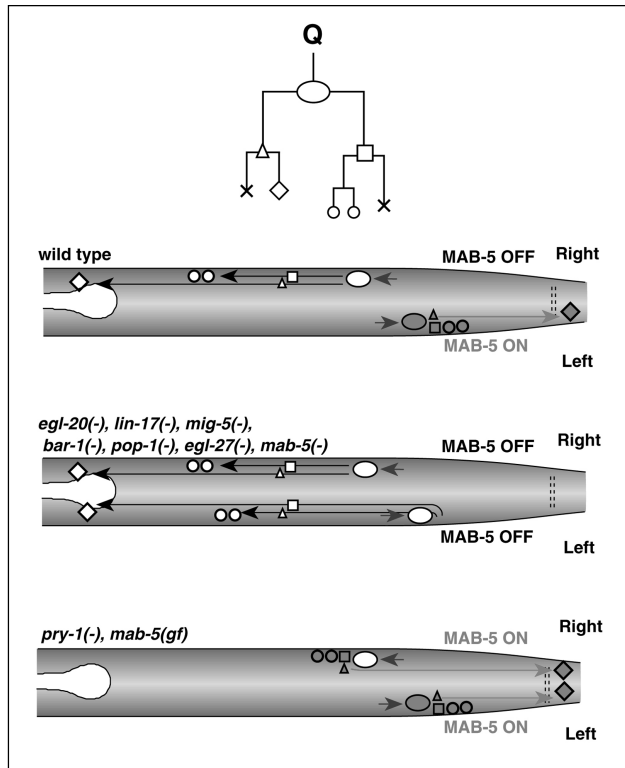


Figure 1. A canonical Wnt pathway controls QL.d migration. The Q neuroblast lineage is shown on top. Both QL and QR (ovals) divide to generate three neurons (diamond and circles) and two cells that undergo programmed cell death (X). Migrations of the QL.d and QR.d in wild-type and mutant animals are shown below. A dorsal view is depicted so that both left and right sides are visible. Soon after hatching QL begins to migrate posteriorly, expresses *mab-5* (shaded) and divides. The QL.d continue to express *mab-5* and migrate posteriorly. Soon after hatching, QR begins to migrate anteriorly and divides. The QR.d do not express *mab-5* and continue to migrate anteriorly. Final positions of the QL.d and QR.d are indicated. In *egl-20*, *lin-17*, *mig-5*, *bar-1*, *pop-1*, *egl-27* and *mab-5* mutants, QL migrates posteriorly, but does not express *mab-5*. Subsequently, the QL.d do not express *mab-5* and migrate anteriorly. In *pry-1* and *mab-5* mutants QL, QR and their descendants all express *mab-5* and migrate posteriorly. After Whangbo, J and Kenyon, C. (1999) A Wnt signaling system that specifies two patterns of cell migration in *C. elegans*. *Mol Cell*. 4: 851-8, with permission from Elsevier Science

mined. This should turn out to be an important discovery as, *mom-3* mutations, which also have defects in endoderm specification and spindle orientation (see below), were shown to be allelic to *mig-14* mutations; indicating that this unknown protein may function more generally in Wnt signaling. I will subsequently refer to this protein as MIG-14/MOM-3.

Another gene that functions in the control of QL.d migration is *egl-27*. Mutations in *egl-27* also cause the anterior migration of the QL.d as well as the loss of *mab-5* expression in the QL lineage.<sup>29</sup> EGL-27 contains a region of similarity to MTA, a factor overexpressed in metastatic cells.<sup>30</sup> Further analysis of the EGL-27 sequence has revealed several regions of similarity to proteins involved in chromatin remodeling.<sup>31</sup> MTA1 has been shown to be a subunit of the NURD complex that displays ATP-dependant chromatin-remodeling and histone

deacetylase activities.<sup>32</sup> Although *egl-27* appears to function upstream of *mab-5*, it is not clear whether it functions upstream, downstream or in parallel with *pop-1*.

## Embryonic Endoderm Introduction

At the four-cell stage of *C. elegans* embryogenesis, the posterior-most blastomere, P<sub>2</sub>, polarizes its anterior neighbor, EMS, and induces it to form endoderm (Fig. 2). After reorienting its spindle (see below), the EMS blastomere subsequently divides to generate the MS cell, which generates mesoderm, and the E cell, that generates all the endoderm, or intestine, of the animal. Blastomere isolation and reconstitution experiments demonstrated that the P<sub>2</sub> blastomere induces the EMS blastomere to generate the E cell fate<sup>33</sup> and that the position of contact between P<sub>2</sub> and EMS establishes which side of the EMS cell is partitioned in the E cell.<sup>34</sup>

The nature of the P<sub>2</sub>-to-EMS signal was revealed by genetic screens that identified several maternally expressed genes required for differentiated E and MS cell fates.<sup>7,8,35,36</sup> These screens identified five *mom* genes (for *more* mesoderm), *lit-1* (for *loss of intestine*) and *pop-1* (for *posterior pharynx* defective). Mutations in the *mom* genes and in *lit-1* cause the EMS blastomere to generate two MS-like daughter blastomeres, thus more mesoderm is produced and endodermal cell fates are lost. Mutations in *pop-1* cause the opposite effect, the EMS blastomere generates two E-like blastomeres, thus mesodermal fates (including the posterior pharynx) are lost and excess endodermal cell fates are produced. Three of the *mom* genes and *pop-1* encode components of a Wnt pathway: *mom-1* encodes the *C. elegans* Porcupine homolog, *mom-2* encodes a Wnt, *mom-5* encodes a Frizzled homolog and *pop-1* encodes the TCF/LEF homolog<sup>7,8,35</sup> (Tables 1 and 2). Blastomere isolation and recombination experiments demonstrated that *mom-1*, *mom-2* and *mig-14/mom-3* function in the signaling cell, P<sub>2</sub>, whereas *mom-4* functions in the receiving cell EMS.<sup>8</sup> This makes sense for *mom-1/Porc* and *mom-2/Wnt* and suggests that *mig-14/mom-3* functions in expression or secretion of the Wnt signal. The genes *mom-4* and *lit-1* encode components of a mitogen-activated protein kinase (MAPK) pathway: *mom-4* encodes a MAPK kinase related to vertebrate TAK1 and *lit-1* encodes a MAPK-like protein that is 70% identical to the *Drosophila* tissue-polarity gene *nemo* and its vertebrate homolog nemo-like kinase (NLK).<sup>19,37,38</sup> This MAPK pathway functions with the Wnt pathway to downregulate POP-1 protein levels (see below). As mentioned above, the molecular identity of *mig-14/mom-3* has yet to be determined.

Other Wnt pathway genes required for embryonic endoderm induction in *C. elegans* were identified by sequence similarity and their roles in endoderm induction were revealed by RNA-mediated interference (RNAi) experiments.<sup>39</sup> *wrm-1/β-catenin* (for wormadillo), *apr-1/APC* (for APC-related), *sgg-1/GSK-3* (for shaggy-like) and *kin-19* (a homolog of casein kinase I) were all shown to be required for endoderm induction by RNAi.<sup>7,40,41</sup> However, one cautionary note is required here: with the exception of *wrm-1*, *lit-1* and *pop-1*, mutation or RNAi of any of these genes only produces partially penetrant defects in endoderm specification that ranges from only 2–5% for *mom-5* to 88% for *mom-2*.<sup>7,8,40</sup> Genetic redundancies exist for several components (*mom-2*, *mom-5*, *sgg-1* and possibly *mom-4*), which might explain why mutations in these genes do not cause fully penetrant defects. Along these lines, it is not yet known whether any of the three *C. elegans* Dishevelled proteins is involved in endoderm induction. However, *mig-5* and *dsh-2* (C27A2.6) messages are enriched in oocytes, as are the other Wnt components involved in endoderm induction,<sup>42</sup> suggesting that they might function together in endoderm induction. Genetic redundancies do not appear to exist for other components (*apr-1* and *mom-1*) that do not have fully penetrant Mom defects, however. The low penetrance Mom defects observed in several *mom* mutants might be caused by the existence of a redundant pathway that specifies endoderm development.

