Chapter 8

Analysis of Wnt Signaling During *Caenorhabditis elegans* Postembryonic Development

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Abstract

Wnts play a central role in the development of many cells and tissue types in all species studied to date. Like many other extracellular signaling pathways, secreted Wnt proteins are involved in many different processes; in *C. elegans* these include: cell proliferation, differentiation, cell migration, control of cell polarity, axon outgrowth and control of the stem cell niche. Perturbations in Wnt signaling are also key factors in cancer formation, and therefore of interest to oncobiologists. Wnts are secreted glycoproteins, which bind to Frizzled transmembrane receptors and signal either through, or independently of β-catenin. Both β-catenin-dependant (Wnt/β-catenin) and -independent pathways function during postembryonic development in *C. elegans* and allow Wnt researchers to explore aspects of Wnt signaling both in common with other organisms and unique to the nematode. **Chapter 9** in **Volume 2** discusses various processes controlled by Wnt signaling during *C. elegans* embryonic development; this chapter discusses Wnt-controlled processes that occur during postembryonic development, including an overview of methods used to observe their function.

Key words: *C. elegans*, Wnt signaling, cell polarity, cell fate specification, cell migration, cell lineage.

1. Introduction

1.1. The Wnt Pathway in C. elegans

The nematode *C. elegans* contains many components of the Wnt signaling pathway, most with homologous counterparts conserved in other organisms. *C. elegans* has five genes encoding the secreted Wnt ligand: *lin-44*, *egl-20*, *mom-2*, *cwn-1*, and *cwn-1*. These proteins signal through the Frizzled family of Wnt receptors: LIN-17, MOM-5, MIG-1 and CFZ-2, and Disheveled proteins: MIG-5, DSH-1, and DSH-2. The many Wnt signaling components then function through the four β-catenin proteins

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BAR-1, WRM-1, HMP-1, and SYS-1. In the Wnt/β-catenin pathway, activation of the Wnt pathway causes Disheveled to inactivate GSK3 which frees β-catenin from the "destruction complex," allowing β-catenin to translocate to the nucleus where it complexes with TCF/LEF family members to activate target genes. β-catenin independent Wnt pathways include the Wnt/calcium and Wnt/Jnk or PCP pathways that signal through frizzled type receptors and disheveled, but function independent of β-catenin.

This chapter focuses on Wnt signaling during *C. elegans* postembryonic development and is organized by specific Wnt-controlled processes. These postembryonic processes could be used to assay potential Wnt function during development. The following discusses the known Wnt components of each developmental process and the methods employed to observe and assay it.

1.2. Methods to Detect Wnt Signaling in C. elegans

Wnts have been shown to be involved in the developmental processes of differentiation, polarity, and migration. *C. elegans* has proven to be an excellent genetic model organism because of its rapid generation time, small size, known cell lineage and hermaphroditic mating, making the identification and characterization of genes involved in developmental processes rather easy. In order to design a genetic screen to isolate genetic mutations that affect a specific biological process of interest, namely the Wnt pathway, one must develop an assay that accurately determines whether the process has been disrupted. Knowledge of *C. elegans* lineages, cell positioning, cell migrations, and anatomy during wild-type development is essential in developing such an assay. Many *C. elegans* screens begin with a population of wild-type hermaphrodites exposed to ethyl methane sulphonate (EMS). EMS induces random mutations in the sperm and oocytes of wild type hermaphrodites. Hermophroditic fertilization will generate a heterozygous F1 individual, one quarter of whose progeny will become homozygous for any given mutation, (for the art and design of screens, see ref. *1)*. One can also analyze the development of a known Wnt mutant and discover an alternative process controlled by a given Wnt protein.

Introduction of double-stranded RNA (dsRNA) has the ability to interfere with gene function in *C. elegans (2, 3)*. dsRNA with sequence homology to an endogenous gene or transgene induces silencing of the corresponding gene by a process called RNA interference (RNAi). RNAi is an excellent tool for studying gene function and dissecting the role of genes in specific developmental pathways *(4)*, in our case the Wnt pathway. A full *C. elegans* RNAi library was generated by Julie Ahringer's group and is available (www.geneservice.co.uk/products.rnai/Celegans.jsp); the library covers an estimated 87% of the genome.

An initial RNAi screen of chromosome I increased the number of genes with phenotypes from 70 to 378 on Chromosone I *(4)*, showing the power and efficiency of such a screen. Since then, many additional RNAi screens have continued to identify new gene functions, including those in the Wnt pathway *(5)*.

