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A novel noncanonical Wnt pathway is involved in the regulation of the asymmetric B cell division in *C. elegans*

Mingfu Wu, Michael A. Herman *

Program in Molecular, Cellular and Developmental Biology, Division of Biology, Kansas State University, Manhattan, KS 66506, USA

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Abstract

The polarities of several cells that divide asymmetrically during *Caenorhabditis elegans* development are controlled by Wnt signaling. LIN-44/Wnt and LIN-17/Fz control the polarities of cells in the tail of developing *C. elegans* larvae, including the male-specific blast cell, B, that divides asymmetrically to generate a larger anterior daughter and a smaller posterior daughter. We determined that WRM-1 and the major canonical Wnt pathway components: BAR-1, SGG-1/GSK-3 and PRY-1/Axin were not involved in the control of B cell polarity. However, POP-1/Tcf is involved and is asymmetrically distributed to the B daughter nuclei, as it is in many cell divisions during *C. elegans* development. Aspects of the B cell division are reminiscent of the divisions controlled by the planar cell polarity (PCP) pathway that has been described in both *Drosophila* and vertebrate systems. We identified *C. elegans* homologs of Wnt/PCP signaling components and have determined that many of them appear to be involved in the regulation of B cell polarity. Specifically, MIG-5/Dsh, RHO-1/RhoA and LET-502/ROCK appear to play major roles, while other PCP components appear to play minor roles. We conclude that a noncanonical Wnt pathway, which is different from other Wnt pathways in *C. elegans*, regulates B cell polarity.

Keywords: B cell polarity; Planar cell polarity; *C. elegans*; Asymmetric cell division; Wnt signaling; Rho

Introduction

Wnt signaling pathways function in almost all animals in diverse developmental processes (Cadigan and Nusse, 1997; Veeman et al., 2003; Nelson and Nusse, 2004). At least three major conserved Wnt signaling pathways have been recognized: Wnt/β-catenin, Wnt/calcium and Wnt/planar cell polarity (PCP) (Nelson and Nusse, 2004). In the canonical, or Wnt/β-catenin pathway, Wnt ligands act through Frizzled (Fz) receptors and Dishevelled (Dsh) to antagonize the degradation of β-catenin, allowing β-catenin to translocate to the nucleus and complex with Tcf/Lef factors to activate or repress the expression of specific genes. The noncanonical Wnt/calcium and PCP pathways do not signal through β-catenin (Veeman et al., 2003; Nelson and Nusse, 2004). In *Drosophila*, the Wnt/PCP pathway regulates the orientation of hairs on the wing and dorsal thorax as well as the polarity of ommatidia in the eye (Mlodzik, 1999; Tree et al., 2002). In addition, Wnt/PCP has been found to regulate cell movements during vertebrate gastrulation and other biological processes (Veeman et al., 2003; Fanto and McNeill, 2004). The PCP pathway contains six core genes, *Fz*, *Dsh*, *Flamingo/Fmi*, *Van Gogh* or *Strabismus/Stbm*, *Diego/Dgo* and *Prickle/Pk*. PCP pathways that control bristle, hair and ommatidial polarity in *Drosophila* share these six molecules, but each tissue has its own specific downstream components and an unknown upstream signal (Tree et al., 2002). The PCP and Wnt/β-catenin pathways share the Fz receptor and the cytoplasmic transduction molecule Dsh but are activated by different Wnts or unknown factors and signal through different downstream components. Although Dsh is involved in both PCP and Wnt/β-catenin pathways, domains within the Dsh molecule display different specificities (Axelrod et al., 1998; Boutros et al., 1998). The asymmetric localization of the core PCP molecules is critical to planar polarity and the inhibition of Wnt/β-catenin signaling. In cells of the *Drosophila* pupal wing, Fz and Dsh are localized to the distal membranes, where the hair forms, whereas the Stbm and Pk are found in the proximal membranes and Fmi and Dgo are found in both (Wodarz and Nusse, 1998; Jenny et al., 2003; Strutt, 2003).

The Wnt/β-catenin and Wnt/PCP pathways are conserved throughout the animal kingdom (Fanto and McNeill, 2004).

* Corresponding author. Fax: +1 785 532 6653.
E-mail address: mherman@ksu.edu (M.A. Herman).

