

tcl-2 encodes a novel protein that acts synergistically with Wnt signaling pathways in *C. elegans*

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Abstract

Mutations in *tcl-2* cause defects in the specification of the fates of the descendants of the TL and TR blast cells, whose polarity is regulated by *lin-44/Wnt* and *lin-17/frizzled*, during *Caenorhabditis elegans* development. In wild-type animals, POP-1/TCF/LEF, is asymmetrically distributed to the T cell daughters, resulting in a higher level of POP-1 in the nucleus of the anterior daughter. The POP-1 asymmetric distribution is controlled by *lin-44* and *lin-17*. However, in *tcl-2* mutants, POP-1 is equally distributed to T cell daughters as is observed in *lin-17* mutants, indicating that, like *lin-17*, *tcl-2* functions upstream of *pop-1*. In addition, *tcl-2* mutations cause defects in the development of the gonad and the specification of fate of the posterior daughter of the P12 cell, both of which are controlled by the Wnt pathway. Double mutant analyses indicate that *tcl-2* can act synergistically with the Wnt pathway to control gonad development as well as P12 descendant cell fate specification. *tcl-2* encodes a novel protein. A functional *tcl-2::gfp* construct was weakly expressed in the nuclei of the T cell and its descendants. Our results suggest that *tcl-2* functions with Wnt pathways to control T cell fate specification, gonad development, and P12 cell fate specification.

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Introduction

The Wnt signaling pathway regulates numerous developmental processes, including the control of cell fate, cell proliferation, and cell polarity, during the development of many animals and is conserved from *Hydra* to mammals (Cadigan and Nusse 1997; Hobmayer et al., 2000; Wodarz and Nusse 1998). The Wnts are members of a large family of secreted glycoproteins that function as signaling molecules. The canonical pathway is activated after a Wnt protein binds to Frizzled, a seven-transmembrane-domain receptor (Bhanot et al., 1996; Kennerdell and Carthew, 1998). Inside the cell, Dishevelled protein (Dsh) is then activated, which antagonizes the inhibitory action of a large protein complex containing Axin, APC, and GSK3, leading to the stabilization of β -catenin, which accumulates in both the

cytoplasm and the nucleus, where it interacts with TCF/LEF transcription factors to activate the target genes. In the absence of Wnt signaling, β -catenin is phosphorylated by GSK3, resulting in its degradation through the ubiquitin/proteasome pathway (Aberle et al., 1997). A noncanonical Wnt pathway that does not involve β -catenin controls the orientation of cells within the plane of the developing epithelium in *Drosophila* as well as the cellular movements of convergent extension that occur during gastrulation in *Xenopus* and zebrafish. This has been termed “planar cell polarity” (Wong and Adler, 1993), and the molecular pathway involved has been dubbed the planar cell polarity, or PCP, pathway. Fz and Dsh appear to function within these noncanonical PCP/Wnt pathways, perhaps also involving the c-Jun N-terminal kinase (JNK) pathway (Kühl et al., 2001; Wallingford et al., 2001). Both Frizzled (Strutt, 2001a) and Dsh (Axelrod, 2001) have been shown to localize asymmetrically to the distal edge of the cell. Subsequently, a pathway that involves a RhoA GTPase homolog

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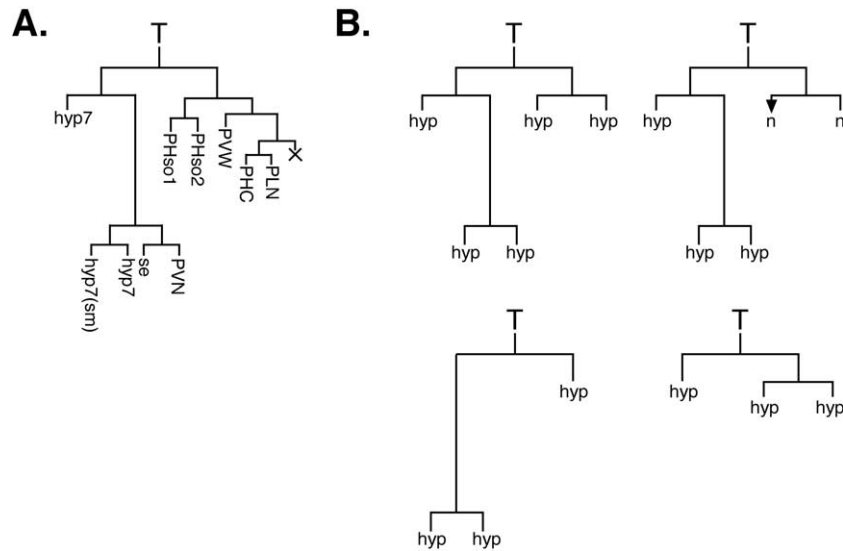


Fig. 1. Cell lineages of the T cell in wild-type and *tcl-2* hermaphrodites. (A) Wild-type hermaphrodite T cell lineage. Anterior is to the left. The fates of many different cells in this lineage can be distinguished by nuclear morphology using DIC microscopy (Herman and Horvitz, 1994; Sulston and Horvitz, 1977). 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 are neural cells and have similar nuclear morphologies. x indicates programmed cell death. The seam cell (se) is a specialized hypodermal cell. (B) We analyzed T cell lineages on both sides of three *mh15*, three *n3170*, and two *os40* mutants (16 total T cell lineages). Arrows indicate that a particular cell was sometimes observed to divide in a subset of the lineages. Two *mh15* lineages and two *os40* lineages had the T cell division pattern shown in the upper left. One *mh15* lineage and three *n3170* lineages had T cell division pattern shown in the upper right; however, in one *n3170* lineage, the T.pa cell divided to generate two neural cells. Two *mh15* and two *n3170* lineages had the T cell division pattern shown in the lower left. One *mh15*, two *os40*, and one *n3170* had the T cell division pattern shown in the lower right; however, in the *n3170* lineage, T.p did not divide. hyp, hypodermal cell; n, neural cell.

may organize the cytoskeleton (Strutt, 2001b). Other components involved in the PCP pathway include the atypical cadherin Flamingo/Starry night (Chae et al., 1999; Usui et al., 1999), the ankyrin repeat protein Diego (Feiguin et al., 2001), Prickle (Gubb et al., 1999), and the integral membrane protein Van Gogh/Strabismus (Darken et al., 2002; Park and Moon, 2002; Wolff and Rubin, 1998). Finally, another noncanonical Wnt pathway that functions by regulating calcium levels (the Wnt/Ca²⁺ pathway) also functions in controlling convergent extension (Kühl et al., 2001), although how this pathway interacts with the PCP pathway is not yet clear. In *Caenorhabditis elegans*, both canonical and noncanonical Wnt pathways, that appear to be distinct from the PCP pathway, control different developmental processes, such as asymmetric cell division, cell fate specification, vulva induction, somatic gonad development, and cell migration (reviewed by Korswagen, 2002 and Herman, 2003).

In *C. elegans*, LIN-44/Wnt controls the asymmetric division of the TL and TR cells that are located bilaterally in the tail (Herman and Horvitz, 1994; Herman et al., 1995). Mutation in *lin-44* causes the reversal of T cell polarity. Mutations in *lin-17/frizzled* cause a loss of T cell polarity (Sawa et al., 1996; Sternberg and Horvitz, 1988). To explain the different effects of *lin-44* and *lin-17* mutations on the asymmetric T cell division, Sawa and his colleagues (1996) proposed that a second signal, perhaps another Wnt from a source anterior to the T cells, functions through LIN-17 to

specify the polarity of the T cell division. Such a signal has not been identified, however. POP-1, a homolog of TCF/LEF, is also required for asymmetric T cell division, as loss of zygotic *pop-1* function causes a phenotype similar to that observed in a *lin-17* mutant (Herman, 2001; Siegfried and Kimble, 2002). The Wnt pathway that controls the asymmetric division of the T cells shares some components with the canonical Wnt pathway, but may not require other components such as a β -catenin, suggesting that it is a noncanonical Wnt pathway (Herman, 2001). The orientations of the divisions of the TL and TR cells are reminiscent of the orientation of epithelial cells in *Drosophila* that are controlled by the noncanonical Wnt/PCP pathway (discussed above).