2. Migration of the Descendants of the QL Neuroblast

The QL and QR neuroblasts are cells born on the left and right side of *C. elegans* at the same relative anterior/posterior positions. During the first larval stage (L1) QL and QR generate similar progeny, three neurons and two cells that undergo programmed cell death. The QR descendants, referred to collectively as the QR.d, migrate anteriorly and the QL descendants (QL.d) migrate posteriorly. The migration of the QL.d is dependent on the HOX gene *mab-5*; cells that express *mab-5* migrate posteriorly, whereas cells with no *mab-5* expression migrate anteriorly *(6)*. The expression of *mab-5* and therefore the migration of QL.d and QR.d is controlled by Wnt signaling *(7–9)*. Quantification of QL neuroblast migration is an excellent tool for studying the Wnt/β-catenin pathway in potential mutants, as all components of the pathway are involved. **Fig. 8.1** illustrates QL migration and shows the phenotypes of known members of the pathway. Positions of cells in the Q lineage are recorded based on their location relative to the adjacent epidermal cells or V cells. During wild-type development QR migrates a short distance from its birth position between V4 and V5, divides and its descendants, which do not express *mab-5*, continue to migrate anteriorly toward V1. The QL cell migrates a short distance, begins to express *mab-5*, divides and QL.ap migrates to the tail. QL.paa and QL.pap do not migrate.

Loss-of-function mutations in *mig-14/Wls* (10), *egl-20/Wnt*, *lin-17/Fz*, *mig-1/Fz*, *mig-5/Dsh* and *bar-1/β-catenin*, *pop-1/Tcf*, and *mab-5/Hox*, all cause the loss of *mab-5* expression in the QL lineage and resultant anterior migration of both the QL.d and QR.d, overexpression of *C. elegans* TCF, POP-1, also causes the anterior migration of the QL.d. In addition, mutations in the Axin homologs *pry-1* and *axl-1* cause posterior migrations of QR.d whereas overexpression of *pry-1*, *axl-1*, or *sgg-1* an ortholog of GSK-3 causes anterior migration of QL.d, suggesting they function negatively in this pathway *(9, 11, 12)*. Interestingly, it is not the level of Wnt signal that determines the migratory route but the sensitivity to the EGL-20 signal, such that signaling is activated in the QL.d but not in the QR.d *(13)*.

