Author's correction

The print version incorrectly cites 'Maloof and Kenyon, 1998' instead of 'Maloof et al., 1999'.

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

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SUMMARY

In *Caenorhabditis elegans*, Wnt signaling pathways are important in controlling cell polarity and cell migrations. In the embryo, a novel Wnt pathway functions through a β-catenin homolog, WRM-1, to downregulate the levels of POP-1/Tcf in the posterior daughter of the EMS blastomere. The level of POP-1 is also lower in the posterior daughters of many anteroposterior asymmetric cell divisions during development. I have found that this is the case for of a pair of postembryonic blast cells in the tail. In wild-type animals, the level of POP-1 is lower in the posterior daughters of the two T cells, TL and TR. Furthermore, in *lin-44/Wnt* mutants, in which the polarities of the T cell divisions are frequently reversed, the level of POP-1 is frequently lower in the anterior daughters of the T cells. I have used a novel RNA-mediated interference technique to interfere specifically with *pop-1* zygotic function and have determined that *pop-1* is required for wild-type T cell polarity. Surprisingly, none of the three *C. elegans* β-catenin homologs appeared to function with POP-1 to control T cell polarity. Wnt signaling by EGL-20/Wnt controls the migration of the descendants of the QL neuroblast by regulating the expression the Hox gene *mab-5*. Interfering with *pop-1* zygotic function caused defects in the migration of the QL descendants that mimicked the defects in *egl-20/Wnt* mutants and blocked the expression of *mab-5*. This suggests that POP-1 functions in the canonical Wnt pathway to control QL descendant migration and in novel Wnt pathways to control EMS and T cell polarities.

Key words: *C. elegans*, Wnt, β-catenin, Tcf, POP-1

INTRODUCTION

The Wnt signaling pathway is one of the major signaling pathways controlling animal development (reviewed by Cadigan and Nusse 1997; Wodarz and Nusse 1998). The Wnts are a conserved family of secreted glycoproteins that function as signaling molecules in many different developmental processes. Genetic and molecular studies have determined the probable order of action of several components of a Wnt pathway that affects segment polarity in *Drosophila*: Porcupine (Porc) is required for synthesis or secretion of the Wnt protein, Wingless (Wg). Wg then can act through the Frizzled 2 (Fz2) receptor (Bhanot et al., 1996; Kennerdell and Carthew, 1998). Inside the cell, Dishevelled protein (Dsh) is then activated and antagonizes the action of Zeste-white3 kinase (a homolog of glycogen synthase kinase 3, GSK3), which results in the stabilization of Armadillo (Arm, a homolog of β-catenin), causing it to accumulate in both the cytoplasm and the nucleus, where it appears to interact with Pangolin (a homolog of Tcf) to activate target genes. Recently, a more complex picture of Wnt signaling has emerged. In short, it appears that activated Dsh interacts with Axin (Kishida et al., 1999), which is part of a large protein complex consisting of GSK3, the adenomatous

polyposis coli protein (APC), β-TrCP/Slimb (Marikawa and Elinson, 1998) protein phosphatase 2a (Hsu et al., 1999) and other proteins that function to promote the degradation of β-catenin (reviewed by Arias et al., 1999; Miller et al., 1999). Components of this pathway have been shown to function in dorsal axis formation in *Xenopus* (Moon et al., 1997), as well as in the development of several forms of cancer (Nusse and Varmus, 1982; Lejeune et al., 1995; Morin et al., 1997; Rubinfeld et al., 1997; Polakis, 1999).

In the early *C. elegans* embryo, Wnt signaling controls the polarity of the EMS blastomere. MOM-2 is a Wnt signal produced in the four-cell embryo by the P2 blastomere, which polarizes the EMS blastomere and thereby confers distinct fates on the EMS daughter cells: E, which gives rise to endoderm, and MS, which gives rise to mesoderm (Rocheleau et al., 1997; Thorpe et al., 1997). Other components of the Wnt pathway also function in this process, including MOM-1 (Porc), MOM-5 (Frizzled), APR-1 (APC), SGG-1 (GSK3) (Schlesinger et al., 1999) and WRM-1 (β-catenin). Mutation of any of these genes causes both EMS daughters to adopt an MS-like fate (Rocheleau et al., 1997; Thorpe et al., 1997; Schlesinger et al., 1999). However, mutations that affect the Tcf homolog POP-1 cause a phenotype opposite to that

expected from the canonical Wnt pathway: both daughters adopt an E-like fate (Lin et al., 1995). The level of POP-1 has been shown to be higher in the anterior MS cell than in the posterior E cell, which receives a Wnt signal; this suggests that Wnt signaling acts to downregulate POP-1 levels in the E cell (Lin et al., 1998). Recently, components of a mitogen-activated protein kinase (MAPK) pathway including LIT-1 (Nemo-like kinase, NLK) and MOM-4 (transforming growth-factor-β-activated kinase, TAK1) have been shown to function with the Wnt signaling pathway to downregulate POP-1 in the E cell (Meneghini et al., 1999; Rocheleau et al., 1999; Shin et al., 1999). The downregulation of this protein and the interaction with MAPK signaling suggest that this Wnt signaling pathway has unique features.

Wnt signaling also controls cell polarities in the tail of developing *C. elegans* larvae. Mutations in the Wnt gene *lin-44* cause the polarities of certain cells that divide asymmetrically in the tail of the animal – the B, TL and TR cells – to be reversed (Herman and Horvitz, 1994). LIN-44 is expressed in the epidermal cells at the tip of the tail, which are posterior to the cells whose polarities are affected by *lin-44* mutations. Mosaic analysis has demonstrated that *lin-44* functions in the same cells in which it is expressed, suggesting that LIN-44 is secreted by the epidermal cells at the tip of the tail and affects the polarity of asymmetric cell divisions that occur more anteriorly in the tail (Herman et al., 1995). Mutations in the Frizzled-related gene *lin-17* lead to a loss of polarity in the same cells in which *lin-44* mutations cause a reversal of polarity (Sternberg and Horvitz, 1988; Sawa et al., 1996), suggesting that LIN-17 may be the receptor for LIN-44 signal. One model to explain the difference in phenotypes of mutations affecting a putative ligand (LIN-44) and its receptor (LIN-17) is that there is a second signal, perhaps another Wnt, emanating from a source anterior to the B, TL and TR cells (Sawa et al., 1996); there is yet no evidence for such a signal, however. *lit-1* mutants also display defects in T cell polarity (Rocheleau et al., 1999), suggesting that there may be a similarity between the control of EMS and T cell polarities.