Both *lin-44* and *lin-17* are also involved in specifying the fate of the P12 cell. (Herman and Horvitz, 1994; Jiang and Sternberg, 1998; Sternberg and Horvitz, 1988). P11 and P12 are the most posterior pair of ventral nerve cord precursors that migrate from their lateral locations into the ventral nerve cord and divide. The P11 cell is usually on the left side and P12 cell on the right (Sulston and Horvitz, 1977). Before they enter the ventral cord, both P11 and P12 cells have the potential to express the P12 fate (Sulston and White, 1980). Mutations in either *lin-44* or *lin-17* can cause P12 to express the P11 cell fate (Herman and Horvitz, 1994; Jiang and Sternberg, 1998; Sternberg and Horvitz, 1988). Mutations in other Wnt pathway genes, *mig-5/Dsh* and *bar-1/ β -catenin*, also cause the same cell fate transformation (Eisenmann and Kim, 2000; Guo, 1995), whereas *pry-*

l/Axin mutations cause the opposite transformation (Howard and Sundaram, 2002), indicating that the Wnt signaling pathway is involved in the specification of P12 cell fate. In addition, the LET-60/Ras signaling pathway also participates in controlling P12 cell fate specification (Aroian and Sternberg, 1991; Clark et al., 1992; Fixsen et al., 1985). For example, mutations in *let-23/EGFR* and *let-60/Ras* cause a P12-to-P11 transformation. Moreover, the Ras and Wnt pathways appear to act synergistically in P12 cell fate specification (Jiang and Sternberg, 1998).

The Wnt signaling pathway also controls development of the somatic gonad. After hatching, the four-cell gonad primordium contains two somatic gonad precursor cells, Z1 and Z4, on each end, and two germline precursor cells, Z2 and Z3, in the middle (Kimble and Hirsh, 1979). The hermaphrodite gonad forms two U-shaped arms, each with its own proximal–distal (PD) axis (reviewed by Hubbard and Greenstein, 2000). The first division of Z1 and Z4 establishes these PD axes. This division is asymmetric; daughters adopting distal fates are located at the ends, and daughters adopting proximal fates are located in the middle. Mutations in *lin-17* disrupt the asymmetric divisions of Z1 and Z4, resulting in both Z1 and Z4 producing daughter cells that adopt proximal cell fates (Sternberg and Horvitz, 1988). Loss of *wrm-1/β-catenin* and *pop-1* functions also caused this defect, termed Sys (*Symmetrical sisters*), indicating that Wnt signaling is involved in this asymmetric division (Miskowski et al., 2001; Siegfried and Kimble, 2002). However, it remains mysterious that no *C. elegans* Wnt genes have been shown to control somatic gonad development.

In this study, we isolated several mutations defining a new gene, *tcl-2* (*T cell lineage defective*), that cause defects in the asymmetric T cell division leading to defects in the specification of the fates of the T cell daughters. We show that *tcl-2* encodes a novel protein, which is weakly expressed in the nuclei of T cells and certain T cell descendants. In addition, we found that *tcl-2* mutants also have defects in the divisions of Z1 and Z4 leading to the abnormal development of gonad axes and P12 cell fate specification. Moreover, *tcl-2* acts synergistically with *lin-44* and *lin-17* to control gonad development as well as P12 cell fate specification. A role for LIN-44 in the development of the gonadal axes was unexpected, as neither *lin-44* nor any of the other *C. elegans* Wnt genes has been shown to be involved in gonad development. We provide evidence that *tcl-2* functions with Wnt pathways to control T cell fate specification, gonad development, and P12 cell fate specification.

Materials and methods

General methods and strains

Nematodes were cultured and manipulated by standard techniques (Sulston and Hodgkin, 1988), and standard no-

menclature was used (Horvitz et al., 1979). The strains used in this study were all derived from the Bristol strain N2 (Brenner, 1974), with the exception of CB4586 strain that was used for genetic mapping (Hodgkin and Doniach, 1997). The following alleles and rearrangements were used: LGI, *fog-3(q470)*, *hT2[qIs48]*, *lin-17(n671, n698)*, *lin-44(n1792)*, *pop-1(q624, q645)*, *sys-1(q544)*, *unc-29(e1072)*; LGII, *let-23(sy97)*, *unc-4(e120)*, *dpy-10(e128)*, *unc-52(e444)*, *mnC1*; LGIV, *unc-17(e245)*, *daf-1(m40)*, *dpy-9(e12)*, *tcl-2(mh15, n3170, os14, os40)*; LGV, *him-5(e1490)*. Strains carrying the following GFP markers were also used: *qIs19[lag-2::GFP]* (Blelloch et al., 1999), *syIs50[cdh-3::GFP]* (Pettitt et al., 1996), and *mhEx50[tlp-1::GFP]* (Zhao et al., 2002).

Isolation of *tcl-2* alleles and genetic mapping

The *mh15* and *n3170* alleles were isolated in a screen for Pdy (*Phasmid dye-filling defective*) mutants among the F₂ progeny of hermaphrodites mutagenized with ethyl methanesulfonate (EMS) (Brenner, 1974). The *os14* and *os40* alleles were isolated in a screen for Psa (*Phasmid socket absent*) mutants among the F₂ progeny of hermaphrodites mutagenized with EMS (Sawa et al., 2000). *tcl-2* was mapped to LGIV (data not shown). Three-factor crosses further defined the map position of *tcl-2*. From heterozygotes of genotype + *dpy-9 unc-17/tcl-2* + +, 31/31 Unc non-Dpy and 0/34 Dpy non-Unc recombinants segregated *tcl-2*. From heterozygotes of genotype + *tcl-2 unc-17/daf-1* + +, 15/15 Unc non-Tcl and 0/14 Tcl non-Unc recombinants segregated *daf-1*. In addition, we further limited the physical region that contains *tcl-2* by mapping relative to single nucleotide polymorphisms (Wicks et al., 2001). Briefly, CB4856 males were crossed to *tcl-2(mh15) unc-17(e245)* hermaphrodites: F₁ cross progeny were placed on individual petri plates, and Unc non-Tcl and Tcl non-Unc recombinants were collected for subsequent polymorphism analysis in the F₂ generation. PCR products amplified from each recombinant were analyzed by restriction enzyme digestion followed by agarose gel electrophoresis (some primer sequences were kindly provided by S. Wicks; others are available upon request).

Cell lineage analysis

Living animals were followed and cell lineages were determined as previously described (Sulston and Horvitz, 1977). T cell lineages were followed from early L1 through the mid or late L2 stage. Phasmid dye-filling as an indicator of normal T cell lineage was scored as previously described (Herman and Horvitz, 1994). Z1 and Z4 lineages were followed from mid-L1 through mid or late L2 stage.

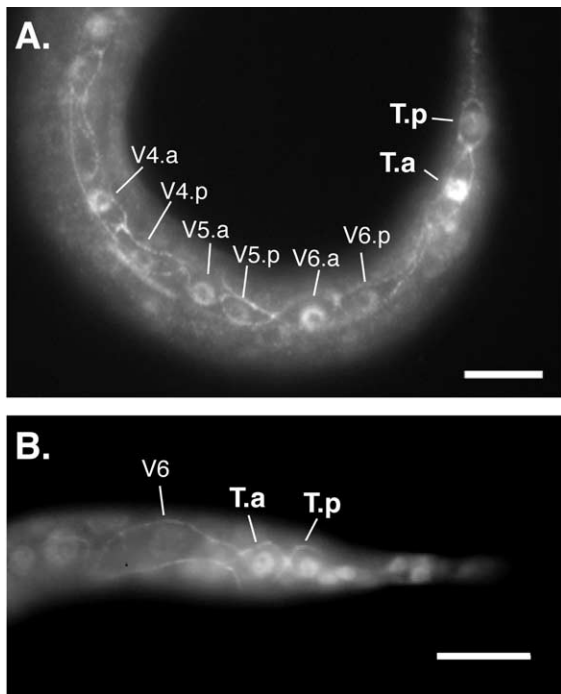


Fig. 2. POP-1 expression in T.a and T.p of wild-type and *tcl-2* mutants. Whole-mount larvae 5–7 h post-hatching were stained with monoclonal anti-POP-1 antibodies and MH27 monoclonal antibodies, which outline hypodermal cells. The V cell (V) and T cell (T) daughters are indicated. (A) In wild-type animals, 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 (Herman, 2001). (B) In *tcl-2* animals, the levels of POP-1 are equal in both T.a and T.p ($n = 28$). In this animal, V6 is in the process of dividing; thus, POP-1 expression is not observed in the V6 nucleus. In other V cell daughters of other *tcl-2* animals the level of POP-1 was higher in Vn.a than in Vn.p (data not shown). Scale bars, 10 μm .