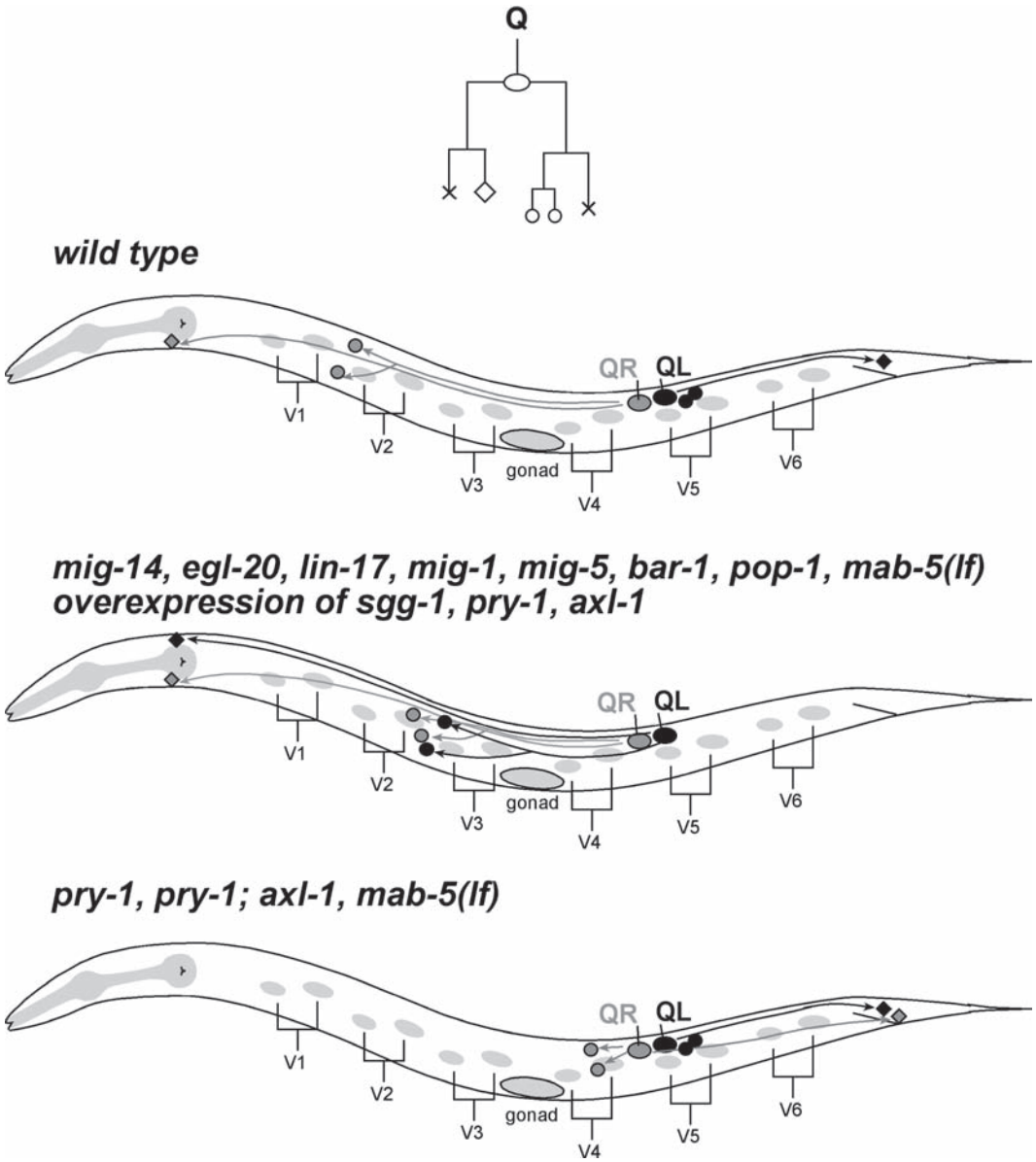

Fig. 8.1. Wnt/β-catenin signaling controls migration of the Q descendents. The Q neuroblast lineage is shown on top. QL (black oval) and QR (dark gray oval) each divide to generate three neurons (diamond and circles) and two cells that undergo programmed cell death (X). Migrations of the QL.d and QR.d in wild-type and mutant animals are shown below. Both are projected onto the views of the left side of a mid-L1 hermaphrodite larva. Positions of the gonad primordium (outlined large light gray oval) and V cell daughters (small light gray ovals) are shown and used for spatial references. The initial positions of QL and QR and the final positions of the QL.d and QR.d are indicated.

Analysis of QL neuroblasts requires Nomarski differential interference contrast (DIC) microscopy and an understanding of *C. elegans* anatomy. In addition, *mec-7::gfp* is expressed in one of the migrating cells in each Q lineage: AVM (QR.paa) and PVM

(QL.paa) and can be used to score or screen for Q migration defects *(14)*.

3. Vulval Fate Specification and Vulval Development

Twelve P cells; six cells on each side of the worm, migrate to the ventral midline, interdigitate and divide. The six central cells P3.p to P8.p express the Hox gene *lin-39*, determining them to be the vulval precursor cells, the VPCs. The six VPC cells are multipotent and can adopt any of three vulval fates: 1°, 2°, or 3° (reviewed in ref. *15)*. During the L3 stage, an inductive signal from the anchor cell in the overlying gonad activates receptor tyrosine kinase (RTK) pathway that specifies P6.p to take on the 1° fate. P6.p then signals neighboring P5.p and P7.p through a Notch-related pathway to specify them to take on the 2° fate. Wnt signaling appears to regulate the competence of the VPCs to participate in vulval development as well as the polarity of P7.p (reviewed in refs. *15* and *16)*. Mutations in Wnt pathway components that promote signaling such as *bar-1/β-catenin*, *mig-14/Wls* and *pop-1/Tcf* causes fewer VPCs to adopt vulval cell fates, whereas mutations in components that inhibit signaling such as *pry-1Axin* and *apr-1/APC* cause too many VPCs to adopt vulval fates. Redundancy is a key feature of Wnt signaling in vulval development. For example, another Axin, AXL-1, was recently found to function redundantly in the pathway *(12)*. In addition, all five Wnt signaling proteins seem to play a role in VPC specification, with one, CWN-1 functioning antagonistically to two others, LIN-44 and MOM-2 *(17)*. Loss of vulval cell fates results in a Vulvaless (Vul) defect and too many VPCs participating in vulval development results in a Multivulva (Muv) defect in which several protuberances form along the ventral surface of the animal, both of which can be observed in the compound or dissecting microscopes. In addition, VPC fates can be monitored by the expression of several cell-type-specific markers. These include: *egl-17::gfp* for the 1° cell fate, *ceh-2::gfp* and *cdh-3::gfp* for the 2° cell fate *(18)*.