Wnt signaling also controls certain cell migrations during *C. elegans* development. Mutations in the Wnt gene *egl-20* cause defects in the migrations of the descendants of the QL neuroblast (Harris et al., 1996; Maloof et al., 1999). The proper migration of the QL neuroblast descendants depends upon the expression and function of the Hox gene *mab-5* in the migrating cells (Kenyon, 1986; Salser and Kenyon, 1992), which in turn depends upon *egl-20* function (Harris et al., 1996). Other components of the Wnt signaling pathway, including LIN-17 and a β-catenin-related protein encoded by *bar-1* (Eisenmann et al., 1998), also control the migrations of the QL descendants by regulating the expression of *mab-5* (Harris et al., 1996; Maloof et al., 1999).

Thus, Wnt signaling is involved in several functions during *C. elegans* development, and many Wnt pathway components have been identified; however, it is not clear to what extent pathway components are shared in each Wnt-controlled process. For example, POP-1 is asymmetrically distributed at many divisions during postembryonic development, and its asymmetric distribution in some of these divisions is dependent upon *lin-17* function (Lin et al., 1998). However, *pop-1* function has only been examined in the context of the control of EMS polarity. I have found that POP-1 protein is also asymmetrically distributed in the daughters of the TL and TR cells. In addition, I have used a novel RNA mediated interference (RNAi) technique to specifically interfere with *pop-1* zygotic function during postembryonic development, in order to show that *pop-1* function is necessary for wild-type T cell polarity and wild-type migration of the QL neuroblast descendants. The involvement of *pop-1* and *lit-1* in the control of T cell polarity suggests other components involved in the control of EMS polarity, such as WRM-1, may also be involved in the control of T cell polarity. Surprisingly, I found that neither WRM-1 nor any of the other *C. elegans* β-catenin homologs appear to function in the control of T cell polarity. However, the Wnt signaling pathway that controls QL descendant migration, unlike the pathway controlling EMS polarity, seems to be similar to the canonical Wnt signaling pathway, demonstrating that not all Wnt pathways in *C. elegans* are unusual.

MATERIALS AND METHODS

General methods and strains

Nematodes were cultured by standard techniques (Sulston and Hodgkin, 1988). The following mutations were used. *LGI*, *lin-44(n1792)* and *lin-17(n3091)*; *LGII*, *egl-27(n170, mn553)*; *LGIII*, *unc-32(e189)* and *unc-119(e2498)*; *LGIV*, *muIs2[mab-5::lacZ; unc-31(+)]*; *LGV*, *rde-1(ne219)*; *LGX*, *bar-1(ga80)*; and *LG?*, *huIs4[hsp16::ΔN-POP-1; rol-6(su1006)]*; *teEx1 [hsp16::pop-1, unc-119(+)]*.

Cell lineage analysis, T cell polarity and scoring of QL descendant migrations

Living animals were observed using Nomarski optics; cell nomenclature and cell lineage analysis were as previously described (Sulston and Horvitz, 1977). N.x refers to both daughters of cell N. An 'n' in the name of cell is used to refer to a series of numbered blast cells. Fates of the T cell descendants were determined by nuclear morphologies (Herman and Horvitz, 1994) and were used as an indicator of T cell polarity as previously described (Herman et al., 1995). The QL.pa daughters were scored in the late L1 stage as described previously (Harris et al., 1996).

Immunofluorescence with anti-POP-1 antibodies

Staged early L1 animals (2-5 hours after hatching for QL.x, or 5-7 hours after hatching for T.x) were stained with anti-POP-1 and MH27 monoclonal antibodies as described previously (Lin et al., 1998) with minor modifications to allow for the handling of L1 larvae rather than embryos. Specifically, L1 larvae were washed off plates in double distilled H_2O, washed in 2% formaldehyde fixative solution and resuspended in an appropriate amount of fixative solution to yield approximately 100 larvae per 15 μl solution.

RNA mediated interference (RNAi)

RNAi was performed according to (Fire et al., 1998). Templates for RNA synthesis were pRL160 (kindly provided by R. Lin) for *pop-1*, subclones of cDNAs (kindly provided by Y. Kohara) for *wrm-1* and *hmp-2*, and a subclone of cDNA clone pDE250 for *bar-1* (kindly provided by D. Eisenmann).

To analyze the zygotic effects of double-stranded RNA interference (RNAi) on zygotic but not maternal expression of *pop-1, hmp-2* and *wrm-1*, a 'zygotic RNAi' scheme was devised. This scheme depended upon the recent isolation of the RNAi-resistant mutant *rde-1(ne219)* (Tabara et al., 1999). *rde-1(ne219)* is a recessive loss-of-function mutation that confers maternal and zygotic resistance to RNAi, such that the progeny of hermaphrodites injected with dsRNAs are not

affected, but the progeny of *rde-1/+* hermaphrodites are susceptible to RNAi. In order to interfere specifically with zygotic function, dsRNA corresponding to a particular gene was injected into *rde-1* hermaphrodites, which were crossed with wild-type males; the *rde-1/+* progeny were then examined for defects. The *rde-1* mutation causes the maternal contribution of mRNA to be resistant to dsRNA injection; however, since *rde-1(ne219)* is recessive, and the dsRNA persists and is inherited by the progeny of the injected hermaphrodite, zygotically expressed mRNA becomes susceptible in the *rde-1/+* progeny of injected *rde-1* mothers. The *rde-1* progeny of injected but non-mated *rde-1* hermaphrodites served as controls. This scheme is termed 'zygotic RNAi'. In practice, *unc-32; rde-1* hermaphrodites were injected with dsRNA for 'gene X', and crossed with wild-type males; the non-Unc-32 male and hermaphrodite cross progeny (genotype *unc-32/+; rde-1/+; gene X(RNAi)*) were then scored in the late L1 stage for defects in T cell polarity and QL descendant migration.

To analyze the level of POP-1 protein in *pop-1* zygotic RNAi L1s, staged early L1 progeny from approximately 30 *rde-1* hermaphrodites, which were each injected with *pop-1* dsRNA and mated to wild-type males, were stained with anti-POP-1 antibodies (see above).

Heat-shock experiments

Animals that carried an integrated transgenic array, *huIs4*, which contains the *rol-6(su1006)* marker and a truncated *pop-1* fragment encoding POP-1 (45-438) cloned into the heat-shock promoter vector pPD49.78 (Korswagen et al., 2000) were heat-shocked at hatching for 1 hour at 33°C. Animals that carried an extrachromosomal array, *teEx1*, which contains the *unc-119(+)* marker and the full-length *pop-1* cDNA cloned into pPD49.78 (R. Lin, personal communication), were heat-shocked at the 1.5-fold or twofold stages of embryogenesis for 30 minutes at 33°C, or at hatching for 1 hour at 33°C. Heat-shocked and non-heat-shocked control animals were scored in the late L1 stage for defects in T cell polarity and QL descendant migration.