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Recent work by Park et al. (2004) demonstrated that during *Caenorhabditis elegans* ventral closure, MOM-5/Frizzled is localized within cells in a manner similar to *Drosophila* Frizzled during planar polarity and dorsal enclosure. This suggests that the PCP pathway might also be conserved in *C. elegans*. During *C. elegans* larval development, LIN-44/Wnt is expressed in the tail hypodermal cells and regulates cell polarities of the TL, TR, B, U, and F cells which lie further anterior in the tail. In males, the B cell divides asymmetrically to generate a larger anterior–dorsal daughter cell, B.a, and a smaller posterior–ventral daughter, B.p. B.a divides to produce 40 cells and generates male copulatory spicules, and B.p divides to produce 7 cells (Sulston et al., 1980). In *lin-44* mutant males, B cell polarity is reversed (Herman and Horvitz, 1994; Herman et al., 1995), while in *lin-17* mutant males, B cell polarity is lost (Sternberg, 1988; Sawa et al., 1996). While genes specifically involved in T cell polarity have been isolated and studied (Sawa et al., 2000; Zhao et al., 2002, 2003), the pathway that is activated by LIN-44 and LIN-17 to regulate B cell polarity has not been elucidated.

Here, we begin to define the Wnt pathway that controls B cell polarity. We determined that β-catenin homologs WRM-1 and BAR-1 as well as SGG-1/GSK-3, PRY-1/Axin and DSH-2/Dsh did not appear to be involved in the control of B cell polarity. However POP-1, the sole *C. elegans* TCF homolog, is involved, and GFP::POP-1 (Siegfried et al., 2004) is asymmetrically distributed to the B.a and B.p cell nuclei. We also identified putative *C. elegans* homologs of Wnt/PCP signaling components and have determined that many of them appear to be involved in the regulation of B cell polarity. We show that, in addition to LIN-44 and LIN-17, MIG-5/DSH, RHO-1/RhoA and LET-502/Rock play major roles in the control of B cell polarity, and other PCP components play minor roles. In addition, we show that LIN-17/Fz is expressed in the T and B cells, whereas MIG-5/Dsh is expressed in the B cell. We conclude that a noncanonical Wnt or a PCP-like pathway, which is different from other Wnt signal pathways in *C. elegans*, regulates B cell polarity.

Materials and methods

General methods and strains

Nematodes were cultured and manipulated by standard techniques (Brenner, 1974). N2 was used as the wild-type strain. The following mutations were used:

Linkage Group I (LGI): *tag-15(gk106)*, *pry-1(mu38)*, *lin-17(n671)*, *pop-1 (q645, q624)*, *lin-44(n1792)*, *let-502(h392)*, *dpy-5(e61)*, *unc-29(e1072)*;
LGII: *mig-5(ok280)*, *dsh-2(or302)*, *mIn1[mIs14 dpy-10(e128)]*, *rrf-3 (pk1426)* (Simmer et al., 2002);
LGIII: *wrm-1(ne1982ts)*, *cdh-3(pk77, pk87)*, *unc-32(e189)*, *Y48G9A.4 (ok460)*, *lit-1(or131ts)*, *unc-119(e2498)*, *cyk-1(t1611)*;
LGIV: *unc-44(e1260, e1197)*, *jnk-1(gk7)*, *unc-43(n498)*, *him-8(e1489)*, *cyk-4(t1689)*;
LGV: *unc-42(e270)*, *rde-1(ne219)*, *cdh-6(tm306)*, *him-5(e1490)*;
LGX: *qIs74[gfp::pop-1]*, *B0410.2a(ok1142)*, *jkk-1(km2)*, *bar-1(ga80)*.

Strains were obtained from the *C. elegans* Genetics Center (University of Minnesota) or from *C. elegans* Gene Knockout Consortium. *qIs74*, which contains *gfp::pop-1* (Siegfried et al., 2004), was used to observe POP-1 expression.

RNAi

RNAi was performed according to Fire et al. (1998). dsRNA was synthesized using MEGAscript® (Ambion) and cDNA clones (provided by Dr. Yuji Kohara, NIG, Mishima, Japan) or genomic DNA was used as templates. PCR primers used for dsRNA synthesis are available upon request.

To bypass the RNAi maternal lethal effects of *pop-1*, *rho-1*, *dsh-2*, *hmr-1*, *hmp-2*, *lit-1*, *wrm-1*, *mlc-4*, *apr-1*, *sys-1*, and *sgg-1*, a zygotic RNAi scheme was used (Herman, 2001). Similarly, *rho-1* or *cyk-4* dsRNA was injected into *unc-42(e270) rde-1(ne219)*; *mhIs9* to determine their effect on cytokinesis. In some cases, dsRNAs were injected into the RNAi hypersensitive *rrf-3* mutant.