The polarities of divisions of the P5.p and P7.p cells, which take on 2° fate, are mirror symmetric relative to the center of the vulva. Wnt signaling causes P7.p to have a polarity opposite to that of P5.p. This includes the cell division pattern as well as nuclear level of POP-1/Tcf in the P7.p daughters (see **Section 5**; ref. *19)*. Mutations in *lin-17* and *lin-18/Ryk* cause P7.p to have the same polarity as P5.p. Three Wnts, LIN-44, MOM-2 and CWN-2, function redundantly through LIN-17 and LIN-18 to control P5.p and P7.p polarities. Specifically, LIN-44 appears to

function through LIN-17 and MOM-2 functions through LIN-18 to regulate a common process *(20)*.

4. P12 Fate Specification

P11 and P12 are the most posterior pair of ventral nerve cord precursors and are located laterally in the worm at hatching. In wild-type animals P12 is usually on the right side and P11 on the left. During the L1 stage, P11 and P12 migrate into the ventral nerve cord and divide, each generating a different patterned lineage. Either cell can adopt the fate of P12 before migration *(21)*. Similar to the case for vulval precursor cell fate specification, the Wnt and RTK/Ras pathways interact to regulate the specification of P12 fate. The genes: *lin-3*, *let-23*, and *let-60* of the Ras signaling pathway and *lin-44*, *lin-17*, and *bar-1* of the Wnt pathway as well as the Hox genes *mab-5* and *egl-5* are all involved *(22)*. Observation of cell lineages is the most accurate method to score P12 cell fate. However, P11.p does not divide in hermaphrodites, whereas P12.p divides to generate a cell that dies (P12.pp) and a hypodermal cell (P12.pa) with a unique morphology, which can be used as a marker of P12 fate. In addition, a fragment from the *egl-5* promoter region has been shown to drive *gfp* expression within the P12 lineage but not the P11 lineage *(23)*, and can be used a marker for P12 cell fate.

5. The Control of Cell Polarity and Asymmetric Protein Localization

Wnt signals control the polarities of several cells during *C. elegans* development: The EMS blastomere (see **Chapter 9**), the T and B cells in the tail, V5 in the lateral epidermis, and the Z1 and Z4 cells in the developing gonad (reviewed in refs. *16* and *24)*. One key advantage to using *C. elegans* in studies of cell polarity is the ability to observe division and lineage specification in the living worm. Such studies require no special equipment, protocols, or methods to assay Wnt-controlled processes.

POP-1/Tcf is asymmetrically localized during most asymmetric cell divisions in *C. elegans*, with anterior daughter having higher nuclear levels of POP-1 than posterior daughters *(25)*. This is assayed either by immunolocalization with a POP-1 monoclonal antisera *(25)* or expression of a GFP::POP-1 transgene (**Fig. 8.2**; ref. *26)*. WRM-1/β-catenin, LIT-1/MAPK and POP-1/Tcf, are all asymmetrically distributed to the T-cell daughters