Expression of *mab-5::lacZ*

Staged early L1 animals (2-5 hours after hatching) were stained for β-galactosidase expression according to Salser and Kenyon (Salser and Kenyon, 1992). In the case of *pop-1* zygotic RNAi animals, *unc-32/+; rde-1/+* cross progeny were checked for the *pop-1* zygotic RNAi effect by scoring the positions of the QL.pa daughters; younger sibs were staged and stained for β-galactosidase expression. Animals containing *huIs4* that were heat-shocked at hatching were allowed to recover for 1-2 hours after heat-shock, prior to being stained for β-galactosidase expression.

RESULTS

POP-1 is asymmetrically distributed to the T cell daughters

The level of POP-1 has been shown to be higher in the anterior daughters of many anteroposterior, asymmetric cell divisions that occur during embryonic and postembryonic development. In *lin-17* males, however, some sister tail seam cells were shown to have equal levels of POP-1 (Lin et al., 1998). To determine whether POP-1 levels are asymmetric in the division of wild-type and *lin-17* T cells, early L1 larvae were fixed and stained with anti-POP-1 monoclonal antibody (Lin et al., 1998) and MH27 monoclonal antibody, which recognizes adherens junctions (Francis and Waterston, 1991). The levels of POP-1 were higher in the anterior T cell daughter in wild-type animals (28/28; Fig. 1A). In *lin-17* animals, which display a loss of T cell polarity, the level of POP-1 was high in both T cell

daughters in 71% of divisions (Fig. 1B), higher in the posterior T cell daughter in 8% of divisions and higher in the anterior T cell daughter in 17% of divisions (*n*=65). However, in *lin-44* animals, in which the polarity of the T cell is frequently reversed (Herman and Horvitz, 1994), the level of POP-1 was higher in the posterior T cell daughter in 71% of divisions (Fig. 1C), equal in 19% of divisions and higher in the anterior T cell daughter in 10% of divisions (*n*=86). The proportion of animals with each staining pattern correlated with the effects of *lin-17* and *lin-44* mutations on T cell polarity (Herman and Horvitz, 1994; Sawa et al., 1996). These results suggest that the LIN-44/WNT signal, acting through LIN-17/FZ receptor, controls T cell polarity by affecting the distribution of POP-1.

pop-1 function is required for proper T cell polarity

The only existing *pop-1* mutation, *pop-1(zu189)*, causes maternal-effect lethality (Lin et al., 1995) and has no zygotic effect on T cell polarity. RNA-mediated interference (RNAi) of *pop-1* causes embryonic lethality, making it impossible to examine postembryonic defects (Lin et al., 1998; Rocheleau et al., 1999). However, an RNAi-resistant mutant, *rde-1(ne219)*, has been recently isolated (Tabara et al., 1999), which has made possible a scheme for interfering specifically with the zygotic function of *pop-1*, or any other maternally acting gene (Tabara et al., 1999). This scheme is termed 'zygotic RNAi' (see Materials and Methods). If *pop-1* functions in T cell polarity as it does in EMS polarity, then according to a simple model based upon the POP-1 localization results, one would predict that removal of *pop-1* function would result in both T cell daughters taking on the neural T.p fate. However, contrary to the prediction of this simple model, in *pop-1* zygotic RNAi animals, both T.a and T.p often expressed T.a-like hypodermal cell fates. T cells in control animals all showed normal polarities (Table 1). Cell lineage analysis of the T cell confirmed these results (Fig. 2). Thus *pop-1* zygotic RNAi results in a loss of T cell polarity. Staining of *pop-1* zygotic RNAi animals with anti-POP-1 antibody demonstrated that zygotic RNAi reduced POP-1 levels below detection (Fig. 1D). Specifically, 72% (*n*=18) of T.x cells in *pop-1* zygotic RNAi animals did not stain with antibody, which correlates well with the 71% percent of symmetric T cell divisions observed in *pop-1* zygotic RNAi animals (Table 1). Thus, a loss of T cell polarity is observed both in *lin-17* mutants, in which POP-1 levels are high in both T.a and T.p, and in *pop-1* zygotic RNAi animals, in which POP-1 levels are reduced below detection.

Overexpression of truncated Tcf proteins that lack the β-catenin interaction domain have been shown to function as potent dominant negative mutants and have been used to interfere with Tcf function (Molenaar et al., 1996; Clevers and van de Wetering, 1997). Overexpression of a POP-1 construct lacking the first 44 amino acid residues, ΔN-POP-1, has been shown to inhibit activation of a Tcf reporter gene by full-length POP-1 and BAR-1 (Korswagen et al., 2000). I used animals containing a transgene that expressed ΔN-POP-1 from the *hsp16* heat-shock promoter (Korswagen et al., 2000) to test *pop-1* function in T cell polarity. Non-heat-shocked animals showed normal T cell polarities, but in animals heat-shocked for 1 hour at hatching, both T.a and T.p often expressed T.a-like hypodermal cell fates (Table 1). Thus, by two different methods I have demonstrated that *pop-1* is involved in the control of T cell polarity. Taken together, the results suggest

that low but non-zero POP-1 levels, as in T.p in wild-type animals, results in a neural T.p-like cell fate, and that either high POP-1 levels, as in T.a in wild-type animals and in both T.a and T.p in *lin-17* animals, or very low POP-1 levels or

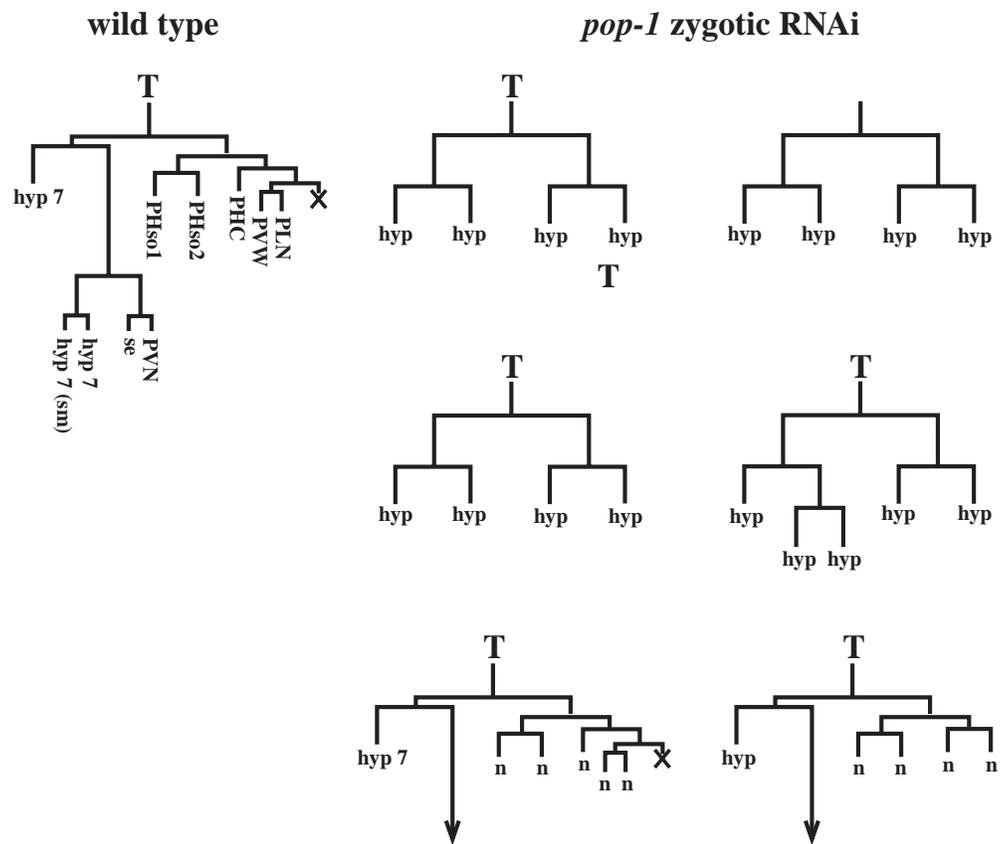activity (as caused by zygotic RNAi or overexpression of ΔN-POP-1), results in hypodermal T.a-like cell fates.