Expression constructs

A *lin-17::gfp* construct that fused *gfp* to the end of *lin-17* coding sequence, similar to the functional *Drosophila* Fz-GFP (Strutt, 2001), was constructed from three fragments: a 14,450-bp *Hind*III-*Kpn*I fragment from pSH6 (Sawa et al., 1996), a 4373-bp *Kpn*I-*Hind*III fragment from pPD95.75 (a gift from A. Fire, Stanford University, CA), which includes *gfp*, and the last 705 bp coding sequence of *lin-17* cDNA amplified from yk1130b08.

gfp was amplified from pPD95.75 and inserted between the *mig-5* coding sequence and the *mig-5* stop codon to generate the *mig-5::gfp* construct with 5520 bp upstream and 1035 bp downstream regulatory sequences. The *mig-5* coding sequence (2499 bp) and upstream sequence was amplified from genomic DNA T05C12, so was the downstream sequence.

The *lin-17::gfp* and *mig-5::gfp* constructs were microinjected at a concentration of 10 ng/μl and 15 ng/μl, respectively, with the co-injection marker pPDMM0166 [*unc-119* (+)] at a concentration of 40 ng/μl, into *unc-119 (e2498)*; *him-5(e1490)* or *mig-5(ok280)*; *unc-119(e2498)*; *him-5(e1490)* hermaphrodites (Maduro and Pilgrim, 1995). Transgenic extrachromosomal arrays containing *lin-17::gfp* were integrated into the genome using a UV irradiation-based method (Mello et al., 1991) to generate *mhIs9*, which was backcrossed five times before phenotypic analysis.

Cell lineage and polarity analysis

Living animals were observed using Nomarski optics; cell nomenclature and cell lineage analysis were as previously described (Sulston and Horvitz, 1977). N.x refers to both daughters of cell N. Fates of the T and B cell descendants were determined by nuclear morphology and size; orientation to the body axis (Herman and Horvitz, 1994) was used as an indicator of T and B cell polarities, as previously described (Herman et al., 1995). However, in this study, B cell polarity was scored any time after the B cell division, and since the difference in B.a and B.p nuclear sizes was not obvious until 25 min after division, a small percentage of control animals were scored as having a loss of B cell polarity (Table 1). Phasmid dye filling was also used as an indicator of T cell polarity (Herman and Horvitz, 1994).

Orientation of the spindle during the division of the B cell was determined using the rectum as a reference. Micrographs of the B.x nuclei were analyzed by measuring the angle formed between the rectum and a line that bisected the B.x nuclei.

Results

LIN-17/Fz is localized to the membranes of the B and T cells

mhIs9 males contain a *lin-17::gfp* construct that was expressed in the membranes of the T, B cells and their descendants as well as the F, P11, P12 and vuval precursor cells (Figs. 1A–C, data not shown). *mhIs9* rescued the *lin-17* T and B cell polarity defects. Only 4% (n = 54) of T cells and 7% (n = 54) of B cells displayed polarity defects in *lin-17; mhIs9* animals, while 99% (n = 70) of T cells and 79% (n = 58) of B cells displayed polarity defects in *lin-17* animals. Only 2%

(n = 101) of *mhIs9* males showed a B cell polarity defect. Thus, the *lin-17::gfp* construct was functional.

Neither AJM-1::GFP nor MH27 antibody staining (Mohler et al., 1998) visualized the B cell membrane (data not shown). However, as LIN-17::GFP appeared to be localized to the cell membrane, we used it to mark the membranes of the B cell and its descendants (data not shown).

