Cell, Vol. 97, 717–726, June 11, 1999, Copyright ©1999 by Cell Press

WRM-1 Activates the LIT-1 Protein Kinase to Transduce Anterior/Posterior Polarity Signals in *C. elegans*

Christian E. Rocheleau,[1,9] Jun Yasuda,[1,2,9] Tae Ho Shin,[1,9] Rueyling Lin,[3] Hitoshi Sawa,[4,5] Hideyuki Okano,[4,6] James R. Priess,[2,7] Roger J. Davis,[1,2] and Craig C. Mello[1,8]

[1]University of Massachusetts Medical School
Program in Molecular Medicine
Worcester, Massachusetts 01605
[2]Howard Hughes Medical Institute
[3]Department of Molecular Biology and Oncology
UT Southwestern Medical Center
Dallas, Texas 75235
[4]Department of Neuroanatomy
Biomedical Research Center
Osaka University Medical School
[5]PRESTO
[6]CREST
Japan Society and Technology Corporation
Suita, 565-0871
Japan
[7]Fred Hutchinson Cancer Research Center
Seattle, Washington 98109
and Zoology Department
University of Washington
Seattle, Washington 98195

Summary

During *C. elegans* development, Wnt/WG signaling is required for differences in cell fate between sister cells born from anterior/posterior divisions. A β-catenin-related gene, *wrm-1*, and the *lit-1* gene are effectors of this signaling pathway and appear to downregulate the activity of POP-1, a TCF/LEF–related protein, in posterior daughter cells. We show here that *lit-1* encodes a serine/threonine protein kinase homolog related to the *Drosophila* tissue polarity protein Nemo. We demonstrate that the WRM-1 protein binds to LIT-1 in vivo and that WRM-1 can activate the LIT-1 protein kinase when coexpressed in vertebrate tissue culture cells. This activation leads to phosphorylation of POP-1 and to apparent changes in its subcellular localization. Our findings provide evidence for novel regulatory avenues for an evolutionarily conserved Wnt/WG signaling pathway.

Introduction

The early blastomeres of the *Caenorhabditis elegans* embryo initiate region-specific patterns of development through several mechanisms, including position-dependent cell–cell interactions and the asymmetric expression of maternally provided transcription factors (for review, see Schnabel and Priess, 1997). Despite the various mechanisms that lead to region-specific development, several experimental and genetic studies have suggested that cells throughout the embryo share a common mechanism for linking cell division to cell fate (Mello et al., 1992, 1994; Kaletta et al., 1997; Lin et al., 1998). Part of this process appears to involve POP-1, a protein related to vertebrate TCF (T cell factor)/LEF (lymphoid enhancer factor) transcription factors (Lin et al., 1995, 1998). Most of the cell divisions in all regions of the early embryo are oriented along the anterior–posterior (AP) axis, and essentially all of these divisions result in AP daughter cells with different fates (Sulston et al., 1983). Antibodies specific for POP-1 show a higher level of nuclear staining in the anterior daughters of AP divisions than in the posterior daughters, and genetic studies have shown that POP-1 function is required for several of the early AP differences in cell fate (Lin et al., 1995, 1998).

POP-1 activity and localization is regulated by a group of genes called *mom* genes. Cloning of several *mom* genes revealed that each of these genes encodes a protein clearly related to known components of the Wnt/Wingless (Wnt/WG) signaling pathway defined in vertebrates, *Drosophila*, and *C. elegans*. These proteins include MOM-1 (Porcupine), MOM-2 (Wnt/WG), and MOM-5 (Frizzled) (Rocheleau et al., 1997; Thorpe et al., 1997). In reverse genetic studies, the inhibition of a *C. elegans* homolog of β-catenin/Armadillo (called WRM-1) and of APR-1, an adenomatous polyposis coli (APC) homolog, also resulted in defects resembling *mom* mutants (Rocheleau et al., 1997). In most models for the Wnt/WG signaling pathway, β-catenin is a principle effector of signaling, and genetic studies of the *mom* genes and *wrm-1* in the *C. elegans* embryo are consistent with this view (Rocheleau et al., 1997; for review, see Cadigan and Nusse, 1997; Han, 1997). However, there is one important difference in the roles of WRM-1 and β-catenin. In the Wnt/WG model, β-catenin enters the nucleus in response to signaling and stimulates transcriptional activator function of TCF/LEF proteins. Thus, the loss of β-catenin and TCF/LEF would result in similar phenotypes (for review, see Cavallo et al., 1997; Kuhl and Wedlich, 1997; Willert and Nusse, 1998). However, studies on the *C. elegans* embryo have shown that the loss of WRM-1 has the opposite consequences in numerous cell fate decisions than the loss of POP-1 (TCF/LEF); loss of WRM-1 causes both AP sisters to adopt anterior fates (Rocheleau et al., 1997; Lin et al., 1998), while loss of POP-1 causes both sisters to adopt posterior fates (Lin et al., 1995, 1998). Thus, a critical objective for understanding how the Wnt/WG pathway functions in *C. elegans* is to elucidate the role of WRM-1 in relation to the rest of the signaling components.

In the present study, we show that the Wnt/WG pathway in *C. elegans* involves the *C. elegans* polarity gene *lit-1* (Kaletta et al., 1997) and that *lit-1* encodes a protein related to the *Drosophila* tissue polarity protein Nemo (Choi and Benzer, 1994). We show that WRM-1 can bind to LIT-1 and can activate a LIT-1-dependent kinase activity. Finally, we show that the WRM-1/LIT-1 kinase

[8]To whom correspondence should be addressed (e-mail: craig.mello@ummed.edu).
[9]These authors contributed equally to the paper.

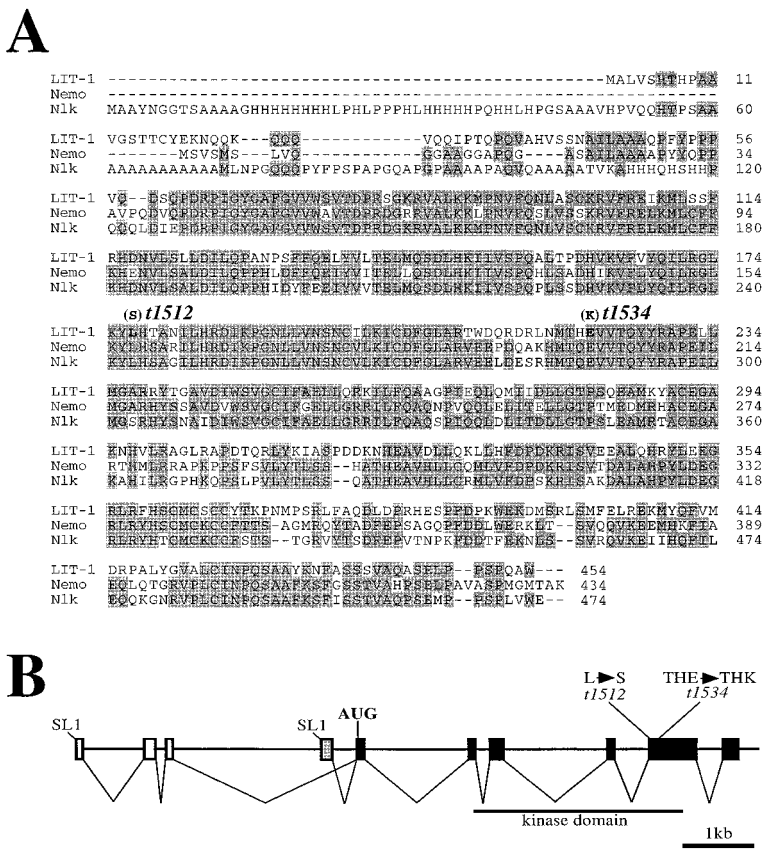

Figure 1. LIT-1 Encodes a Homolog of *Drosophila* Nemo and Mouse Nlk

(A) Alignment of LIT-1 amino acid sequence (LIT-1a, see below) with *Drosophila* Nemo form II and mouse Nlk (Nemo-like kinase). Identical residues are shaded in gray. The conserved residues altered in *lit-1(t1512)* (leucine 177 to serine) and in *lit-1(t1534)* (glutamate 222 to lysine) are bold in the LIT-1 sequence.

