Development 129, 443-453 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
DEV3556

POP-1 controls axis formation during early gonadogenesis in *C. elegans*

Kellee R. Siegfried[1] and Judith Kimble[1–4,*]

[1]Laboratory of Genetics, [2]Department of Biochemistry, [3]Laboratory of Molecular Biology, and [4]Howard Hughes Medical Institute, University of Wisconsin-Madison, Madison, WI 53706-1544 USA
*Author for correspondence (e-mail: jekimble@facstaff.wisc.edu)

Accepted 23 October 2001

SUMMARY

The shape and polarity of the *C. elegans* gonad is defined during early gonadogenesis by two somatic gonadal precursor cells, Z1 and Z4, and their descendants. Z1 and Z4 divide asymmetrically to establish the proximal-distal axes of the gonad and to generate regulatory leader cells that control organ shape. In this paper, we report that *pop-1*, the *C. elegans* TCF/LEF-1 transcription factor, controls the first Z1/Z4 asymmetric division and hence controls proximal-distal axis formation. We have identified two *pop-1(Sys)* alleles (for symmetrical sisters) that render the Z1/Z4 divisions symmetrical. The *pop-1(q645)* allele is fully penetrant for the Sys gonadogenesis defect in hermaphrodites, but affects male gonads weakly; *pop-1(q645)* alters a conserved amino acid in the β-catenin binding domain. The *pop-1(q624)* allele is weakly penetrant for multiple defects and appears to be a partial loss-of-function mutation; *pop-1(q624)* alters a conserved amino acid in the HMG-box DNA binding domain. Zygotic *pop-1(RNAi)* confirms the role of *pop-1* in Z1/Z4 asymmetry and reveals additional roles of *pop-1*, including one in leader cell migration. Two other Wnt pathway regulators, *wrm-1* and *lit-1*, have the same effect as *pop-1* on Z1/Z4 asymmetry. Therefore, *wrm-1* and *lit-1* are required for *pop-1* function, rather than opposing it as observed in the early embryo. We conclude that POP-1 controls the Z1/Z4 asymmetric division and thereby establishes the proximal-distal axes of the gonad. This control over proximal-distal polarity extends our view of Wnt signaling in *C. elegans*, which had previously been known to control anterior-posterior polarities.

Key words: POP-1, *C. elegans*, Gonadogenesis, Axis formation, Wnt signaling

INTRODUCTION

Early steps in organogenesis include the specification of organ precursor cells, formation of an organ primordium, and establishment of organ polarity. We have begun to analyze early organogenesis in the nematode *C. elegans*, where its development can be analyzed in living animals at the level of individual cells. Our focus has been development of the gonad, which, like many organs, consists of an epithelial tube. The *C. elegans* gonad is a symmetrical organ in hermaphrodites and an asymmetrical one in males. Although a common genetic program underlies gonadogenesis in the two sexes (e.g. Blelloch et al., 1999; Friedman et al., 2000; Greenwald et al., 1983), early events must be controlled in a sex-specific manner to generate gonads with distinct polarity. More generally, this problem can be viewed as a paradigm for how developmental regulators can be modified to create distinct structures, a fundamental problem in evolutionary biology.

Gonadogenesis has been described in detail at the cellular level for both sexes [reviewed by Hubbard and Greenstein (Hubbard and Greenstein, 2000)]. The gonadal primordium contains two somatic gonadal precursor cells, Z1 and Z4 (Fig. 1A), which flank two germline precursor cells, Z2 and Z3 (not shown in Fig. 1). The first division of Z1 and Z4 establishes the proximal-distal (PD) axes of the organ. The hermaphrodite gonad will develop two ovo-testes, each with a PD axis; the male gonad will have a single testis, and therefore a single PD axis (Hirsh et al., 1976; Kimble and Hirsh, 1979). These PD axes are determined by the daughters of Z1 and Z4: after this first division the daughters with distal fates reside at the poles and the daughters with proximal fates are located centrally in both sexes (Fig. 1B). This arrangement persists in hermaphrodites throughout gonadogenesis. In males, however, the two proximal daughters migrate anteriorly and the anterior distal daughter is displaced posteriorly (Fig. 1C, left). The resultant structure (Fig. 1C, right) places the two proximal daughters at the anterior and the two distal daughters at the posterior end, transforming the symmetrical structure with two opposing PD axes into an asymmetrical structure with a single PD axis. In this way, the developing gonads acquire sex-specific polarities after a single division of Z1 and Z4.

In addition to establishment of organ polarity, other key events occur during early gonadogenesis that are crucial for morphogenesis. First, cells are generated that organize the developing gonad. Gonad shape is controlled by ‘leader cells’, which reside at the growing tips and govern elongation (Kimble and White, 1981) (Fig. 1D-G, red). In hermaphrodites, leader cells derive from the Z1/Z4 distal daughters, and are called distal tip cells (DTC) (Fig. 1D). In males, by contrast, the Z1/Z4 proximal daughters generate cells with leader

potential, but lateral signaling directs one to differentiate as a leader cell, called a linker cell (LC), and the other to become a vas deferens (VD) precursor cell (Kimble, 1981) (Fig. 1E). Therefore, the number and position of leader cells is controlled by the asymmetric allocation of leader potential at the Z1/Z4 division in both sexes and lateral signaling in males.

In addition to specification of leader cells, two other regulatory functions are assigned early in gonadogenesis. In both sexes, the Z1/Z4 distal daughters (or granddaughters) signal the germline to proliferate (Kimble and White, 1981). In hermaphrodites, the germline proliferation and leader functions reside in the same cell (the DTC), whereas in males, they are allocated to separate cells: germline control in the DTCs and leader function in the linker cell (LC). Another regulatory cell specified during early gonadogenesis is the anchor cell (AC), which is specific to hermaphrodites and arises from the proximal Z1/Z4 daughters (Fig. 1D). The two cells competent to become the AC use lateral signaling (arrows, Fig. 1D) to make one AC and one ventral uterine (VU) precursor cell (Kimble, 1981). The AC induces vulval development in the ventral hypodermis (Kimble, 1981) and also induces nearby uterine precursors to take on the π cell fate (Newman et al., 1995).

Another key event, which completes early gonadogenesis, is formation of a 'somatic primordium'; the blast cells in this primordium generate the somatic gonadal structures of the adult (Kimble and Hirsh, 1979). In hermaphrodites, all somatic gonadal cells, except the DTCs, migrate centrally and coalesce to create a hermaphrodite-specific somatic gonadal primordium (SPh) during the late L2 and early L3 stage (Fig. 1D). In males, all somatic gonadal precursor cells, except the DTCs, cluster anteriorly to form the male-specific somatic gonadal primordium in early L2 (SPm) (Fig. 1E). These sex-specific somatic primordia (SP) prefigure the shape and pattern of the adult somatic gonadal structures.

Several genes are critical for early gonadogenesis [reviewed by Hubbard and Greenstein (Hubbard and Greenstein, 2000)]. Of particular importance for this work are *lin-17* (Sternberg and Horvitz, 1988) and *sys-1* (Miskowski et al., 2001). Both *lin-17* and *sys-1* affect the asymmetric division of Z1 and Z4. In *lin-17* nonsense mutants and *sys-1* strong loss-of-function mutants, Z1 and Z4 can divide symmetrically and generate two daughters that adopt the same, proximal fate (Fig. 1F,G). This is the Sys phenotype, for 'symmetrical sisters' (Miskowski et al., 2001). The *lin-17* gene encodes a *frizzled*-like receptor (Sawa et al., 1996), implicating Wnt signaling in control of Z1/Z4 asymmetric divisions. The *sys-1* gene encodes a novel protein, providing no additional clue to biochemical function (J. Miskowski, A. Kidd and J. K., unpublished). Although the *sys-1* and *lin-17* mutant phenotypes are similar, they are not identical (Miskowski et al.,