Table 1
B cell polarity defects

Wnt component[a]	Genotype	Relative nuclear sizes of B.a and B.p (%)				
		n	B.a > B.p (normal)	B.a = B.p (loss)	B.a < B.p (reversed)	Polarity defect[b]
None	*wild-type*	62	92	8	0	−
	rde-1/+	34	95	5	0	−
	rrf-3	49	98	2	0	−
Wnt	*lin-44(n1792)*	49	16	12	71	+
Fz	*lin-17(n671)*	58	21	69	10	+
Dsh	*mig-5(ok280)*	117	45	50	5	+
	mig-5(zygotic RNAi)	107	76	23	1	+
	dsh-1(RNAi)	37	97	3	0	−
	dsh-2	59	90	10	0	−
	dsh-1(RNAi) mig-5(ok280)	24	42	50	8	+
	dsh-2(RNAi) mig-5(ok280)	32	37	50	13	+
	dsh-1 dsh-2 mig-5(zygotic RNAi)[c]	35	61	29	0	+
Tcf	*pop-1(q645)*	25	64	32	4	+
	pop-1(q624)	39	77	21	2	+
Nlk	*lit-1(zygotic RNAi)*	57	91	9	0	−
	lit-1(or131) at 25°C	86	79	21	0	+
Ds/Fat[d]	*hmr-1*	49	84	16	0	+
	cdh-1(RNAi)	69	97	3	0	−
	cdh-3(pk77)	45	89	11	0	−
	cdh-3(pk87)	42	86	14	0	−
	cdh-4(RNAi)	78	87	13	0	−
Stbm	*B0410.2a*	62	81	19	0	+
Fmi	*rrf-3; cdh-6 (RNAi)*	40	87	13	0	−
	cdh-6(tm306)	39	93	7	0	−
Pk	*B0496.8 (RNAi)*	88	93	7	0	−
	rrf-3; ZK381.5(RNAi)	66	83	17	0	+
	tag-15(gk106)	45	80	20	0	+
	tag-15(gk210)	60	88	12	0	−
	tag-15(gk106); B0496.8 (RNAi); ZK381.5(RNAi)	50	76	24	0	+
RhoA	*rho-1(zygotic RNAi)*	68	28	72	0	+
Rock	*let-502*	50	48	52	0	+
	let-502; rrf-3(RNAi)	32	30	70	0	+
Daam1/Dia[d]	*Y48G9A.4*	55	85	15	0	−
	rrf-3; F56E10.2 (RNAi)	41	87	13	0	+
	cyk-1	32	94	6	0	−
	mlc-4(or253)	64	72	28	0	+
Jnk	*jnk-1(gk7)*	52	94	4	2	−
Jkk	*jkk-1(km2)*	40	97	3	0	−
CamKII	*unc-43(n498)*	50	92	8	0	−
	unc-43(n498n1179)	30	97	3	0	−
	unc-43(n498n1186)	45	93	7	0	−

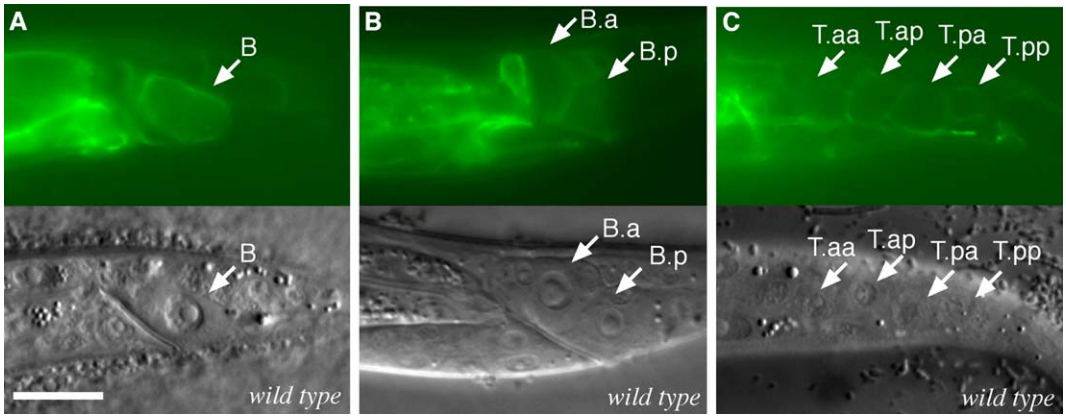

Fig. 1. LIN-17::GFP is expressed in and localized to the membranes of the B and T cells and their descendants. Anterior is left and ventral is down, bars are 10 μm in all subsequent figures. Fluorescence is shown above and corresponding DIC images below. LIN-17::GFP is expressed in the membrane (arrows) of the (A) wild-type B cell, (B) wild-type B.a and B.p cells and (C) wild-type T cell descendants.

POP-1/Tcf is involved in the regulation of B cell polarity and functions downstream of LIN-44/Wnt and LIN-17/Fz

The relative difference in B daughter cell nuclear size was used to determine the polarity of the B cell division. Wild-type males exhibit normal polarity with B.a being larger than B.p, while *lin-44* males primarily display reversed polarity with B.p being larger than B.a, and *lin-17* males display a loss of polarity with B.a and B.p being equal size. We examined the relative sizes of B daughters to determine that 32% (n = 25) of *pop-1 (q645)* and 21% (n = 39) of *pop-1(q624)* males displayed a loss of B cell polarity (Table 1 and Fig. 2A). The penetrance of the B cell defect caused by these nonnull *pop-1* alleles is comparable to the T cell defects of 40% and 19% for *pop-1(q645)* and *pop-1 (q624)*, respectively (Siegfried and Kimble, 2002). The *pop-1 (q624)* mutation alters a conserved amino acid in the HMG box DNA-binding domain and causes many defects at low penetrance, as one expects of a typical partial loss-of-function allele. However, the *pop-1(q645)* mutation alters a conserved amino acid in the ß-catenin binding domain and causes a highly penetrant gonad defect but other defects at low penetrance, suggesting that it may alter residues specifically involved in hermaphrodite gonadogenesis (Siegfried and Kimble, 2002). The low penetrance of the *pop-1(q645)* B cell defect might also be explained by the observation that *C. elegans* ß-catenin homologs *bar-1* and *wrm-1* are not involved in the control of B cell polarity (see below).