(B) Schematic representation of *lit-1* genomic structure. Two SL1 *trans*-spliced isoforms are shown. The longer transcript, *lit-1a*, is predicted to contain three noncoding exons (white boxes) in front of the predicted start codon. The second transcript, *lit-1b*, starts at an alternatively spliced exon (gray box) and contains two in-frame methionine codons upstream of the ATG for *lit-1a*. Black boxes indicate predicted coding exons from which the amino acid sequence in (A) was deduced. The exons containing the kinase domain are also indicated. Positions of the *lit-1(t1512)* and *lit-1(t1534)* lesions are shown.

complex precipitated from vertebrate cells can phosphorylate POP-1 in vitro.

Results

The *lit-1* Gene Encodes a Putative Protein Kinase Implicated in Mom (Wnt/WG) Signaling

We have used an RNA-mediated reverse genetic approach (RNAi) to ask whether polarity genes identified in other systems had Mom-related functions in *C. elegans* (Rocheleau et al., 1997 and our unpublished data). We found a completely penetrant Mom phenotype resulted from RNAi targeted to a *C. elegans* homolog of the *Drosophila* polarity gene *nemo* (Table 1 and Experimental Procedures). We noted that the physical map position of the *nemo* homolog was consistent with the genetic position of *lit-1*. Previous studies (Kaletta et al., 1997) had shown that mutations in the *lit-1* gene cause at least one defect that is identical to that of *mom* mutants, that is, the transformation of intestinal precursors into mesodermal precursors. We found that a yeast artificial chromosome (YAC) with *C. elegans* DNA containing the *nemo* homolog completely rescued *lit-1(t1512)* (see Experimental Procedures). These observations suggested that the *nemo* homolog might be the *lit-1* gene. We sequenced the *nemo* homolog in the *lit-1* mutants *lit-1(t1512)* and *lit-1(t1534)* and found a mutation in each (Figure 1). Taken together, the proximity of the respective physical and genetic map positions of the *nemo* and *lit-1* genes, the similarity of their RNAi and mutant phenotypes, and the existence of point mutations predicted to alter conserved residues of the Nemo-like protein in each *lit-1* mutant lead us to conclude that *lit-1* is the *C. elegans nemo* gene.

LIT-1 is highly homologous to *Drosophila* Nemo (Choi and Benzer, 1994) and mouse Nlk (Nemo-like kinase) (Brott et al., 1998) within its predicted kinase domain and also within a conserved C-terminal region. The N-terminal region is most divergent between LIT-1, Nemo, and Nlk (Figure 1A). *lit-1* can encode at least two different 5′ splice variants (Figure 1B). The *lit-1(t1512)* mutation changes a conserved leucine in the predicted kinase domain, and the *lit-1(t1534)* mutation changes a glutamate to a lysine in a regulatory motif believed to be important for the activation of related kinases (THE to THK; Figure 1) (Payne et al., 1991).

Previous genetic tests have shown that mutations in *pop-1* are epistatic to mutations in *lit-1* (Kaletta et al., 1997); however, whether POP-1 localization and/or levels are regulated by *lit-1*(+) activity had not been determined. We immunostained *lit-1(t1512)* mutant embryos for POP-1 and found that POP-1 protein levels appear equal in the nuclei of sister cells resulting from AP divisions (Figure 2). Thus, a reduction or loss of *lit-1*(+) activity appears to have the same effect on POP-1 asymmetry as previously described mutations in the *mom* genes (Rocheleau et al., 1997; Thorpe et al., 1997; Lin et al., 1998).

To determine if *lit-1* had genetic interactions with the *mom* genes, we examined the phenotypes of *lit-1* mutant embryos in which *mom* gene activity was removed by

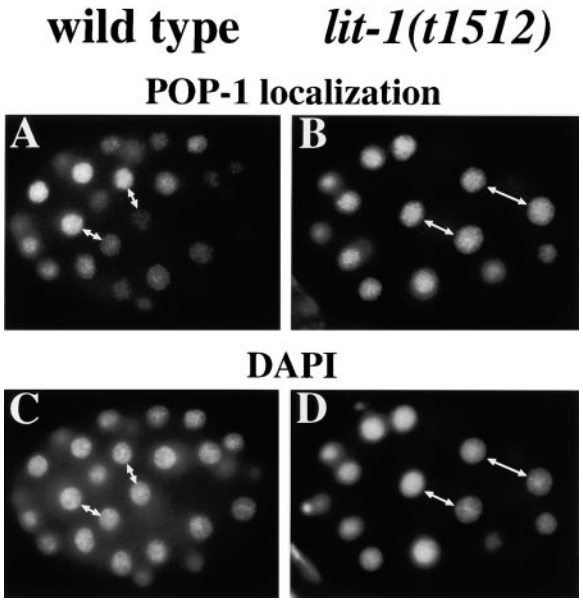

Figure 2. POP-1 Is Localized Symmetrically in *lit-1(t1512)*

Top panels show immunofluorescence staining of POP-1 in either wild-type (A) or *lit-1(t1512)* mutant embryos (B). The anterior of the embryo is to the left and the posterior to the right. Double-headed arrows indicate pairs of anterior/posterior sisters. In wild-type embryos, the anterior cells show higher levels of nuclear POP-1 immunostaining than do their posterior sisters. *lit-1(t1512)* embryos show equal, high levels of staining in the nuclei of both anterior and posterior sisters. The embryos as well as their mothers were kept at restrictive temperature for *lit-1(t1512)*. The lower panels show corresponding DAPI staining of the nuclei in either wild type (C) or *lit-1(t1512)* (D).

mutation or by RNAi. We found that *lit-1* mutants strongly enhance the polarity defects associated with *mom-2 (Wnt/WG)*, *mom-5 (frizzled)*, and *apr-1* (APC related) (Table 1). For example, double mutants between the temperature-sensitive *lit-1(t1512)* allele and a partially penetrant *mom-2(ne141)* allele produced a fully penetrant Mom phenotype even at permissive temperature for *lit-1(t1512)* (Table 1). Consistent with previous analysis of the *pop-1(zu189)* mutant (Kaletta et al., 1997), we found that *pop-1(RNAi)* was fully epistatic to *lit-1 (RNAi)* and to *lit-1(t1512)* (Table 1). In summary, we conclude that Wnt/WG signaling in the early embryonic divisions requires *lit-1*(+) to downregulate *pop-1*(+) activity.

LIT-1 Functions in Multiple Wnt/WG–Related Signaling Events in *C. elegans*

We found that a transgene expressing a fusion protein consisting of LIT-1 and green fluorescent protein (GFP) fully rescues the *lit-1(t1534)* mutant and partially rescues the more severe *lit-1(t1512)* mutant (see Experimental Procedures). Although GFP fluorescence could not be detected in early embryos, fluorescence was detected in the nuclei of most embryonic cells beginning around the 100 cell stage. Faint GFP was also visible in the cytoplasm of some embryonic and larval cells (data not shown). In larvae, GFP::LIT-1 was detected in numerous cells, some of which had been shown previously to contain POP-1 (Lin et al., 1998). These results suggest a possible role for LIT-1 in larval cell fate decisions involving POP-1.