C. elegans β-catenin homologs may not function in the control of T cell polarity

In the canonical Wnt pathway, binding of a Wnt to a Frizzled receptor at the cell surface causes β-catenin to be localized to the nucleus where it interacts with Tcf to activate expression of target genes. There are three β-catenin homologs in *C. elegans,* BAR-1, WRM-1 and HMP-2. Only one of these, BAR-1, interacts specifically with POP-1 (Korswagen et al., 2000). *bar-1* mutants exhibit defects in QL.d migration (Maloof et al., 1999) and in the specification of vulval cell fates (Eisenmann et al., 1998). However, T cell polarity is normal in *bar-1* mutants, *bar-1(RNAi)* animals and *bar-1* zygotic RNAi animals (Table 1). Zygotic RNAi was also used to determine whether WRM-1 or HMP-2 play roles in T cell polarity. T cell polarity was normal in both *wrm-1* and *hmp-2* zygotic RNAi animals (Table 1). Both *wrm-1* and *hmp-2* dsRNA preparations were also injected into wild-type hermaphrodites, and the respective previously reported RNAi phenotypes were observed (Rocheleau et al., 1997; Costa et al., 1998), demonstrating that the dsRNA was active in each case. In addition, the *wrm-1* zygotic RNAi hermaphrodites displayed abnormal vulval development, and the males displayed abnormal tail morphologies. Furthermore, *hmp-2* zygotic RNAi males displayed an abnormal bulge anterior to the anus. Finally, I tested whether the three *C. elegans* β-catenin homologs might function redundantly in T cell polarity by using zygotic RNAi to simultaneously interfere with the functions of *bar-1*, *wrm-1* and *hmp-2*. T cell polarity was normal in all the *hmp-2; wrm-1; bar-1* zygotic RNAi and control animals (Table 1). In addition, in 23% of the animals in which QL.d migration was scored (*n*=61), the QL.pa daughters were found anterior to V3.p, indicating a defect in QL.d migration and demonstrating that the *bar-1* dsRNA was active. Both TL and TR had normal polarities in all 14 animals that displayed defects in QL.d migration. Thus, none of the known *C. elegans* β-catenin homologs appeared to function in the control of T cell polarity. There are possible explanations for the absence of a T cell polarity defect. First, although defects caused by *bar-1* and *wrm-1* zygotic RNAi were observed in some larval cells, expression from these genes may not be completely eliminated in the daughters of the T cells, even though *pop-1* zygotic RNAi caused defects in these daughters. Second, since zygotic RNAi, like regular RNAi, may be more or less effective on different genes within
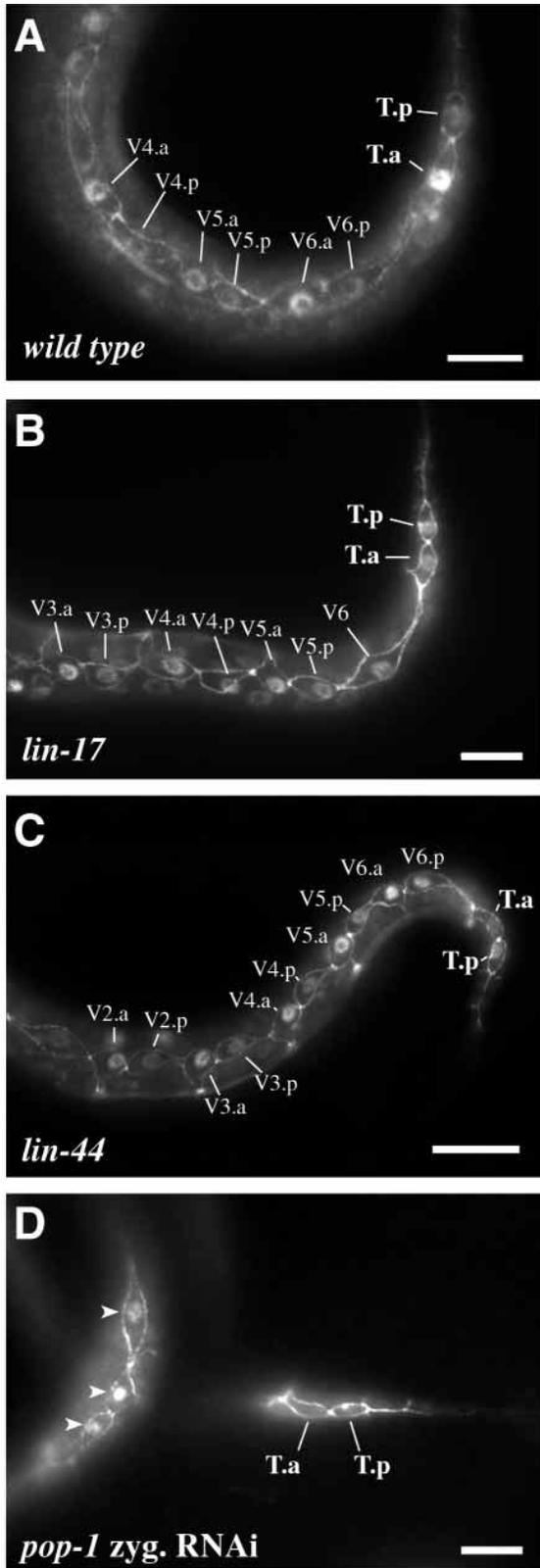
Fig. 1. POP-1 is asymmetrically distributed at the T cell division. POP-1 expression in T.a and T.p. Whole-mount larvae 5-7 hours post-hatching, were stained with monoclonal anti-POP-1 antibodies and MH27 monoclonal antibodies, which outline hypodermal cells. The V cell (V) and T cell (T) daughters are indicated. (A) In wild-type animals, the level of POP-1 is higher in Vn.a than in the Vn.p, and also higher in T.a than in T.p. (B) In *lin-17* animals, the levels of POP-1 are high in both T.a and T.p. (C) In *lin-44* animals, the level of POP-1 is higher in Vn.a than in the Vn.p, but is higher in T.p than in T.a. (D) In *pop-1* zygotic RNAi animals, POP-1 is often not detected in T.a and T.p. POP-1 was detected in the Vn.x cells in 26% (*n*=229) of *pop-1* zygotic RNAi animals; arrowheads indicate POP-1-positive Vn.x nuclei in the lateral hypodermis of an adjacent *pop-1* zygotic RNAi animal. Scale bars: 10 μm.