We constructed a *lin-44 pop-1* double mutant to determine the functional order of POP-1 and LIN-44 in the regulation of B cell polarity. The B cell defect of *lin-44 pop-1* males is similar to that of *pop-1* males, and 51% displayed a loss of polarity (Table 2 and Fig. 2B). Thus, *pop-1*/*Tcf* functions downstream of *lin-44 /Wnt* in the control of B cell polarity, as it does in the control of T cell polarity and other Wnt signaling pathways (Lin et al., 1998; Herman, 2001; Herman and Wu, 2004).

To determine the effect of *pop-1* B cell polarity defects on B cell fate, we followed the B cell lineage of 18 *pop-1* males (Figs. 2F–H). While B.a was larger than B.p 25 min after the B cell division in all the wild-type males examined (n = 7), in six of 18 *pop-1* males, the B daughters were of equal size (loss of polarity), and in another three, B.p was larger than B.a even 1 h after the B cell division (polarity reversal). However, the relative nuclear sizes of the B.a and B.p nuclei sometimes changed just before B.a or B.p divided. In the three *pop-1* males with reversed B cell polarities, the B.a and B.p nuclei become equal in size before they divided, the B.p cell divided with abnormal pattern and abnormal timing, and the B.axx cells were abnormally oriented (Figs. 2F, G). In the six worms that displayed a loss of polarity, B.p divided earlier than in wild-type males (Fig. 2F, H). Thus, defects in B cell lineage were observed in all nine *pop-1* males in which the relative sizes of the B.a and B.p nuclei were abnormal. Although not severe, the B cell lineage defect in *pop-1* males suggests that the B.p cell

Notes to Table 1:

The relative sizes of daughter nuclei of B cell division, B.a and B.p, of late L1 or early L2 stage males were scored using Nomarski microscopy. n, number of males scored. B.a > B.p, the B.a nuclear size was larger than that of B.p; B.a = B.p, the nuclear size of B.a was the same as that of B.p; B.a < B.p, the nuclear size of B.a was smaller than that of B.p.

B cell polarity defects in mutants and RNAi males were compared to the eight percent defect observed in the wild-type background or the two percent defect observed in *rrf-3* males and defects in zygotic RNAi males were compared to the 5% defect observed in *rde-1*/+ males. −, the difference was not significant indicating the gene was not involved in the control of B cell polarity; +, the difference was significant, suggesting minor involvement; ++, the difference was significant and also greater than the defect observed in *pop-1(q645)* males, which we considered to be an indication of a major role in the control of B cell polarity.

[a] Wnt components: *Drosophila* or vertebrate homologs.

[b] A Chi-squared test was used to determine whether the defect was significantly different from the relevant genetic background at the 0.05 level.

[c] dsRNA of all three Dsh homologs in the *wild-type* background caused severe embryonic lethality (84%, n = 108), results of zygotic RNAi are shown.

[d] It was difficult to tell the difference between the homologs of Daam1 and Dia or Ds and Fat in *C. elegans*, so their homologs are listed together. In some cases, it was difficult to identify *C. elegans* orthologs; so several potential homologs were tested.