We found GFP::LIT-1 expression in a larval cell called the T cell and in its descendants; these cells were of interest because proper T cell development has been shown to involve a Wnt/WG–like signaling pathway (Herman et al., 1995; Sawa et al., 1996). To ask whether *lit-1*(+) activity was required in this pathway, we examined the development of the T cell in temperature-sensitive *lit-1(t1512)* mutants shifted to restrictive temperature during late embryogenesis. We observed several defects in T cell development that were similar to those caused by mutations in the *lin-17* gene (Sternberg and Horvitz, 1988), which encodes a protein related to *Drosophila* Frizzled (Table 2; Sawa et al., 1996). For example, *lit-1* animals exhibit a 33% frequency of symmetric T cell division, a frequency similar to that seen in weak *lin-17* mutants. As with *lin-17* mutations, the *lit-1* mutations suppressed the reversed polarity phenotype of *lin-44 (Wnt/WG)* mutants (see Table 2). These results suggest that *lit-1* is involved in the T cell *lin-17/lin-44* signaling system and appears to function downstream of *lin-44 (Wnt/WG)*.

Table 1. Genetic Analysis of Endoderm Specification in *lit-1*

Embryo Type	% Embryos Lacking Endoderm (n)
lit-1(RNAi)	100 (544)
lit-1(t1534)	2 (195)
lit-1(t1512)	0 (216) 15°C
	96 (473) 25°C
mom-2(ne141)	53 (304)
mom-2(ne141);lit-1(t1534)	100 (225)
mom-2(ne141);lit-1(t1512)	100 (235) 15°C
	100 (220) 25°C
mom-5(RNAi)	2 (511)
mom-5(RNAi);lit-1(t1534)	97 (458)
mom-5(RNAi);lit-1(t1512)	60 (105) 15°C
	98 (259) 25°C
apr-1(RNAi)	49 (603)
apr-1(RNAi);lit-1(t1534)	98 (219)
pop-1(RNAi)	0 (123)
pop-1(RNAi);lit-1(RNAi)	0 (62)
pop-1(RNAi);lit-1(t1512)	0 (75) 15°C
	0 (235) 25°C

Strains containing the temperature-sensitive mutation *lit-1(t1512)* were cultured at 25°C, the restrictive temperature, and 15°C, the permissive temperature, as indicated at right. All other strains were cultured at room temperature (~22°C).

Table 2. Genetic Analysis of T Cell Polarity in *lit-1*

		Polarity of T Cell Division		
Genotype	n	% Normal	% Symmetric	% Reversed
lin-44(n1792)	85	6	14	80
lin-17(mn589)	62	56	31	13
lin-17(n3091)	61	8	82	10
lit-1(t1512)	91	67	33	0
lin-44(n1792); lit-1(t1512)	66	3	85	12
lin-17(mn589); lit-1(t1512)	64	2	98	0
lin-17(n3091); lit-1(t1512)	81	0	100	0

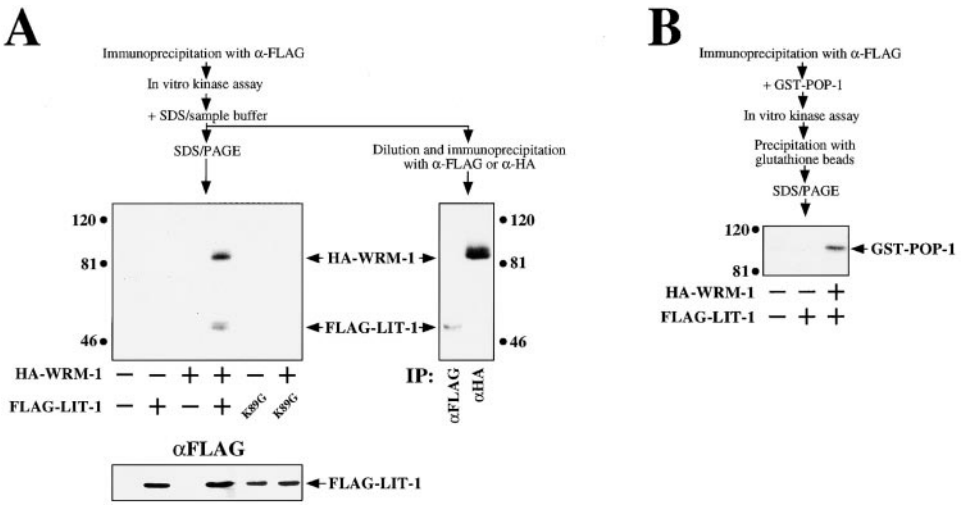

Figure 3. LIT-1 Immunoprecipitates Can Phosphorylate LIT-1, WRM-1, and GST-POP-1

(A) LIT-1-dependent phosphorylation of LIT-1 and WRM-1 requires coexpressed WRM-1. COS-7 cells were transfected as indicated at the bottom of the gel, and in vitro kinase assays were performed on FLAG-LIT-1 immunoprecipitates. The anti-FLAG immunoblot (lower panel) shows that FLAG-LIT-1 and kinase-inactive FLAG-LIT-1 K89G were expressed at comparable levels. Two radioactive bands were identified as WRM-1 and LIT-1 by immunoprecipitation using either the anti-FLAG or anti-HA antibody.

(B) In vitro phosphorylation of GST-POP-1 by the LIT-1 immunoprecipitation complex. Cell lysates from COS-7 cells transfected as indicated beneath the gel were subjected to immunoprecipitation with the anti-FLAG antibody followed by in vitro kinase assays with bacterially expressed GST-POP-1 protein as a substrate. GST-POP-1 was precipitated from the kinase reaction with glutathione–Sepharose. This precipitate contained a phosphorylated protein with the electrophoretic mobility expected for GST-POP-1. A faint comigrating band corresponding to a small amount of nonspecifically phosphorylated GST-POP-1 was precipitated with the glutathione–Sepharose (first two lanes and data not shown). GST alone, without the fusion to POP-1, was not a substrate for phosphorylation by the LIT-1 immunoprecipitates (data not shown).

LIT-1-Dependent Kinase Is Activated by WRM-1 and Phosphorylates POP-1

Mouse Nlk has been shown to have an apparent autophosphorylation activity when expressed in mammalian cell culture (Brott et al., 1998). We failed to detect LIT-1 kinase activity when an epitope-tagged LIT-1 was expressed and immunoprecipitated from vertebrate cells. However, if WRM-1 was coexpressed with LIT-1, two proteins present in the LIT-1 immunoprecipitation complex could be phosphorylated; these proteins were identified as LIT-1 itself and WRM-1 (Figure 3A). This kinase activity requires the putative ATP-binding site in the LIT-1 kinase domain, as a point mutation at this position (K89G) abolished the phosphorylation of both LIT-1 and WRM-1. Thus, WRM-1 is both a substrate for and an activator of LIT-1-dependent kinase activity. We next asked if POP-1 could be phosphorylated by the activated LIT-1 kinase. We found that a bacterially expressed GST-POP-1 protein can be phosphorylated by the LIT-1 immunoprecipitation complex and that phosphorylation required WRM-1 and an intact LIT-1 kinase domain (Figure 3B and not shown).