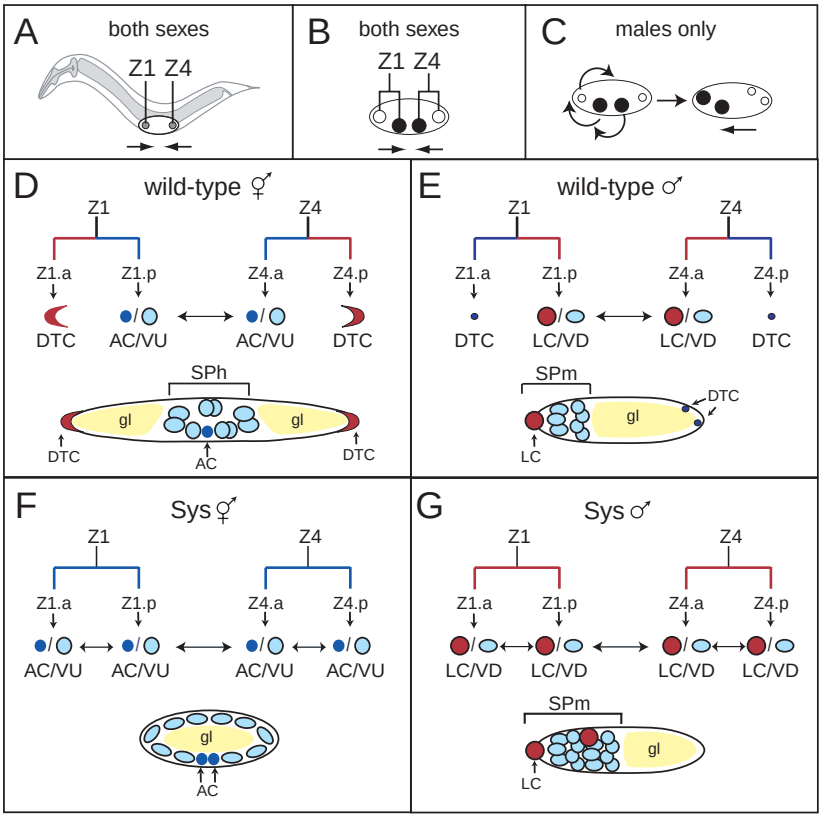

Fig. 1. Early gonadogenesis in wild-type and Sys mutant animals. See Kimble and Hirsh (Kimble and Hirsh, 1979) for more detail about wild type and see Miskowski et al. (Miskowski et al., 2001) for more detail about *sys-1* mutants. (A) The gonadal primordium is morphologically indistinguishable in hermaphrodites and males (primordium symmetry represented by arrows beneath figure). The somatic gonadal precursors, Z1 and Z4, reside at the poles of the primordium; germline precursors, Z2 and Z3 (not shown), reside centrally between Z1 and Z4. (B) Z1 and Z4 divide asymmetrically in both sexes. When first born, their daughters are positioned with the same symmetry as the earlier primordium (arrows point towards proximal regions). In hermaphrodites, this symmetry is retained throughout gonadogenesis. (C) In males, the Z1/Z4 daughters rearrange (left figure, curved arrows) to generate an asymmetric structure (right figure, single arrow points towards proximal pole). (D) Fates of Z1/Z4 daughters in wild-type hermaphrodites. Z1.a and Z4.p give rise to DTCs (red), which have leader function and control germline proliferation. Z1.p and Z4.a give rise to AC/VU cells. Lateral signaling results in selection of one precursor as an AC (dark blue) and the other as a ventral uterine precursor (VU), one of nine somatic gonadal blast cells (light blue) that form the somatic gonadal primordium in hermaphrodites (SPh). gl, germline. (E) Fates of Z1/Z4 daughters in wild-type males (and most *sys-1* mutant males). Z1.a and Z4.p give rise to DTCs (blue), which control germline proliferation, but do not have leader function. Z1.p and Z4.a give rise to cells with potential to become either a linker cell (LC) or a vas deferens blast cell (VD). Lateral signaling results in selection of one of these precursors as an LC (red), which has leader function; the other becomes a VD, one of seven somatic gonadal blast cells (light blue) that form the somatic gonadal primordium (SPm). gl, germ line. (F) Fates of Z1/Z4 daughters in *sys-1* mutant hermaphrodites. All four Z1/Z4 daughters can give rise to AC/VU cells, and no DTCs are made. Furthermore, no SPh forms; instead, a ring of somatic gonadal cells encircles the germline (gl) at late L2/early L3. (G) Fates of Z1/Z4 daughters in 30% of *sys-1* mutant males. Z1 and Z4 do not make DTCs; instead extra LCs and extra somatic gonadal cells are observed by late L2.

2001; Sternberg and Horvitz, 1988). Whereas *lin-17* null mutants have a low penetrant (~30%) Sys gonadal phenotype in both sexes, *sys-1* is fully penetrant in hermaphrodites, but poorly penetrant (~30%) in males. In addition, *lin-17* is involved in asymmetric divisions in many tissues; however, the post-embryonic effects of *sys-1* appear to be limited to the gonad. Therefore, these two genes overlap functionally, but are also distinct.

In this work, we introduce the *sys-2* locus. We find that *sys-2* is allelic with *pop-1*, a TCF/LEF-1 transcription factor that activates genes in response to Wnt signaling (Lin et al., 1995). We therefore have dropped the *sys-2* gene name, but retained the Sys name to describe Z1/Z4 division defects typical of *sys-1*, *lin-17* and *pop-1(Sys)* mutants. The two *pop-1(Sys)* alleles are both missense mutations, one in the β-catenin binding domain and one in the HMG box. The phenotypes of these two alleles are distinct, both from each other and from those of *pop-1(RNAi)* progeny. To explore the pathway controlling Z1/Z4 asymmetry, we analyzed components of the canonical Wnt signaling pathway and components of a Map kinase pathway that regulate POP-1 function. We find that animals lacking either the β-catenin homologue *wrm-1* or the nemo-like kinase (NLK) homologue *lit-1* have typical Sys gonadal defects. Similarly, loss of *lit-1* in a post-embryonic cell, called the T cell, also results in the same phenotype as loss of *pop-1* (Herman, 2001). These results contrast to what has been found in the *C. elegans* embryo and in vertebrates, where *lit-1* opposes *pop-1* function (Ishitani et al., 1999; Kaletta et al., 1997; Meneghini et al., 1999; Rocheleau et al., 1999).

MATERIALS AND METHODS

Strains

Animals were grown at 20°C unless otherwise noted. All strains were derivative of Bristol strain N2 (Brenner, 1974). The following mutations have been described previously (Hodgkin, 1997) (and cited references). *LGI*: *pop-1(zu189)*, *lin-44(n1792)*, *mom-4(or39)* (Thorpe et al., 1997), *mom-5(or57)* (Thorpe et al., 1997), *fog-1(q187)*, *unc-11(e47)*, *mek-2(q425)*, *mec-8(e398)*, *lin-17(sy277)* (Sawa et al., 1996), *tDf3*, *tDf4*; *LGIII*: *unc-32(e189)*, *lin-12(n137gf,sd n720lf)*, *lit-1(t1512)* (Kaletta et al., 1997), *lit-1(t1534)* (Kaletta et al., 1997), *lit-1(or131)* (Meneghini et al., 1999); *LGIV*: *egl-20(n585)*, *rde-1(ne219)* (Tabara et al., 1999); *LGV*: *mom-2(or42)* (Thorpe et al., 1997); *LGX*: *bar-1(ga80)* (Eisenmann and Kim, 2000), *mom-1(or10). hT2[qIs48]* was used as a dominant green balancer chromosome. *qIs48* is an insertion of *ccEx9747* (contains *pes-10::GFP*, *myo-2::GFP*, and a gut enhancer driving GFP) onto *hT2*. In addition, the following markers were used: *qIs19* and *qIs56* are *lag-2::GFP* insertions (Blelloch et al., 1999), and *syIs50* is a *cdh-3::GFP* insertion (Pettitt et al., 1996).

Isolation and genetic characterization of *sys-2* mutations

Two *pop-1* alleles were isolated in an F_2 screen following mutagenesis with ethyl methane sulfonate (EMS). These two mutations failed to complement and mapped to the left arm of chromosome *I* between *lin-17* and *mek-2* by 3-factor and deficiency mapping. The deficiency *tDf4* complemented *pop-1(q645)* and *tDf3* failed to complement *pop-1(q645)*. From *pop-1/mek-2 unc-11*, 18/18 Unc non-Mek progeny carried *pop-1(Sys)*. We used the deletion allele *lin-17(sy277)* as a molecular marker for mapping. From *pop-1 unc-11/ lin-17*, 53/53 Unc non-Sys carried the *lin-17* deletion. We also failed to separate *pop-1(zu189)* from *pop-1(q645)*: from *zu189/q645 unc-11*, 46/46 Unc-non Sys were Mel.

pop-1(q624) is recessive: wild-type males were crossed into *pop-1(q624) mec-8/hT2[qIs48]*; all cross-progeny were wild type (*n*=91). *pop-1(q645)* shows minor dominance: wild-type males were crossed into *pop-1(q645) mec-8/hT2[qIs48]*; 1% of *pop-1(q645)/+* cross progeny had one gonad arm (*n*=77). In addition, whole broods were scored from *pop-1(q645)/+*: two broods were scored at 25°C, and three at 20°C. At 25°C, 24% had no gonad arms, 2% had one gonad arm, and 72% had two gonad arms. At 20°C, 26% had no gonad arms and 73% had two gonad arms. At both temperatures about 1% of embryos were dead. Because *pop-1(q624)* can be L1 lethal, we scored total survivors from eggs laid during an 8 hour time period, rather than whole broods. From a heterozygous *pop-1(q624)/+* parent, 6% arrested or died as L1, and from a homozygous *pop-1(q624)* parent 65% arrested or died as L1. Animals that did not arrest as L1 grew to adulthood; gonad arms were scored in survivors (Table 1). This scheme was also used to assay lethality for *pop-1(q645)/tDf3* and *pop-1(RNAi)*. To assay lethality in *pop-1(q624)/tDf3*, we crossed *pop-1(q624)/hT2[qIs48]* females (feminized by *fog-1(RNAi)*) with *tDf3/hT2[qIs48]* males. Fewer than one *pop-1(q624)/tDf3* (non-green) survivor was seen per mother; gonad arms were scored in survivors (Table 1).

Male fertility was assayed for *pop-1(q645)* males. Non-green males, obtained by crossing *pop-1(q645)/hT2[qIs48]* XX hermaphrodites with *pop-1(q645)/hT2[qIs48]* XO males, were crossed, either singly or in groups of five, to five to seven *unc-11 fog-1* females. From single male matings, 0/15 males mated. From group matings, 5/7 crosses produced offspring; the genotype of these F_1 offspring was verified by analysis of F_2 progeny.

Cell lineage and laser ablation

Cell lineages were examined by standard methods (Sulston and Horvitz, 1977). Laser ablations were done as described previously (Bargmann and Avery, 1995) using a Micropoint Ablation Laser System (Photonics Instruments, Inc. Arlington, IL). *pop-1(q645)* homozygotes were obtained as non-green L1s from *pop-1(q645)/hT2[qIs48]* parents. Either Z2 or Z3 was ablated in these animals prior to lineage analysis to reduce complexity; this ablation does not affect the Z1/Z4 lineage. *pop-1(RNAi)* males were selected for lineage by presence of a large B cell. For Z1.a/Z4.p ablations and Z1.p/Z4.a ablations, cells were ablated soon after they were born. Ablations were verified by DIC 1-2 hours post-operative.