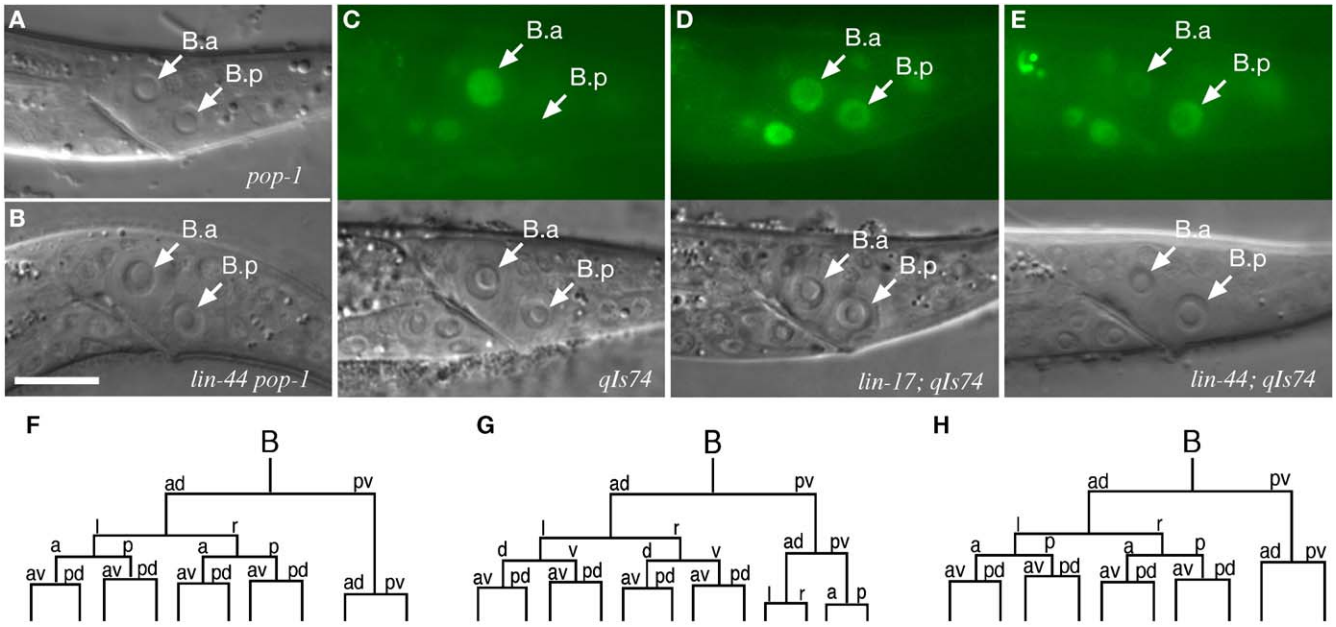

Fig. 2. POP-1 is involved in the control of B cell polarity, and its asymmetric distribution to the B.a and B.p cell nuclei is controlled by *lin-44* and *lin-17*. (A) The nuclear size of B.a can be equal to B.p in *pop-1* mutant males and (B) in *lin-44(n1792) pop-1(q645)*. Panels C–E show fluorescence above and corresponding DIC images below. Asymmetric distribution of GFP::POP-1 to the B.a and B.p nuclei (arrows) is normal in (C) *wild type*, lost in (D) *lin-17* and reversed in (E) *lin-44* males. Wild-type B cell lineage (F). *pop-1* mutant B cell lineages observed in 3/18 (G) and 6/18 males (H).

fate is abnormal. This is consistent with the observation that 40% (n = 55) of *pop-1* males had crumpled or shortened spicules. However, prior to division, the B cell itself is likely to be normal in *pop-1* mutants as the pattern of B.a divisions was normal (Figs. 2G, H) and LIN-17::GFP is expressed in the B cell (data not shown).

POP-1 is asymmetrically distributed to anterior–posterior daughters of most asymmetric cell divisions during *C. elegans* development. At several cell divisions, the asymmetric distribution of POP-1 is controlled by Wnt signaling (Lin et al., 1998; reviewed by Herman and Wu, 2004). To examine whether POP-1 is asymmetrically localized to the nuclei of the B daughter cells, we used an integrated array, *qIs74*, that contains a *gfp::pop-1* construct (Siegfried et al., 2004). In *qIs74* males, GFP::POP-1 is asymmetrically distributed to the nuclei of the B.a and B.p cells (Fig. 2C), with the level of GFP::POP-1 being higher in the B.a nucleus than in the B.p nucleus (100%, n = 27). In order to confirm that POP-1 asymmetric distribution to the B cell daughters is regulated by Wnt signaling, we examined GFP::POP-1 localization in *lin-44* and *lin-17* mutants. The levels of

Table 2
Pathway analysis

Components	Genotype	Relative nuclear sizes of B.a and B.p (%)			
		n	B.a > B.p (normal)	B.a = B.p (loss)	B.a < B.p (reversed)
Wnt	*wild-type lin-44(n1792)*	62	92	8	0
		49	16	12	71
Fz	*lin-17(n671) lin-17lin-44*	58	21	69	10
		48	17	77	6
Dsh	*mig-5(ok280) lin-44;mig-5*	117	45	50	5
		46	13	70	17
RhoA	*rho-1 (zygotic RNAi)*	68	28	72	0
	lin-44 rho-1 (zygotic RNAi)	40	27	68	5
Drok	*let-502(RNAi);rrf-3*	32	30	70	0
	lin-44; let-502(RNAi)	27	26	70	4
Tcf	*pop-1(q645) pop-1lin-44*	25	64	32	4
		47	11	51	39

The relative sizes of daughter nuclei of B cell division, B.a and B.p, of late L1 or early L2 stage males were scored as in Table 1.