WRM-1 and LIT-1 Form a Stable Complex

The experiments described above indicate that the LIT-1 immunoprecipitate contained sufficient amounts of WRM-1 to be detected after the in vitro phosphorylation reaction using radiolabeled ATP. Although this may

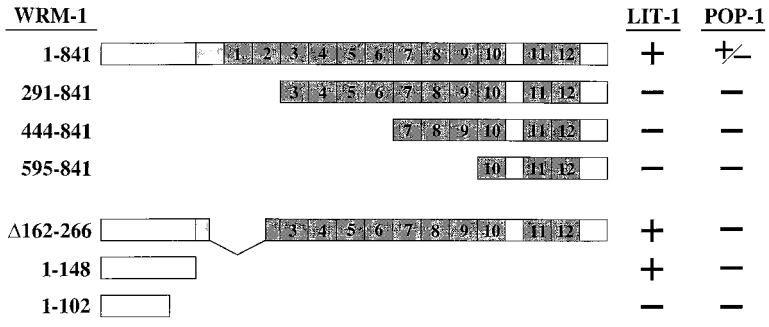

Figure 4. Two-Hybrid Analysis of WRM-1, LIT-1, and POP-1 Interactions

Full-length WRM-1 and a series of truncation proteins (schematically diagrammed) were cloned into the GAL4 activation domain vector, pACT2, and tested for interactions with POP-1 and LIT-1 in the GAL4 DNA-binding domain vector pAS1. LIT-1 interacts with full-length WRM-1. An N-terminal 148 amino acid region in WRM-1 is sufficient to bind LIT-1. POP-1 interacts weakly with full-length WRM-1 but not with any of the truncated proteins we tested. (+/−) indicates growth on 5 mM but not on 10 mM 3-AT medium. (+) indicates growth on 10 mM 3-AT. The shaded boxes represent the 12 conserved Armadillo (ARM) motifs. A lightly shaded box represents a region with weak homology to the consensus ARM motif and may represent a 13th repeat.

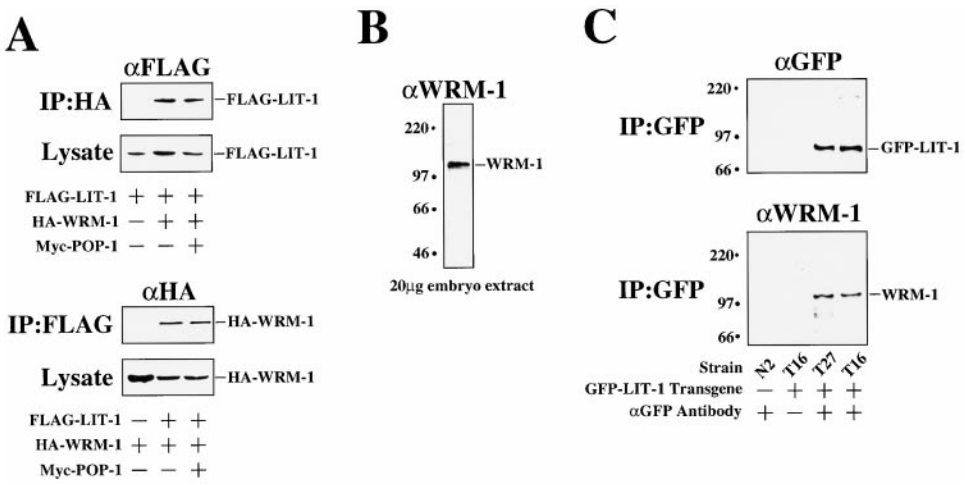

Figure 5. LIT-1 and WRM-1 Form a Stable Complex

(A) FLAG-LIT-1 and HA-WRM-1 coimmunoprecipitate from vertebrate cells. COS-7 cells were transfected with a combination of plasmids expressing FLAG-LIT-1, HA-WRM-1, and Myc-POP-1, as indicated at the bottom of the gel. HA-WRM-1 or FLAG-LIT-1 was immunoprecipitated with monoclonal antibodies against the respective epitope tags, and the presence of coprecipitated FLAG-LIT-1 or HA-WRM-1 was determined by immunoblotting using the anti-FLAG or anti-HA antibody, respectively. Similar experiments failed to detect coprecipitation of Myc-POP-1 with either FLAG-LIT-1 or HA-WRM-1 (data not shown).

(B) Immunodetection of WRM-1 from embryo extracts. Immunoblotting with a monoclonal antibody, P3C8, raised against bacterially expressed WRM-1 protein (see Experimental Procedures), revealed a single band of approximately 110–120 kDa in *C. elegans* embryo extracts. An independent monoclonal antibody, P5D6, as well as an affinity-purified polyclonal antiserum, all raised against WRM-1 expressed in *E. coli*, recognized a band that apparently comigrated with the 110–120 kDa band reactive to P3C8 (data not shown).

(C) GFP-LIT-1 associates with WRM-1 in vivo. Protein extracts were prepared from two independent transgenic worm strains homozygous for the *lit-1(t1534)* mutation and rescued by GFP::LIT-1 (see Experimental Procedures). GFP-LIT-1 was immunoprecipitated using a monoclonal anti-GFP antibody (3E6) from embryo extracts and visualized by immunoblotting using a second monoclonal anti-GFP antibody (7.1/13.1) (top panel). Coprecipitation of WRM-1 was determined by immunoblotting using the anti-WRM-1 monoclonal antibody P3C8 (bottom panel). P3C8 detected a single major band with the mobility expected for endogenous WRM-1 protein. Immunoprecipitation using extracts prepared from nontransgenic wild-type strain (N2) did not yield coprecipitated WRM-1. Neither GFP-LIT-1 nor WRM-1 was precipitated in the absence of the anti-GFP antibody.

represent a transient interaction between the kinase and the substrate, apparent activation of LIT-1 kinase by WRM-1 raises the possibility that the two proteins may form a stable complex.

To examine how WRM-1 might activate LIT-1, we asked if WRM-1 could bind LIT-1 directly. In the yeast two-hybrid assay, we found that the full-length WRM-1 could interact with LIT-1 (Figure 4). We mapped the minimal interaction domain in WRM-1 to a small N-terminal region (Figure 4). Similarly, we found that a bacterially expressed N-terminal WRM-1 protein, containing the first 214 amino acids, was sufficient to bind LIT-1 that had been translated in vitro (data not shown). The binding domain in LIT-1 has not been mapped in detail; however, neither the first 378 nor the last 74 amino acids were sufficient to bind WRM-1 (data not shown). Interactions between WRM-1 and LIT-1 were also observed in transfected vertebrate cells by reciprocal coimmunoprecipitation experiments (Figure 5A). This interaction was apparently unaffected by the presence of POP-1 (Figure 5A).

To ask if WRM-1 can associate with LIT-1 in the *C. elegans* embryo, we prepared embryo extracts from transgenic GFP::LIT-1 strains and used a monoclonal antibody against GFP to precipitate GFP::LIT-1. The immunoprecipitate was analyzed for the presence of coprecipitated WRM-1 by immunoblotting. A monoclonal antibody raised against bacterially expressed WRM-1 (Experimental Procedures) detected a single band in the GFP::LIT-1 immunoprecipitate that has approximately the correct electrophoretic mobility to be WRM-1 (Figures 5B and 5C). A second monoclonal antibody against WRM-1 recognized an apparently identical band in GFP::LIT-1 (data not shown). We conclude that WRM-1 forms a stable complex with GFP::LIT-1 in vivo.