Mutant rescue and RNAi

To test for cosmid rescue, *pop-1(q645) unc-11(e47)/hT2* hermaphrodites were injected with 9 ng/μl BF8 cosmid and 100 ng/μl pRF4 (Mello and Fire, 1995). Fertile Rol Uncs were found among the F_2, and shown genetically to be homozygous for *pop-1(q645)*.

pop-1 template DNA for double stranded RNAi synthesis was made by PCR from genomic DNA, using primers with T7 sites at both ends. There are no other genes in the *C. elegans* genome that could be targeted by this RNA. Template DNA for other Wnt pathway members (except *bar-1* and *mom-5*, see below) was made by RT-PCR to the 5′ end of the gene. *bar-1* and *mom-5* template was made by PCR from genomic DNA. PCR products were subcloned and amplified using primers with T7 promoter sites at the 5′ ends. Double stranded RNA was produced using the MEGAscript T7 Kit (Ambion Inc.). All dsRNA products included at least 650 bp of exon sequence. RNA to each gene was injected into N2 and *unc-32 rde-1* at both 2.5 and 1 mg/ml. Injected animals were crossed with N2 males or males carrying GFP reporters. We also injected *lin-44(n1792)* mutants with *egl-20*, *cwn-1*, or *cwn-2* dsRNA, each at 1 mg/ml. In addition, we soaked *lin-44(n1792)* mutants for 2 days (with *E. coli*) in the following dsRNA combinations: *mom-2* and *egl-20*; *mom-2* and *cwn-1*; *mom-2* and *cwn-2*. Each RNA was at 1 mg/ml in M9.

Molecular analysis

Exons of *pop-1* were sequenced from *q624* and *q645* homozygotes. Template DNA for sequencing was made by amplifying *pop-1* exons

from homozygotes using Expand™ High Fidelity PCR System (Boehringer Mannheim). At least three independent PCR reactions were sequenced from each mutant, and two independent RT-PCR reactions were sequenced from *pop-1(q645)* mutants. Big-Dye Terminator Ready Reaction Mix was used for sequencing reactions (PE/Applied BioSystems). Reactions were run by the Blattner Lab sequencing service, Department of Genetics, UW-Madison. Protein alignments were made using PILEUP from GCG Wisconsin Package v.10 (Genetic Computer Group, Madison, WI).

RESULTS

In a screen for mutants defective in early gonadogenesis, we identified two mutations, *sys-2(q624)* and *sys-2(q645)*, that defined the *sys-2* locus (see Methods). Genetic mapping placed *sys-2* near *pop-1*, and three-factor-mapping failed to separate *sys-2* from *pop-1* (see Methods). The only *pop*-1 mutation available at the time was *pop-1(zu189)*, a maternal effect lethal allele that disrupts maternal, but not zygotic, *pop-1* (Lin et al., 1995; Morse and Bass, 1999). Although *pop-1(zu189)/sys-2* transheterozygotes were not Sys, the mutations might be allelic, if *pop-1(zu189)* only affected maternal functions and *pop-1(q645)* primarily affected zygotic functions. To determine whether *pop-1* might indeed influence early gonadogenesis, we used RNA-mediated interference (RNAi). We circumvented the *pop-1* maternal effect lethality by injecting double stranded *pop-1* RNA into a *rde-1* homozygote, which is defective in RNAi (Tabara et al., 1999), then mated with wild-type males to generate *rde-1/+* cross-progeny, which are zygotically sensitive to RNAi. This technique has been dubbed 'zygotic RNAi' (Herman, 2001). The *pop-1(RNAi); rde-1/+* cross-progeny had numerous problems, including typical Sys gonadal defects (see below). We therefore suspected that *sys-2* was actually *pop-1*. To test this idea, we first attempted mutant rescue and found that the *pop-1*-bearing cosmid BF8 (Lin et al., 1995) rescued *sys-2(q645)* to fertility (see Materials and Methods). We next sequenced all *pop-1* exons from *sys-2(q624)* and *sys-2(q645)* mutants and found a single nucleotide change in each (Fig. 2A). We conclude that *sys-2* is allelic with *pop-1*, and refer to the locus as *pop-1* henceforth.

The *pop-1* gene encodes a protein related to the TCF/LEF-1 transcription factor (Lin et al., 1995). Like other TCF-related proteins, POP-1 contains a conserved N-terminal β-catenin binding domain and a more central HMG box that binds DNA [reviewed by Roose and Clevers (Roose and Clevers, 1999)] (Fig. 2A). The *pop-1(q645)* mutation carries a nucleotide substitution predicted to change an aspartic acid (D) to a glutamic acid (E) (Fig. 2B); this mutation resides within the *pop-1* β-catenin binding domain and alters an amino acid conserved in all known TCF/LEF-1 proteins, including nematode, fly, and vertebrate homologues. The *pop-1(q624)* mutation possesses a nucleotide change in the region encoding the HMG box; the predicted amino acid change in this case also affects a conserved amino acid (Fig. 2C). Therefore, both *pop-1(Sys)* mutations alter critical, although distinct, portions of the protein.

Early gonadogenesis in *pop-1(Sys)* hermaphrodites

The *pop-1(Sys)* hermaphrodites exhibit typical Sys gonadal defects. First, whereas wild-type hermaphrodites have two DTCs (Fig. 3A), *pop-1(q645)* mutants have none (Fig. 3B, Table 1). Similarly, *pop-1(q624)* mutants can lack gonadal arms and DTCs (Table 1, not shown). Second, whereas wild-type hermaphrodites possess a single AC (Fig. 3D), *pop-1(Sys)* mutants often have two or sometimes 3 AC (Fig. 3E, Table 1). Third, whereas wild-type hermaphrodites assemble an SPh (Fig. 3A), this structure is never seen in *pop-1(q645)* hermaphrodites. Instead, a ring of somatic gonadal cells surrounds the germline at the L2/L3 stage (Fig. 3B, arrowheads).

The presence of two ACs could be explained either by an aberrant lineage or a defect in lateral signaling. Normally, Z1 and Z4 each produces a cell with potential to become an AC, and one is selected for the AC fate by lateral signaling (Fig. 1D, see Introduction). Lateral signaling is mediated by the LIN-12/Notch signaling pathway, and in *lin-12* mutants two ACs are observed (Greenwald et al., 1983; Seydoux and Greenwald, 1989). To test if *pop-1(Sys)* mutants are defective in lateral signaling, we examined *pop-1(q645); lin-12(0)* double mutants and found that they made from four to ten ACs (Table 1). If *pop-1* controlled polarity of the Z1/Z4 division

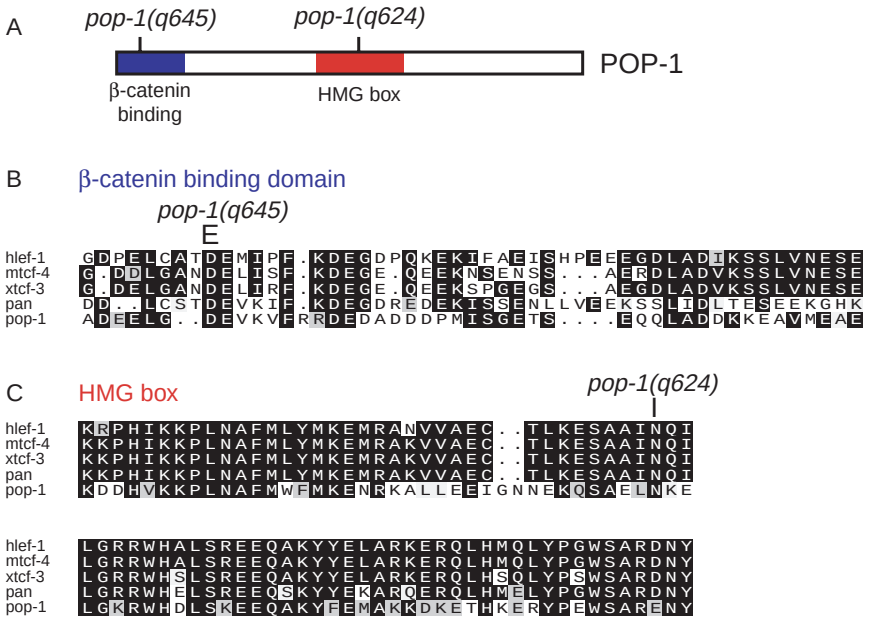

Fig. 2. Molecular basis of *pop-1(Sys)* mutations. (A) Diagram of POP-1 protein. A β-catenin binding domain resides at the N terminus (blue) and an HMG DNA binding domain is located centrally (red). Sites of *pop-1(Sys)* lesions are marked. (B) *pop-1(q645)* is predicted to change a conserved aspartic acid (D) to glutamic acid (E) in the β-catenin binding domain. (C) *pop-1(q624)* is predicted to change a conserved asparagine (N) to isoleucine (I) in the HMG box. Each alignment includes human LEF-1 (hlef-1), murine TCF-4 (mtcf-4), *Xenopus* TCF-3 (xtcf-3), *Drosophila* Pangolin (pan), and *C. elegans* POP-1.