GFP::POP-1 in the nuclei of the B.a and B.p cells were equal in *lin-17; qIs74* males (82%, $n = 17$) (Fig. 2D), but in *lin-44; qIs74* males, the level of GFP::POP-1 in the B.p. nucleus was higher than that in the B.a. nucleus (67%, $n = 33$) (Fig. 2E). The regulation of GFP::POP-1 levels in the B cell daughters is similar to that of T cell daughters in which POP-1 was also regulated by *lin-44* and *lin-17* (Herman, 2001).

MIG-5/Dsh is expressed in the B cell and its descendants and is involved in the regulation of B cell polarity

Of the three *C. elegans* Dsh homologs, *mig-5* plays the larger role in the control of B cell polarity (Tables 1, 3); 50% of *mig-5(ok280)* males segregating from *mig-5(ok280)/mIn1* mothers displayed a loss of B cell polarity and 5% ($n = 117$) displayed a reversal of B cell polarity (Table 1 and Fig. 3A), while 85% ($n = 60$) of *mig-5(ok280)* animals displayed normal T cell polarity. In addition, *mig-5 (RNAi)* increased the loss of B cell polarity of *lin-44* males from 12% to 48% ($n = 54$) (Fig. 3B) and 70% ($n = 46$) of *lin-44; mig-5(ok280)* males displayed loss of B cell polarity (Table 2), indicating that *lin-44* functions upstream of *mig-5*.

In order to determine whether MIG-5 is expressed in the B cell and its descendants, a *mig-5::gfp* construct was made. *mig-5* animals bearing an extrachromosal array containing *mig-5::gfp* can grow to adulthood, whereas nonarray containing *mig-5* animals arrested as L1 or early L2 larvae, indicating that the construct is functional. Animals that contained *mig-5::gfp* expressed GFP in the B, QL cell and several cells in the nerve ring (Fig. 3C and data not shown). MIG-5::GFP was expressed strongly in the B cell and its descendants (Fig. 3c and data not shown). *mig-5::gfp* also rescued the *mig-5(ok280)* B cell polarity defect; 85% ($n = 33$) of *mig-5* animals that contained *mig-5::gfp* showed normal B cell polarity.

In order to determine whether *mig-5* functions upstream of *pop-1*, a *mig-5(ok280)/mIn1; qIs74* strain was constructed.

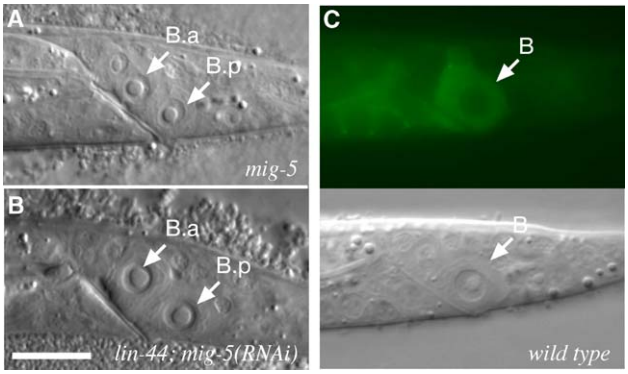

Fig. 3. MIG-5 is involved in the control of B cell polarity, functions downstream of LIN-44 and is expressed in the B cell and its descendants. In *mig-5(ok280)* males (A) and *lin-44(n1792); mig-5(RNAi)* males (B), the size of the B.a nucleus can be equal to the B.p nucleus. (C) *mig-5::gfp* is expressed in the B cell in a punctate pattern.

Interestingly, the levels of GFP::POP-1 in the B cell and its descendants were dramatically reduced in *mig-5; qIs74* males, as well as in the Z1, Z4 and P11/P12 cells (data not shown). GFP::POP-1 was distributed equally to the B daughter nuclei in 31% ($n = 29$) of *mig-5; qIs74* males in which the sizes of the B.a and B.p nuclei were equal, suggesting that MIG-5 functions upstream of POP-1. Inactivation of all three Dsh orthologs by RNAi did not significantly enhance the defect caused by *mig-5 (RNAi)* alone (Table 1), suggesting that the incomplete penetrance of the B cell defect in *mig-5* males is not due to redundant Dsh function.

lit-1 mutants weakly affect B cell polarity, but wrm-1 mutants may not; and neither affect the asymmetric distribution of POP-1 to the B cell daughters

WRM-1 is one of four *C. elegans* β-catenin homologs (Natarajan et al., 2001) and functions with LIT-1/Nemo like

Table 3
Many known Wnt pathway components are not involved in the control of B cell polarity