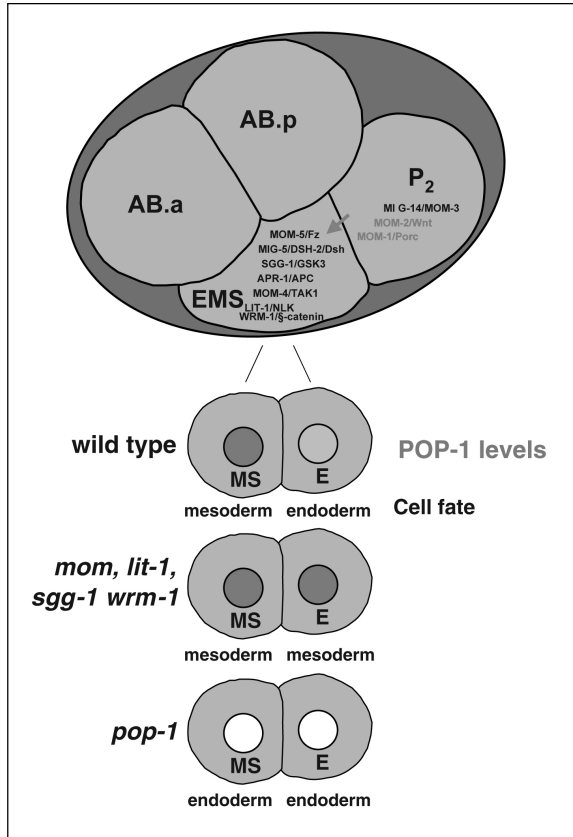


Figure 2. Endoderm induction. In the four-cell *C. elegans* embryo a Wnt signal from the P<sub>2</sub> blastomere polarizes the EMS blastomere and induces endoderm fate. Genes are listed inside the blastomere in which they have been shown to function (top). In wild type embryos, this results in a high level of POP-1 protein in the anterior MS blastomere nucleus and a low level in the E blastomere nucleus. Effects of mutations or RNAi on POP-1 localization and EMS daughter fates are indicated (bottom). See text for details.

### Unusual Aspects of Wnt Signaling during Endoderm Induction

In the absence of MOM-2/Wnt signal, POP-1/TCF appears to repress the E cell fate. The level of POP-1 has been shown to be higher in the anterior MS cell than in the posterior E cell, which receives a Wnt signal and in *pop-1* mutants both daughters adopt the E cell fate (Fig. 2).<sup>35,43</sup> Thus endoderm induction appears to be achieved by inhibition of a repressor. In the MS cell POP-1 is required to repress endoderm fate. At least for the endoderm-specific GATA-like transcription factor gene, *end-1*,<sup>44</sup> this appears to be achieved by POP-1 functioning to recruit a complex of the histone deacetylase (HDAC) HDA-1 and UNC-37/Groucho.<sup>45</sup> In this respect, POP-1 functions as a canonical TCF/LEF factor by recruiting HDAC and Groucho to function as corepressors in the absence of a Wnt signal.<sup>46,47</sup> Interestingly, the *C. elegans* p300 homolog CBP-1 is required positively in the E cell for *end-1* transcription and endoderm fate.<sup>45,48</sup> Although in mammalian cells CBP/p300 can interact with  $\beta$ -catenin/TCF to activate transcription,<sup>49</sup> neither POP-1 nor WRM-1/ $\beta$ -catenin has been reported to interact with CBP-1 in *C. elegans*. The involvement of the MAPK pathway components MOM-4/TAK

and LIT-1/NLK make this pathway unusual. In the E cell, the Wnt and MAPK components function together to repress POP-1; although exactly how this is achieved is not yet clear. In vertebrate cells NLK has been shown to phosphorylate TCF-4, which interfered with DNA binding by the TCF-4/ $\beta$ -catenin complex.<sup>38</sup> The *C. elegans* proteins, WRM-1 and LIT-1 have been shown to interact in tissue culture cells and together can phosphorylate POP-1. Thus Wnt and MAPK pathways might collaborate to phosphorylate POP-1, which could lead to its degradation, accounting for the repression of POP-1 function. However, it is unlikely that POP-1 and WRM-1 form a complex that binds DNA as their vertebrate counterparts do, as WRM-1 and POP-1 have only been shown to interact weakly in yeast two-hybrid assays<sup>18,19</sup> and failed to interact in a co-immunoprecipitation assay.<sup>17</sup> Interestingly, when POP-1 is expressed alone in vertebrate COS cells it is localized to the nucleus; but when POP-1 is coexpressed with WRM-1 and LIT-1, it becomes cytoplasmic.<sup>19</sup> Thus WRM-1 and LIT-1 appear to regulate POP-1 nuclear localization which might cause a perceived lowering of POP-1 levels by dilution in the cytoplasm or alternatively, selective degradation in the cytoplasm. Thus POP-1 must be regulated differently during endoderm induction in *C. elegans* than is TCF-4 in vertebrate cells. WRM-1 and LIT-1 appear to interact, somehow leading to a lowering of POP-1 levels in the E blastomere by an unknown mechanism.

Another very unusual aspect of the Wnt pathway involved in endoderm induction is that APR-1/APC and SGG-1/GSK3 appear to act positively in the transduction of the Wnt signal leading to the negative regulation of POP-1/TCF (Fig. 9). In the absence of Wnt signaling, the *Drosophila* and mammalian homologs of APC and GSK3 function negatively by promoting the degradation of  $\beta$ -catenin. A WRM-1/ $\beta$ -catenin/POP-1 complex might be targeted for degradation by the functions of APR-1 and SGG-1. How Wnt signaling might activate rather than inhibit SGG-1/GSK3 function is not clear;<sup>50</sup> especially in light of the possibility that two Dsh proteins might function redundantly in endoderm induction. The positive role of APR-1 is also curious. However, APR-1 is only 1186 amino acids, much smaller than the 2843 amino acid human APC protein,<sup>7</sup> suggesting APR-1 might have a divergent function in endoderm induction. Other data suggest a more canonical, negative role, for APR-1 in the Wnt signaling pathway involved in vulval development (see below). Determination of WRM-1 localization patterns as well as the investigation of the role of Dsh in this pathway is needed to learn how this unusual Wnt pathway establishes endoderm fates.

## Wnt Signaling Might Directly Target the Cytoskeleton to Control Mitotic Spindle Orientation

During EMS division, the nucleus and centrioles are initially aligned along the left-right axis. Just before mitosis, the EMS nucleus and centrioles rotate about 90° and align along the anterior-posterior axis (Fig. 3). Signaling from P<sub>2</sub> also induces this spindle rotation. If EMS is isolated, no rotation occurs. However, when placed in contact with P<sub>2</sub>, the EMS nuclear complex rotates and aligns along an axis perpendicular to the plane of contact.<sup>51</sup> Spindle rotation and endoderm specification by the signal from P<sub>2</sub> can be uncoupled, however. EMS loses competence to respond to P<sub>2</sub> to orient its spindle before it loses competence to polarize endoderm fate. For example, the spindle orients randomly when P<sub>2</sub> is placed in contact with EMS late in the EMS cell cycle.

In the 8-cell *C. elegans* embryo, there are four descendants of the AB blastomere. The mitotic spindle of three of these descendants are oriented in parallel, but the spindle of the fourth descendant, AB.ar, is oriented perpendicular to the others. The orientation of the AB.ar spindle is defective in some, but not all, of the *mom* mutants. Specifically, mutations in *mom-1*, *mom-2*, *mom-3*, *mom-5* and *sgg-1(RNAi)* cause defects in AB.ar spindle orientation, whereas mutations in *pop-1*, *apr-1* and *wrm-1* do not.<sup>7,8,40</sup> By contrast with the effects on endoderm specification, the defects in AB.ar spindle orientation are highly penetrant. Although it was not

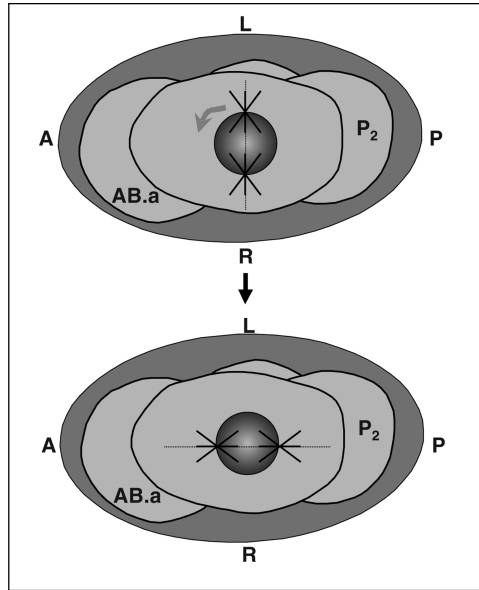


Figure 3. In the four-cell embryo, a Wnt signal from the P<sub>2</sub> blastomere orients the EMS mitotic spindle. A ventral view is depicted. The EMS spindle is initially aligned along the left (L)-to-right (R) axis (top). Approximately 30 seconds prior to nuclear envelope breakdown the EMS spindle rotates about 90° and becomes aligned along the Anterior (A)-to-posterior (P) axis (bottom).