In vertebrates and *Drosophila*, proteins related to *C. elegans* WRM-1 and POP-1 form a stable complex (see Discussion). In the yeast two-hybrid assay, we detected only weak interactions between POP-1 and full-length WRM-1 and no interactions with any of the truncated forms of WRM-1. Similarly, we were unable to detect POP-1 in immunoprecipitates of WRM-1 or LIT-1 from vertebrate cells expressing these proteins (data not shown). These findings suggest that WRM-1 does not form a stable complex with POP-1.

LIT-1 and WRM-1 May Regulate the Localization of POP-1

We have shown that WRM-1 can exist in a complex with LIT-1 in vivo and can also activate a LIT-1-dependent kinase that phosphorylates GST-POP-1 in vitro. These findings raise the possibility that LIT-1 and WRM-1 may form an active kinase complex that regulates POP-1 activity or localization. We therefore decided to address the consequences of WRM-1/LIT-1 regulation of POP-1 upon expression in vertebrate cells. We found that in

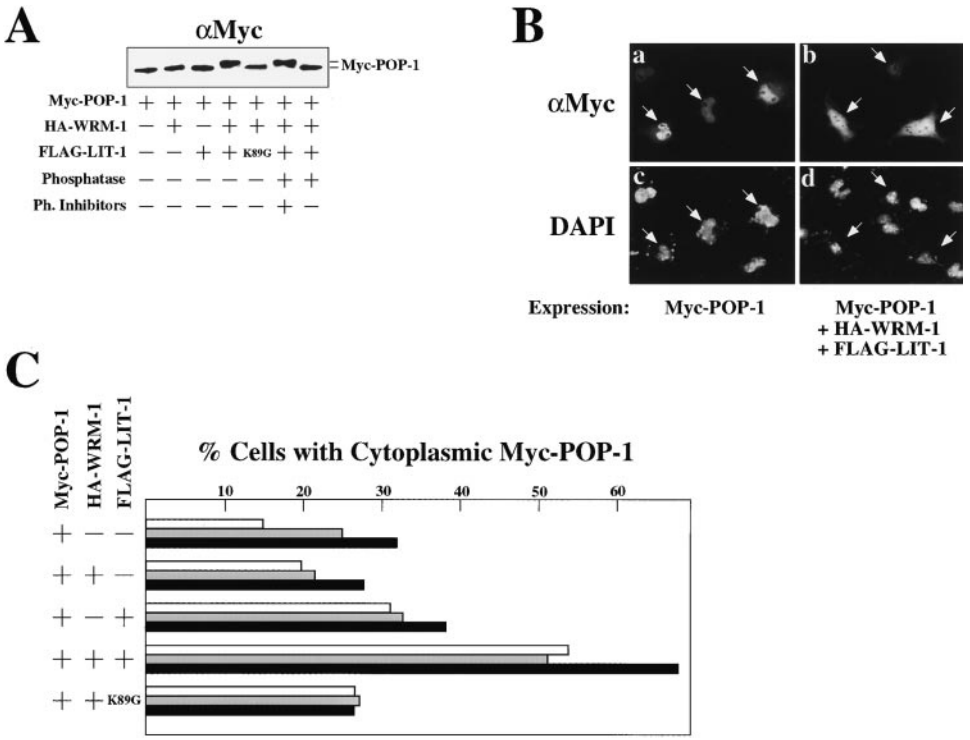

Figure 6. Regulation of POP-1 by LIT-1/WRM-1 in Vertebrate Cells

(A) POP-1 phosphorylation depends on WRM-1 and kinase-active LIT-1. COS-7 cells were transfected with Myc-POP-1 and combinations of HA-WRM-1, FLAG-LIT-1, and FLAG-LIT-1 K89G. Two forms of Myc-POP-1 were detected, and the appearance of the slower migrating form was dependent on coexpression of HA-WRM-1 and kinase-active FLAG-LIT-1. Treatment of the sample with phosphatase converted the slow migrating band to the faster form, and this conversion was blocked by adding phosphatase inhibitors (last two lanes), indicating that the slower mobility reflects phosphorylation of POP-1.

(B and C) Cytoplasmic levels of Myc-POP-1 are increased by WRM-1/LIT-1. (B) COS-7 cells transfected with POP-1 alone (left panels) or with POP-1, WRM-1, and LIT-1 (right panels) were fixed and stained for Myc-POP-1 by immunofluorescence (B_a and B_b) and for nuclei with DAPI (B_c and B_d). The arrows indicate individual transfected cells in each field of view (B_a and B_b) and the corresponding nuclei (B_c and B_d). (C) Graphic representation showing the percentages of transfected cells with predominantly cytoplasmic Myc-POP-1. Sets of three bars represent three independent transfection experiments. The total numbers of cells scored are 2127 for Myc-POP-1, 1563 for Myc-POP-1/HA-WRM-1, 1367 for Myc-POP-1/FLAG-LIT-1, 1744 for Myc-POP-1/FLAG-LIT-1/HA-WRM-1, and 1454 for Myc-POP-1/FLAG-LIT-1 K89G/HA-WRM-1. In parallel experiments, the localization of an endogenous nuclear protein, MKK7, was monitored by immunofluorescence microscopy. The pattern of MKK7 localization was not affected in these transfected populations (data not shown), indicating that the effects on POP-1 are not due to alterations in protein nuclear localization in general.

transfected vertebrate cells, POP-1 became hyperphosphorylated, as represented by a change in electrophoretic mobility, when LIT-1 and WRM-1 were coexpressed but not when the kinase-inactive form of LIT-1 was substituted for LIT-1 (Figure 6A). When expressed alone in the vertebrate cells, POP-1 is primarily nuclear, as it is in anterior daughters of AP divisions in *C. elegans* embryos (Figures 6B and 6C; see Figure 2). However, when POP-1 is coexpressed in vertebrate cells with both LIT-1 and WRM-1, it becomes prominent in the cytoplasm (Figures 6B and 6C). This redistribution of POP-1 required both an intact kinase domain in LIT-1 and the coexpression of LIT-1 and WRM-1, suggesting that it is mediated by the kinase activity of the LIT-1/WRM-1 complex.

Discussion

Activation of the LIT-1 Protein Kinase

In *C. elegans*, embryonic cells that divide AP show high levels of immunostaining for POP-1 in the nuclei of anterior daughters and relatively low levels of nuclear POP-1 staining in posterior daughters (Lin et al., 1995, 1998). The MOM proteins, WRM-1, and APR-1 (Rocheleau et al., 1997; Thorpe et al., 1997; Lin et al., 1998) and LIT-1 (our present study) are all required for the low levels of nuclear POP-1 in posterior daughters. We have shown here that *lit-1* encodes a protein related to the *Drosophila* Nemo kinase (Choi and Benzer, 1994) and mouse Nlk (Brott et al., 1998). The observation that mutations present in *lit-1(t1512)* and *lit-1(t1534)* alleles alter conserved residues in the predicted kinase domain suggests that the kinase activity of LIT-1 is essential for POP-1 asymmetry. Consistent with this idea, we have shown that LIT-1 kinase activity causes phosphorylation of POP-1. This kinase activity also promotes phosphorylation of both LIT-1 and WRM-1 and is dependent on WRM-1 (β-catenin).

There are several phenotypic similarities in embryos depleted of LIT-1 and WRM-1. Nearly all such embryos fail to differentiate intestinal cells and lack POP-1 asymmetry (Kaletta et al., 1997; Rocheleau et al., 1997; this study). In contrast, at least 20% of the embryos produced by all other *mom* mutants differentiate intestinal

cells and retain POP-1 asymmetry (Rocheleau et al., 1997; Thorpe et al., 1997; Lin et al., 1998). Furthermore, almost all *mom* mutants have defects in mitotic spindle orientation that are not observed in embryos lacking either *wrm-1*(+) or *lit-1*(+) (Rocheleau et al., 1997; Thorpe et al., 1997). These observations support the view that LIT-1 and WRM-1 might function downstream of the other MOM proteins in a closely related event that generates POP-1 asymmetry.