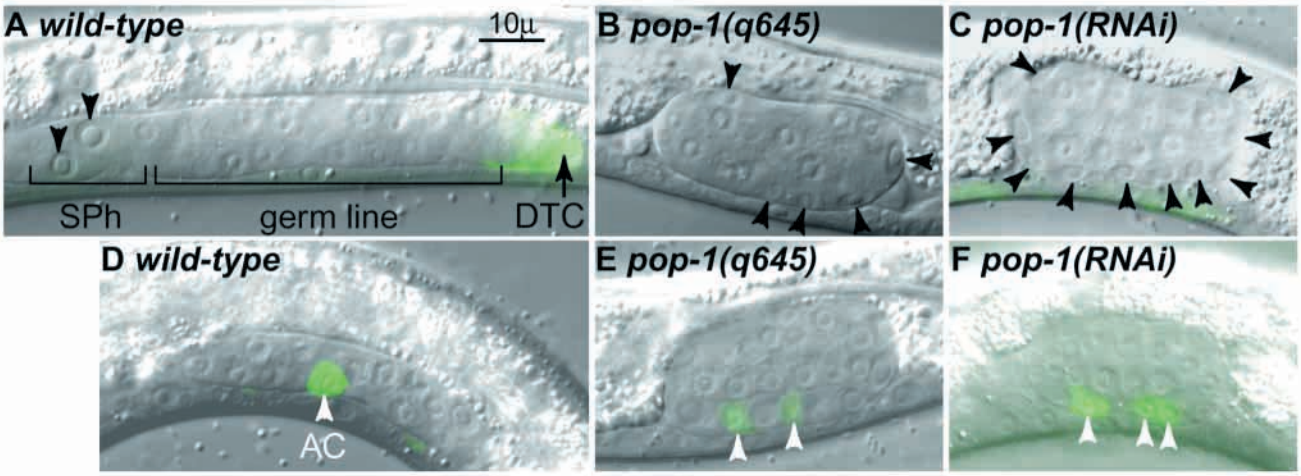

Fig. 3. Defects in *pop-1(Sys)* hermaphrodite gonad. (A-C) Animals carrying *lag-2::GFP*. Animals in A and C carry *qIs56[lag-2::GFP]*, animal in B carries *qIs19[lag-2::GFP]*. Both transgenes have similar GFP expression in the DTC, but *qIs56* has brighter GFP expression in the ventral nerve cord than *qIs19*. (A) Wild-type L3, SPh and one gonadal arm are shown. The DTC caps the end of the elongating arm and expresses *lag-2::GFP* brightly (arrow). Somatic gonadal cells within the SPh (black arrowheads) cluster centrally. (B,C) *pop-1(q645)* and *pop-1(RNAi)* animals have no gonadal elongation and no bright *lag-2::GFP* expression. Somatic gonadal cells are arranged around the gonadal periphery (black arrowheads). (D-F) Animals expressing *cdh-3::GFP*. (D) Wild-type L3. The AC marker *cdh-3::GFP* is expressed in a single cell (white arrowhead). (E,F) *pop-1(q645)* and *pop-1(RNAi)* L3 animals can have multiple ACs (white arrowheads).

and not subsequent divisions, a maximum of four ACs would be predicted in *pop-1(q645); lin-12(0)* double mutants. However, the production of more than four ACs suggests that *pop-1* functions at multiple points of the Z1/Z4 lineage. We conclude that lateral signaling is functional in *pop-1(Sys)* mutants and limits the number of ACs made.

We next examined the early Z1/Z4 lineage in *pop-1(q645)* hermaphrodites (Fig. 4). Normally, Z1 and Z4 generate daughters with different fates (Fig. 4A). However, in *pop-1(q645)* mutants, Z1 and Z4 appear to generate daughters with equivalent fates (Fig. 4B). Although some variability was seen (see legend to Fig. 4), all Z1/Z4 daughters could divide twice, a pattern normally restricted to proximal daughters. Furthermore, all four daughters could produce AC/VU cells, as shown by laser ablation and assaying vulva formation. When Z1.p and Z4.a were ablated in *pop-1(q645)* hermaphrodites, a vulva was nearly always formed (9/10); similarly, when Z1.a and Z4.p were ablated, a vulva was made (6/6). However, when Z1 and Z4 were ablated, no vulva was formed (5/5). Therefore, the *pop-1(Sys)* Z1/Z4 daughters are equivalent with respect to their pattern of cell divisions and generation of AC/VU cells.

Early gonadogenesis in *pop-1(Sys)* males

Unlike *pop-1(Sys)* hermaphrodite gonads, *pop-1(Sys)* male gonads can develop normally, and these males can be fertile (see Materials and Methods). To examine *pop-1(Sys)* males at a cellular level, we scored the production of regulatory cells and SPm formation. In wild-type males, the distal Z1/Z4

Table 1. Defects in *pop-1(Sys)* hermaphrodites

	% hermaphrodites with *x* number gonadal arms†				% hermaphrodites with *x* number anchor cells‡					L1 lethal§
Genotype*	2	1	0	*n*	1	2	3	≥4	*n*	% (*n*)
+/+	100	0	0	>100	100	0	0	0	45	0 (>100)
pop-1(q645)	0	0	100	158	14	70	16	0	44	0 (>100)
pop-1(q645)/tDf3	0	0	100	79			nd			20 (24)
pop-1(q645); lin-12(0)	0	0	100	24	0	0	0	100¶	24	nd
pop-1(q624)	28	45	26	117			nd			6 (144)**
pop-1(q624)††	44	42	17	125	72	28	0	0	36	65 (147)
pop-1(q624)/tDf3	0	0	100	26			nd			≥90 (>100)
pop-1(q624)/pop-1(q645)	0	6	94	79			nd			nd
pop-1(RNAi)	0	0	100	56	14	62	24	0	21	27 (165)

*Mutants are homozygotes derived from heterozygous mothers of genotype *pop-1(Sys)/ hT2[qIs48]*, except where noted.
†Gonadal arms scored by Nomarski optics.
‡Anchor cells scored using *cdh-3::GFP* marker.
§In experiments requiring crosses, both males and hermaphrodites were scored.
¶Number AC per animal: 4AC (2); 5AC (8); 6AC (5); 7AC (1); 8AC (6); 10AC (2).
**Percent lethality from heterozygous mothers of genotype *pop-1(q624)/ +*.
††*pop-1(q624)* homozygotes derived from *pop-1(q624)* homozygous mother.

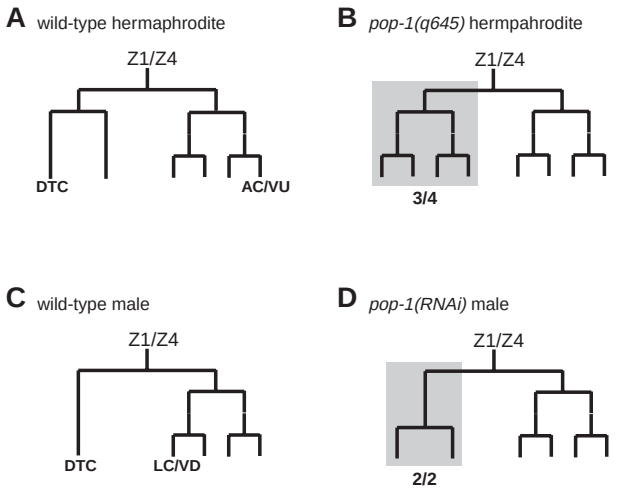

Fig. 4. Early lineage of Z1 or Z4 in wild-type and *pop-1* defective animals. **(**A-D) Vertical lines represent cells; horizontal lines represent divisions. Z1/Z4 represents Z1 or Z4; left daughter is distal; right daughter is proximal. DTC, distal tip cell; AC/VU, anchor cell or ventral uterine precursor; LC/VD, linker cell or vas deferens precursor. Only cell divisions in L1 and L2 were observed. (A) Wild-type hermaphrodite. The distal daughter divides once and gives rise to a DTC; the proximal daughter divides twice and gives rise to an AC/VU. (B) *pop-1(q645)*. Two hermaphrodites were watched from hatching until L2 lethargus. Z1/Z4 distal daughters divided twice, in three out of four cases; no DTCs were made. (C) Wild-type male. The distal daughter does not divide and becomes a DTC; the proximal daughter divides twice and gives rise to an LC/VD. (D) *pop-1(RNAi)* male. Z1/Z4 distal daughters both migrated anteriorly and divided once, albeit later than their proximal sisters. Later divisions were not followed.

daughters become DTCs, which are tiny and express *lag-2::GFP* weakly (Fig. 5A,B). A functional assay for male DTCs is germline proliferation: in the absence of DTCs the germline has a germline proliferation defect (Glp) (Kimble and White, 1981). We found that most *pop-1(Sys)* males made a robust germline, indicating that at least one DTC was made (Table 2). The production of DTCs in *pop-1(Sys)* males was corroborated by *lag-2::GFP* (Table 2), an assay that permits direct visualization of DTCs, but suffers from faint staining and an apparent enhancement of the Glp phenotype by the transgene (Table 2). Nonetheless, we found that 50% of *pop-1(Sys)* males made two DTCs (not shown). The wild-type male linker cell expresses *lag-2::GFP* intensely (Fig. 5A,B) (Blelloch et al., 1999). Similarly, all *pop-1(Sys)* males had at least one, and *pop-1(q645)* males sometimes had two LCs (Table 2; Fig. 5C,D). Furthermore, SPm formation was grossly normal in all *pop-1(q645)* males, but ~30% contained extra cells (Fig. 5D) (*n*=17). We conclude that *pop-1(Sys)* male gonads have typical Sys defects, but at low penetrance.