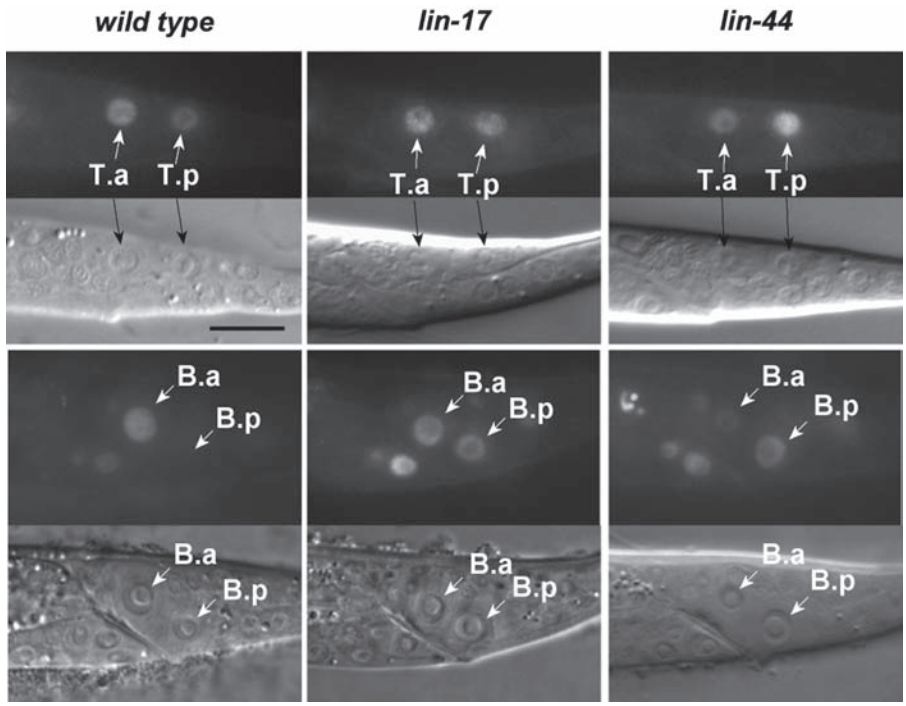

Fig. 8.2. Asymmetric nuclear localization of POP-1/Tcf is controlled by Wnt signaling. Nuclear distribution of GFP::POP-1 is shown for the T-cell (upper) and B-cell (lower) daughters in *wild type*, *lin-44*, and *lin-17* animals carrying *qIs74.* For each pair of cells in each strain, fluorescence and corresponding DIC images are shown. The nuclear levels of GFP::POP-1 are higher in the anterior T- or B-cell daughter than in the posterior daughter in *wild-type* animals, are equal in both daughters in *lin-17* mutants and are higher in the posterior T- or B-cell daughter than in the anterior daughter in *lin-44* mutants. Bar equals 10 μm in all panels.

(8, *27*, *28)*. WRM-1 interacts with and activates LIT-1 kinase, which phosphorylates POP-1 and regulates its subcellular localization *(29*, *30)*. The asymmetric distribution of POP-1 to nuclei in the early embryo is controlled by differential nuclear export mediated by the 14-3-3 protein PAR-5 and nuclear exportin homolog IMB-4/CRM-1 *(31*, *32)*. Furthermore, LIT-1 modification of POP-1 was shown to be required for its asymmetric nuclear distribution in a process that required WRM-1 *(31)*. Both LIT-1 and WRM-1 were also differentially localized in a reciprocal pattern to that of POP-1, with higher levels in the posterior E cell nucleus *(31*, *32)*. WRM-1 was also localized to the anterior cortex of the anterior MS cell in a process that required MOM-5/Fz *(32)* and both WRM-1 and LIT-1 were localized to the anterior cortex before and during postembryonic lateral hypodermal V5 cell division *(28)*. Nakamura et al. proposed that

Wnt and Src signaling leads to the phosphorylation and retention of WRM-1 in the posterior E nucleus, where it phosphorylates POP-1 in a LIT-1-dependent manner *(32)*. Thus asymmetric nuclear retention of WRM-1 appears to drive the control of cell polarity during the EMS and T-cell divisions. Asymmetric cortical localizations of several proteins were recently shown to be involved in generating WRM-1 nuclear asymmetry in the V5 and T-cell divisions. Specifically, APR-1/APC, WRM-1, LIT-1, and PRY-1/Axin were anteriorly localized, whereas LIN-17, DSH-2, and MIG-5/Dsh were posteriorly localized *(28, 33–35)*. Anterior cortical localization of LIT-1 and WRM-1 appear to inhibit the anterior nuclear localization of WRM-1, leading to higher relative WRM-1 levels in the posterior nucleus, which in turn leads to low POP-1 nuclear level *(34)*.

SYS-1 is another β-catenin homolog that functions to control the polarities of the somatic gonad precursor cells by interacting with POP-1 to control cell fates *(36, 37)*. SYS-1 interacts with POP-1 leading to the activation of gene expression and appears to play a positive role in the specification of cell fates *(37)*. SYS-1 is also asymmetrically localized during the EMS, T and somatic gonad precursor (SGP) cell divisions in a pattern opposite to that of POP-1 in a process that requires LIN-17 and Dsh function *(38)*. Thus low POP-1 and high SYS-1 nuclear levels lead to the activation of target genes, such as CEH-22/Hox in the SGPs *(39)*. Interestingly, SYS-1 nuclear asymmetry is not controlled by WRM-1 and possibly not the other anteriorly localized cortical proteins either, suggesting another mechanism may be operating *(38)*. It is not yet clear whether SYS-1 plays a role in WRM-1 localization.