Immunofluorescence with anti-POP-1 antibodies

Synchronized early L1 animals (2–5 h after hatching for QL.x, or 5–7 h after hatching for T.x) were collected and stained with anti-POP-1 and MH27 monoclonal antibodies as described previously (Herman, 2001).

Molecular analysis

Standard molecular biology methods (Sambrook et al., 1989) were followed unless otherwise indicated. The sequences of all mutant alleles were determined from PCR-amplified genomic DNA by using primers that amplified candidate genes in the *tcl-2* region. Primers used to search for DNA sequence changes and polymorphisms were designed based on the wild-type genomic nucleotide sequence (*C. elegans* Genome Consortium, 1998). DNA sequence data were analyzed by using the Vector NTI software (Informax, Inc). Sequence changes were further confirmed by additional independently isolated PCR products.

Germline transformation and *tcl-2* rescue

DNAs were microinjected into the mitotic germline of hermaphrodites (Mello and Fire, 1995). For rescue experiments, pRF4 [a plasmid containing the semidominant *rol-6*(*su1006*) allele] was coinjected as a marker. Yeast genomic DNA from strains containing the desired YAC DNAs was isolated using a Qiagen Blood and Cell Culture DNA Kit (Qiagen, Inc) and injected at the concentration of 40–100 $\text{ng}/\mu\text{l}$. PCR fragments were amplified from yeast genomic DNA containing Y38C1 using primers based on sequence data from WormBase (Stein et al., 2001) and injected at the concentration of 10–30 $\text{ng}/\mu\text{l}$. DNAs were injected into *tcl-2*(*mh15*) or *tcl-2*(*n3170*)/*dpy-9*(*e12*); *him-5*(*e1490*). We scored each line for phasmid dye-filling, gonad defects, or protruding vulvae and sometimes all.

tcl-2 cDNA clone isolation

Two cDNA clones, yk643h11 and yk604f8, that contain the full *tcl-2* coding region, including the 3'-UTR with potential polyadenylation signal AATAAAA, followed by poly(A) tail, were kindly provided by Yuji Kohara (National Institute of Genetics, Japan). A cDNA clone containing the 5' end of the *tcl-2* transcript was isolated by the reverse transcription polymerase chain reaction (RT-PCR) on total RNA isolated from mixed-stage wild-type animals using the transplanted leader SL1 primer (Krause and Hirsh, 1987) and an internal primer by the ThermoScript RT-PCR system (Life Technologies).

tcl-2 RNA-mediated interference (RNAi)

RNAi was performed as described previously (Fire et al., 1998). A DNA fragment that corresponded to the first 1028 bp of the *tcl-2* cDNA was amplified and used as template to synthesize the double strand RNA (dsRNA) by using a MAXIscript T7 kit (Ambion). dsRNA was injected into adult hermaphrodite gonad at the concentration of at least 100 $\text{ng}/\mu\text{l}$.

Expression experiments

A *tcl-2::gfp* fusion that contained 4 kb of sequence 5' to the ATG start site, the entire *tcl-2* open reading frame, all *tcl-2* introns, and *gfp* fused in frame with the last codon of *tcl-2* was created by recombinant PCR using yeast genomic DNA containing Y38C1 and GFP expression vector pPD95.75 (a gift from A. Fire, Carnegie Institute, Baltimore, MD) as templates. The junction of the gel-purified recombinant *tcl-2::gfp* was confirmed by sequence analysis and was used for microinjection. The cosmid C45D10, containing *unc-29*(+), was used as a coinjectable marker (Lackner et al., 1994). *mhEx56* is a transgenic array generated in the *unc-29* background containing the *tcl-2::gfp* fusion and C45D10 that rescues the phasmid dye-filling

defect of *mh15* and *os14* alleles. Similar results were obtained with another independently generated array, *mhEx57*. Transgenic animals were mounted on a 2% agarose pad containing 10 mM sodium azide as an anesthetic and examined by using a Zeiss Axioplan microscope equipped with epifluorescence.

Results

tcl-2 mutants have T cell lineage defects

The phasmids are sensory structures located bilaterally in the tail, each of which consists of two neurons, a sheath cell, and two socket cells, providing an opening to the environment through which fluorescent dyes can enter and fill the neurons (Hedgecock et al., 1985). The phasmid socket cells, PHso1 and PHso2, are generated from the asymmetric division of the T cell (Fig. 1A). During the L1 stage, the T cell divides asymmetrically to generate daughters that adopt different cell fates; T.a divides to generate four hypodermal cells and one neuron, while T.p divides to generate five neural cells and one cell that undergoes programmed cell death (Fig. 1A). In *lin-44* mutants, the asymmetric division of the T cell is reversed with respect to the body axis: T.a generates five neural cells and T.p generates four hypodermal cells as well as one neuron (Herman and Horvitz, 1994). In *lin-17* mutants, the asymmetry of the T cell division is lost: both T.a and T.p generate hypodermal cells (Sternberg and Horvitz, 1988). The reversal and loss of asymmetry of the T cell division in *lin-44* and *lin-17* mutants causes the phasmid socket cells to be misplaced or absent, which blocks dye-filling in phasmid neurons (Herman and Horvitz, 1994; Sawa et al., 1996).

Screens for Pdy and Psa animals resulted in the isolation of four mutations that defined the *tcl-2* locus (see Materials and methods). All four alleles failed to complement each other for the Pdy or Psa defects, indicating that all were alleles of *tcl-2*. All four *tcl-2* alleles are recessive (Table 1, and data not shown). The Pdy and Psa defects led us to examine the T cell lineage. Analysis of the T cell lineages showed that the fates of the T.a and T.p cells were variably defective in each *tcl-2* mutant (Fig. 1B). We found four basic patterns of defective cell lineages from which we conclude that *tcl-2* mutations most often result in the loss of T.a and T.p cell divisions and the loss of neural cell fates (Fig. 1B).

The distributions of POP-1 and TLP-1 are disrupted in a tcl-2 mutant

The level of POP-1/TCF is higher in the nuclei of anterior daughters of many anteroposterior asymmetric cell divisions that occur during embryonic and postembryonic development (Lin et al., 1995, 1998). Herman (2001) recently demonstrated that POP-1 is also required to control

Table 1
tcl-2 mutant phenotype

Genotype	% Protruding vulva	% Spew ^a	n	% Phasmid dye-filling ^b	n
Wild type	0	0	101	98 ^c	472
<i>tcl-2(mh15)</i>	15	3	108	0	169
<i>tcl-2(n3170)</i>	44	11	126	1	104
<i>tcl-2(os14)</i>	38	10	82	0	111
<i>tcl-2(os40)</i>	50	12	108	0	114
<i>tcl-2(RNAi)</i>	6	1	183	21	129
<i>tcl-2(n3170)/+</i>	1	0	142	97	114
<i>tcl-2(os14)/+</i>	0	0	123	97	107

^a Animals display a rupture at the site of the vulva (Eisenmann and Kim, 2000).

^b Phasmid dye-filling was used as an indicator of normal T cell division (Herman and Horvitz, 1994). There is one phasmid on each side of the animal.

^c Data from Herman and Horvitz (1994).

the asymmetric T cell division. In wild-type animals, POP-1 is asymmetrically distributed to the T cell daughters: the level of POP-1 is high in the nucleus of anterior T cell daughter, T.a, and lower in the nucleus of the posterior T cell daughter, T.p. However, *lin-44* mutations cause the level of POP-1 to be high in T.p and lower in T.a and *lin-17* mutations cause the level of POP-1 to be high in both T.a and T.p, which correlates with the effects of these mutations on the T cell lineage (Herman, 2001). Since mutations in *tcl-2* also disrupt the T cell lineage, we asked whether asymmetric distribution of POP-1 in the T cell daughters requires TCL-2 function. To test this, we fixed and stained early L1 larvae using anti-POP-1 monoclonal antibody (Lin et al., 1998) and MH27 monoclonal antibody, which recognizes adherens junctions (Francis and Waterston, 1991). We found that the level of POP-1 was equal in both T cell daughters in all the animals we examined ($n = 28$) (Fig. 2B). This is similar to the POP-1 distribution observed in *lin-17* mutants and suggests that TCL-2 might function before POP-1 to control the asymmetric T cell division and subsequent specification of daughter cell fates.