clear whether the defects in AB.ar spindle orientation were direct effects of Wnt signaling in P<sub>2</sub> or any other blastomere, they suggested that Wnt signals might also be involved in the orientation of the EMS spindle.<sup>40</sup>

The same genes that affect AB.ar spindle orientation also affect EMS spindle orientation. The results of blastomere isolation and reconstitution experiments using wild-type and mutant blastomeres indicated that upstream components *mom-1/Porc*, *mom-2/Wnt*, *mom-5/Fz* and *sgg-1/GSK3* affect both EMS spindle orientation and endoderm specification; whereas downstream components *apr-1/APC*, *wrm-1/β-catenin* and *pop-1/TCF* only affect endoderm induction.<sup>40</sup> Thus *sgg-1* appears to be a branch point in these pathways (Fig. 4).<sup>50</sup> In addition, because the EMS spindle rotates quickly after P<sub>2</sub> signals and still rotates if transcription is blocked, it appears that Wnt signaling in *C. elegans* may directly target the cytoskeleton.<sup>50</sup> This may also occur in other systems as there is evidence of Wnt signaling affecting axonal cytoskeletal elements (reviewed in ref. 52) as well as gap junction patterns.<sup>53</sup>

## Wnt and Ras Pathways Control P12 Cell Fate

The P11 and P12 cells are located laterally in the animal at hatching and are the most posterior pair of postembryonic ventral nerve cord precursors. The P12 cell usually is on the right side and P11 on the left. Midway through the first larval stage they migrate into the ventral nerve cord and divide.<sup>1</sup> Before they enter the ventral cord, either cell can adopt the P12 cell fate, as killing either with a laser microbeam prior to its migration results in the remaining cell adopting the P12 cell fate.<sup>54</sup> Multiple pathways and genes can affect this cell fate determination. The genes of the *lin-3/let-23/let-60* pathway, the *C. elegans* Ras signaling pathway that functions in vulval development (see below), the Wnt pathway and the Hox genes *mab-5* and *egl-5* are all involved. Mutations in several genes including *let-23*, *lin-44/Wnt* and

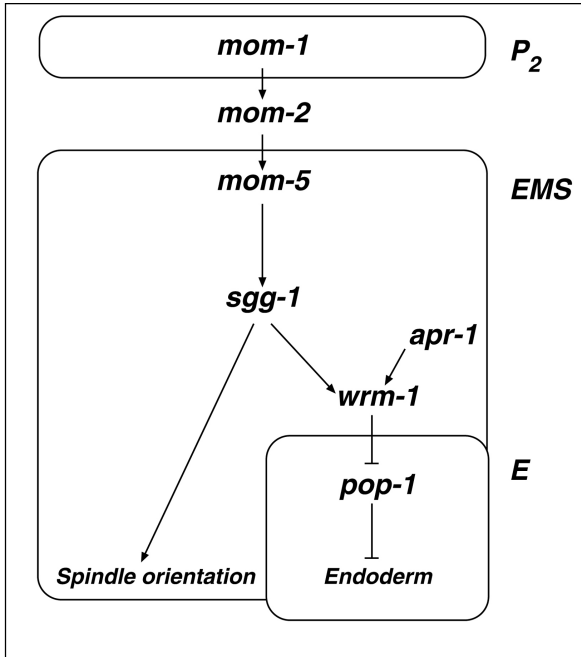


Figure 4. A Wnt signal from the P<sub>2</sub> blastomere induces both endoderm fate and spindle orientation in the EMS blastomere. See text for details. Reprinted with permission from Elsevier Science from Thorpe, CJ, et al. (2000) Wnt signalling in *Caenorhabditis elegans*: regulating repressors and polarizing the cytoskeleton. Trends Cell Biol. 10: 10-17.

*egl-5* cause a P12 to P11 cell fate transformation. Mutations in the Wnt pathway genes *mig-14/mom-3*, *mig-5/Dsh*, *bar-1/β-catenin* and *lin-17/Fz* also cause this cell fate transformation,<sup>28,55</sup> while mutations in *pry-1* cause the opposite transformation (D. Eisenmann, personal communication). Interestingly, a moderate reduction in *lin-3/EGF* function enhances the P12 to P11 cell fate transformation of *lin-44* mutants, but a *lin-17* mutation does not. This indicates that *lin-3* and *lin-44* function in separate pathways and *lin-44* and *lin-17* function in the same pathway for P12 cell fate specification.<sup>55</sup> Additional genetic evidence suggests that LIN-44 and LIN-3 functions are required at different times during development for P12 cell fate specification. LIN-44 appears to be required during late embryogenesis and LIN-3 is required during early L1 stage, thus LIN-44 may function before LIN-3. One possibility is that the LIN-44/Wnt pathway functions to make both cells competent to receive the LIN-3/EGF signal.<sup>55</sup> It is also possible that these two pathways both function to regulate the expression of the target gene, *egl-5*, which is required for P12 cell fate (Fig. 9). Finally, although it appears that P12 cell fate competence/specification is controlled by a canonical Wnt pathway, it is not clear what other Wnt signaling components are involved. The examination of recently isolated *pop-1* alleles that affect postembryonic development will more definitively establish whether this is a canonical Wnt pathway.

## Wnt Signaling in Vulval Development

*C. elegans* vulval development has been extensively studied as an excellent example of induction of cell fates in one tissue by a cell in an overlying tissue (reviewed by Greenwald<sup>56</sup>).

For proper vulval cell fate specification to occur, cells must integrate information from both a Ras/MAPK pathway and a Wnt pathway. Vulval development can be broken down into four processes: the generation of the vulval precursor cells (VPCs) that will divide to give rise to the vulva; determination of particular vulval cell fates; execution of a particular vulval cell fate; and morphogenesis of the vulva, where the 22 cells that are produced organize, migrate and rearrange to form the functional vulva that connects the uterus to the outside (Fig. 5).<sup>57</sup>

The generation stage begins when six cells from the each side of the animal (the 12 P cells) migrate to the ventral midline, interdigitate and divide. The posterior daughters become the 12 Pn.p cells, P1.p-P12.p. The six central cells, P3.p-P8.p express the Hox gene *lin-39* and become VPCs, forming the Vulval Equivalence Group.<sup>58-60</sup> The other cells, P1.p, P2.p, P9.p-P11.p do not express *lin-39*, adopt the F (fused) fate and fuse with the surrounding epidermal syncytium in the L1 stage. Later, in the L3 stage, P3.p adopts the F fate about 50 percent of the time.

The six VPCs, P3.p-P8.p, are multipotent, able to adopt any of the three vulval fates: 1°, 2° or 3°.<sup>54,61,62</sup> During the late L2 and early L3 individual VPC fates are determined based on extracellular signals. The pattern of VPC fates adopted by P3.p-P8.p is usually 3°-3°-2°-1°-2°-3°. The anchor cell (AC) in the somatic gonad secretes a LIN-3/EGF signal, that induces P6.p, which is closest to the AC, to adopt the 1° fate.<sup>62-64</sup> Activation of a Ras pathway that includes LET-23/RTK, LET-60/Ras and MPK-1/MAPK specifies the 1° cell fate.<sup>65-67</sup> P6.p then signals laterally to its immediate neighbors, P5.p and P7.p, causing them to adopt the 2° fate<sup>68</sup> via activation of the LIN-12/Notch receptor.<sup>69</sup> VPCs that do not receive a signal from the anchor cell or a neighbor adopt the uninduced 3° fate.