Fig. 2. Cell lineages of the T cell in wild-type and *pop-1* zygotic RNAi hermaphrodites. Wild-type hermaphrodite T cell lineage is shown on the left. The fates of many different cells in this lineage can be distinguished by nuclear morphology using Nomarski microscopy (Sulston and Horvitz, 1977; Herman and Horvitz, 1994). The hyp7 cells T.aa, T.apaa and T.apap join the hypodermal syncytium, but T.apaa has a smaller nucleus and is designated hyp7(sm). PHso1, PHso2, PVW, PHC and PLN have similar neuronal-type nuclear morphologies. The × indicates apoptosis. The seam cell (se) is a specialized hypodermal cell. The T.ap cell continued to divide beyond the L1 stage in some animals, as it does in wild-type animals, but was not followed, indicated by the arrow. Six T cell lineages were followed through the L1 stage: one T cell divided in a wild-type pattern in the L1 stage, four T cells showed a loss of T polarity (T.ap divided in one of these) and one T cell showed a loss of T.p polarity.

wild type **pop-1 zygotic RNAi**

the same cell, it is still possible that one or more of these genes may play a role in the control of T cell polarity. Despite these caveats, >99% of the *C. elegans* genome sequence is known and no other β-catenin homologs have been identified, suggesting that although T cell polarity is controlled by a Wnt signal (LIN-44) which acts through a Tcf factor (POP-1), a β-catenin-like protein may not be involved.

pop-1 functions in a canonical Wnt pathway that controls QL.d migration

The migratory neuroblasts QL and QR are left/right homologs that each generate three neurons and two cells that undergo apoptosis. The migrations of these cells and their descendants are left/right asymmetric. The QR cell and its descendants migrate anteriorly, whereas the QL neuroblast migrates posteriorly and divides. The posterior daughter, QL.p, stops

Table 1. Polarity of the T cell division

| | | Percentage of T cells | |
| | | Normal | Symmetric |
Genotype	*n*		
Wild type	>100	100	0
rde-1; pop-1(RNAi)	112	100	0
rde-1/+; pop-1(RNAi)	220	29	71
hsp16::ΔN-POP-1, no heat shock	58	100	0
hsp16::ΔN-POP-1, heat shock	96	39	61
hsp16::POP-1, no heat shock	50	94	6
hsp16::POP-1, heat shock at 1.5 fold	16	94	6
hsp16::POP-1, heat shock at 2 fold	40	95	5
hsp16::POP-1, heat shock at hatching	132	98	2
rde-1/+; wrm-1(RNAi)	128	100	0
rde-1/+; hmp-2(RNAi)	42	100	0
bar-1	136	100	0
bar-1(RNAi)	64	100	0
rde-1/+; bar-1(RNAi)	110	100	0
rde-1; wrm-1(RNAi); hmp-2(RNAi); bar-1(RNAi)	96	100	0
rde-1/+; wrm-1(RNAi); hmp-2(RNAi); bar-1(RNAi)	170	100	0

Fates of the T cell descendants were determined by nuclear morphologies according to Herman and Horvitz (Herman and Horvitz, 1994) and were used as an indicator of T cell polarity as previously described (Herman et al., 1995).

n, number of T cells scored.

Normal polarity: T.aa and T.ap expressed hypodermal cell fates, and the T.p descendants expressed neural cell fates.

Symmetric: both T.a and T.p descendants expressed hypodermal cell fates.

migrating and generates the PVM and SDQL neurons, and a cell that undergoes apoptosis. The anterior daughter, QL.a, continues a posterior migration and divides to generate a cell
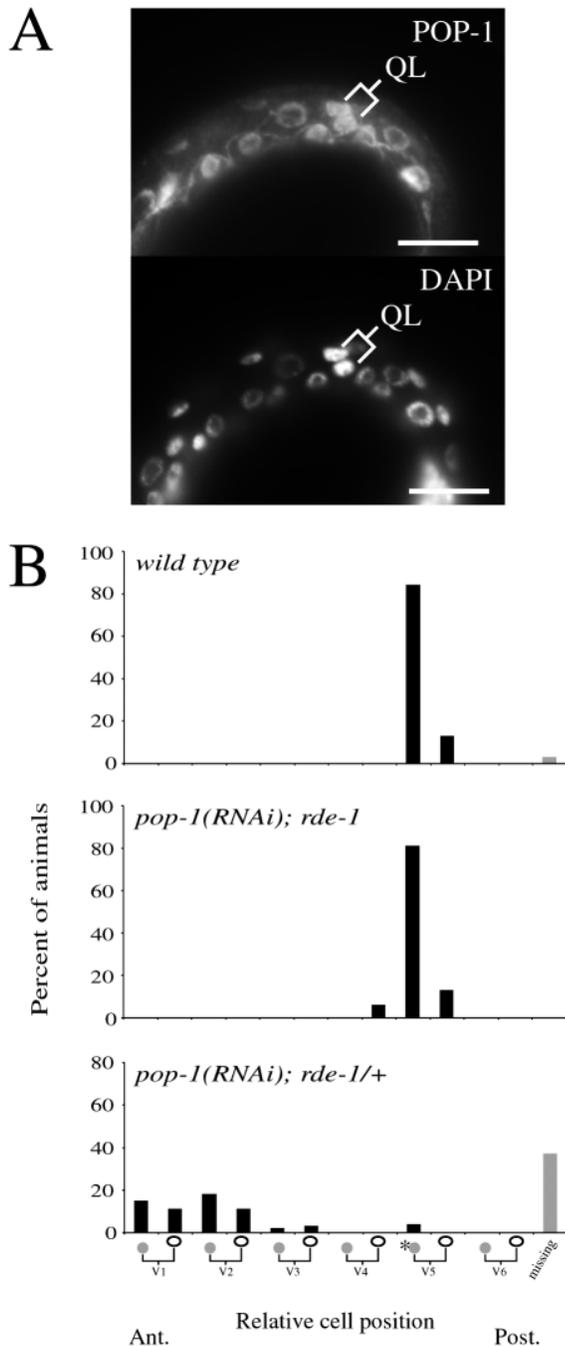
Fig. 3. *pop-1* functions in the control of QL.d migration. (A) POP-1 is expressed in QL.a and QL.p in wild-type animals. Whole-mount larvae 2-5 hours post-hatching were stained with monoclonal anti-POP-1 antibodies and DAPI, which stains DNA. Scale bars: 10 µm. (B) *pop-1* zygotic RNAi causes abnormal anterior migrations of the QL.d. Final positions of the QL.pa daughters were scored (Harris et al., 1996) in wild-type (*n*=32), *rde-1; pop-1(RNAi)* hermaphrodites (*n*=16) and *rde-1/+; pop-1(RNAi)* animals (*n*=54). The asterisk above V5.a marks the birthplace of QL. Black bars indicate the proportion of cells at each position. Gray bars indicate the proportion of missing cells.