6. T-cell Polarity

In hermaphrodites the TL and TR cells collectively known as T cells lie in the tail on each side of the animal. They divide asymmetrically to give an anterior daughter T.a that generates primarily epidermal cells, and a posterior daughter; T.p which divides to generate neural cells (**Fig. 8.3**). Mutations in *lin-44/Wnt* cause the asymmetric division of T to be reversed *(40)*. Among the cells generated by T.p are the phasmid sockets, which are glial cells that extend out to the surface of the tail and whose presence can be assayed by their ability to allow the phasmid neurons, PHA and PHB, to take up the lipophilic dye DiO. Phasmid dye filling is used as an indicator of normal T-cell lineage, and is an easy assay to screen for mutants in the laboratory. Briefly, worms are

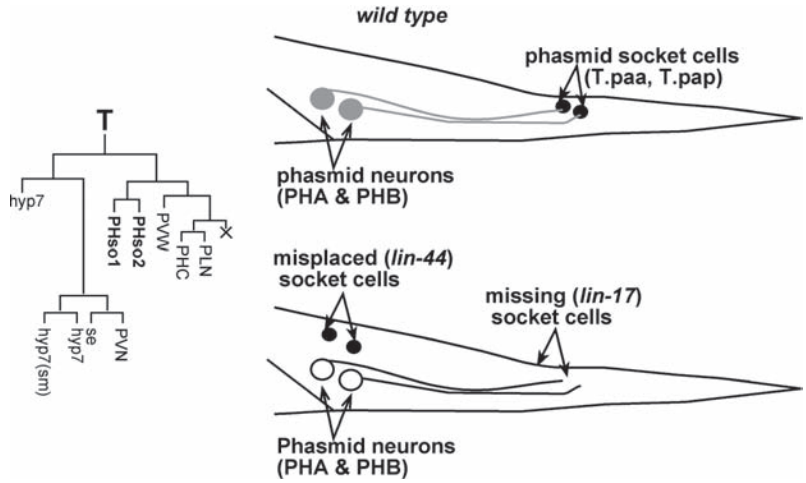

Fig. 8.3. Dye-filling and position of the phasmid socket cells can be used to score -ell polarity. The T-cell lineage is shown on the left and schematics illustrating the positions of the phasmid neurons and the socket cells in *wild type* (above) as well as *lin-44* and *lin-17* (below) on the right. Shading indicates the dye filling status of the phasmid neurons that can be determined by DiO filling. While DiO filling can be done at any life stage, it is easiest to observe in adult hermaphrodites. The phasmid socket cell nuclei can be recognized by their nuclear morphology and position as the most posterior neuronal nuclei on the lateral sides of the animal. They can be seen anytime after their birth in the mid-L1 stage but are easiest to find in L3 animals.

soaked in a 10 µg/mL solution of DiO (in M9 buffer) on a shaker for 2 hours, rinsed multiple times with M9 buffer and plated on *E. coli* OP50 coated NGM plates. After a period of time feeding to clear excess dye from the gut, worms are scored with FITC fluorescence for the presence (WT) or absence of socket cells; such worms are said to be Phasmid dye (Pdy)-filling defective. A more accurate indication of T-cell lineage and polarity defects is the position of the phasmid socket cells, T.paa and T.pap, by DIC optics. This defect is called Psa for phasmid socket cell absent *(41)*.

lin-17/Fz, is also involved in the control of T-cell polarity. Whereas mutations in *lin-44* cause a reversal of polarity, mutations in *lin-17* causes a loss of polarity and both daughters adopt epidermal fates. Mutations in other known Wnt components, *wrm-1*, *lit-1*, *sys-1*, *pop-1* as well as *egl-27*, *tcl-2* and *tlp-1*, also cause a loss of T-cell polarity *(8, 29, 37, 42–44)*. The difference in the phenotype between ligand (LIN-44) and receptor (LIN-17) is of interest and has yet to be explained, one possibility is there is an additional anterior signal working with LIN-17.