The role of Wnt signaling in vulval development appears to be to maintain LIN-39 expression during VPC determination. LIN-39 is required twice during vulval development: first during the generation step to specify VPC versus F cell fate and second during the determination step to specify 1° versus 2° versus 3° cell fates, where it might make P3.p-P8.p competent to receive signals from the anchor cell. For example, mutations that eliminate *lin-39* function cause P3.p-P8.p to adopt the F fate rather than VPC fate;<sup>58,59</sup> while mutations that partially reduce *lin-39* function allow some VPC fates to be specified, but these VPCs often adopt 3°, F or 1°/2° hybrid cell fates.<sup>60,70</sup> LIN-39 levels in the VPCs are regulated by the Ras pathway: reduction-of-function mutations in *lin-3/EGF*, *let-23/RTK* and *let-60/Ras* cause a decrease in LIN-39 expression, while gain-of-function mutations in *let-60* cause an increase in LIN-39 expression.<sup>60</sup> Like in *lin-39* mutants, P3.p-P8.p in *bar-1/β-catenin* mutants often adopt F cell fates and VPC fate specification is defective, indicating a role for Wnt signaling in vulval development.<sup>71</sup> In addition, there is a loss of LIN-39 expression during the late L2 stage in *bar-1* mutants. Furthermore, *lin-39* expressed from the *bar-1* promoter rescues *bar-1* mutants, suggesting that *bar-1* function is required for *lin-39* expression. Thus Wnt signaling may also specify the competence of these cells to respond Ras pathway signaling as with P12 cell fate. It is likely that the Wnt and Ras pathways both impinge upon LIN-39 expression during VPC fate determination (Fig. 5C). Double mutant analyses suggest *bar-1* functions after *let-60*, *mpk-1/MAPK* and *mek-2/MAPKK* but prior to, or in parallel with, the transcription factors *lin-1/ETS* or *lin-31/WH* (Winged helix protein).<sup>71</sup> Interestingly, when expressed from the *bar-1* promoter, both *wrm-1* and *hmp-2* can rescue a *bar-1* mutant, suggesting the other *C. elegans* β-catenins can also function in the vulval development Wnt pathway when overexpressed in the VPCs. This is quite surprising given that HMP-2 does not normally function in any signaling pathways and does not interact with POP-1 in yeast two-hybrid or co-immunoprecipitation assays. Perhaps HMP-2 can interact with POP-1 when overexpressed in worm cells or interacts with POP-1 via another factor not present in yeast or cultured cells.<sup>18</sup>

Other factors implicated in the Wnt pathway that functions during VPC determination include *mig-14/mom-3*, *pry-1/Axin* and *apr-1/APC*. *mig-14/mom-3* mutants display defects in VPC generation and determination similar to those observed in *bar-1* mutants, indicating that

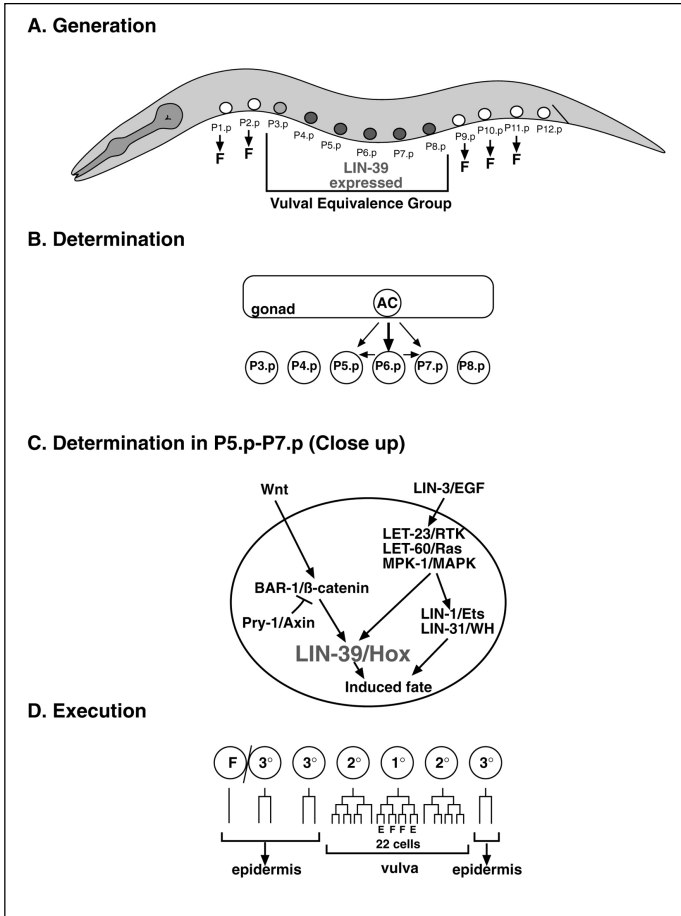


Figure 5. Vulval development in *C. elegans*. Three of the four stages of vulval development are diagrammed. A) Generation of P1.p–P12.p during the L1 stage. Expression of LIN-39/Hox in P3.p–P8.p causes them to take on the VPC fate instead of fusing with the epidermal syncytium, or F fate, as do P1.p, P2.p and P9–11.p. B) Determination of particular vulval cell fates occurs in response to several extracellular signals: an apparently graded signal from the anchor cell (AC) in the overlying gonad, lateral signals between the Pn.p cells and an inhibitory signal from the surrounding epidermal syncytium (not shown). As a result, P6.p adopts the 1° vulval cell fate, P5.p and P7.p adopt the 2° vulval cell fates and P3.p, P4.p and P8.p adopt non-vulval 3° cell fates. P3.p adopts the non-VPC F fate and fuses with the epidermal syncytium 50% of the time in wild-type animals. C) An interaction between Wnt and Ras pathways impinge on LIN-39 expression, which is required for P5.p–P7.p to take on induced vulval cell fates. Neither a Wnt ligand nor a Fz receptor have yet to be identified. The RTK/MAPK pathway also induces the expression of LIN-1/Ets and LIN-31/WH, transcription factors required for the induced vulval cell fate. D) Execution of vulval cell fates by the vulval precursor cells occurs in the L3 stage. The patterns of cell division of P3.p–P8.p are shown. The vulE (E) and vulF (F) pattern of cell fates among 1° descendants is also indicated. This pattern appears to be generated by Ras signaling from the AC to the inner 1° descendants and Wnt signals via LIN-17/Fz between the inner and outer 1° descendants. After refs. 28 and 71.

it is also involved in regulating LIN-39 expression. However, it is not known whether *mig-14/mom-3* mutations affect LIN-39 levels as do *bar-1* mutations.<sup>18</sup> *pry-1* mutants display an overinduction of vulval cell fates, where too many cells adopt 1° or 2° VPC fates. This VPC determination defect is opposite to that observed in *bar-1* mutants,<sup>72</sup> suggesting that *pry-1/Axin* functions as a negative regulator of Wnt signaling involved in vulval development, as it does in other animals. In addition, expression of a stabilized BAR-1 protein, produced by removing the amino-terminal consensus GSK-3 phosphorylation sites ( $\Delta$ N-BAR-1) also caused an overinduction of vulval cell fates. Furthermore, double mutant analyses indicated that the Overinduced defect of *pry-1* mutants was dependant upon *bar-1*, *pop-1* and *lin-39*, but not upon *mig-14/mom-3* (Fig. 9). Interestingly, mutations that reduced Ras signaling did not affect the overinduction of vulval cell fates caused by loss of *pry-1* function and BAR-1 stabilization, suggesting that Wnt signaling can directly affect LIN-39 expression.<sup>72</sup> Thus the Wnt pathway that functions during VPC determination appears to be canonical, and this pathway interacts with a RTK/Ras pathway to regulate *lin-39* Hox gene expression (Fig. 12.5C).

The role of *apr-1/APC* in this pathway is less clear, however. Loss of *apr-1* function specifically in P3.p-P8.p by expression of an antisense *apr-1* transgene driven by the VPC-specific *lin-31* promoter causes defects in the regulation of LIN-39 expression and function similar to those observed in *bar-1* mutants,<sup>73</sup> suggesting that both *apr-1* and *bar-1/ $\beta$ -catenin* functions are required positively for LIN-39 expression. This would be similar to the situation in the EMS blastomere, where the loss of *apr-1* and *wrm-1* function have similar effects. However, the overinduction of vulval cell fates caused by loss of *pry-1/Axin* function or BAR-1 stabilization was enhanced by *apr-1(RNAi)*, indicating that the overinduction does not require *apr-1* function, and that APR-1 may negatively regulate Wnt signaling, as it does in the canonical pathway.<sup>72</sup> Further experiments are required to reconcile these results on the role of *apr-1* in vulval development.