that undergoes apoptosis and the PQR neuron, which continues to migrate posteriorly until it reaches its characteristic position in the tail (Sulston and Horvitz, 1977). The direction of Q cell descendant migration is controlled by the Hox gene *mab-5*. In *mab-5* gain-of-function mutants, which ectopically express *mab-5* (Hedgecock et al., 1987; Salser and Kenyon, 1992), both QL and QR descendants migrate posteriorly, just as the QL descendants do in wild-type animals, whereas in *mab-5* loss-of-function (lf) mutants QL and QR descendants all migrate anteriorly (Kenyon, 1986; Salser and Kenyon, 1992).

Wnt signaling controls the migration of the QL descendants (referred to collectively as the QL.d) by regulating the expression of *mab-5* (Harris et al., 1996; Maloof et al., 1999). Specifically, mutations in *egl-20/Wnt*, *lin-17/Fz*, or *bar-1/β-catenin* all cause anterior migration of the QL.d, as well as loss of *mab-5* expression in the QL lineage (Sawa et al., 1996; Eisenmann et al., 1998; Maloof et al., 1999). Staining of wild-type animals with anti-POP-1 monoclonal antibody revealed that POP-1 is expressed in the QL lineage (Fig. 3A), indicating that *pop-1* might play a role in the control of QL.d migration. The function of *pop-1* in the control of QL.d migration was assessed by scoring the positions of the QL.d in *pop-1* zygotic RNAi animals. The QL.pa daughters were found at normal positions, near V5.a, in both wild-type and control animals. However in 60% of the *pop-1* zygotic RNAi animals, the QL.pa daughters were found anterior to V3.p (Fig. 3B). In addition, in 37% of *pop-1* zygotic RNAi animals, the QL.pa daughters could not be found (Fig. 3B), suggesting that *pop-1* zygotic RNAi might cause a mis-specification of QL cell fate similar to that observed in *egl-27* animals (Herman et al., 1999).

The inhibition of *pop-1* function results in a QL.d migration defect similar to that seen in other Wnt pathway mutants, *egl-20, lin-17* and *bar-1*. This suggests that *pop-1* functions in QL.d migration to activate the target gene *mab-5*. To determine whether *pop-1* function is required for *mab-5* expression, a *mab-5::lacZ* reporter construct that mimics the expression of the endogenous *mab-5* gene (Salser and Kenyon, 1992) was used. Expression of the *mab-5::lacZ* reporter was analyzed in *pop-1* zygotic RNAi animals (Fig. 4). Whereas β-galactosidase expression was invariably observed in the QL daughter cells of control animals (*n*=22), 86% of *pop-1* zygotic RNAi animals failed to express β-galactosidase in the QL daughter cells (*n*=21; Fig. 4). In addition, overexpression of the ΔN-POP-1 construct described above also caused a QL.d migration defect similar to that seen in Wnt pathway mutants and inhibited *mab-5* expression in the QL daughters (Korswagen et al., 2000). Thus, *pop-1* appears to function to activate *mab-5* expression in response to a canonical Wnt pathway that controls QL.d migration.
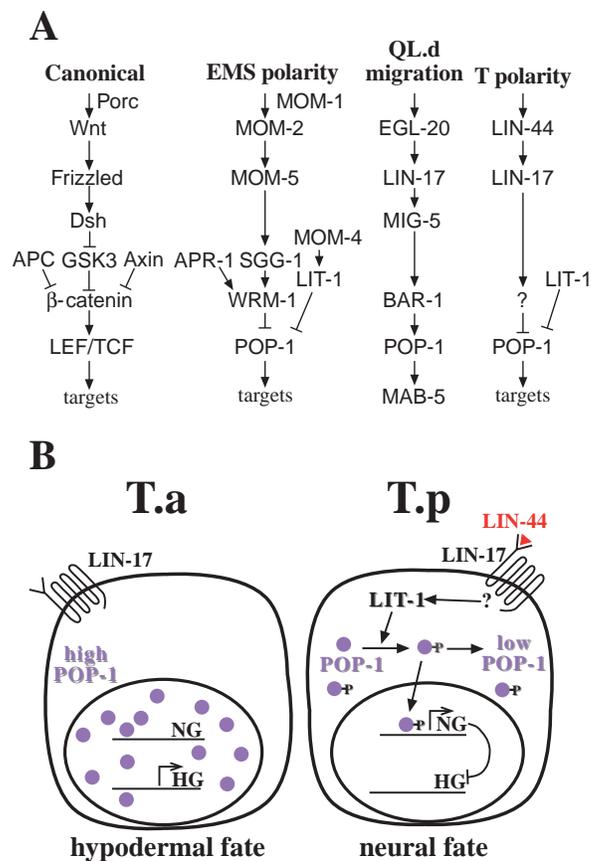
POP-1 functions differently in T cell polarity and QL.d migration