A potential target of the *lin-44/Wnt* pathway in the T cell division is *tlp-1*, which encodes a C2H2 zinc finger protein (Zhao et al., 2002). In wild-type animals, *tlp-1* expression is often asymmetric; it is expressed in the T.p cell, but not in the T.a cell. However, in *lin-44* mutants, the asymmetry of *tlp-1* expression is often reversed, and in *lin-17* mutants, *tlp-1* is rarely expressed, indicating that *tlp-1* functions downstream of *lin-44* and *lin-17* to specify T cell daughter fates. Similar to what we have observed in *lin-17* mutants (Zhao et al., 2002), we rarely observed *tlp-1::gfp* expression in the T cell daughters in *tcl-2* mutants. Specifically, after the division of the T cell, only 4% of T cell lineages showed GFP expression ($n = 74$); 0% showed GFP expression in T.a alone, 2.7% showed expression in T.p alone, and 1.4% showed expression in T.a and T.p, suggesting that like *lin-17*, *tcl-2* functions upstream of *tlp-1*.

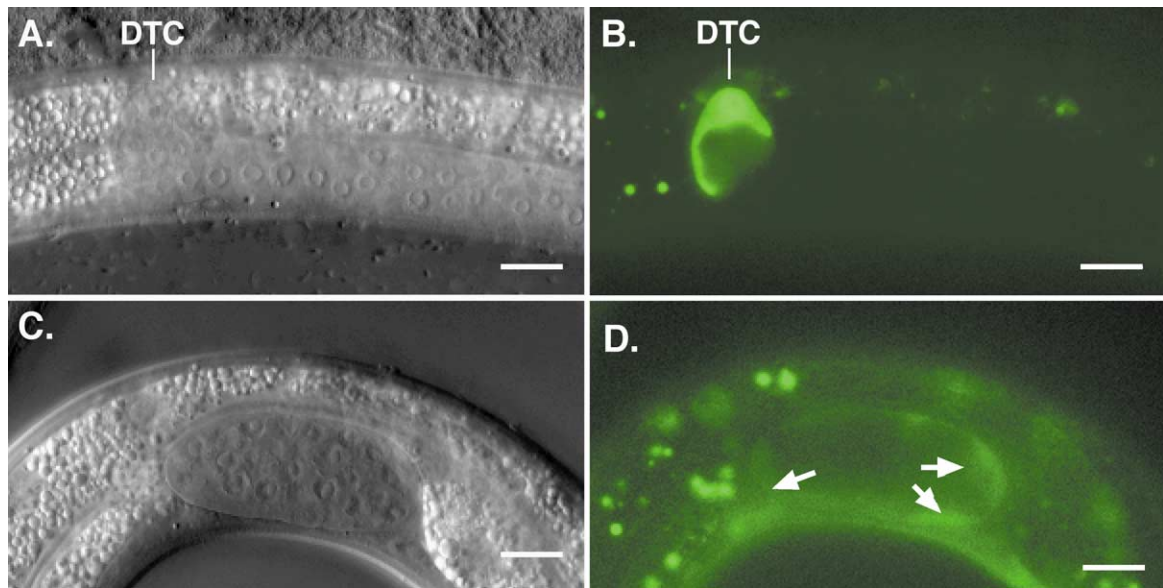


Fig. 3. *tcl-2* hermaphrodites display gonad defects. (A) Wild-type L3 hermaphrodite, DTC at the end of the anterior gonad arm, the posterior gonad arm is not shown. (B) Same animal as in (A) with intense *lag-2::gfp* expression in DTC. (C) *tcl-2(os14)* L3 hermaphrodite. (D) The same animal as in (C), only weak *lag-2::gfp* expression was observed in the somatic cells (arrows). Scale bars, 10 μm .

tcl-2 genetically interacts with the Wnt signaling pathway to control somatic gonad development

In wild-type hermaphrodites, the adult gonad forms two equivalent U-shaped arms at the center of the body, each with its own proximal–distal axis. During early gonadogenesis, several regulatory cells are generated from the division of two somatic gonad precursor cells, Z1 and Z4. Distal tip cells (DTCs), located at the end of each gonad arm, are required for the formation of each U-shaped arm and the proliferation of germline (Kimble and White, 1981). These DTC cells are generated from distal Z1/Z4 daughters. The third regulatory cell, the anchor cell (AC), is generated from proximal Z1 or Z4 daughters, and is required for induction of the vulva and the uterine π cell fates (Kimble, 1981; Newman et al., 1995). While characterizing the *tcl-2* mutants, we observed that *tcl-2* hermaphrodites display abnormal gonad morphologies, such as the loss of one or both gonad arms. The lack of gonad arm extension could be caused by defects in the divisions of the somatic gonad precursors Z1 and Z4. The morphology of the four-cell gonadal primordium at hatching was normal in *tcl-2* mutants (data not shown). Furthermore, *lag-2::gfp*, a marker of the somatic gonad precursors, was expressed normally in both Z1 and Z4 cells in 96% of *tcl-2(os14)* mutants ($n = 73$), suggesting that the initial specification of Z1/Z4 cell fates is normal. At the L3 stage in wild-type hermaphrodites, intense expression of *lag-2::gfp* is restricted to the distal tip cells (DTCs) (Miskowski et al., 2001) (Fig. 3A and B). However, at the same stage in *tcl-2* mutant hermaphrodites that display gonad arm extension defects, intense expression of *lag-2::gfp* in the DTCs was not observed (Fig. 3C and D). We observed weak *lag-2::gfp* expression in

somatic gonad cells, similar to that observed in *sys-1* mutants (Miskowski et al., 2001), suggesting that *tcl-2* mutants cause gonad defects reminiscent of *sys* mutants (Fig. 3D). These defects indicate that either the DTCs were not produced from the normal Z1/Z4 divisions or they were produced but not correctly specified. Mutations in several Wnt signaling components, including *lin-17*, *wrm-1/ β -catenin*, and *pop-1/Tcf*, result in loss of DTC and *lag-2::gfp* expression (Siegfried and Kimble, 2002). However, unlike these mutants, which can generate extra anchor cells (ACs) (Siegfried and Kimble, 2002), we have not observed extra ACs in *tcl-2* mutants as assayed with the AC marker gene, *cdh-3::gfp* (data not shown). In addition, animals with both *sys-1* and a mutation in any one of the other *sys* genes in heterozygous condition display gonad defects, suggesting that these genes function in a common pathway (K. Siegfried and J. Kimble, personal communication). However, animals with both *sys-1* and *tcl-2* in heterozygous condition did not display gonad defects (Table 2), suggesting *tcl-2* functions in a separate pathway.

To understand the basis of the gonad defects, we examined Z1 and Z4 cell lineages in six *tcl-2(n3170)* hermaphrodites. We observed that the divisions of Z1 and Z4 were delayed in all *tcl-2* animals. In five *tcl-2* animals, Z1 or Z4 divided once before L1 lethargus, and in one Z1 and Z4 started to divide in the early L2 (Fig. 4D), whereas in wild-type animals, Z1 and Z4 divide twice before L1 lethargus (Fig. 4A). In the three animals, the Z1 and Z4 divisions were delayed, but the patterns were the same as wild-type. The remaining three animals displayed loss of division defects shown in Fig. 4B–D.