In summary, during vulval development, the Wnt pathway collaborates with the Ras pathway, to specify which vulva cell fate, 1°, 2° or 3° each VPC adopts. Thus, as in QL.d migration and P12 cell fate determination processes in the worm, the Wnt pathway that functions in vulval development regulates the expression of a Hox gene. At present, it appears that this Wnt pathway is canonical, however the functions of many components have yet to be determined. For example, no Wnt gene has been found to affect this process nor has a Fz receptor been identified. Exactly how this Wnt pathway interacts with the Ras pathway is also not clear. Both Wnt and Ras signaling may function together to instruct VPC fates (Fig. 5C). Alternatively, Wnt signaling may function permissively for the VPC to be competent to receive Ras signals, for example to achieve a certain level of LIN-39 expression that is increased by Ras signaling, leading to induced vulval cell fates.<sup>72</sup>

Finally, Wnt signaling has also been implicated in the execution and morphogenesis of the 1° cell fate. The 1° cell generates a specific pattern of vulval cells: four outer vulE cells and four inner vulF cells (Fig. 5D). This pattern appears to be generated by Ras signaling from the AC to the inner 1° descendants and Wnt signals via LIN-17/Fz between the inner and outer 1° descendants ensure the proper differentiation of vulE and vulF cell fates.<sup>74</sup>

## An Unusual Wnt Pathway Controls T Cell Polarity

Wnt signals control the polarities of several cells during *C. elegans* development. Mutations in *lin-44/Wnt* cause the polarities of the B and T cells in the tail to be reversed (Fig. 6A).<sup>5</sup> Mutations in *lin-17/Fz* cause the loss of polarity in these same cells;<sup>75,76</sup> suggesting that LIN-17 is the LIN-44 receptor. LIN-44 is expressed in the epidermal cells at the tip of the developing tail, which is posterior to the cells affected by *lin-44* mutations.<sup>6</sup> Although both *lin-44* and *lin-17* mutations affect the polarities of the same cells, the phenotypes are slightly different,

which is unexpected for a receptor-ligand pair. One possibility is that a second signal, probably another Wnt, emanates from an anterior source, causing the reversal of polarity observed in *lin-44* mutants. Unfortunately, as of yet, there is not evidence for this second signal. *egl-20/Wnt* is expressed in cells just anterior to the T cells that are in a perfect position to the source of such an anterior signal.<sup>26</sup> However, *egl-20* mutants have no effect on T cell polarity even when in combination with *lin-44* mutations, indicating that EGL-20 is not the anterior signal (M.H., unpublished).

POP-1 is also involved in the control of T cell polarity. Recall that in the embryo, Wnt signaling functions with components of a MAPK pathway to lower the levels of POP-1 in the posterior daughter of the EMS blastomere. The level of POP-1 is lower in the posterior daughters of many anteroposterior asymmetric cell divisions during development<sup>43</sup> and is also true of the T cell divisions. In wild-type animals, the level of POP-1 is lower in the posterior T cell daughter. Furthermore, in *lin-44* mutants, the level of POP-1 was lower in the anterior T cell daughter, reflecting the reversal of T cell polarity. Interfering with *pop-1* zygotic function by RNAi or expression of the dominant negative  $\Delta$ N-POP-1 construct causes a loss of T cell polarity similar to that observed in *lin-17* mutants (Fig. 6A).<sup>27</sup> Further, mutations in *pop-1* that cause defects in gonad development (see below) also cause T cell polarity defects.<sup>77</sup> Therefore, a Wnt, Frizzled and TCF are required for proper T cell polarity. However, the pathway that controls T cell polarity appears to be unusual. *lit-1*, which functions in the control of EMS polarity, also appears to act in the control of T cell polarity. Mutations in *lit-1* cause the loss of T cell polarity and *lit-1* is expressed in the T cells.<sup>19</sup> Thus there are some similarities in the control of T cell and EMS polarities. Surprisingly, none of the three *C. elegans*  $\beta$ -catenin homologs appear to function with POP-1 to control T cell polarity.<sup>27</sup> This is a major difference and it is not clear what, if any, other proteins might interact directly with POP-1 in the control of T cell polarity. The identification of which of the other known Wnt signaling components function in T cell polarity may help sort this out.

*lin-44* expressed from a heat-shock promoter, which presumably causes near uniform expression, can rescue the T cell polarity defects of *lin-44* mutants, suggesting that LIN-44 is a permissive rather than an instructive signal for T cell polarity.<sup>6</sup> This is similar to how *egl-20/Wnt* is thought to function in the control of V5 polarity<sup>78</sup> (see below). However, whereas expression of *egl-20* in the head of the animal can rescue V5 polarity defects of *egl-20* mutants,<sup>78</sup> similar expression of *lin-44* does not rescue the T cell polarity defects of *lin-44* mutants (M.H. unpublished). This suggests that LIN-44 acts as an instructive signal. *lin-44* expressed from the heat-shock promoter might not be processed or secreted uniformly, perhaps due to asymmetric expression of *mom-1/Porc* or *mig-14/mom-3*.

The Wnt signaling pathway that controls T cell polarity may exert its effects on transcription via chromatin remodeling complexes. Specifically, mutations in *egl-27*, which encodes a protein with similarity to a factor isolated as a component of the NURD complex (see above), cause the loss of T cell polarity. However it is not clear whether EGL-27 functions in the NURD complex during the T cell division or at any other time. In addition, mutations in genes encoding *C. elegans* homologs of components of the SWI/SNF complex also cause a loss of T cell polarity.<sup>79</sup> The SWI/SNF complex was originally identified in yeast as being required for activation of genes involved in mating-type switching and sucrose fermentation. *Drosophila* and vertebrate SWI/SNF complexes can be involved in either activation or repression of transcription (reviewed by Vignali<sup>80</sup>). Interestingly, components of the *C. elegans* SWI/SNF complex interact genetically with *egl-27*, suggesting that both play roles; it is not clear whether they have the same or distinct targets, however.

Mutations in *tlp-1* also cause defects in T cell asymmetry. *tlp-1* is asymmetrically expressed in the posterior T cell daughter and encodes a C2H2 Zinc finger protein that is likely to function as a transcription factor required for neural and other cell fates. This suggests that

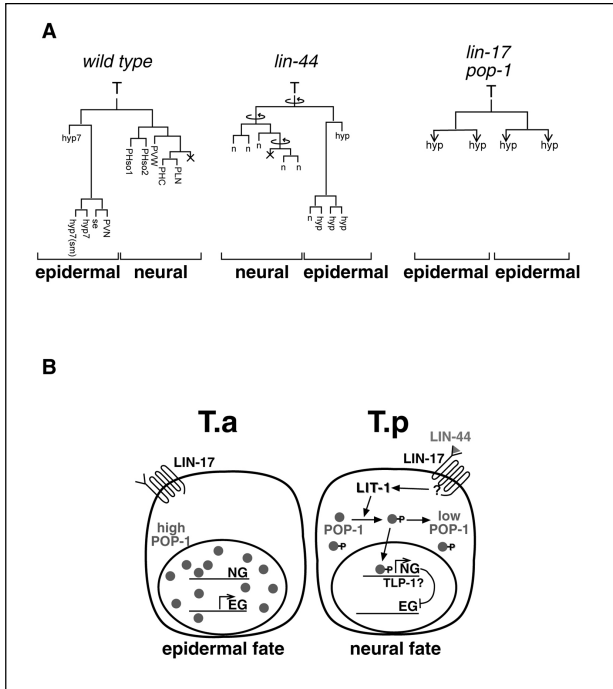


Figure 6. Control of T cell polarity by an unusual Wnt pathway. A) Representative hermaphrodite T cell lineages in wild type, *lin-44*, *lin-17* and *pop-1* mutants. In wild-type animals T.a generates epidermal cell fates and T.p generates neural cell fates. This is reversed in *lin-44* mutants. Cell fates are distinguished by nuclear morphologies.<sup>1,5</sup> *hyp7* cells join the epidermal syncytium, including T.apaa which has a smaller nucleus (sm). *Phso1*, *Phso2*, *PVW*, *PHC* and *PLN* have neural nuclear morphologies. X indicates programmed cell death. Seam (se) is a specialized epidermal cell. Downward arrows indicate variable numbers of epidermal cells can be generated. B) A model for T cell polarity. LIN-44/Wnt (triangle) binds to LIN-17/FZ on the posterior surface of the T cell before division, although for simplicity it is shown binding to T.p. The signal is transduced through unknown factors (?) leading to the activation of LIT-1, which functions to phosphorylate POP-1 (circles), perhaps in combination with an unknown factor. Some of the modified POP-1 is degraded leading to lower POP-1 levels, and some may activate expression of neural-specific genes (NG). A neural-specific gene then represses epidermal-specific (EG) genes in T.p. TLP-1 might be a target gene that gets activated in T.p. The T.a cell takes on a default epidermal fate. This might occur through the constitutive expression of epidermal-specific genes that promote epidermal fate. Without modification, the high levels of POP-1 in T.a may be nonfunctional. Modified from Herman, M.A. (2001) Development, 128: 581-590, *C. elegans* POP-1/TCF function in a canonical Wnt pathway that controls cell migration and in a noncanonical Wnt pathway that control cell polarity, with permission from Company of Biologists, Ltd.