7. Gonad Polarity

The gonad of *C. elegans* hermaphrodites is bilaterally symmetric and lies in the center of the developing animal. The somatic gonad develops from two somatic gonad precursor (SGP) cells, Z1 and Z4, on the ends of the gonad primordium that each generates one of the bilaterally symmetric gonad arms. Each SGP divides asymmetrically along the proximal-distal axis with distal cell fates lying at the ends of the primordium and proximal fates lying in the center. The SGPs divide three times in the first larval stage to generate the 12-cell gonad primordium. Ten of these cells have invariant fates, and two become the distal tip cells (DTCs) on each end, which leads the elongation of the gonad arms. The remaining two cells, Z1.ppp and Z4.aaa have variable cell fates, with one becoming the anchor cell (AC) and the other a ventral uterine precursor (VU). However, which cell becomes the AC and which becomes the VU varies from animal to animal *(45)*.

Although a Wnt has not yet been shown to control the asymmetric SGP divisions, Wnt pathway components are involved. Specifically, mutations in *lin-17/Fz*, *wrm-1/*β*-catenin*, *sys-1/*β-catenin, *lit-1/Nlk* and *pop-1/TCF*, lead to a loss of asymmetry and the generation of two daughters with proximal fates *(36*, *46*, *47)*. The SGP daughters exhibit POP-1 nuclear asymmetry, with higher nuclear GFP::POP-1 in proximal daughters than in distal ones, which is lost in Wnt pathway mutants *(26)*. SYS-1 is also asymmetrically localized during the EMS, T and somatic gonad precursor (SGP) cell divisions in a pattern opposite to that of POP-1 in a process that requires LIN-17 and Dsh function *(38)*. Thus low POP-1 and high SYS-1 nuclear levels lead to the activation of target genes, such as CEH-22/Hox in the SGPs *(39)*. Interestingly, SYS-1 nuclear asymmetry is not controlled by WRM-1 and possibly not the other anteriorly localized cortical proteins either, suggesting another mechanism may be operating *(38)*. However, it is not yet clear whether SYS-1 plays a role in WRM-1 localization. Asymmetric nuclear localization of GFP::POP-1 and asymmetric expression of CEH-22::GFP can be used to monitor the polarities of the SGP divisions.

8. V5 Polarity

Six epidermal V cells along each side of the postembryonic larvae in *C. elegans* divide asymmetrically. Each anterior daughter Vn.a becomes a syncytial cell and has high POP-1 levels, and each posterior daughter Vn.p becomes a seam cell and continues to divide;

these cells have low nuclear POP-1 levels. The polarity of the V5 cell in the posterior lateral epidermis is controlled by *egl-20/Wnt*. The polarity of the V5 division is reversed in approximately 50% of *egl-20/Wnt* mutants. EGL-20 has been shown to be a permissive rather than an instructive signal for V5 polarity as *egl-20* expressed from a heat shock promoter and a pharynx-specific promoter can rescue V5 polarity. Furthermore, the reversal of polarity seen in *egl-20* mutants seems to require a lateral signal functioning through *lin-17/Fz* and *pry-1/Axin* suggesting an interaction of more than one Wnt pathway *(48)*.

The asymmetric division or polarity of V5 can be visualized by Nomarski differential interference contrast microscopy in L1 stage animals, or by POP-1 localization. Individuals are scored to determine whether Vn.a fuses with the epidermal syncytium and whether Vn.p divides to generate proliferative seam cells. An interaction of Wnt pathways functions to control V cell polarity. Therefore, using the V cells as an assay for Wnt signaling could elucidate a number of molecular signaling components.

9. Postereid and Male Ray Formation

Wnt signaling also plays a role in interactions that occur among the lateral epidermal cells that generate sensilla. In hermaphrodites and males, descendants of the V5 cells, V5.pa, generate the postderid sensilla. Postderid formation by V5.pa is regulated by interactions among the lateral epidermal cells *(49–52)* that regulate *mab-5* expression *(53)*. Specifically, killing the V cells anterior or posterior of V5, causes extopic expression of *mab-5* and V5.pa makes a seam cell instead of a postdereid *(53)*. Ectopic expression of *mab-5* occurs by activation of a Wnt pathway that includes *egl-20/Wnt*, *lin-17/Fz*, *bar-1/β-catenin* and *pry-1/Axin (9)*.