pop-1(RNAi) gonadal defects

Given that the *pop-1(Sys)* alleles are missense mutations, we next examined the effect of *pop-1(RNAi)* on gonadogenesis. For most genes, RNAi drastically reduces mRNA to specific genes and can mimic the null phenotype (Fire et al., 1998; Rocheleau et al., 1997). As described above, we bypassed the requirement for maternal *pop-1* by using an RNAi-resistant, *rde-1* mutant mother. We note that the zygotic *pop-1(RNAi)* phenotype may not be equivalent to the zygotic null phenotype, since we do not know how early during embryogenesis the wild-type copy of *rde-1* confers sensitivity to RNAi. The zygotic *pop-1(RNAi); rde-1/+* progeny showed typical Sys

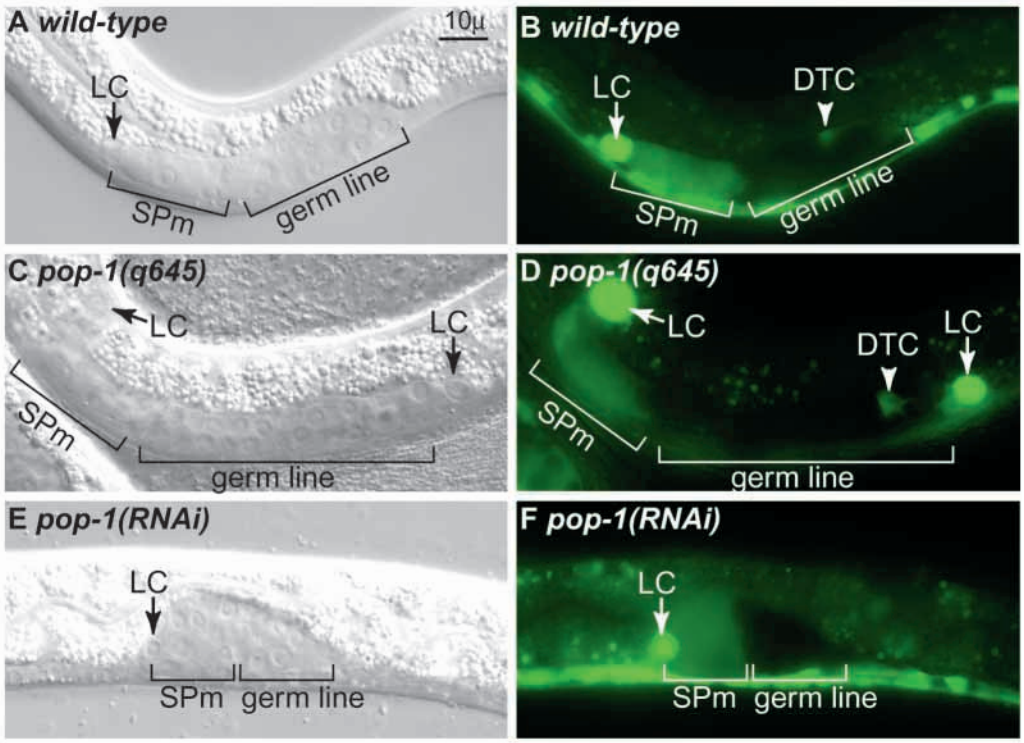

Fig. 5. Defects in *pop-1(Sys)* and *pop-1(RNAi)* male gonads. All animals carry *qIs56[lag-2::GFP]*, which expresses brightly in the LC and weakly in DTCs and other somatic gonadal cells. LC, linker cell; SPm, somatic primordium of male; DTC, distal tip cell. Extents of SPm and germline are indicated by brackets. (A,B) Wild-type L2 male. Single LC leads migration of the single gonadal arm. The SPm resides directly behind the LC and follows its migration. Two DTCs are present, but only one is seen in this focal plane (arrowhead). (C,D) *pop-1(q645)* male in L2 lethargus or early L3. Two LCs are present. The SPm appears normal: the somatic gonadal cells cluster to the anterior and follow the migrating LC. Only one DTC is present (arrowhead). (E,F) *pop-1(RNAi)* L2 male. Gonad polarity and organization are normal: the SPm is established at the anterior directly behind the LC. However, no DTCs are present and the LC has not migrated. The lack of LC migration is accompanied by a widening of the developing gonad.

Table 2. Gonadogenesis defects in *pop-1(Sys)* males

	Glp†		No DTCs‡		Extra LCs§	
Genotype*	%	*n*	%	*n*	%	*n*
+/+	0	>100	nd		0	>100
pop-1(q624)	4	102	nd		nd	
pop-1(q645)	5	105	nd		15	54
+/+; lag-2::GFP	0	43	0	43	0	43
pop-1(q624); lag-2::GFP	9	32	6	32	0	15
pop-1(q645); lag-2::GFP	15	58	17	58	21	39
pop-1(RNAi); lag-2::GFP	not scored¶		95	44	33	24

*Mutants are homozygotes derived from heterozygous mothers of genotype *pop-1(Sys)/hT2[qIs48]*.

†Scored by Nomarski optics; Glp animals have small germline composed of only sperm.

‡Scored by GFP fluorescence; DTCs observed as faint staining cells at posterior.

§When scored by Nomarski, extra LCs observed by morphology of LC; when scored by GFP fluorescence, extra LCs observed as intense staining with typical LC morphology.

¶Gonadal organization too poor to score germline fates.

gonadal defects at high penetrance in both sexes: hermaphrodites were 100% Sys and males were 95% Sys, as judged by lack of DTCs (Table 1 and 2, Fig. 3C,F, Fig. 5F). The percentage of males with extra LCs was lower, but this may reflect the effect of lateral signaling. The high Sys penetrance permitted lineage analysis of a *pop-1(RNAi)* male, which had not been practical with the weakly penetrant *pop-1(Sys)* mutants. In contrast to wild type, where Z1.a and Z4.p never divide (Fig. 4C), Z1.a and Z1.p both divided in a *pop-1(RNAi)* male (Fig. 4D). Interestingly, although *pop-1(RNAi)* males show a penetrant Sys defect, the somatic gonadal cells still cluster anteriorly as normal. Therefore, the anterior-posterior polarity of this organ is independent from the PD axis.

In addition to the early Z1/Z4 Sys defect, *pop-1(RNAi)* males have another gonadal defect not seen in *pop-1(Sys)* mutants: the gonadal arm does not elongate, even though the LC is correctly specified, as assayed by normal morphology and expression of *lag-2::GFP* (Fig. 5E,F). This failure in gonadal elongation results in a severely malformed gonad. Furthermore, *pop-1(RNAi)* males usually die in L3 or L4 owing to tail defects (see below), making their analysis beyond L2 difficult.

Non-gonadal defects of *pop-1(Sys)* mutants and *pop-1(RNAi)* progeny

Both *pop-1(Sys)* mutants and *pop-1(RNAi)* progeny exhibit non-gonadal defects in addition to the gonadal defects described above. Most severe is L1 lethality, which is found among *pop-1(q624)* mutants and zygotic *pop-1(RNAi)* animals, but is rare or non-existent among *pop-1(q645)* progeny (Table 1). The dead L1s exhibit various defects, including a detached pharynx, misshapen body, and incorrect assembly of the gonadal primordium (not shown). Among *pop-1(RNAi)* survivors, most hermaphrodites develop to adulthood, whereas most males die during L3 or L4. Virtually all *pop-1(RNAi)* animals are uncoordinated, and nearly all males exhibit tail defects. *pop-1(RNAi)* and *pop-1(Sys)* hermaphrodites often have a protruding vulva, and fertile *pop-1(q624)* hermaphrodites are frequently egg-laying defective.

Table 3. Comparison of *pop-1* effects on Z1/Z4 and T cell lineages

	% Z1/Z4 defective*,‡		
Allele	Hermaphrodite	Male	% T cells defective†,‡
q624	51% (234)	22% (64)	19% (94)
q645	100% (316)	37% (118)	40% (86)
RNAi	100% (112)	97% (88)	95% (86)

*% somatic gonadal precursor cells (Z1 and Z4) that do not generate a DTC.

†% T cells that do not produce phasmids.

‡Number of Z1 and Z4 or T cell lineages scored is shown in parentheses.

To monitor a non-gonadal defect that depends on Wnt signaling (Herman et al., 1995; Sawa et al., 1996), we assayed T cell polarity in both *pop-1(Sys)* mutants and *pop-1(RNAi)* animals. In wild-type hermaphrodites, the posterior T cell daughters give rise to the phasmid socket cells and the anterior daughters give rise to primarily hypodermis (Sulston and Horvitz, 1977). In the absence of Wnt signaling components, both daughters generate hypodermis and no phasmids are made (Herman, 2001; Herman and Horvitz, 1994; Herman et al., 1995). We therefore scored phasmids in *pop-1(Sys)* mutants and *pop-1(RNAi)* animals using a dye-filling assay (Herman and Horvitz, 1994). In both *pop-1(Sys)* mutants, fewer than normal phasmids were made, whereas in *pop-1(RNAi)* animals, almost none were found (Table 3). Therefore, both alleles affect T cell polarity in addition to Z1/Z4 polarity. Interestingly, the percentage of *pop-1(Sys)* mutants with T cell lineage defects was roughly equivalent to the percentage of *pop-1(Sys)* males with Z1/Z4 defects, both of which were substantially lower than the percentage of *pop-1(Sys)* hermaphrodites with Z1/Z4 defects (Table 3).

Genetic analysis of *pop-1(Sys)* mutants

The *pop-1(q624)* and *pop-1(q645)* mutants are similar in phenotype, but not identical. Furthermore, neither phenotype is equivalent to that of zygotic *pop-1(RNAi)*. We therefore explored the nature of the *pop-1(Sys)* mutations by standard genetic tests. We first tested whether either *pop-1(Sys)* mutation might be null. When *pop-1(q624)* was placed over a *pop-1* deficiency, its penetrance became considerably more severe, both with respect to the Sys gonadal defect and L1 lethality (Table 1). Therefore, *pop-1(q624)* is clearly not a null and is likely to be a partial loss-of-function mutation.