Each *tcl-2* allele caused a gonad defect. The *os14*, *os40*, and *n3170* mutants displayed a similar and more penetrant

Table 2
tcl-2 interacts with the Wnt and Ras pathways

Genotype	Gonad defects ^a		P12.p to P11.p ^b	
	%	<i>n</i>	%	<i>n</i>
Wild type	0	132	0	132
<i>lin-44(n1792)</i>	0	75	17 ^c	35
<i>lin-17(n671)</i>	30	69	29 ^d	41
<i>lin-17(n698)</i>	6	68	0 ^e	46
<i>lin-17(n671); lin-44(n1792)</i>	24	75	28 ^d	54
<i>tcl-2(mh15)</i>	7	83	13	30
<i>tcl-2(os14)</i>	33	84	22	23
<i>tcl-2(os40)</i>	34	85		ND
<i>tcl-2(n3170)</i>	30	83	24	21
<i>lin-44(n1792); tcl-2(mh15)</i>	33 ^g	84	83 ^h	18
<i>lin-17(n698); tcl-2(mh15)</i>	83 ⁱ	85	55 ^j	22
<i>lin-17(n671); tcl-2(mh15)</i>	77 ^k	96		ND
<i>lin-17(n671); tcl-2(n3170)</i>	76 ^l	29		ND
<i>pop-1(q624)^f</i>	58	50	0	49
<i>pop-1(q624)/+</i>	0	49		ND
<i>pop-1(q624)/+; tcl-2(mh15)</i>	98	62		ND
<i>pop-1(q645)/+; tcl-2(os14)/+</i>	2	41		ND
<i>sys-1(q544)/+; tcl-2(os14)/+</i>	0	58		ND
<i>let-23(sy97)</i>		ND	44 ^d	50
<i>let-23(sy97); tcl-2(mh15)</i>		ND	85 ^m	26

Note. All *P* values were calculated by using Fisher's Exact test. ND, not determined.

^a Gonad defects were defined as abnormal morphology or a gonad with one or both arms failing to extend. Animals were scored at late L3 or early to mid-L4 stage.

^b P11.p and P12.p fates were scored under the Nomarski optics according to the nuclear morphologies and positions of P11.p and P12.pa cells at late L2 and early L3 stage. Only animals with normal Pn.p cell numbers were scored.

^c Data from Herman and Horvitz (1994).

^d Data from Jiang and Sternberg (1998).

^e Data from Jiang and Sternberg (1999).

^f Mutants are derived from *pop-1(q624)/hT2[qIs48]*

^g *P* < 0.0001, compared with additive effect of 7%.

^h *P* < 0.0001, compared with additive effect of 28%.

ⁱ *P* < 0.0001, compared with additive effect of 13%.

^j *P* = 0.00016, compared with additive effect of 13%.

^k *P* < 0.0001, compared with additive effect of 35%.

^l *P* = 0.015, compared with additive effect of 51%.

^m *P* = 0.0013, compared with additive effect of 51%.

defect than did *mh15* (Table 2). To test whether *tcl-2* interacts with the genes involved in the Wnt signaling pathway that controls the Z1 and Z4 divisions, we constructed double mutant strains. Whereas 6% of *lin-17(n698)* and 7% of *tcl-2(mh15)* animals displayed gonad defects, we observed that 83% of *lin-17(n698); tcl-2(mh15)* double mutant animals displayed gonad defects (Table 2), indicating a synergistic interaction between these mutations. Moreover, the weak gonad defects of *tcl-2(mh15)* mutants are significantly enhanced by one mutant copy of *pop-1(q624)* (Table 2). In addition, double mutant combinations between the *tcl-2(n3170)* allele, which causes a more severe gonad defect than does the *mh15* allele, and the null *lin-17(n671)* allele displayed 76% gonad defects, also demonstrating synergy between these mutations. Finally, in contrast to each single mutant, double mutant combinations between *tcl-2(mh15)* and the null *lin-44(n1792)* allele displayed strong defects in gonad development (Table 2), while we found no synergy in gonad defects between *lin-44(n1792)* and *lin-17(n671)* (Table 2). This is surprising because LIN-44/WNT is thought to function as a short-range signal, and *lin-44* is expressed far posterior in the tip of tail cells (Herman et al., 1995). Together, these data suggest that *tcl-2* might have functions that overlap with the Wnt signaling pathway involved in gonad development.

tcl-2 genetically interacts with the Wnt signaling pathway to specify P12 cell fate

Six pairs of P cells are located bilaterally in wild-type animals at hatching. During the L1 stage, they migrate into the ventral nerve cord, and are numbered P1 to P12 from anterior to posterior; P11 and P12 are the most posterior pair (Sulston and Horvitz, 1977). In hermaphrodites, P11 divides to generate P11.a, that divides to give rise to three neurons, as well as P11.p, which does not divide but fuses with the surrounding hypodermal syncytium, hyp7. P12 divides to generate P12.a, which has the same division pattern as P11.a, and P12.p, which divides once to generate P12.pa, which becomes a small hypodermal cell, and P12.pp, which undergoes programmed cell death.

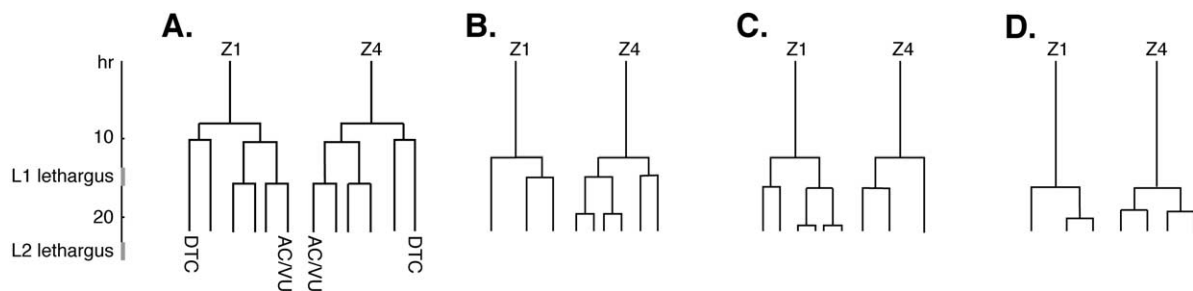


Fig. 4. Cell lineages of the Z1 and Z4 cells in wild-type and *tcl-2* hermaphrodites. (A) Wild-type hermaphrodite Z1 and Z4 cell lineages (Kimble and Hirsh, 1979). Time scale of development is shown in hours on the left. Z1.a and Z4.p each divide once to generate DTCs. Z1.p and Z4.a each divide twice to generate to an AC/VU cell, one of which becomes the AC (Kimble and Hirsh, 1979). DTC, distal tip cell; AC/VU, anchor cell/ventral uterine precursor. (B–D) Three *tcl-2(n3170)* hermaphrodites Z1 and Z4 cell lineages.

We observed defects in the specification of the P12.p cell in *tcl-2* mutants, which suggest the specification of the P12 cell fate might be defective. In addition, *tcl-2* mutants also are missing Pn.p cells and have extra Pn.p divisions. In various animals, we observed that any of the P(1–12).p cells could be missing or undergo extra divisions. Specifically, 7% ($n = 42$) of *tcl-2(mh15)* hermaphrodites were missing Pn.p cells, and 21% displayed extra Pn.p divisions; among those *tcl-2(mh15)* mutants with normal Pn.p numbers, 13% ($n = 30$; Table 2) had a P11.p-like cell in the position of P12.pa cell and 7% had two P12.pa like cells, suggesting that both P12.p-to-P11.p and P11.p-to-P12.p cell fate transformations occur in *tcl-2* mutants. The *os14* and *n3170* alleles also caused 55% ($n = 33$) and 45% ($n = 31$) of Pn.p cells to be missing and 35 and 16% extra Pn.p divisions, respectively. Furthermore, the degree of P12.p-to-P11.p transformation among *os14* and *n3170* mutants with normal Pn.p numbers was higher than *mh15* mutants, 22% and 24%, respectively (Table 2). However, in contrast to *mh15* mutants, we did not observe P11.p-to-P12.p cell fate transformations in *os14* or *n3170* mutants, suggesting that this cell fate transformation might be specific to *mh15* mutants.

Mutations in either *lin-44* or *lin-17* can also cause P12.p-to-P11.p cell fate transformations (Herman and Horvitz, 1994; Sternberg and Horvitz, 1988). We observed that *tcl-2* strongly interacts with *lin-44* and *lin-17* to specify P12 cell fate. Null alleles, *lin-44(n1792)* and *lin-17(n671)*, caused 17 and 29% P12.p-to-P11.p transformations, respectively; while *lin-17(n698)*, a weak allele, did not cause a transformation (Table 2). Since the level of defective animals in *lin-17 lin-44* double mutants was nearly identical to that of *lin-17* alone, *lin-17* is epistatic to *lin-44* (Jiang and Sternberg, 1998; Table 2). Interestingly, we observed that *tcl-2(mh15)* significantly enhances the P12.p-to-P11.p transformations of both *lin-44(n1792)* and *lin-17(n698)* mutants to 83 and 55%, respectively (Table 2). However, we never observed P11.p-to-P12.p transformations in *lin-17(n698)*; *tcl-2(n3170)* double mutants. Finally, we were unable to score the P12.p-to-P11.p transformation in *lin-17(n671)*; *tcl-2(n3170)* or *lin-17(n671)*; *tcl-2(mh15)* double mutants due to the high frequency of animals with abnormal Pn.p numbers, 82% ($n = 35$) in the case of *lin-17(n671)*; *tcl-2(n3170)*.