WRM-1 can be coimmunoprecipitated with LIT-1 from *C. elegans* extracts, providing evidence that WRM-1 and LIT-1 may form a complex in vivo. Using the yeast two-hybrid system, we have shown that the N-terminal region of WRM-1 is sufficient to bind LIT-1. This region is outside of the canonical 12 "Armadillo" (ARM) repeats that are known to be involved in protein–protein interactions in WRM-1-related proteins (for reviews, see Cavallo et al., 1997; Kuhl and Wedlich, 1997; Willert and Nusse, 1998). If LIT-1 binds in vivo to only the N-terminal region of WRM-1, it may leave the ARM repeat region available for interactions with other proteins involved in signaling. The phosphorylation of WRM-1 observed when bound to LIT-1 may in turn alter the binding properties of the complex to promote binding or release of factors involved in POP-1 regulation.

WRM-1, LIT-1, and POP-1

When POP-1 is coexpressed with both LIT-1 and WRM-1 in vertebrate cells, POP-1 becomes phosphorylated; this phosphorylation requires the putative ATP-binding site in LIT-1. Thus, an attractive possibility is that activated LIT-1 phosphorylates POP-1 directly. Consistent with this idea, the LIT-1 complex immunoprecipitated from transfected cells can phosphorylate GST-POP-1 in vitro. In *C. elegans* embryos, WRM-1 and LIT-1 appear to downregulate *pop-1*(+) activity in posterior daughters of AP divisions and cause a reduction in the apparent nuclear level of POP-1 in these daughters. It is not yet known whether the difference in POP-1 observed after immunostaining results primarily from changes in protein levels, subcellular distribution, or modification of the immunoepitope (see Lin et al., 1998). The findings described here suggest a possible model to explain POP-1 regulation. We propose that in *C. elegans*, the primary function of WRM-1 may be to activate LIT-1, which then results in the phosphorylation of POP-1. As in other systems, Wnt signaling might serve to increase levels of WRM-1 (β-catenin). Phosphorylation of POP-1 by LIT-1 might directly inactivate POP-1 in the nucleus or stimulate the nuclear export or cytoplasmic retention of POP-1. The observation that POP-1 protein, which is nuclear in mammalian cells when expressed alone, accumulates in the cytoplasm when coexpressed with WRM-1 and LIT-1 would be consistent with this latter possibility.

Wnt/WG Signaling and Tissue Polarity

The *lit-1* homolog *nemo* was identified in *Drosophila* by a mutant with altered patterns of rotation in the ommatidia of the compound eye (Choi and Benzer, 1994) and is considered to be part of the "tissue-polarity" pathway (Zheng et al., 1995). This pathway involves the Frizzled protein (Vinson and Adler, 1987; Vinson et al., 1989), which together with Frizzled 2 (Bhanot et al., 1996) has recently been implicated as a receptor for *Drosophila* Wingless (Bhat, 1998; Kennerdell and Carthew, 1998; Müller et al., 1999). However, there are no known requirements for WRM-1-related or POP-1-related proteins in the tissue-polarity pathway.

Specification of AP differences in the blastomeres of early *C. elegans* embryos might involve the chance convergence of a tissue-polarity pathway involving LIT-1 and a largely separate Wnt/WG pathway. Perhaps consistent with this view, the genetics of the *mom* genes is complex and suggests a pathway involving multiple branches (see Rocheleau et al., 1997). However, we have shown here that LIT-1 functions in at least one additional developmental event that also involves a Wnt/WG pathway: the T cell fate decision. The observation that LIT-1/Nemo/NLK appears to be an integral part of at least two Wnt/WG–mediated cell fate decisions in *C. elegans* raises the interesting possibility that members of this highly conserved protein kinase family may also have roles in Wnt/WG signaling in other systems.

Comparison with Wnt/WG Signaling in Other Organisms

Current models for Wnt/WG signaling in vertebrates and *Drosophila* suggest that signaling stabilizes the β-catenin/Armadillo protein, making it available for binding to TCF/LEF–related transcription factors. The β-catenin-TCF/LEF complex in turn activates Wnt/WG target genes in the nucleus (for review, see Cavallo et al., 1997; Kuhl and Wedlich, 1997; Willert and Nusse, 1998). WRM-1 (β-catenin) and POP-1 (TCF/LEF), rather than working as cofactors, appear to have opposing functions (for review, see Cadigan and Nusse, 1997; Han, 1997); WRM-1 negatively, rather than positively, regulates POP-1 (TCF/LEF).

Findings reported here point to possible explanations for the reversed genetic relationship between WRM-1 and POP-1. We have shown that rather than binding stably with POP-1, WRM-1 is required for POP-1 phosphorylation. It is easy to imagine several possible molecular models based on these observations. For example, the LIT-1-related kinases Nemo and Nlk may phosphorylate the corresponding POP-1 homologs in vertebrates and *Drosophila*. In the context of a stable association with β-catenin/Armadillo, this phosphorylation could lead to positive rather than negative regulation.

Multiple Upstream Branches for Polarity Signaling in *C. elegans*

Understanding how upstream signals control WRM-1 and LIT-1 will require much more genetic and biochemical investigation. β-catenin and its related proteins, Plakoglobin, Armadillo, and *C. elegans* HMP-2 and BAR-1 (Costa et al., 1998; Eisenmann et al., 1998) are all reported to associate with cell junctions. The interaction of β-catenin and Plakoglobin with cell adhesion molecules such as E-cadherin appears to influence their signaling properties (reviewed by Klymkowsky and Parr, 1995; Gumbiner, 1997; Bullions and Levine, 1998), and a recent study suggests that integrin-linked kinase (Novak et al., 1998) regulates both β-catenin levels and LEF-1 transcriptional activity. The apparent complexity of AP polarity signaling in *C. elegans* embryos (Rocheleau et al.,

1997) could reflect the existence of multiple signals that converge on WRM-1. The identification of LIT-1 as a coeffector in polarity signaling increases the possibilities and raises the question of whether upstream activators may directly target LIT-1 kinase activation.

In summary, the current study increases the repertoire of activities associated with β-catenin-related proteins. In addition, these findings suggest a possible novel target via regulation of LIT-1/Nemo/Nlk kinase activity for controlling the transcriptional activity of TCF/LEF–related transcription factors. It will now be interesting to follow the pathway upward from WRM-1 and LIT-1 to identify how MOM-5/LIN-17/Frizzled and potentially other cell surface receptors activate signaling.

Experimental Procedures

Strains and Alleles

The Bristol strain N2 was used as the standard wild-type strain. The marker mutations, deficiencies, and balancer chromosomes used are listed by chromosome as follows: *LGIII*: *unc-32(e189)*, *lit-1(t1512)*, *lit-1(t1534)*, *eT1(III; V)*, *qC1*; *LGIV*: *him-3(e1147)*; *LGV*: *dpy-11(e224)*, *mom-2(ne141)*. *C. elegans* culture and genetics were as described in Brenner (1974).

Microinjection

RNAi was performed as described in Fire et al. (1998) and Rocheleau et al. (1997). The *lit-1* cDNA clone yk457d2 was used to prepare dsRNA.