A similar simple conclusion could not be made for *pop-1(q645)*. This allele is fully penetrant for the Sys gonadal defect, as is *pop-1(q645)/tDf3* (Table 1). However, in contrast to *pop-1(q624)/tDf3* animals, which never made gonadal arms, a few *q624/q645* hermaphrodites did possess gonadal arms (Table 1), suggesting that *pop-1(q645)* is a strong loss-of-function allele with respect to DTC production, but not a null. Intriguingly, L1 lethality was not seen in *pop-1(q645)* mutants and was far less severe in *pop-1(q645)/tDf3* than *pop-1(q624)/tDf3* animals (Table 1). The full penetrance of *pop-1(q645)* with respect to hermaphrodite gonadogenesis contrasts sharply with its extremely weak effect on L1 viability. We speculate that the *pop-1(q645)* mutation may affect a regulatory region essential for hermaphrodite gonadogenesis, but not for most other *pop-1* functions (see Discussion).

L1 lethality was observed among *pop-1(q624)* mutants

Table 4. Wnt pathway components and regulators controlling Z1/Z4 asymmetry

Wnt pathway component	Gene	Method*	Sys ♀†	Sys ♂‡
Porcupine	*mom-1*	mutant	+	—
Wnt signal	*lin-44*	RNAi, mutant	—	—
	egl-20	RNAi, mutant	—	—
	mom-2	RNAi, mutant	—	—
	cwn-1	RNAi	—	—
	cwn-2	RNAi	—	—
Frizzled receptor	*lin-17*	RNAi, mutant	+	+
	cfz-1	RNAi	—	—
	mom-5	RNAi, mutant	—	—
β-catenin	*bar-1*	RNAi, mutant	—	—
	wrm-1	RNAi	+	+
nemo-like kinase	*lit-1*	mutants	+	+
TGFβ-activated kinase	*mom-4*	mutant	—	nd

*See Materials and Methods for specific alleles and RNAi.

†+, Sys hermaphrodites observed (DTCs assayed by arm elongation or *lag-2::GFP*; extra ACs scored using *cdh-3::GFP* or by DIC optics).

—, no Sys animals observed.

‡+, Sys males observed (DTCs assayed by germline proliferation; extra LCs scored using DIC optics or *lag-2::GFP* expression). Typically, males were only scored in RNAi experiments.

derived from a heterozygous mother (6%, *n*=144), but was more frequent among *pop-1(q624)* mutants derived from a homozygous mother (65%, *n*=147). Therefore, maternal *pop-1* product plays a role that is crucial for L1 survival in addition to its previously known role in controlling early embryogenesis (Lin et al., 1995).

Wnt signaling and gonadogenesis

The *pop-1* gene encodes a key transcription factor acting at the end of the Wnt pathway in *C. elegans* [reviewed by Wodarz and Nusse (Wodarz and Nusse, 1998)]. To determine what other Wnt signaling components might be involved in early gonadogenesis in nematodes, we used a combination of RNAi and available mutants to look for Sys defects. Table 4 summarizes our results. We detected no gonadogenesis defect for any of the five known Wnt homologues, by using RNAi for each of the five, or by analyzing mutants of three of them, or by double and triple RNAi (see Materials and Methods). However, 3% of animals mutant for the *porcupine* homologue, *mom-1*, had only one gonadal arm (*n*=72). Therefore, a Wnt ligand may be involved in Z1/Z4 polarity. We next examined three *frizzled* receptor homologues (Table 4). Previous work showed that a *lin-17* nonsense mutant has a low penetrance Sys phenotype (Sternberg and Horvitz, 1988). Using RNAi, we confirmed this effect. RNAi directed against either of the other two *frizzled* receptor homologues, *mom-5* and *cfz-1*, did not yield Sys defects. In addition, *mom-5* mutants do not show Sys defects. Therefore, LIN-17 is the only *frizzled* receptor known to function in the early gonadal lineage, and its role is not essential. In RNAi experiments, genes with known mutant phenotypes exhibited expected defects (for example, *mom-2(RNAi)* produced 100% dead eggs when wild-type animals were injected), suggesting that RNAi of these genes was working to some extent.

We also tested two β-catenin homologues in the *C. elegans* genome. RNAi of *bar-1*, a β-catenin that binds the β-catenin binding domain of POP-1 (Korswagen et al., 2000; Natarajan et al., 2001), does not give a Sys phenotype; nor does a *bar-1* null mutant. However, RNAi of *wrm-1*, a second β-catenin homologue that weakly binds full length POP-1 (Korswagen et al., 2000; Natarajan et al., 2001; Rocheleau et al., 1999), gave a 100% penetrant Sys phenotype in both males and hermaphrodites (Table 4). LIT-1 functions with WRM-1 to phosphorylate POP-1 and regulates POP-1 function and localization (Meneghini et al., 1999; Rocheleau et al., 1999). We found that *lit-1* mutants can exhibit a Sys phenotype in both sexes when grown at 25°C. Therefore, *wrm-1* and *lit-1* loss-of-function both exhibit the same phenotype as *pop-1* loss-of-function in the gonad.

DISCUSSION

POP-1 controls axis formation during gonadogenesis

C. elegans POP-1 belongs to the TCF/LEF-1 family of transcription factors, which are controlled by Wnt signaling [reviewed by Wodarz and Nusse (Wodarz and Nusse, 1998)]. Previous studies implicated Wnt signaling in the control of anterior-posterior (AP) cell polarity in the embryonic EMS blastomere (Lin et al., 1995; Rocheleau et al., 1997; Thorpe et al., 1997) and AP cell polarity in the post-embryonic T blast cells (Herman, 2001; Herman et al., 1995; Sawa et al., 1996), V cells (Whangbo et al., 2000) and Pn.p cells (Sawa et al., 1996; Sternberg and Horvitz, 1988), as well as in the control of AP migration of Q neuroblast descendants (Herman, 2001; Korswagen et al., 2000; Maloof et al., 1999; Whangbo and Kenyon, 1999). Therefore, Wnt signaling is utilized in both embryonic and zygotic development to distinguish anterior versus posterior identities. In this paper, we report that POP-1 is critical during gonadogenesis. Rather than establishing AP polarity, Wnt signaling sets up the PD axes of the gonad.

During normal gonadogenesis, POP-1 governs the asymmetric division of Z1 and Z4, and thereby controls the gonadal PD axes and the generation of regulatory cells essential for subsequent morphogenesis. In the absence of POP-1 function, Z1 and Z4 generate equivalent daughters that both assume a proximal fate. Hermaphrodite gonads lacking *pop-1* lose their PD axes and fail to generate regulatory cells required for gonad elongation and germline proliferation. Male gonads lacking *pop-1* also lose their PD axis – they have no cells with distal fates and therefore no distal end. However, the male gonad acquires an apparently normal polarity, which reflects the male-specific specification of an AP axis in this organ.

How might POP-1 govern Z1/Z4 asymmetry? We suggest two simple models (Fig. 6A), although they are not mutually exclusive and others certainly exist. Both models take advantage of the fact that Z1 and Z4 contact each other ventrally beneath the two germline precursor cells, Z2 and Z3 (F-H. Markussen, L. Mathies, Y. Li, and J. Kimble, unpublished). In the first model (Fig. 6A, left), a Wnt signal polarizes Z1 and Z4, which leads to enhanced POP-1 activity in the distal daughter. Polarization may result from an inhibitory signal in the underlying hypodermis or from a decreased access of the Wnt to proximal regions of Z1/Z4. In

the second model (Fig. 6A, right), no Wnt signal is invoked. Instead, contact between Z1 and Z4 (or contact between Z1/Z4 and the hypodermis) polarizes the cell (for example, excluding LIN-17 from the proximal region), which leads to enhanced POP-1 activity in the distal daughters.

Wnt signaling controls primary axis formation in vertebrates [reviewed by Sokol (Sokol, 1999)] and limb axis formation in vertebrates [reviewed by Johnson and Tabin (Johnson and Tabin, 1997)] and flies [reviewed by Serrano and O'Farrell (Serrano and O'Farrell, 1997)]. In *C. elegans*, POP-1 controls axis formation during early organogenesis by controlling a key cell polarity (this work). In vertebrate primary axis formation, Wnt signaling is restricted to a dorsal developmental field by opposing ventral signals, leading to dorsal-ventral (D-V) axis specification; similar processes control limb DV axis specification [see the following reviews (Johnson and Tabin, 1997; Serrano and O'Farrell, 1997; Sokol, 1999)]. Polarization of Z1 and Z4 in *C. elegans* gonad development may act in a similar fashion: the proximal-distal axis may be determined by restriction of Wnt signaling to the distal Z1 and Z4 daughter cells.