The Ras pathway is also involved in controlling P12 cell fate (Jiang and Sternberg, 1998). We tested whether *tcl-2* functions with the Ras pathway by constructing a *let-23*; *tcl-2* double mutant. Only 10% ($n = 29$) of the *let-23*; *tcl-2(mh15)* animals were missing Pn.p cells, and of those with normal Pn.p, 85% displayed P12.p-to-P11.p transformation, whereas *let-23* single mutant displayed 44% transformation (Jiang and Sternberg, 1998; Table 2). This indicated that *tcl-2* synergistically interacted with Ras pathway and that *tcl-2* might overlap in function with the Ras pathway.

Taken together, the increased penetrances of the gonad defects and P12.p-to-P11.p transformation in *lin-44*; *tcl-2*,

lin-17; *tcl-2* and *pop-1/+*; *tcl-2* double mutants are synergistic rather than an additive combination of the defects caused by each single mutation, implying that *tcl-2* might function with or in parallel to the Wnt signaling pathway. Although it is not clear whether any of the *tcl-2* alleles are null (see Discussion), we observed synergistic interactions with null alleles of both *lin-44* and *lin-17*. Since the Wnt pathway was inactivated by a null mutation in these double mutants, we conclude that *tcl-2* must function, in part, in a pathway parallel to the Wnt pathway. However, we cannot make the same conclusion for the Ras pathway as *let-23(sy97)* is not a null allele. Moreover, null mutations of *let-23* and other Ras pathway components cause lethality, preventing double mutant analyses with these mutations and *tcl-2*.

In addition to the T cell division, Z1 and Z4 division, and P12.p cell fate defects, *tcl-2* mutants have additional abnormalities. These include a Pv1 (Protruding vulva) and extrusion of the gonad through the vulva (spew) (Tables 1 and 2).

tcl-2 encodes a novel protein

We mapped *tcl-2* to the left arm of linkage IV, very close to *daf-1* and *dpy-9* (see Materials and methods; Fig. 5A). “Snip-SNP” mapping (Wicks et al., 2001) defined the physical location of *tcl-2* near clones Y66H1A and K11H12. Microinjection of a YAC clone Y38C1 rescued the Pdy defect of *tcl-2* mutants (Fig. 5A). An 11-kb PCR fragment generated from Y38C1, containing the predicted genes Y38C1AA.4 and Y38C1AA.10, efficiently rescued the Pdy defect of *tcl-2(mh15)* and *tcl-2(n3170)* (Fig. 5A). We found DNA lesions in Y38C1AA.4 in each *tcl-2* mutant (Fig. 5B). *mh15* is a point mutation that changed tyrosine residue 11 to an amber stop codon. *n3170* is a point mutation that changed tryptophan residue 86 to an amber stop codon. *os14* is a splice-site mutation that changed G to A in the donor site of the third intron, potentially resulting in an aberrant protein. *os40* had two changes: one is a point mutation that changed glutamic acid residue 319 to a glutamine residue and the other is a 1-bp deletion that causes a frameshift at codon 329 resulting in a termination codon 13 codons later. Furthermore, RNAi directed against the 5' portion of *tcl-2* region partially phenocopies *tcl-2* mutants, confirming that we have identified the *tcl-2* locus (see Materials and methods; Table 1).

The composite *tcl-2* cDNA contains the SL1 transplanted leader sequence, a 1308-nucleotide open reading frame, a 97-nucleotide 5' untranslated region, a 347-nucleotide 3' untranslated region, and a poly(A) tail, indicating it is full length. Comparison of the cDNA and the genomic sequences was used to determine the exon and intron structure (Fig. 5B). The positions of the five exons in the gene structure are the same as that predicted gene for Y38C1AA.4 by WormBase (Stein et al., 2001). Conceptual translation of the *tcl-2* composite cDNA yielded a predicted

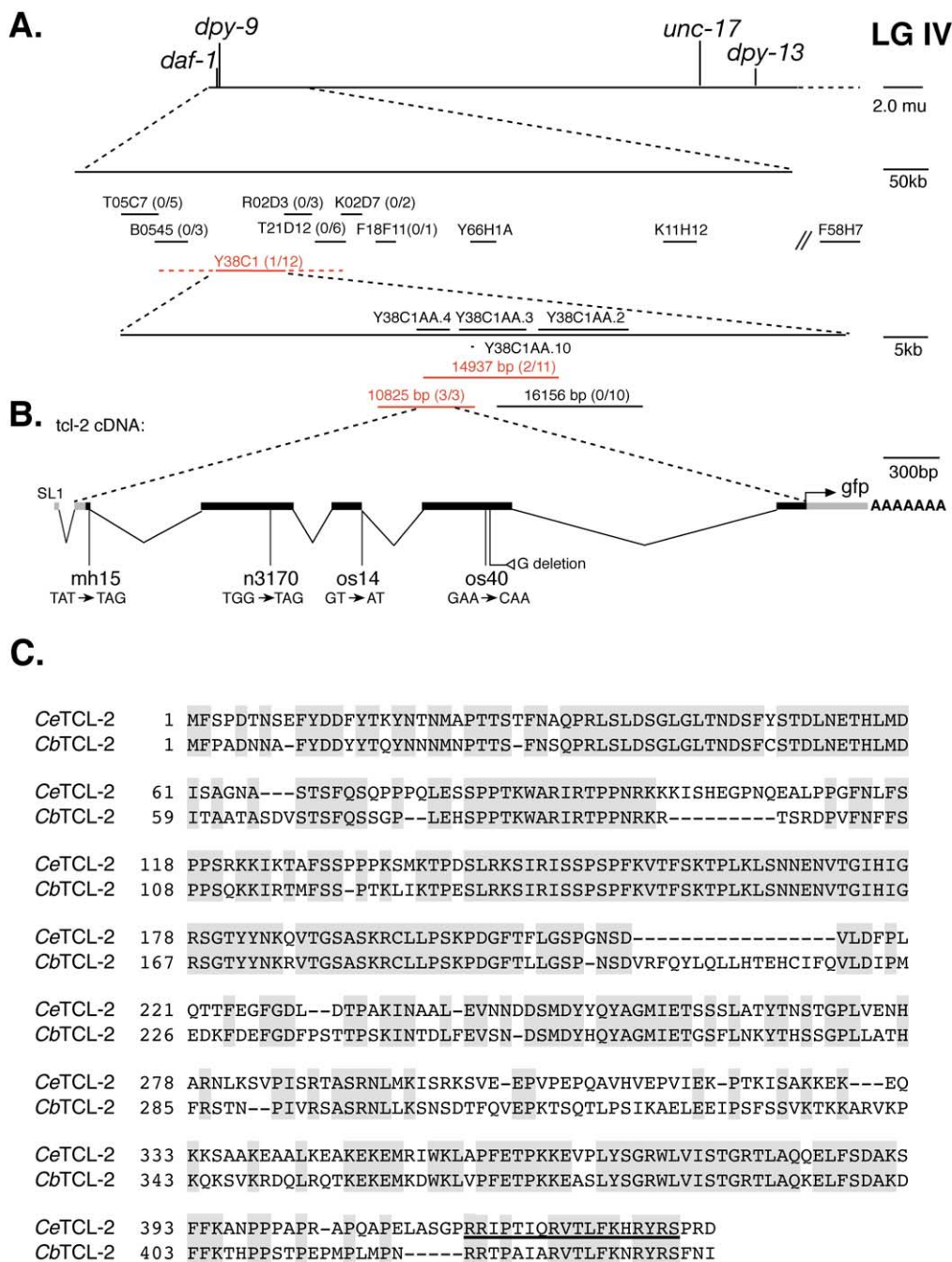


Fig. 5. *tcl-2* cloning and molecular analysis. (A) *tcl-2* was mapped to LG IV. Relative positions of genomic clones tested for ability to rescue *tcl-2* phenotype are shown below the genetic map. Red lines represent genomic clones that rescued the *tcl-2* Pdy defects, and the numbers of rescued transgenic lines (>70% of the transgenic animals were rescued) are indicated in parenthesis. (B) Intron/exon structure of *tcl-2*. Boxes indicate exons: black portions indicate the open reading frame; gray portions indicate the 5'- and 3'-UTR. Position of the SL1 (*trans*-spliced leader sequence) and the poly(A) tail are shown (GenBank Accession No. AY145133). The positions of the base changes in the *mh15*, *n3170*, *os14*, and *os40* are indicated. *os14* is a splice-site mutation. *os40* has two lesions (see text). The position of *gfp* tag is indicated with an arrow. (C) Alignment of the TCL-2 protein with *CbTCL-2*. Identical residues are indicated by shade. *CbTCL-2* is conceptually translated from a gene predicted by the FGNSH (Salamov and Solovyev, 2000; <http://www.softberry.com>) using defaults for *C. elegans* genomic sequences. A bipartite nuclear localization sequences (underlined) was predicted by the PSORT II program (<http://psort.nibb.ac.jp/>).

protein of 435 amino acid residues with a predicted molecular mass of 48 kDa.