TLP-1 functions in the execution of cell polarity decisions. *lin-44* and *lin-17* mutants cause *tlp-1::gfp* expression to be reversed or lost, respectively. Thus *lin-44* and *lin-17* must act upstream of *tlp-1*, suggesting that *tlp-1* may be a target of the Wnt pathway that functions to control T cell polarity.<sup>81</sup>

### A Model for T Cell Polarity

In wild-type animals T.a generates epidermal cell fates and T.p generates neural cell fates (Fig. 6B). Since the polarity of the T cell is determined before it divides,<sup>5</sup> there is a segregation

of cell fates at the T cell division: epidermal fate to T.a and neural fate to T.p. Each cell fate is correlated with a particular level of POP-1/TCF protein: high levels and epidermal fate in T.a and lower levels and neural fate in T.p. Mutations in both *lin-44* and *lin-17* affect POP-1 distribution, but interference with *pop-1* function causes both cells to adopt epidermal fates. One model to reconcile these data is shown in Fig. 6B.<sup>27</sup> LIN-44/Wnt might act through LIT-1/NLK to modify POP-1, which decreases POP-1 levels and at the same time converts POP-1 to an activator that activates neural-specific genes in T.p. The high levels of POP-1 in T.a could be nonfunctional. Specifically, LIN-44 binds to LIN-17/Fz on the posterior end of the T cell before its division and on T.p and its descendants. LIN-44 does not bind to T.a and it accumulates high levels of POP-1 and expresses epidermal-specific genes. This could be the default state, since loss of *pop-1* function also causes T.a to adopt an epidermal fate, and might occur by the constitutive expression of epidermal-specific genes in T.a. Upon binding of LIN-44 to LIN-17, LIT-1 is activated by transduction through unknown components. Activated LIT-1 could then phosphorylate POP-1, leading to the degradation of POP-1, as occurs in the E cell.<sup>50</sup> Some POP-1 function must be required for the neural cell fate since loss of *pop-1* function causes T.p to take on an epidermal fate. A low level of modified, perhaps phosphorylated, POP-1 might function positively without a  $\beta$ -catenin ortholog to activate neural-specific genes, one or more of which could then repress epidermal-specific genes in T.p. The activation of neural-specific genes might require the N-terminal domain of POP-1 as overexpression of  $\Delta$ N-POP-1 causes a loss of neural cell fates. Further work is required to identify other factors that are involved and how they interact in this unusual Wnt pathway.

## Gonad Polarity

Wnt signaling also controls asymmetric cell divisions that occur during the development of the *C. elegans* gonad. In this case the plane of asymmetry is proximal-distal (P-D), rather than anterior-posterior as with the EMS blastomere and the T cell. *C. elegans* gonadogenesis starts with the specification of the somatic gonad precursor cells, then the gonad primordium forms and gonad polarity is established (reviewed by Hubbard and Greenstein<sup>82</sup>). The gonad primordium contains four cells: the somatic gonadal precursor cells, Z1 and Z4 on each end and the germline precursor cells, Z2 and Z3 in the middle. The hermaphrodite gonad develops two arms, each containing an ovo-testes with its own (P-D) axis. The male gonad develops into a single testis with a single P-D axis.<sup>83</sup> In each sex, the P-D axes are set up by the first divisions of Z1 and Z4, descendants with distal fates lying at the ends and descendants with proximal fates lying in the middle. This arrangement is maintained in hermaphrodites, but in males cell migrations result in the generation of a single P-D axis (Fig. 7).

Mutations in *lin-17/Fz* cause a loss of asymmetry such that both Z1 and Z4 daughters generate proximal cell fates<sup>75</sup>, implicating Wnt signaling in this asymmetric cell division. This defect has been termed Sys (for symmetric sisters) and additional mutants with this defect have been isolated, some of which turned out to be alleles of *pop-1/TCF*.<sup>77</sup> The Wnt pathway that controls Z1 and Z4 polarity includes *mom-1/Porc*, *lin-17* (and no other *C. elegans* Frizzleds), *lit-1/NLK*, *wrm-1/ $\beta$ -catenin* (and not *bar-1*) and *pop-1* since interference with these gives the same Sys phenotype. None of the *C. elegans* Wnt genes appeared to be involved, although the possibility of redundancy has not been ruled out.

The fact that *pop-1* appears to play a positive role in Wnt signaling in the gonad is similar to the situation in the T cell (although *wrm-1* may not be involved in the T cell) but contrasts the situation in the embryo where *pop-1* has a phenotype opposite to that of *lit-1* and *wrm-1*. This suggests that the regulation of polarity in Z1, Z4 and T may be similar, but different, from EMS. Siegfried and Kimble<sup>77</sup> propose two models for how WRM-1 and LIT-1 might function to positively regulate POP-1 function in the gonad. In the first, WRM-1 binds to POP-1 in a