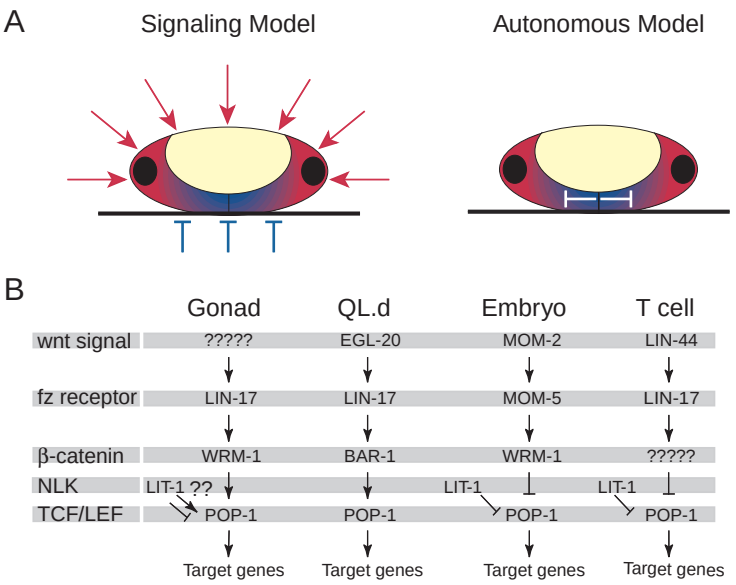

Fig. 6. Models for control of Z1/Z4 asymmetry. (A) Two models by which Z1 and Z4 might be polarized. See text for explanation. (B) Pathways of POP-1 control. See text for explanation.

Identification of two distinct *pop-1(Sys)* mutations

Prior to this paper, only one *pop-1* mutation had been reported. This allele, *pop-1(zu189)*, is a transposon insertion into the *pop-1* 3′ untranslated region that abolishes maternal *pop-1* mRNA, but leaves zygotic *pop-1* mRNA intact (Lin et al., 1995; Morse and Bass, 1999). The *pop-1(zu189)* allele identifies a regulatory site for maternal *pop-1* mRNA, but does not shed light on regulatory regions within POP-1 protein itself. In this paper, we report two *pop-1(Sys)* mutations that change the *pop-1* coding region and have dramatic effects on gonadogenesis. Our characterization of these alleles as well as zygotic *pop-1(RNAi)* suggests that one, *pop-1(q624)*, is a typical partial loss-of-function mutation and that the other, *pop-1(q645)*, is unusual. The *pop-1(q624)* defects are found at low penetrance, are similar to those observed by *pop-1(RNAi)* (e.g. L1 lethality, Sys gonads, T cell defects), and become more penetrant when the allele is placed over a deficiency. This mutation alters a conserved amino acid in the HMG box DNA binding domain which is conserved specifically in TCF/LEF-1 type HMG proteins (Laudet et al., 1993), suggesting that the *pop-1(q624)* mutation may affect either recognition of the TCF/LEF-1 consensus sequence or DNA binding affinity, thereby lowering POP-1 activity.

Unlike the weak *pop-1(q624)* mutation, *pop-1(q645)* is fully penetrant for the Sys gonadal defect in hermaphrodites, but exhibits no L1 lethality. Therefore, *pop-1(q645)* alters a residue essential for hermaphrodite gonadogenesis, but not for other *pop-1*-dependent events. The *pop-1(q645)* mutation alters an aspartic acid to glutamic acid in the β-catenin binding domain. This amino acid is conserved in all TCF/LEF-1 homologues and, through analysis of vertebrate homologues, has been shown to be essential for β-catenin binding and for β-catenin and TCF/LEF-1 to activate transcription of target genes (Graham et al., 2000; Hsu et al., 1998; von Kries et al., 2000). Although two missense mutations affecting the β-catenin binding domain of the *D. melanogaster* TCF/LEF-1 homologue, Pan, have been isolated, neither of these two mutations affects residues conserved among all TCF/LEF-1 homologues (Brunner et al., 1997). As the only mutation isolated thus far in a developmental system that changes a highly conserved amino acid in the β-catenin binding domain, the *pop-1(q645)* missense mutation may shed new light on TCF/LEF-1 function during development. It is striking that the β-catenin homologue critical for Z1/Z4 asymmetry is WRM-1, which does not bind the POP-1 β-catenin binding domain (Korswagen et al., 2000; Natarajan et al., 2001; Rocheleau et al., 1999). We speculate that the POP-1 β-catenin binding site may interact with a regulator in Z1 and Z4 that differentially affects that tissue.

Z1/Z4 asymmetry, POP-1 control and sexual dimorphism?

Because *pop-1(q645)* mutant hermaphrodites are always sterile while mutant males can be fertile, we looked for sex-specific defects in *pop-1(Sys)* mutants and *pop-1(RNAi)* animals. We found that POP-1 controls Z1/Z4 asymmetry in both sexes. Unlike *pop-1(RNAi)*, which has equivalent strong effects on both sexes, the two *pop-1(Sys)* mutations affect hermaphrodite gonadogenesis more strongly than male gonadogenesis (Table 3). This difference in the effect of *pop-1* mutations may reflect a more stringent requirement for *pop-1* activity in the hermaphrodite gonad, it may reflect a more stringent dependence of the hermaphrodite gonad on correctly establishing the PD axes, or it may reflect a sex-specific regulation of POP-1. If sex-specific regulation occurs, the unusual *pop-1(q645)* mutation may identify the POP-1 β-catenin binding domain as the site of that regulation.

PD axis specification is required for SP formation in hermaphrodites, but not in males. In *pop-1(Sys)* and *pop-1(RNAi)* hermaphrodites, SPh formation is disrupted resulting in a disorganized organ and sterility. However, in *pop-1(Sys)* and *pop-1(RNAi)* males, early cell rearrangements

that establish the AP axis of the male gonad occur as normal, and overall organ polarity appears normal. Therefore, AP axis specification in the male gonad does not require proper specification of the PD axis.

The POP-1 pathway controls Z1/Z4 asymmetry

We surveyed Wnt signaling components and additional regulators of POP-1 for control of Z1/Z4 asymmetry and found that a decrease in either WRM-1, a β-catenin homologue, or LIT-1, a nemo-like kinase (NLK) homologue, led to Z1/Z4 defects similar to those of decreased POP-1 activity. Furthermore, previous work showed that the *lin-17* receptor is involved, albeit with lower penetrance (Sawa et al., 1996; Sternberg and Horvitz, 1988). Surprisingly, we identified no Wnt signal as critical for Z1/Z4 polarity. This negative result may mean that a Wnt signal is not required, that the signal is encoded by redundant *wnt* genes, or that it is not sensitive to RNAi. At present, we cannot distinguish among these possibilities. Our models (Fig. 6A) leave open the possibility that a Wnt signal may exist.

Fig. 6B summarizes the pathway of POP-1 control in the gonad and compares that control to what has been observed in other *C. elegans* tissues. In Z1/Z4, WRM-1 and LIT-1 are both required for POP-1 activity. All three genes are required for the distal fate. By contrast, WRM-1 and LIT-1 antagonize POP-1 activity in the embryo (Kaletta et al., 1997; Meneghini et al., 1999; Rocheleau et al., 1999) and LIT-1, but not WRM-1, is involved in controlling T cell polarity (Herman, 2001; Rocheleau et al., 1999).

We envision two explanations for how WRM-1 (a β-catenin) and LIT-1 (NLK) may act to enhance POP-1 activity in the gonad. One simple explanation is that WRM-1 and LIT-1 may positively regulate POP-1; this idea is based on classical results that β-catenins are TCF co-activators [reviewed by Wodarz and Nusse (Wodarz and Nusse, 1998)], and that NLK is complexed with TCF and β-catenin (Ishitani et al., 1999). The WRM-1 β-catenin does not bind the POP-1 β-catenin binding domain per se, but weakly binds full length POP-1 (Korswagen et al., 2000; Natarajan et al., 2001; Rocheleau et al., 1999). Perhaps WRM-1 interacts with POP-1 in Z1/Z4 and serves there as a coactivator. Such a physical interaction might be stabilized by another binding protein, such as LIT-1, which is known to bind POP-1 (Natarajan et al., 2001; Rocheleau et al., 1999). A second possibility is that WRM-1 and LIT-1 negatively regulate POP-1, reducing its level to an active state; this rather counterintuitive model was proposed by Herman (Herman, 2001) to explain how POP-1 might be regulated in the T cells. If the Herman model holds true in Z1/Z4 as well, then WRM-1/LIT-1 may downregulate POP-1 in the distal daughters and thereby bring POP-1 to the appropriate level to drive distal fates. The first model predicts that POP-1 will be more abundant in the distal daughters, whereas the Herman model predicts POP-1 to be more abundant in the proximal daughters. Unfortunately, POP-1 antibodies currently available do not reliably stain the L1 gonad, although hypodermal and other tissues stain well (K. Siegfried, unpublished). We have therefore been unable to distinguish between these two models.

We gratefully acknowledge additional participants of the gonadogenesis screen in which both *pop-1* mutants were isolated: Laura Mathies, Finn-Hugo Markussen, Peggy Kroll-Conner, and Lisa Friedman. We also thank Yulin Zhang for technical assistance; Laura Mathies, Trey Kidd, and Jennifer Miskowski for stimulating discussions during the course of this work; and Laura Mathies, Eric Haag and Matthew Harris for insightful comments on this manuscript. Thanks to Jonathan Pettitt for the integrated *cdh-3::GFP* and Mark Edgley for *ccEx9747*. Some strains were provided by the *Caenorhabditis* Genetics Center. K. S. was supported by an NIH predoctoral training grant to the Laboratory of Genetics; J. K. is an investigator with the Howard Hughes Medical Institute.

REFERENCES

Bargmann, C. I. and Avery, L. (1995). Laser killing of cells in *Caenorhabditis elegans*. In *Caenorhabditis elegans: Modern Biological Analysis of an Organism*, (ed. H. F. Epstein and D. C. Shakes), pp. 225-250. San Diego: Academic Press, Inc.

Blelloch, R., Santa Anna-Arriola, S., Gao, D., Li, Y., Hodgkin, J. and Kimble, J. (1999). The *gon-1* gene is required for gonadal morphogenesis in *Caenorhabditis elegans*. *Dev. Biol.* **216**, 382-393.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71-94.