In males, different types of sensilla, the sensory rays, are also 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 *(54)*. V4 can generate rays in the absence of V5 and V6 and the number of rays produced by V5 increases in the absence of with V4 or V6, however postderid no postderid in formed *(49, 51, 52)*.*mab-5* is required for generation of the V5- and V6-derived rays. 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 *(55)*. However, following the killing of V6, Wnt signaling involving *egl-20Wnt*, *lin-17/Fz*, and *bar-1/β-catenin* is required for V5 to take on the V6 fate *(53)*. Furthermore, *pry-1* mutations cause

ectopic *mab-5* expression (which requires *bar-1*) leading to loss of the postderid and ectopic ray formation *(9)*. This Wnt pathway appears to be inhibited by cell contacts and requires *dpy-22/sop-1/mdt-12* and *sop-3/mdt-1.1*, which encode homologs of the transcriptional Mediator complex components MED12/TRAP230 and MED1/TRAP220, respectively *(56–58)*. The targets of this pathway, either direct or indirect, include *pal-1/caudal* and the Hox genes *mab-5* and *egl-5*.

10. B-cell Polarity

Male tail development involves complex postembryonic cell lineages many involving regulated asymmetric cell divisions. The B, Y, U, and F cells of the male tail divide postembryonically transforming the simple posterior tube of the worm into a complex array or spicules, postcloacal sensillae, proctodeum, and the gubernaculum. Remodeling of this region occurs during late L3 and L4 and results from a series of asymmetric cells divisions and complex cell-cell interactions. The polarity of the B cell and the B-cell lineage is a well-studied tool for the analysis of asymmetric cell divisions. The B cell divides asymmetrically with a large anterior-dorsal daughter B.a and a smaller posterior-ventral daughter B.p (**Fig. 8.2**). B.a divides to produce 40 cells and generates male copulatory spicules, and B.p divides to produce 7 cells *(54)*. Furthermore, B-cell polarity is controlled by Wnt signaling. In *lin-44* mutant males B-cell polarity is reversed *(40, 59)*, while in *lin-17* mutant males B-cell polarity is lost *(46, 60)*.

B-cell polarity is controlled differently than T-cell polarity in that a planar cell polarity (PCP)-like pathway is involved. There are some similarities, such as the involvement of the asymmetrical distribution of POP-1/Tcf. However, there are clear differences. Specifically, MIG-5/Dsh, RHO-1/RhoA, and LET-502/ROCK appeared to play major roles, while other PCP components appeared to play minor roles *(61)*. Furthermore, none of the five *C. elegans* β-catenin homologs *(62)* plays a role in B-cell polarity. While disruption of *wrm-1/β-catenin*, *lit-1/MAPK*, or *sys-1β-catenin* functions caused T-cell polarity defects, including the distribution of GFP::POP-1; little or no B-cell polarity defect was observed and neither *lit-1* nor *wrm-1* affected the asymmetric distribution of GFP::POP-1 to the B.a and B.p cells *(61)*. In addition, the Wnt/β-catenin pathway does not function in the control of B-cell polarity *(61)*. Finally, LIN-17/Fz and MIG-5/Dsh were asymmetrically localized during the B-cell division. Asymmetric localization of LIN-17::GFP was dependent upon LIN-44/Wnt and MIG-5 function whereas asymmetric

localization of MIG-5::GFP was dependent upon LIN-44 and LIN-17. This suggests that a Wnt/PCP-like pathway is involved in the regulation of B-cell polarity *(63)*.

11. Conclusions

In *C. elegans*, Wnt pathways control the migration, polarity and fate decisions of many cells. One key advantage of using *C. elegans* in Wnt research is the ability to observe developmental processes as they occur in the living animal. Some of the developmental processes we have discussed share many components and features of the Wnt pathways first described in *Drosophila* and vertebrates, others share some molecular components but signal in a different manner; the method of target gene activation therefore seems to differ among some Wnt pathways. It is clear that *C. elegans* has a conserved Wnt/β-catenin pathway that functions during QL.d migration and vulval development. In addition, in the case of the regulation of cell polarity in *C. elegans*, Wnt pathway components can be asymmetrically localized by at least two different conserved mechanisms. Exactly how asymmetric protein regulation leads to the generation of cell polarity is not yet clear. Finally, the components involved in each developmental process are still being identified. The approaches described here may allow the reader to participate in the search for new Wnt pathway components in *C. elegans*.

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