Transformation rescue of *lit-1(t1512)* was performed using YAC Y26C10, which contains a large segment of *C. elegans* genomic DNA, including the *nemo* homolog. The GFP gene was inserted into Y26C10 by using homologous recombination in yeast. First, a GFP::sup4° cassette was engineered to contain the yeast ochre suppressor tRNA, sup4°, embedded within a synthetic *C. elegans* intron. This GFP::sup4° cassette was then used to create vectors, in which GFP is flanked by short 5′- and 3′-flanking sequences of the *nemo* homolog. This configuration allowed homologous recombination in yeast to drive the formation of an in-frame N- or C-terminal insertion of GFP in the *nemo* homolog in Y26C10. Recombinant yeast strains were selected by virtue of suppression of the ochre mutation *ade2-1* present in the host yeast strain AB1380 (*MATa ade2-1 can1-100 lys2-1 trp1 ura3 his5*). The N-terminal GFP insertion resulted in full rescue of *lit-1(t1534)* and partial rescue of *lit-1(t1512)*. Rescue of *lit-1(t1534)* by GFP::LIT-1 was abolished by RNAi targeting the GFP tag, suggesting that the tagged gene is responsible for *lit-1* rescue in this strain. The YAC DNA was prepared for injection by making total yeast genomic DNA from the YAC-bearing strain and purifying the DNA over a Qiagen column (Qiagen). In each experiment, 200 μg/ml of total yeast DNA was mixed with 100 μg/ml of the dominant *rol-6* marker plasmid pRF4 (Mello et al., 1991; Mello and Fire, 1995). Approximately 50 separate transgenic strains were made with each construct, and 1 in 5 were found to have incorporated the coinjected YAC sequences.

Molecular Analysis and Plasmids

Coding sequences in *lit-1* and mutant alleles were determined by sequencing RT-PCR products as described in Rocheleau et al. (1997). The *lit-1* cDNA sequences differ slightly from the genome center's GeneFinder predictions for the corresponding open reading frame W06F12.1 and are detailed in the GenBank accession numbers given in this paper.

For expression in COS-7 cells, full-length WRM-1 (Rocheleau et al., 1997), LIT-1, and POP-1 (Lin et al., 1995) were tagged at the N terminus with HA, FLAG, and Myc epitopes, respectively, and cloned in vector pCDNA3 (Invitrogen). Point mutations and truncations of the full-length genes were constructed using protocols described in Ausubel et al. (1997). The vectors pACT2 and pAS1 were used for the two-hybrid assays (Clontech).

Cell Culture, Transfection, and Immunoprecipitation-Kinase Assay

COS-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. Transient transfections were performed using lipofectamine (Life Technologies). Cells were harvested 48 hr after transfection following serum starvation for 12 hr by lysis in Triton X-100 lysis buffer (20 mM Tris-HCl [pH 7.4], 137 mM NaCl, 10% glycerol, 1% Triton X-100, 25 mM β-glycerophosphate, 2 mM pyrophosphate, 1 mM sodium orthovanadate, 2 mM EDTA, 1 mM PMSF, and 10 μg/ml leupeptin). The immunoprecipitation-kinase assay was performed as described previously (Whitmarsh et al., 1997), with or without added purified GST-POP-1 (1 μg).

Immunoprecipitation and Phosphatase Treatment

Immunoprecipitation of tagged proteins from COS-7 cells was performed using a mouse anti-FLAG antibody, M2, a rat anti-HA antibody, 3F10 (Roche Molecular Biochemicals), or a mouse anti-Myc antibody, 9E10. The phosphatase treatment of immunoprecipitated Myc-POP-1 was performed as described previously (Papavassiliou and Bohmann, 1992). For immunoprecipitation from the *C. elegans* extracts, $\sim 2.5 \times 10^6$ embryos were homogenized in buffer (25 mM HEPES-NaOH [pH 7.4], 140 mM NaCl, 1 mM DTT, 10% glycerol, 25 mM β-glycerophosphate, 1 mM sodium orthovanadate, 2 mM PMSF, 5 μg/ml aprotinin, 4 μg/ml E-64, 2 μg/ml pepstatin and 5 μg/ml leupeptin) using a stainless steel homogenizer and lysed with 1% NP-40. Approximately 1 mg of protein extract was used for immunoprecipitation with a mouse monoclonal anti-GFP antibody 3E6 (Quantum Biotechnologies). Following the immunoprecipitation, WRM-1 was visualized by immunoblotting using a monoclonal antibody, P3C8 (1/10 dilution). We estimate that 1%–2% of the total WRM-1 protein present in the extract coimmunoprecipitated with GFP::LIT-1 under the conditions described here. This number may reflect a significant underestimate, as the conditions for immunoprecipitating GFP and for maintaining the WRM-1/LIT-1 complex were not optimized.

Microscopy

Cellular differentiation in mutant and *lit-1(RNAi)* embryos was analyzed as described previously (Mello et al., 1992; Rocheleau et al., 1997). For example, *lit-1(RNAi)* embryos were analyzed for endoderm differentiation using both cell lineage analysis and light microscopy. Briefly, the E cell lineage was examined in six embryos, and in each case Ea and Ep were found to divide prematurely, with timing similar to the mesodermal precursors MSa and MSp. The descendants of E failed to gastrulate, and after 6 hr, when E descendants in wild type would normally begin to exhibit gut birefringence, instead exhibited evidence of pharyngeal differentiation (see Mello et al., 1992). In all respects, the terminal phenotype of *lit-1(RNAi)* embryos is indistinguishable from *lit-1(t1512)* embryos. The POP-1 mABRL2 antibody and the staining procedure were described by Lin et al. (1998). Analysis of postembryonic phenotype of *lit-1* was done as follows. Homozygous *lit-1(t1512)* animals grown at 15°C were allowed to lay eggs at 15°C for 3 hr. Then the adults were removed and the eggs incubated at 25°C for 14 to 16 hr. The T cell polarity was scored in late L1 larvae as described by Herman and Horvitz (1994).

Immunofluorescence microscopy for COS-7 cells was performed essentially as described (Tournier et al., 1999) except for the following modifications. Cells were fixed in 1×PBS (pH 7.2), 3% paraformaldehyde for 10 min at room temperature, followed by permeabilization in methanol for 10 min at −20°C. Myc-POP-1 was detected by a monoclonal antibody, 9E10. Endogenous MKK7 was visualized as described by Tournier et al. (1999).

Two-Hybrid Assay

A yeast strain, HF7c (*MATa*, *his3-200*, *trp1-901*, *leu2-3112, gal4-542*, *gal80-538*, *LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3*, *URA3::GAL4$_{17\text{-}mer\ \times 3}$-CYC1$_{TATA}$-lacZ*), was transfected with one of the pACT2 WRM-1 plasmids or pACT2 together with either pAS1 LIT-1, pAS1 POP-1, or pAS1. Two-hybrid interactions were determined by colony formation on −Trp/−Leu/−His medium containing 5 to 25 mM 3-AT (3-amino-1,2,4-triazole, Sigma).

Antibody Production

Monoclonal antibodies were generated against a full-length, His-tagged WRM-1 fusion protein. RBF/Dn mice (Jackson Laboratory) were injected subcutaneously with 100 μg of fusion protein and boosted at 2 week intervals according to published protocols (Wayner and Carter, 1987) in the Hybridoma Production Facility at the Fred Hutchinson Cancer Research Center.