Brunner, E., Peter, O., Schweizer, L. and Basler, K. (1997). *pangolin* encodes a Lef-1 homologue that acts downstream of Armadillo to transduce the Wingless signal in *Drosophila*. *Nature* **385**, 829-833.

Eisenmann, D. M. and Kim, S. K. (2000). Protruding vulva mutants identify novel loci and Wnt signaling factors that function during *Caenorhabditis elegans* vulva development. *Genetics* **156**, 1097-1116.

Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E. and Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806-811.

Friedman, L., Santa Anna-Arriola, S., Hodgkin, J. and Kimble, J. (2000). *gon-4*, a cell lineage regulator required for gonadogenesis in *Caenorhabditis elegans*. *Dev. Biol.* **228**, 350-362.

Graham, T. A., Weaver, C., Mao, F., Kimelman, D. and Xu, W. (2000). Crystal structure of a β-catenin/Tcf complex. *Cell* **103**, 885-896.

Greenwald, I. S., Sternberg, P. W. and Horvitz, H. R. (1983). The *lin-12* locus specifies cell fates in Caenorhabditis elegans. *Cell* **34**, 435-444.

Herman, M. A. (2001). *C. elegans* POP-1/TCF functions in a canonical Wnt pathway that controls cell migration and in a noncanonical Wnt pathway that controls cell polarity. *Development* **128**, 581-590.

Herman, M. A. and Horvitz, H. R. (1994). The *Caenorhabditis elegans* gene *lin-44* controls the polarity of asymmetric cell divisions. *Development* **120**, 1035-1047.

Herman, M. A., Vassilieva, L. L., Horvitz, H. R., Shaw, J. E. and Herman, R. K. (1995). The C. elegans gene *lin-44*, which controls the polarity of certain asymmetric cell divisions, encodes a Wnt protein and acts cell nonautonomously. *Cell* **83**, 101-110.

Hirsh, D., Oppenheim, D. and Klass, M. (1976). Development of the reproductive system of *Caenorhabditis elegans*. *Dev. Biol.* **49**, 200-219.

Hodgkin, J. (1997). Appendix 1. Genetics. In *C. elegans II* (ed. D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess), pp. 881-1047. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Hsu, S.-C., Galceran, J. and Grosschedl, R. (1998). Modulation of transcriptional regulation by LEF-1 in response to Wnt-1 signaling and association with β-catenin. *Mol. Cell. Biol.* **18**, 4807-4818.

Hubbard, E. J. A. and Greenstein, D. (2000). The *Caenorhabditis elegans* gonad: A test tube for cell and developmental biology. *Dev. Dyn.* **218**, 2-22.

Ishitani, T., Ninomiya-Tsuji, J., Nagai, S.-i., Nishita, M., Meneghini, M., Barker, N., Waterman, M., Bowerman, B., Clevers, H., Shibuya, H. et al. (1999). The TAK1-NLK-MAPK-related pathway antagonizes signalling between β-catenin and transcription factor TCF. *Nature* **399**, 798-802.

Johnson, R. L. and Tabin, C. J. (1997). Molecular models for vertebrate limb development. *Cell* **90**, 979-990.

Kaletta, T., Schnabel, H. and Schnabel, R. (1997). Binary specification of the embryonic lineage in *Caenorhabditis elegans*. *Nature* **390**, 294-298.

Kimble, J. (1981). Alterations in cell lineage following laser ablation of cells in the somatic gonad of *Caenorhabditis elegans*. *Dev. Biol.* **87**, 286-300.

Kimble, J. and Hirsh, D. (1979). The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. *Dev. Biol.* **70**, 396-417.

Kimble, J. E. and White, J. G. (1981). On the control of germ cell development in *Caenorhabditis elegans*. *Dev. Biol.* **81**, 208-219.

Korswagen, H. C., Herman, M. A. and Clevers, H. C. (2000). Distinct β-catenins mediate adhesion and signalling functions in *C. elegans*. *Nature* **406**, 527-532.

Laudet, V., Shehelin, D. and Clevers, H. (1993). Ancestry and diversity of the HMG box superfamily. *Nucleic Acids Res.* **21**, 2493-2501.

Lin, R., Thompson, S. and Priess, J. R. (1995). *pop-1* encodes an HMG box protein required for the specification of a mesoderm precursor in early C. elegans embryos. *Cell* **83**, 599-609.

Maloof, J. N., Shangbo, J., Harris, J. M., Jongeward, G. D. and Kenyon, C. (1999). A Wnt signaling pathway controls Hox gene expression and neuroblast migration in *C. elegans*. *Development* **126**, 37-49.

Mello, C. and Fire, A. (1995). DNA transformation. In *Caenorhabditis elegans: Modern Biological Analysis of an Organism* (ed. H. F. Epstein and D. C. Shakes), pp. 451-482. San Diego: Academic Press, Inc.

Meneghini, M. D., Ishitani, T., Carter, J. C., Hisamoto, N., Ninomiya-Tsuji, J., Thorpe, C. J., Hamill, D. R., Matsumoto, K. and Bowerman, B. (1999). MAP kinase and Wnt pathways converge to downregulate an HMG-domain repressor in *Caenorhabditis elegans*. *Nature* **399**, 793-797.

Miskowski, J., Li, Y. and Kimble, J. (2001). The *sys-1* gene and sexual dimorphism during gonadogenesis in *Caenorhabditis elegans*. *Dev. Biol.* **230**, 61-73.

Morse, D. P. and Bass, B. L. (1999). Long RNA hairpins that contain inosine are present in *Caenorhabditis elegans* poly(A)$^+$ RNA. *Proc. Natl. Acad. Sci. USA* **96**, 6048-6053.

Natarajan, L., Witwer, N. E. and Eisenmann, D. M. (2001). The divergent *Caenorhabditis elegans* β-catenin proteins BAR-1, WRM-1 and HMP-2 make distinct protein interactions but retain functional redundancy *in vivo*. *Genetics* **159**, 159-172.

Newman, A. P., White, J. G. and Sternberg, P. W. (1995). The *Caenorhabditis elegans lin-12* gene mediates induction of ventral uterine specialization by the anchor cell. *Development* **121**, 263-271.

Pettitt, J., Wood, W. B. and Plasterk, R. H. A. (1996). *cdh-3*, a gene encoding a member of the cadherin superfamily, functions in epithelial cell morphogenesis in *Caenorhabditis elegans*. *Development* **122**, 4149-4157.

Rocheleau, C. E., Downs, W. D., Lin, R., Wittmann, C., Bei, Y., Cha, Y.-H., Ali, M., Priess, J. R. and Mello, C. C. (1997). Wnt signaling and an APC-related gene specify endoderm in early C. elegans embryos. *Cell* **90**, 707-716.

Rocheleau, C. E., Yasuda, J., Shin, T. H., Lin, R., Sawa, H., Okano, H., Priess, J. R., Davis, R. J. and Mello, C. C. (1999). WRM-1 activates the LIT-1 protein kinase to transduce anterior/posterior polarity signals in *C. elegans*. *Cell* **97**, 717-726.

Roose, J. and Clevers, H. (1999). TCF transcription factors: molecular switches in carcinogenesis. *Biochim. Biophys. Acta* **97456**, M23-M37.

Sawa, H., Lobel, L. and Horvitz, H. R. (1996). The *Caenorhabditis elegans* gene *lin-17*, which is required for certain asymmetric cell divisions, encodes a putative seven-transmembrane protein similar to the *Drosophila* Frizzled protein. *Genes Dev.* **10**, 2189-2197.

Serrano, N. and O'Farrell, P. H. (1997). Limb morphogenesis: connections between patterning and growth. *Curr. Biol.* **7**, R186-R195.

Seydoux, G. and Greenwald, I. (1989). Cell autonomy of *lin-12* function in a cell fate decision in C. elegans. *Cell* **57**, 1237-1245.

Sokol, S. Y. (1999). Wnt signaling and dorso-ventral axis specification in vertebrates. *Curr. Opin. Genet. Dev.* **9**, 405-410.

Sternberg, P. W. and Horvitz, H. R. (1988). *lin-17* mutations of *Caenorhabditis elegans* disrupt certain asymmetric cell divisions. *Dev. Biol.* **130**, 67-73.

Sulston, J. E. and Horvitz, H. R. (1977). Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. *Dev. Biol.* **56**, 110-156.

Tabara, H., Sarkissian, M., Kelly, W. G., Fleenor, J., Grishok, A., Timmons, L., Fire, A. and Mello, C. C. (1999). The *rde-1* gene, RNA interference, and transposon silencing in *C. elegans*. *Cell* **99**, 123-132.

Thorpe, C. J., Schlesinger, A., Carter, J. C. and Bowerman, B. (1997). Wnt signaling polarizes an early C. elegans blastomere to distinguish endoderm from mesoderm. *Cell* **90**, 695-705.

von Kries, J. P., Winbeck, G., Asbrand, C., Schwarz-Romond, T., Sochnikova, N., Dell'Oro, A., Behrens, J. and Birchmeier, W. (2000). Hot spots in β-catenin for interactions with LEF-1, conductin and APC. *Nat. Struct. Biol.* **7**, 800-807.

Whangbo, J., Harris, J. and Kenyon, C. (2000). Multiple levels of regulation specify the polarity of an asymmetric cell division in *C. elegans*. *Development* **127**, 4587-4598.

Whangbo, J. and Kenyon, C. (1999). A Wnt signaling system that specifies two patterns of cell migration in *C. elegans*. *Mol. Cell* **4**, 851-858.

Wodarz, A. and Nusse, R. (1998). Mechanisms of Wnt signaling in development. *Annu. Rev. Cell Dev. Biol.* **14**, 59-88.