Expression of full-length *pop-1* from the *hsp16* heat-shock promoter during embryogenesis or at hatching did not cause defects in T cell polarity (Table 1), but did cause a QL.d migration defect. Specifically, in heat-shocked animals containing *teEx1[hsp16::pop-1, unc-119(+)]*, 89% (*n*=35) of the QL.pa daughters were found anterior to V3.p, however in non-heat-shocked animals, only 11% (*n*=19) of the QL.pa daughters were found anterior to V3.p. This suggested that
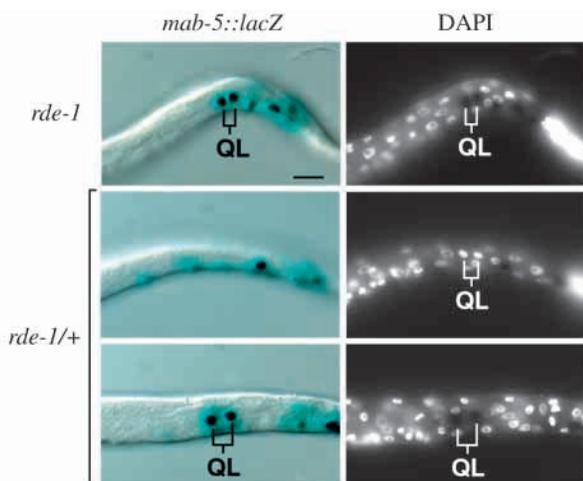
Fig. 4. *pop-1* zygotic RNAi blocks *mab-5* expression in the QL daughters. Whole-mount larvae, 2-5 hours post hatching, were fixed and stained for β-galactosidase expression (Salser and Kenyon, 1992) and DAPI. In the top panel, a *rde-1; muIs2[mab-5::lacZ]; pop-1(RNAi)* control animal exhibits *mab-5::lacZ* reporter gene expression in the QL daughter cells. DAPI staining of the QL.x nuclei is obscured by the β-galactosidase expression. The middle and lower panels show two *rde-1/+*; *muIs2[mab-5::lacZ]; pop-1(RNAi)* progeny of injected *rde-1; muIs2[mab-5::lacZ]*mothers. The middle panels show one of the 18 animals in which the *mab-5::lacZ* reporter gene was not expressed in the QL daughter cells; the bottom panels show one of the three animals in which the *mab-5::lacZ* reporter gene was expressed in the QL daughter cells. In the middle, right-hand panel, DAPI staining of the QL.x nuclei is visible because they are not obscured by β-galactosidase expression. Scale bar: 10 µm.

Fig. 5. *C. elegans* Wnt pathways and a model for T cell polarity. (A) Comparison of the canonical and *C. elegans* Wnt pathways. Known genes involved in EMS polarity, QL.d migration and T cell polarity and their inferred regulatory relationships are shown. The canonical pathway has been simplified to include only the major components. Positive interactions are indicated by arrows, negative interactions by truncated lines. Related proteins are shown at corresponding positions in the figure. APR-1 is shown as influencing WRM-1 independently of SGG-1 in the EMS polarity pathway, since SGG-1 has also been shown to influence EMS spindle orientation, whereas APR-1 does not (Schlesinger et al., 1999). The possibility that either a β-catenin homolog is not involved or that an unknown factor may be involved in the T cell polarity pathway is indicated by a '?'. (B) A model for T cell polarity incorporating the POP-1 localization and zygotic RNAi results. For simplicity, LIN-44/Wnt (red triangle) is shown as binding to LIN-17/FZ on the T.p cell; however, this most likely occurs earlier, on the posterior surface of the T cell before it divides. LIN-44 signal is transduced by unknown factors (?) that may not be components of the canonical WNT pathway, leading to the activation of LIT-1, which may combine with an unidentified factor to phosphorylate POP-1 (violet circles). This modifies POP-1 and leads to the reduction of POP-1 levels in T.p. The modified form of POP-1 may activate expression of neural-specific genes (NG), one or more of which may function to repress hypodermal-specific (HG) genes in T.p. In the absence of LIN-44 signal, T.a may take on a default hypodermal fate, perhaps by the constitutive expression of hypodermal-specific genes that function to promote hypodermal fate. The high levels of POP-1 that accumulate in T.a may be inactive.

POP-1 might function differently in the T.x and QL.d cells. The observation that overexpression of *pop-1* causes QL.d migration defects similar to that seen in *egl-20/Wnt* and Wnt pathway mutants is reminiscent of experiments in *Drosophila* in which overexpression of wild-type Pangolin/dTcf enhanced the phenotype of a weak *wg* allele (Cavallo et al., 1998); in both cases, overexpression of a Tcf homolog antagonized Wnt signaling, suggesting that QL.d migration is controlled by a canonical Wnt pathway. To determine if expression of *pop-1* from the heat-shock promoter affected the distribution of POP-1 in the T.x cells, heat-shocked animals containing *teEx1* were stained with anti-POP-1 antibody. Specifically, when the progeny of hermaphrodites containing the *teEx1[hsp16::pop-1, unc-119(+)]* array were heat-shocked at hatching, 63% (*n*=128) of T.x cells had higher POP-1 levels in T.a than in T.p, 20% contained equal and high levels of POP-1 protein, and 17% contained equal and low levels of POP-1 protein (data not shown). The stained larvae that contained the array could not accurately be differentiated from those that did not; thus, some of the stained animals in the did not contain the array. However, since 77% (*n*=582) of the progeny of *teEx1*-bearing hermaphrodites contained the array, most of the stained animals contained the array. Thus, while in some heat-shocked animals equal levels of POP-1 protein could be observed in T.a and T.p, in most heat-shocked animals the levels of POP-1 were higher in T.a. One possibility is that POP-1 was not expressed in the T.x cells following heat-shock. Alternatively, the mechanism that functions to decrease the levels of POP-1

in T.p may be able to efficiently decrease the levels of POP-1 following increased expression from the heat-shock promoter. Furthermore, this mechanism does not function in the QL.d

cells, suggesting that POP-1 functions differently in T cell polarity and QL.d migration.

DISCUSSION

pop-1 plays a role in two different Wnt-controlled processes during *C. elegans* postembryonic development

pop-1 appears to function in at least two processes during postembryonic development: the control of T cell polarity and the migration of the QL descendants. I have shown that POP-1 is asymmetrically distributed to the T cell daughters, such that in wild type animals the level of POP-1 is higher in T.a than in T.p. However, the level of POP-1 is higher in the posterior T cell daughter in *lin-44* animals and is equally high in the T cell daughters in *lin-17* animals, suggesting that the LIN-44/WNT signal acts through LIN-17/FZ to affect the distribution of POP-1. Furthermore, interference with *pop-1* function caused a loss of T cell polarity, similar to that seen in *lin-17* mutants, suggesting that *pop-1* plays an important role in the control of T cell polarity. Interestingly, *pop-1* appears to play this role without interacting with a β-catenin homolog, as interfering with any or all of the *C. elegans* β-catenin homologs had no effect on T cell polarity.