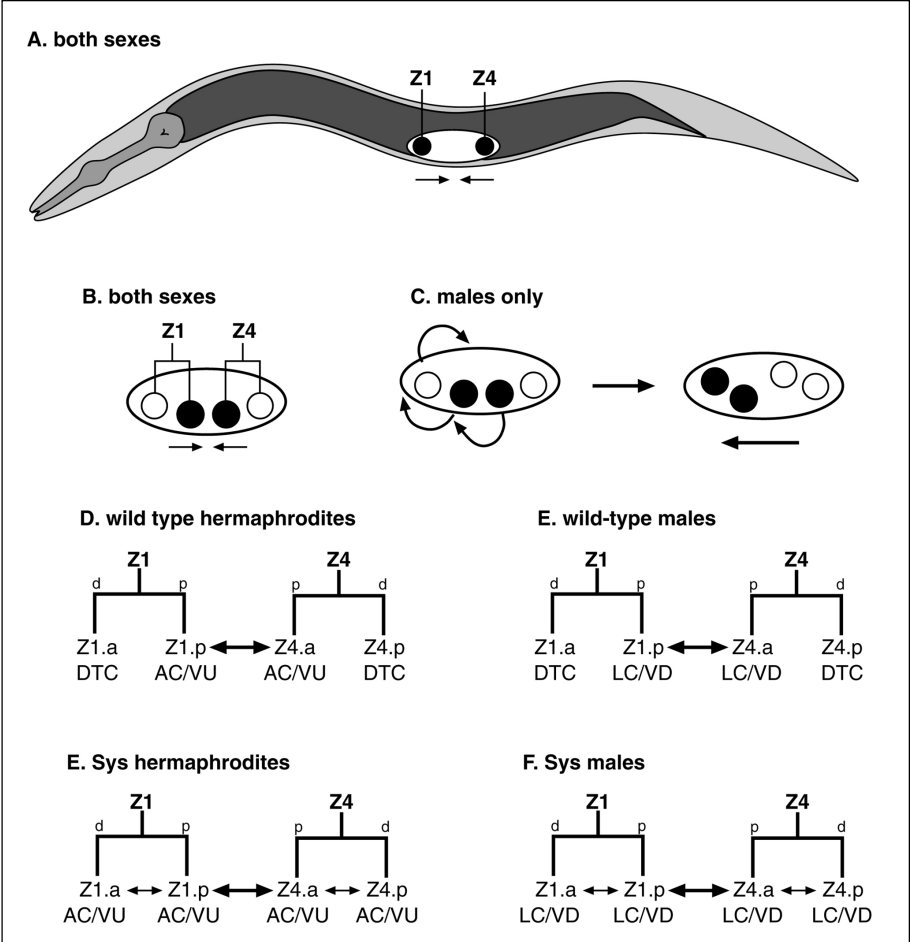


Figure 7. Gonadogenesis in wild-type and *sys* (*pop-1*) mutants. A) The gonad primordium in males and hermaphrodites appears identical. Arrows indicate primordium symmetry. The somatic gonad precursors, Z1 and Z4, are present at each end of the primordium and germline precursors, Z2 and Z3 (not shown) are in the middle. B) Z1 and Z4 divide asymmetrically along the proximal (p) to distal (d) axis in both sexes. This symmetry is retained throughout gonadogenesis in hermaphrodites. C) In males, an asymmetric structure is generated by Z1/Z4 daughter rearrangements. D) In wild-type hermaphrodites Z1.a and Z4.p generate distal tip cells (DTCs), whereas Z1.p and Z4.a generate AC/VU cells, with one anchor cell (AC) and the other as a ventral uterine precursor (VU) as a result of lateral signaling. E) In wild-type males Z1.a and Z4.p generate DTCs, whereas Z1.p and Z4.a generate bipotential cells that can adopt a linker cell (LC) or a vas deferens (VD) fate. One becomes an LC and the other becomes a VD as a result of lateral signaling. F) In *sys* mutant hermaphrodites all four Z1/Z4 daughters can generate AC/VU cells. G) In *sys* mutant males Z1 and Z4 generate bipotential LC/VD cells and no DTCs are made. Modified from Siegfried, KR and Kimble, J. (2002) POP-1 controls axis formations during early gonadogenesis in *C. elegans*. *Development*. 129: 443-453, with permission from Company of Biologists, Ltd.

canonical  $\beta$ -catenin-TCF relationship to activate gene expression leading to the establishment of different proximal-distal cell fates. Although WRM-1 does not bind to the amino terminal domain of POP-1,<sup>17</sup> it appears to bind weakly to full length POP-1. This interaction may be stabilized by LIT-1, which binds to POP-1.<sup>18,19</sup> The second is similar to that proposed for T cell polarity (Fig. 6B).<sup>27</sup> Specifically, WRM-1 and LIT-1 function to modify POP-1, which results in the activation of gene expression. It is not clear whether the action of WRM-1 or LIT-1 also results in decreased POP-1 levels as is proposed in the posterior T cell daughter, or whether a particular level of POP-1 is correlated with a Z1 or Z4 daughter cell fate, as POP-1 antibodies do not stain the gonads of L1 animals.

## Wnt Pathways Might Interact to Control V5 Cell Polarity

*egl-20/Wnt* also controls the polarities of asymmetric cell divisions in the lateral epidermis of the animals. One each side of the animal six epidermal V cells divide asymmetrically along the anteroposterior axis during postembryonic development to generate cuticular and sensory structures. The anterior V cell daughter, Vn.a, becomes a syncytial cell which fuses with the epidermal syncytium (*hyp7*) that covers most of the body of the animal. The posterior V cell daughters, Vn.p, becomes a seam cell and continues to divide (Fig. 8). Sensory structures are generated from V5.p in both sexes and from V6.p in males. Like most anterior-posterior asymmetric cell divisions, POP-1 is expressed at high levels in the Vn.a cells and at low levels in Vn.p cells.<sup>27,43</sup> The polarity of the V5 division is reversed in 50 percent of *egl-20* mutants<sup>25</sup> and seven percent of *mig-14/mom-3* mutants. Like *lin-44/Wnt*, *egl-20* is expressed in a group of cells that are posterior to the cells whose polarity it controls.<sup>84</sup> *egl-20* expressed from a heat-shock promoter, which presumably causes near uniform expression, can rescue the V5 polarity defects of *egl-20* mutants. Surprisingly, expression of *egl-20* from a pharynx-specific promoter (at the anterior end of the animal) can also rescue the V5 polarity defects of *egl-20* mutants, even though the source of EGL-20 signal is on the other side of the target cells and over half the body length away!<sup>78</sup> This suggests that EGL-20/Wnt must be secreted and can diffuse a distance from its source to function and that EGL-20 is a permissive rather than an instructive signal for V5 polarity.<sup>78</sup> What controls the polarity of the other V cells? Mutations in *cam-1*, that encodes a Ror orphan receptor kinase cause polarity reversals in V1.<sup>85</sup> Thus, some signaling system involving Ror kinases may be involved in V cell polarity. Long periods of heat-shock induced higher levels of *hs::egl-20* expression and also caused the polarity defects in the V1-V4 and V6 cells. This indicates that, although these cells do not normally require *egl-20* to establish their polarities, the system that determines their polarities can be disrupted by high levels of EGL-20.<sup>78</sup>

What could be responsible for the V5 polarity reversals observed in *egl-20* mutants? Killing the V6 and T cells with a laser microbeam suppressed the V5 polarity reversals in *egl-20/Wnt* mutants; whereas killing anterior neighboring cells did not. This suggests that a lateral signal from the cells posterior and adjacent to V5 appears to be responsible for the V5 polarity reversals in *egl-20* mutants. LIN-44/Wnt is not involved in this lateral signaling system as mutations in *lin-44* could not suppress the V5 reversals observed in *egl-20* mutants and did not influence the effects of killing posterior cells. Normally, the suppressive effect of killing the posterior cells in *egl-20* mutants is restricted to the side of the animal on which the cells were killed. Curiously, in *lin-44; egl-20* double mutants, this side restriction became uncoupled, suggesting that LIN-44 functions somehow to restrict polarity signals from one side of the animal from affecting the other. LIN-17 does appear to be involved, however, as mutations in *lin-17* suppress the polarity reversals seen in *egl-20* mutants. PRY-1/Axin is also involved as *pry-1* mutations enhance the V5 polarity reversals of *egl-20* mutants. Thus the lateral signal from the cells posterior and adjacent to V5 that appears to be responsible for the V5 polarity reversals in *egl-20* mutants requires *lin-17* and *pry-1*, but not *lin-44*.<sup>78</sup> It appears that lateral

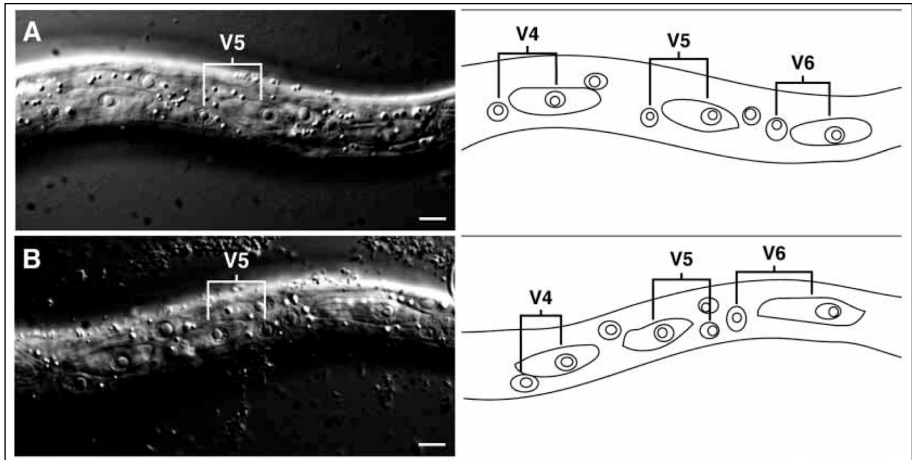


Figure 8. EGL-20 orients the V5 cell division. Nomarski micrographs of L1 larvae are shown on the left, with tracings of the on the right. A) Wild type. The outline of the posterior V5 daughter (V5.p) is visible, which is the seam cell morphology. The anterior daughter (V5.a) has a syncytial morphology: its nucleus is slightly ventral to the V5.p nucleus, and has a grainy nucleoplasm. B) *egl-20* mutant. The polarity of the V5 cell division is reversed: V5.a has the morphology and position of a seam cell and V5.p has the morphology and position of a syncytial nucleus. Bars, 10  $\mu$ m. Reprinted from Whangbo, J, et al. (2000) Multiple levels of regulation specify the polarity of an asymmetric cell division in *C. elegans*. *Development*. 127: 4587-98, with permission from Company of Biologists, Ltd.

signaling occurs through a canonical Wnt pathway, but with only three components identified this is not certain. Whangbo et al.<sup>78</sup> hypothesized that EGL-20 function is required to override the lateral signal. This pathway may involve MIG-14/MOM-3, but additional components have not been identified. They further hypothesize an underlying global system may exist that functions to orient anteroposterior cell polarities in the worm. This system may be responsible for the asymmetric distribution of POP-1 at many anterior-posterior asymmetric cell divisions as well as for the polarities of the EGL-20 and LIN-44 dependant cell divisions. A prediction of this model is that LIN-44 would also function as a permissive signal, this does not appear to be the case, however (M.H., unpublished observations). Finally, the targets of the V5 polarity Wnt pathways are not known.