Database searches of protein and nucleotide databases using *tcl-2* primary sequence revealed no dramatically similar sequences. Thus, *tcl-2* encodes a novel protein. How-

ever, we found that *tcl-2* shares significant homology to a predicted gene from the genomic sequence of the closely related species, *Caenorhabditis briggsae* (Genome Sequencing Center, personal communication). Specifically, TCL-2 and the protein encoding by this gene, *CbTCL-2*

share 70% identity in overall sequence (Fig. 5C). In addition, bipartite nuclear localization signals (NLS) have been predicted within the TCL-2 sequence (Fig. 5C), suggesting that TCL-2 might be a nuclear protein.

TCL-2 expression pattern

A transgene in which *gfp* was fused in frame with *tcl-2* and whose expression was designed to be driven by the *tcl-2* promoter (see Materials and methods; Fig. 5B) rescued the *tcl-2* T cell and gonad defects, but only partially rescued the P1 defects (data not shown). *tcl-2::gfp* is expressed strongly in the cytoplasm of the pharynx muscle cells and several head neurons, probably the IL1s or IL2s, throughout development (Fig. 6A). Several other unidentified neurons in the tail region expressed GFP throughout development. We have not characterized this pattern in detail, as we do not observe defects associated with these cells in *tcl-2* mutants. Although *tcl-2* mutants display gonad, and P12.p cell fate defects, we did not observe GFP expression in these cells or their descendants in L1 animals. In contrast to the cytoplasmic expression we observed in head neurons, we observed weak GFP expression in the nuclei of the T cells and the T cell daughters, T.a and T.p (Fig. 6B and C). The level of GFP expression was equal in both T cell daughters. Expression of *tcl-2::gfp* in the nucleus of T cell and its descendants might account for the role for *tcl-2* in the differentiation of these cells that, when reduced or absent, could lead to the T cell lineage defects we observed in *tcl-2* mutants.

Discussion

tcl-2 mutants are defective in three developmental processes controlled by Wnt signaling

tcl-2 mutants display defects in three developmental processes that are controlled by the Wnt signaling pathway: the asymmetric T cell division controlled by *lin-17*, *lin-44*, and *pop-1*, the asymmetric Z1 and Z4 cell divisions controlled by *lin-17* and *pop-1* as well as the specification of the P12.p cell controlled by *lin-44* and *lin-17*. Mutations affecting other components of the Wnt signaling pathway also cause defects in these processes (Herman, 2003). In addition, we observed that *tcl-2* mutants displayed other defects similar to those observed in *lin-17* and *pop-1* mutants, such as protruding vulvae and extra vulva invaginations. However, there are a number of intriguing differences between mutations in *tcl-2* and these Wnt pathway components. First, mutations in *lin-17* and *pop-1* disrupt the asymmetric division of Z1 and Z4, resulting in Z1 and Z4 symmetric divisions (Siegfried and Kimble, 2002; Sternberg and Horvitz, 1988), while mutations in *tcl-2* result in the delay of Z1 and Z4 division, and loss of division in some cases, suggesting that the gonad defects in *tcl-2* mutants are not

caused by the same Sys defect observed in *lin-17* and *pop-1* mutants. Second, *tcl-2* mutations do not interact with *sys-1* in the same manner as do *pop-1* mutations. Third, mutations in *lin-44* and *lin-17* cause only P12.p-to-P11.p cell fate transformations, while *tcl-2* mutations cause a low level of P11.p-to-P12.p cell fate transformations in addition to P12.p-to-P11.p cell fate transformations, suggesting that mutations in *tcl-2* might destabilize both cell fates. Interestingly, *lin-17*; *tcl-2* double mutants show only an enhancement of the P12.p-to-P11.p cell fate transformation, suggesting that in the absence of Wnt signaling mutations in *tcl-2* might stabilize P11.p cell fate and enhance the expression of P11.p fate by P12.p. The only other gene in which mutations can cause both P12.p-to-P11.p and P11.p-to-P12.p cell fate transformations is the Hox gene *mab-5* (Kenyon, 1986). This suggests that the *tcl-2* may function through *mab-5* to control P12.p cell fate.

What is the null phenotype of tcl-2?

All *tcl-2* mutant alleles display a highly penetrant T cell division defect; however, the penetrance of the other defects was variable: *n3170*, *os14*, and *os40* are more severe than is *mh15* (Tables 1 and 2). Moreover, each allele also displays variable expressivity; for example, the vulva protrusion is often smaller in *mh15* mutants than it is in *n3170*, *os14*, or *os40* mutants. Although no deficiency is available for the *tcl-2* region, cloning the *tcl-2* gene allowed us to examine the loss-of-function mutant phenotypes using RNAi, by which maternal and zygotic gene products can often be reduced or eliminated, often resulting in strong phenotypes associated with null mutations (Fire et al., 1998). We observed that *tcl-2(RNAi)* animals partially phenocopied *tcl-2* mutants, but displayed a lower penetrance of defects than did *tcl-2* mutants. While this suggests that the *tcl-2* alleles are loss-of-function mutations (Table 1), it does not give an indication of the null phenotype. Although the *mh15* mutation causes a stop codon after only 10 amino acid residues, while the *tcl-2(os14, os40 n3170)* alleles could conceptually translate longer products, these latter mutations cause more severe defects, suggesting that *mh15* is not a null allele. A possibility to reconcile this molecular and phenotypic difference is that *tcl-2(mh15)* might give rise to a longer product with partial function by translation initiation from a downstream methionine, located 21 amino acid residues downstream of the predicted start codon. Although in the absence of a deficiency of the locus we cannot unambiguously determine the *tcl-2* null phenotype, all the *tcl-2* alleles are recessive, suggesting that the more severe alleles may approximate a complete loss of *tcl-2* function.

tcl-2 interacts with the Wnt signaling pathway

The shared defects between *tcl-2* and Wnt pathway mutants led us to look for genetic interactions. We found that *tcl-2* can act synergistically with *lin-17* and *lin-44* to control

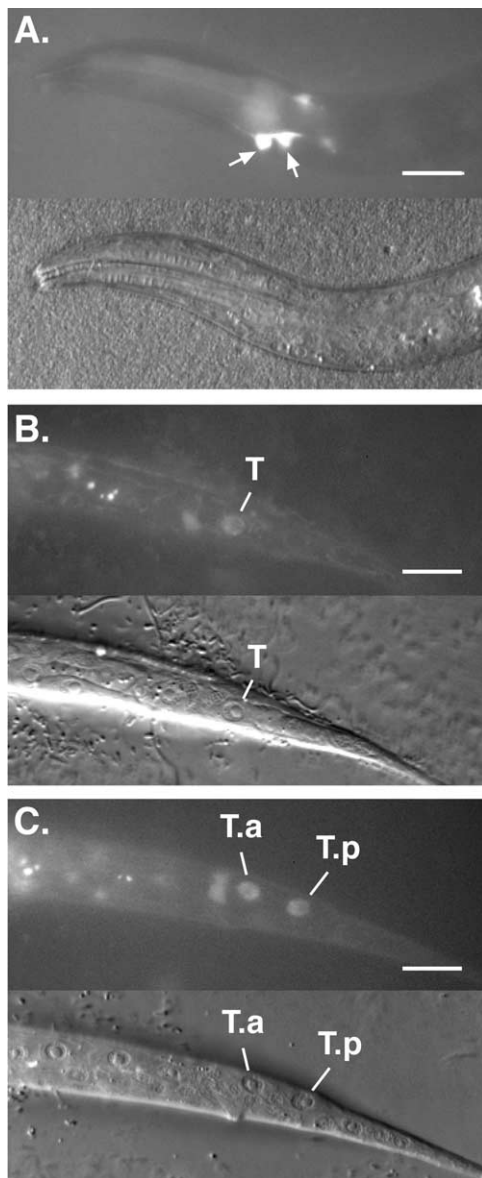


Fig. 6. *tcl-2* expression. (A–C) Fluorescence (upper) and DIC optics (lower). Bars equal 10 μm . (A) GFP expression in the cytoplasm of the pharynx muscle cells and several head neurons, which appeared to be IL1 or IL2 and are indicated by the arrow (B) GFP expression in the nuclei of T cell. (C) Equal GFP expression in the nuclei of T.a and T.p cells.