POP-1 functions in a canonical Wnt pathway to control QL.d cell migrations

I have shown that POP-1 is expressed in the QL daughter cells. Interference with *pop-1* function by zygotic RNAi caused QL.d migration defects and the loss of *mab-5* expression, phenotypes seen in other Wnt pathway mutants, including *bar-1* (Maloof et al., 1999). A Dsh homolog encoded by *mig-5* is also required for wild-type QL.d migration (Antebi et al., 1997). In addition, the effects of *pop-1* zygotic RNAi on QL.d migration and *mab-5* expression in the QL daughters were similar to those caused by overexpression of the dominant negative ΔN-POP-1 construct (Korswagen et al., 2000). BAR-1 is the only *C. elegans* β-catenin homolog that interacts with POP-1; BAR-1 and POP-1 together are able to activate a Tcf reporter gene (Korswagen et al., 2000). Furthermore, overexpression of *pop-1* antagonized Wnt signaling in a manner similar to observations with other Wnt pathways. Therefore, the Wnt pathway that functions to control QL.d migration appears to activate POP-1, which activates expression of the target gene *mab-5*. Thus BAR-1 and POP-1 seem to be part of a canonical Wnt pathway that includes EGL-20/Wnt and LIN-17/FZ (Fig. 5A), demonstrating that such a pathway exists in *C. elegans*. Other canonical Wnt pathway components, such APC and GSK3, are present in *C. elegans*, but their function in QL.d migration has not yet been demonstrated.

C. elegans Wnt pathways

The Wnt pathway that controls polarity of the EMS blastomere and expression of endodermal cell fates during embryogenesis is novel in that, in collaboration with a MAPK pathway, it causes the asymmetric distribution of POP-1 in the MS and E daughter cells. In the canonical Wnt pathway, Wnt signaling functions to cause the accumulation of β-catenin which interacts with Tcf to activate gene expression; however, in this pathway, POP-1 appears to function to repress gene expression, and the role of Wnt signaling appears to be to downregulate the level of POP-1 in the E cell to allow expression of endoderm-specific genes (Fig. 5A). In addition, MOM-4/TAK1 is required to activate LIT-1, which in the presence of the β-catenin homolog, WRM-1, phosphorylates POP-1 (Rocheleau et al., 1999) and downregulates POP-1 levels in the E cell. Components of this pathway also appear to function in vertebrate cells (Ishitani et al., 1999), suggesting that this arrangement is conserved. The interaction of Wnt signaling with other signaling pathways has also been observed in other systems. For example, during the establishment of tissue polarity in *Drosophila*, Wnt signaling appears to interact with RhoA and, through Dsh, with Jun N-terminal kinase (JNK) pathways (Boutros et al., 1998). A Dsh protein involved in EMS polarity has not yet been identified, however.

The pathway that controls T cell polarity also appears to be unusual. T cell polarity is controlled by LIN-44/Wnt, LIN-17/Fz and POP-1/TCF, but a β-catenin homolog may not be involved. There are some similarities between the control of EMS and T cell polarities, as *lit-1* is involved in both; mutation of *lit-1* causes the loss of T cell polarity, and *lit-1* appears to be expressed in the T cells (Rocheleau et al., 1999). However, the lack of involvement of WRM-1 or another β-catenin homolog in T cell polarity raises questions about the similarity of these pathways. The observation that NLK does not interact directly with any Tcf/Lef factors and requires β-catenin to phosphorylate TCF-4 (Ishitani et al., 1999) makes it unlikely that LIT-1 would interact directly with POP-1 in the control of T cell polarity. This suggests that another protein, which is not one of the *C. elegans* β-catenin homologs, may function in a similar way to WRM-1 in the control of T cell polarity. There are other proteins with Arm repeats that, while not very similar to WRM-1, may be able to perform this function. Alternatively, signaling through LIN-44 and LIN-17 may not involve the canonical downstream components. For example, Wnt-5a functions to increase intracellular Ca^{2+} levels in zebrafish (Slusarski et al., 1997) and *Xenopus*, perhaps through interactions with heterotrimeric G-proteins (Sheldahl et al., 1999). However, this Wnt/Ca^{2+} pathway does not appear to regulate Tcf, making it unlikely that T cell polarity is controlled by a similar mechanism.

A model for T cell polarity

In wild-type hermaphrodites, the T.a cell divides to generate a hypodermal cell and a blast cell that gives rise to primarily hypodermal cell fates, whereas the T.p cell divides to generate neural cell fates and a cell that undergoes apoptosis. Based upon the analysis of *lin-44* and *lin-17* mutants, the polarity of the T cell appears to be determined before it divides (Herman and Horvitz, 1994). Thus, it seems that there is an asymmetric segregation of cell fates at the T cell division: hypodermal cell fate is segregated to T.a and neural cell fate is segregated to T.p. The segregation of cell fate is correlated with a particular level of POP-1 protein: a higher level of POP-1 is correlated with hypodermal cell fates, while a lower level of POP-1 is correlated with neural cell fates. The distributions of both cell fate and POP-1 are dependent upon *lin-44* and *lin-17*. On the other hand, reducing POP-1 function even further, by RNAi or expression of ΔN-POP-1, leads to hypodermal cell fates. Based upon these results, I propose a model for the control of T cell

polarity (Fig. 5B). The basic idea is that LIN-44/Wnt signal, acting through LIT-1 kinase, functions to modify POP-1, which results in decreased POP-1 levels and the activation of neural-specific genes in T.p. The high levels of POP-1 in T.a may be nonfunctional. Specifically, LIN-44/Wnt binds to LIN-17/FZ on the posterior portion of the T cell before it divides (and on T.p and its descendants). Without LIN-44 signal, the T.a cell accumulates a high level of POP-1 and expresses hypodermal-specific genes. Surprisingly, the interference with *pop-1* function also causes T.a to take on a hypodermal fate, suggesting that such a fate does not depend upon POP-1 function and may even represent the default state, perhaps achieved by the constitutive expression of hypodermal-specific genes in T.a. In the presence of LIN-44 signal, transduction through LIN-17 and unknown factors, that may not be components of the canonical WNT pathway, leads to the activation of LIT-1, which might lead to the to phosphorylation of POP-1, resulting in the reduction of POP-1 levels in T.p by degradation as may occur in the E blastomere (Thorpe et al., 2000). This may occur by LIT-1 combining with an unidentified factor that performs a function similar to WRM-1 in the embryo. The interference with *pop-1* function also leads to the T.p descendants taking on hypodermal cell fates, suggesting that some *pop-1* function is required for specification of neural cell fates. One possibility is that a low level of a modified, perhaps phosphorylated, form of POP-1 is required for the activation of neural-specific genes, one or more of which might function to repress hypodermal-specific genes in T.p. The observation that overexpression of ΔN-POP-1 also caused the loss of neural cell fates suggests that the N-terminal domain of POP-1 may be necessary for activation of neural-specific genes, perhaps because it becomes modified or it interacts with an unknown factor. The isolation and characterization of additional genes that function in the control of T cell polarity will help to elucidate how this novel Wnt signaling pathway can function through POP-1/Tcf to control cell polarity.

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