## Sensory Ray Formation

Wnt signaling also plays a role in interactions that occur among the lateral epidermal cells that generate sensilla. In hermaphrodites and males, a descendant of the V5 cells, V5.pa, generates the postderid sensillum. In males, a different type of sensilla, the sensory rays, are produced such that each side of the animal generates nine rays: the V5.pp, V6 and T cells generate one, five and three rays, respectively. Interactions among the lateral epidermal cells regulate the numbers of rays that are generated. If the V5 and V6 cells in males were killed with a laser microbeam, the next anterior V cell, V4, generates a number of rays.<sup>54</sup> If either V4 or V6 is killed in a male, V5 makes a number of rays, but no postderid.<sup>86-88</sup> Thus cell interactions regulate cell fates. *mab-5* is required for generation of the V5- and V6-derived rays, but represses postderid formation. If V6 is killed or if *mab-5* is expressed from a heat-shock promoter, the V5 cell takes on the V6 cell fate, producing extra rays but no postderid.<sup>89</sup> However, following the killing of V6, Wnt signaling involving *egl-20*Wnt, *lin-17*/Fz and *bar-1*/ $\beta$ -catenin is required for V5 to take on the V6 fate.<sup>90</sup> Furthermore, *pry-1* mutations cause ectopic *mab-5*

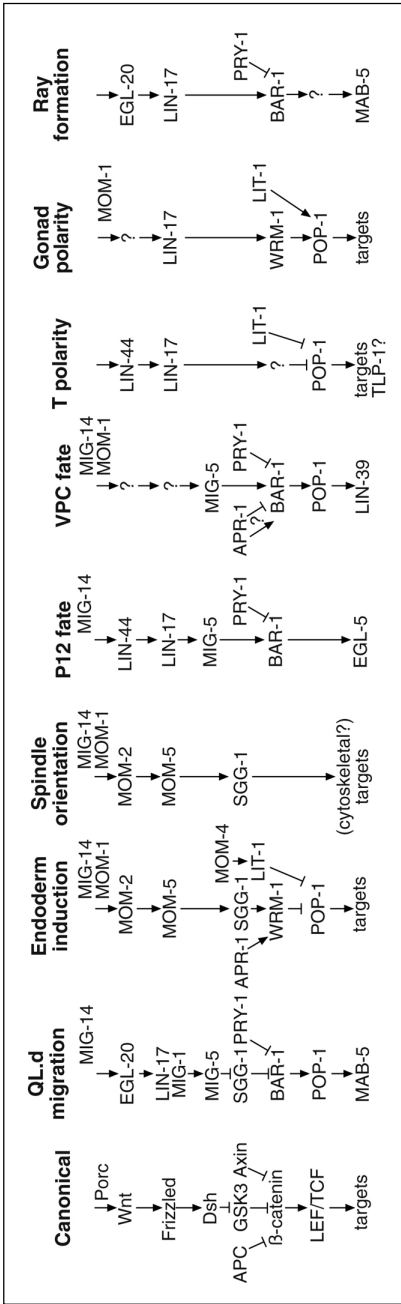


Figure 9. Comparison of the canonical and *C. elegans* Wnt pathways. Known genes involved in QL.d migration, Endoderm induction, EMS spindle orientation, P12 cell fate, VPC cell fate, T cell polarity, gonad polarity and male ray formation and the inferred regulatory relationships are shown. The Wnt pathways that interact the control V5 cell polarity are not shown, as the nature of the interaction is not well understood. MIG-14/MOM-3 is simplified to MIG-14. Only the canonical pathway components with *C. elegans* homologs are shown. APR-1 is shown as influencing WRM-1 independently of SGG-1 in the Endoderm induction pathway, since SGG-1 influences EMS spindle orientations whereas APR-1 does not. “?” indicates that multiple or unknown homologs of a component function in a particular pathway. In the T cell polarity pathway “?” indicates that either a β-catenin homolog is not involved or that an unknown factor may be involved and “TLP-1?” indicates that tlp-1 might not be a direct target. In the gonad polarity pathway “?” indicates the Wnt involved has not been identified. Similarly, in the VPC fate pathway “?” indicates that a Wnt or a Fz involved has not been identified.

expression (which requires *bar-1*) leading to loss of the postderid and ectopic ray formation.<sup>26</sup> Thus a canonical Wnt signaling pathway activates *mab-5* expression in the lateral epidermal cells, but is normally inhibited by cell contacts by a mechanism that is not clear. In the V6 cell, the cell contact inhibition is overcome by the caudal homolog, *pal-1*, which functions to activate *mab-5* expression to specify V6 cell fate.<sup>90</sup>

## Themes and Remaining Questions in Worm Wnt Signaling

Wnt signaling pathways are involved in three major types of process during *C. elegans* development: control of asymmetric cell divisions and cell polarity, cell fate decisions and cell migration. Both canonical and noncanonical Wnt pathways are used in the various processes. The Wnt pathway that controls the migrations of the QL descendants is clearly canonical. The pathways that control P12 cell fate, VPC fate, V5 cell polarity and signaling in the lateral sensilla also appear to be canonical, although several “gaps” remain.

The Wnt pathways involved in the control of cell polarity of EMS (endoderm induction), T and Z1 and Z4 are each unusual, but in different respects. Whereas the pathway that controls V5 polarity may be canonical, the involvement of many of the canonical pathway components have yet to be assessed. POP-1/TCF and LIT-1/NLK function in the EMS, T and Z1/Z4 cell polarity Wnt pathways, but POP-1 is negatively regulated in the EMS blastomere. Just how POP-1 levels and function are regulated in the EMS daughters, as well as the determination the role of Dsh in this regulation, remain as important questions for the control of EMS polarity. In addition, WRM-1/ $\beta$ -catenin functions in EMS and Z1/Z4 cell polarity but may not function in T cell polarity; although the latter is a negative RNAi result that will have to be reexamined once a *wrm-1* mutation is isolated. The isolation of additional components of these pathways and further examination of the regulatory relationships of the components is required to determine the exact extent to which these pathways are similar or different.

For several processes it is difficult to discern whether or not the pathway is canonical. Some of this uncertainty is caused by the lack of information about the involvement of particular Wnt pathway component in each of the processes. This is particularly true of the Wnt pathway involved in vulval cell fate determination, which appears to be canonical in many respects in that PRY-1/Axin, BAR-1/ $\beta$ -catenin and POP-1/TCF appear to be regulated in a canonical manner, but the role of APR-1/APC is unclear as is the identity of the Wnt ligand and Fz receptor that are involved. In order to get a complete picture of Wnt signaling in *C. elegans* the “gaps” that exist in Table 2 need to be filled.

Aside from the types of processes that are regulated by either canonical or noncanonical Wnt signaling, a couple other themes emerge. As is the case in other animals, a target gene of many of the *C. elegans* Wnt pathways is a Hox gene: *mab-5* in the QL.d migration and lateral sensilla pathways, *egl-5* in the P12 cell fate pathway and *lin-39* in the VPC fate pathway. Another possible theme is that for both P12 cell fate and VPC fate, Wnt signaling might control the competence of cells to respond to other signals involving the Ras pathway that control cell fates. However, in the case of VPC fate it is also possible that Wnt and Ras pathways collaborate to specify cell fates.

Finally, MIG-14/MOM-3 is involved in multiple *C. elegans* Wnt signaling pathways (Table 2, Fig. 9), yet the molecular identity of MIG-14/MOM-3 has not been determined. *mig-14/mom-3* maps to a genetic region that appears to be devoid of known Wnt signaling components or regulators, suggesting that it may encode a novel Wnt signaling component. Since *mom-3* functions in P2, the signaling cell, for endoderm induction, it is likely to function in the signaling cell in other signaling events. Thus, MIG-14/MOM-3 appears to function generally in the synthesis, secretion or processing of Wnt proteins that function in many, if not all, *C. elegans* Wnt pathways and may also be conserved in Wnt signaling pathways in other organisms.

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