gonad development as well as P12.p cell fate specification. For example, *tcl-2(mh15)* strongly enhances defects in gonad development and P12.p fate specification associated with the weak *lin-17(n698)* allele (Table 2). Intriguingly, we observed that *lin-44* could enhance the gonad defects in a *tcl-2* background. This is surprising, since *lin-44* mutants do not display any defects in gonad development, as observed by Nomarski optics. Since *lin-44* is expressed in cells at the tip of the tail where LIN-44/WNT is thought to function as a short-range signal (Herman et al., 1995), it is not expected to be involved in the gonad development. However, LIN-44 might function as a signal to the gonad during the 1.5-fold stage of embryogenesis when the gonad primordium and the

tail cells, which begin to express LIN-44 at this time, are in closer proximity. The sort of genetic synergy observed in double mutant combinations with *tcl-2* and *lin-44* or *lin-17* usually suggests that the genes in question function in the same biological pathway or in parallel pathways, depending on the type of alleles that are used. For example, *lin-44 lin-17* double mutants did not show synergy in P12.p fate specification or gonad development. As null *lin-44* and *lin-17* alleles were used in this analysis and other studies (Herman and Horvitz, 1994; Jiang and Sternberg, 1998), we and others concluded that the genes are in the same pathway, and that *lin-17* is epistatic to *lin-44*. Although we do not know whether the alleles of *tcl-2* used are null alleles (see above), we did use *lin-44* and *lin-17* null alleles in our analyses, which allowed us to conclude that *tcl-2* functions, in part, in a parallel pathway. Specifically, the significant increase in penetrance and expressivity of the gonad defect in *lin-17(0); tcl-2* as well as the gonad defect and P12.p-to-P11.p cell fate transformation in *lin-44(0); tcl-2* double mutants, relative to either single mutant, suggests that *tcl-2* functions in parallel with the Wnt pathway. However, since *tcl-2* mutants may retain some function, it remains possible that *tcl-2* has some additional role in Wnt signaling. As *tcl-2* encodes a novel protein, the molecular nature of the interaction between *tcl-2* and the Wnt signaling pathway presently remains unknown. In addition, *tcl-2* can act synergistically with the *let-23* in P12.p cell fate specification, as do *lin-44* and *lin-17* (Jiang and Sternberg, 1998), indicating that *tcl-2* also interacts with Ras signaling. However, since *let-23(sy97)* is not a null allele, we could not determine whether *tcl-2* functions within, or in parallel to, the Ras pathway.

How does tcl-2 regulate the asymmetric T cell division?

Mutations in *lin-44/Wnt* cause the reversal of the asymmetric T cell division, while *lin-17/frizzled* and *pop-1/Tcf* mutations cause the loss of asymmetry. Moreover, activation of the Wnt pathway appears to down-regulate POP-1 levels in the T.p nucleus, while in the absence of Wnt signaling, POP-1 level is higher in the T.a nucleus. Since no β -catenin homolog has been identified to be involved in the control T cell division (Herman, 2001), the Wnt pathway controlling T cell division might be unusual. Herman (2001) proposed a model for T cell polarity: induction of LIN-44/Wnt signal binding to LIN-17 functions through *lit-1* to modify POP-1, perhaps by phosphorylation, resulting in decreased POP-1 levels and the activation of neural-specific genes in T.p. Without LIN-44 signal, the T.a cell accumulates a high level of POP-1 that might be nonfunctional, and expresses hypodermal specific genes. Recently, Maduro et al. (2002) demonstrated that, in the embryo, the asymmetric distribution of POP-1 to anterior and posterior sister nuclei is caused by the redistribution of POP-1 to the cytoplasm in posterior sisters. This redistribution appeared to be the result of a qualitative difference in POP-1 protein in signaled and

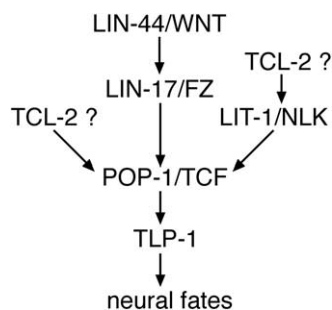


Fig. 7. A genetic model for T.p cell fate specification. Upon transduction of LIN-44 through LIN-17 action in the T.p cell, the level of POP-1 is down-regulated in the nucleus and perhaps modified by LIT-1 (Herman, 2001). In this process, TCL-2 might either regulate POP-1 level in parallel to the Wnt pathway or act through LIT-1, indicated by “?”. Thereafter, the modified form of POP-1 might activate expression of target gene *tlp-1* that is required for T.p to generate neural cell fates.

unsignaled cells that correlated with repressor function and the coalescence of POP-1 into subnuclear puncta in unsignaled, anterior nuclei. Although it is not clear whether these same mechanisms function during the T cell division, the involvement of *lit-1* in both processes suggests they might. In this study, we observed that *tcl-2* also functions to down-regulate level of POP-1 in the T.p cell, suggesting that *tcl-2* functions before POP-1 to control the T cell division. What role might *tcl-2* play in down-regulating the POP-1 level in T.p? Since *tcl-2* appears to be symmetrically expressed in the nuclei of T.a and T.p, any model must explain how a symmetrically localized protein might affect POP-1 asymmetric distribution. One possibility is that TCL-2 is involved in the modification or redistribution of POP-1 protein in the signaled T cell daughter. Alternatively, other asymmetrically distributed protein(s) might exist that function to control POP-1 distribution together with TCL-2. Finally, TCL-2 might be nonfunctional in T.a through modification by other asymmetrically distributed proteins.

The functions of the Wnt signaling pathway seem to be the activation of the responsive gene(s), usually transcription factors, in the nucleus (Nusse, 2002). In *C. elegans*, *mab-5* and *lin-39* encode homeodomain proteins, apparent targets of the Wnt signaling pathway that function in the control of QL migration and vulva induction, respectively (Eisenmann et al., 1998; Gleason et al., 2002; Harris et al., 1996; Korswagen et al., 2002; Maloof et al., 1998). We have previously identified *tlp-1*, which encodes a C2H2 type zinc finger protein, a potential target of *lin-44*-mediated Wnt signaling pathway in the control of the asymmetric T cell division. Furthermore, although *tlp-1* mutations could cause the T.p cell to adopt hypodermal fates, the POP-1 distribution pattern is not altered (M.H., unpublished observations), suggesting that *tlp-1* functions downstream of *pop-1*. We observed that *tcl-2* mutations also affect *tlp-1* expression. Although our experiments do not define the exact point where TCL-2 might function, it must be before POP-1 and

the end point might be to regulate *tlp-1* to promote neural cell fates through the Wnt pathway (Fig. 7).

TCL-2 is not similar to any other proteins in current databases. However, the existence of a *C. briggsae* homolog suggests that function of this protein might be evolutionarily conserved. Since *tcl-2* is involved in three developmental processes that involve the Wnt pathway, and *tcl-2* genetically interacts with *lin-17*, *lin-44*, and *pop-1*, *tcl-2* appears to act in parallel with the Wnt pathway. Alternatively, it remains possible that *tcl-2* has some overlapping function within the Wnt pathway and may encode a novel Wnt pathway component.

Acknowledgments

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