Acknowledgments

We thank Ralf Schnabel for providing the *lit-1* mutant strains; Hiroko Kouike for excellent technical support; and members of our laboratories for helpful discussions. Some strains were obtained from the *C. elegans* Genetic Stock Center, which is funded by a grant from the NIH National Center for Research Support. Thanks to the entire Worm Genome Consortium for providing the clones and sequences that made this work possible. Support for C. C. M. was provided in part by a PEW scholarship, a University of Massachusetts DERC pilot and feasibility grant, and an NIH grant. T. S. is a Schering-Plough fellow of the Life Sciences Research Foundation. J. Y., R. J. D., and J. R. P. were supported by the HHMI and by grants from the NIH. Further support for J. Y. was provided by the Foundation for Promotion of Cancer Research. R. L. was supported in part by a Basil O'Connor Grant from the March of Dimes Foundation.

Received March 25, 1999; revised May 13, 1999.

References

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Smith, J.A., and Struhl, K. (1997). Current Protocols in Molecular Biology (New York: John Wiley and Sons).

Bhanot, P., Brink, M., Samos, C.H., Hsieh, J.-C., Wang, Y., Macke, J.P., Andrew, D., Nathans, J., and Nusse, R. (1996). A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. Nature *382*, 225–230.

Bhat, K.M. (1998). *frizzled* and *frizzled 2* play a partially redundant role in Wingless signaling and have similar requirements to Wingless in neurogenesis. Cell *95*, 1027–1036.

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

Brott, B.K., Pinsky, B.A., and Erikson, R.L. (1998). Nlk is a murine protein kinase related to Erk/MAP kinases and localized in the nucleus. Proc. Natl. Acad. Sci. USA *95*, 963–968.

Bullions, L.C., and Levine, A.J. (1998). The role of β-catenin in cell adhesion, signal transduction, and cancer. Curr. Opin. Oncol. *10*, 81–87.

Cadigan, K.M., and Nusse, R. (1997). Wnt signaling: a common theme in animal development. Genes Dev. *11*, 3286–3305.

Cavallo, R., Rubenstein, D., and Peifer, M. (1997). Armadillo and dTCF: a marriage made in the nucleus. Curr. Opin. Genet. Dev. *7*, 459–466.

Choi, K.W., and Benzer, S. (1994). Rotation of photoreceptor clusters in the developing *Drosophila* eye requires the *nemo* gene. Cell *78*, 125–136.

Costa, M., Raich, W., Agbunag, C., Leung, B., Hardin, J., and Priess, J.R. (1998). A putative catenin-cadherin system mediates morphogenesis of the *Caenorhabditis elegans* embryo. J. Cell Biol. *141*, 297–308.

Eisenmann, D.M., Maloof, J.N., Simske, J.S., Kenyon, C., and Kim, S.K. (1998). The β-catenin homolog BAR-1 and LET-60 Ras coordinately regulate the Hox gene *lin-39* during *Caenorhabditis elegans* vulval development. Development *125*, 3667–3680.

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

Gumbiner, B.M. (1997). Carcinogenesis: a balance between β-catenin and APC. Curr. Biol. *7*, R443–R446.

Han, M. (1997). Gut reaction to Wnt signaling in worms. Cell *90*, 581–584.

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

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

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

Kennerdell, J.R., and Carthew, R.W. (1998). Use of dsRNA-mediated genetic interference to demonstrate that *frizzled* and *frizzled 2* act in the Wingless pathway. Cell *95*, 1017–1026.

Klymkowsky, M.W., and Parr, B. (1995). The body language of cells: the intimate connection between cell adhesion and behavior. Cell *83*, 5–8.

Kuhl, M., and Wedlich, D. (1997). Wnt signaling goes nuclear. Bioessays *19*, 101–104.

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

Lin, R., Hill, R.J., and Priess, J.R. (1998). POP-1 and anterior-posterior fate decisions in *C. elegans* embryos. Cell *92*, 229–239.

Mello, C.C., and Fire, A. (1995). DNA transformation in *C. elegans*. In Methods in Cell Biology Vol. 48, D. Shakes and H. Epstein, eds. (San Diego, CA: Academic Press), pp. 451–482.

Mello, C.C., Kramer, J.M., Stinchcomb, D., and Ambros, V. (1991). Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. EMBO J. *10*, 3959–3970.

Mello, C.C., Draper, B.W., Krause, M., Weintraub, H., and Priess, J.R. (1992). The *pie-1* and *mex-1* genes and maternal control of blastomere identity in early C. elegans embryos. Cell *70*, 163–176.

Mello, C.C., Draper, B.W., and Priess, J.R. (1994). The maternal genes *apx-1* and *glp-1* and establishment of dorsal-ventral polarity in the early C. elegans embryo. Cell *77*, 95–106.

Müller, H.-A.J., Samanta, R., and Wiechaus, E. (1999). Wingless signaling in the *Drosophila* embryo: zygotic requirements and the role of the *frizzled* genes. Development *126*, 577–586.

Novak, A., Hsu, S.C., Leung-Hagesteijn, C., Radeva, G., Papkoff, J., Montesano, R., Roskelley, C., Grosschedl, R., and Dedhar, S. (1998). Cell adhesion and the integrin-linked kinase regulate the LEF-1 and β-catenin signaling pathways. Proc. Natl. Acad. Sci. USA *95*, 4374–4379.

Papavassiliou, A.G., and Bohmann, D. (1992). Dephosphorylation of phosphoproteins with potato acid phosphatase. Methods Mol. Cell. Biol. *3*, 149–152.

Payne, D.M., Rossomando, A.J., Martino, P., Erickson, A.K., Her, J.H., Shabanowitz, J., Hunt, D.F., Weber, M.J., and Strugill, T.W. (1991). Identification of the regulatory phosphorylation sites in pp42/mitogen-activated protein kinase (MAP kinase). EMBO J. *10*, 885–892.

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

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

Schnabel, R., and Priess, J.R. (1997). Specification of cell fates in the early embryo. In *C. elegans* II, D.L. Riddle, T. Blumenthal, B.J. Meyer, and J.R. Priess, eds. (Plainview, NY: Cold Spring Harbor Laboratory Press), pp. 361–382.

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

Sulston, J.E., Schierenberg, E., White, J.G., and Thomson, J.N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. *100*, 64–119.

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

Tournier, C., Whitmarsh, A.J., Cavanagh, J., Barrett, T., and Davis, R.J. (1999). The *MKK7* gene encodes a group of c-Jun NH_2-terminal kinase kinases. Mol. Cell. Biol. *19*, 1569–1581.

Vinson, C.R., and Adler, P.N. (1987). Directional non-cell autonomy and the transmission of polarity information by the *frizzled* gene of *Drosophila*. Nature *329*, 549–551.

Vinson, C.R., Conover, S., and Adler, P.N. (1989). A *Drosophila* tissue polarity locus encodes a protein containing seven potential transmembrane domains. Nature *338*, 263–264.

Wayner, E.A., and Carter, W.G. (1987). Identification of multiple cell surface receptors for fibronectin and collagen in human fibrosarcoma cells possessing unique α and common β subunits. J. Cell Biol. *105*, 1873–1884.

Whitmarsh, A.J., Yang, S-H., Su, M.S.-S., Sharrocks, A.D., and Davis, R.J. (1997). Role of p38 and JNK mitogen-activated protein kinases in the activation of ternary complex factors. Mol. Cell. Biol. *17*, 2360–2371.

Willert, K., and Nusse, R. (1998). β-catenin: a key mediator of Wnt signaling. Curr. Opin. Genet. Dev. *8*, 95–102.

Zheng, L., Zhang, J., and Carthew, R.W. (1995). *frizzled* regulates mirror symmetrical pattern formation in the *Drosophila* eye. Development *121*, 3045–3055.

GenBank Accession Numbers

The accession numbers for the sequences reported in this paper are AF143243 and AF143244.