Genotype	T cell defect		Relative nuclear sizes of B.a and B.p cells (%)				
	n	Plasmid dye filling (%)	*n*	B.a > B.p (normal)	B.a = B.p (loss)	B.a < B.p (Reversed)	B Polarity defect
wild-type	>100	100	62	92	8	0	−
rde-1/+	>100	100	34	95	5	0	−
wrm-1(zygotic RNAi)	114	38	115	95	5	0	−
wrm-1(ne1982ts) at 25°C	60	3	48	92	8	0	−
sys-1(zygotic RNAi)	94	56	52	85	15	0	+
lit-1(zygotic RNAi)	78	12	57	91	9	0	−
lit-1(or131) at 25°C	88	45	86	79	21	0	+
dsh-1(RNAi)	136	100	37	97	3	0	−
bar-1(ga80)	114	87	46	93	7	0	−
hmp-2(zygotic RNAi) [a]	42	100	51	92	8	0	−
pry-1(mu38)	192	99	45	91	9	0	−
sgg-1(zygotic RNAi)	114	100	48	94	6	0	−
apr-1(zygotic RNAi)	86	90	56	96	4	0	−

Phasmid dye filling was used as an indicator of normal T cell polarity (Herman and Horvitz, 1994). There is one phasmid on each side of the animal. The relative sizes of daughter nuclei of B cell division, B.a and B.p, were scored as in Table 1. ND, not determined.

[a] T cell data from Herman (2001).

kinase to control the polarities of the EMS blastomere and the Z1 and Z4 somatic gonad precursor cells. WRM-1 interacts with and activates LIT-1 kinase, which phosphorylates POP-1 and regulates its subcellular localization (Rocheleau et al., 1999; Maduro et al., 2002). SYS-1 is another ß-catenin homolog that functions to control the polarities of the Z1 and Z4 somatic gonad precursor (SGP) cells by interacting with POP-1 to control cell fates (Miskowski et al., 2001; Kidd et al., 2005). *wrm-1* and *lit-1*, but not *sys-1* mutations, also affect the asymmetric distribution of POP-1 in the Z1 and Z4 cells (Siegfried et al., 2004; Kidd et al., 2005). *lit-1* and *sys-1* mutations also caused a loss of T cell polarity (Rocheleau et al., 1999; Siegfried et al., 2004). We used phasmid dye filling (Herman and Horvitz, 1994) as well as nuclear morphologies of the T cell granddaughters (Herman and Horvitz, 1994) to assess the effectiveness of *lit-1(RNAi)*, *sys-1(RNAi)* and *wrm-1(RNAi)* as well as *lit-1(or131ts)* and *wrm-1 (ne1982ts)* mutations. In each case, T cell polarity was defective (Table 3). In addition, T cell daughter nuclei displayed the asymmetric distribution of GFP::POP-1 in only 15% ($n = 34$) of *lit-1(ts); qIs74* animals and only 18% ($n = 28$) of *wrm-1(RNAi)* animals (Figs. 4A–C).

Next, we investigated whether LIT-1, WRM-1 and SYS-1 were also involved in the regulation of B cell polarity and the asymmetric distribution of POP-1 to the B daughter cell nuclei. Disruption of *lit-1* or *sys-1* function caused a minor B cell polarity defect, but disruption of *wrm-1* caused little or no defect (Table 3). Surprisingly, *lit-1* did not affect the asymmetric distribution of GFP::POP-1 to the B.a and B.p cells. All of the *lit-1(RNAi); qIs74* males ($n = 26$) and *lit-1(ts); qIs74* males ($n = 29$) displayed a normal asymmetric distribution of POP-1 to B.a and B.p nuclei (Fig. 4D), even though two of the *lit-1 (RNAi); qIs74* males and four of *lit-1(ts); qIs74* males showed a loss of B cell polarity. In addition, the asymmetric distribution of GFP::POP-1 to the B.a and B.p cell nuclei was normal in *wrm-1(RNAi)* ($n = 33$) males (Fig. 4E). Furthermore, all the *sys-1(zygotic RNAi); qIs74* males that showed a loss of B cell polarity had a normal distribution of GFP::POP-1 to the B cell daughters ($n = 8$). Thus, while *wrm-1, sys-1* and *lit-1* play major roles in the control of T cell polarity and the asymmetric distribution of POP-1 to the T daughters, they played lesser roles in the control of B cell polarity and the asymmetric distribution of POP-1 to the B daughters. This suggests that the pathways that control T and B cell polarities are different.

Canonical Wnt signaling does not control T or B cell polarity

A canonical Wnt pathway has been shown to be involved in the migration of QL neuroblast descendants and other

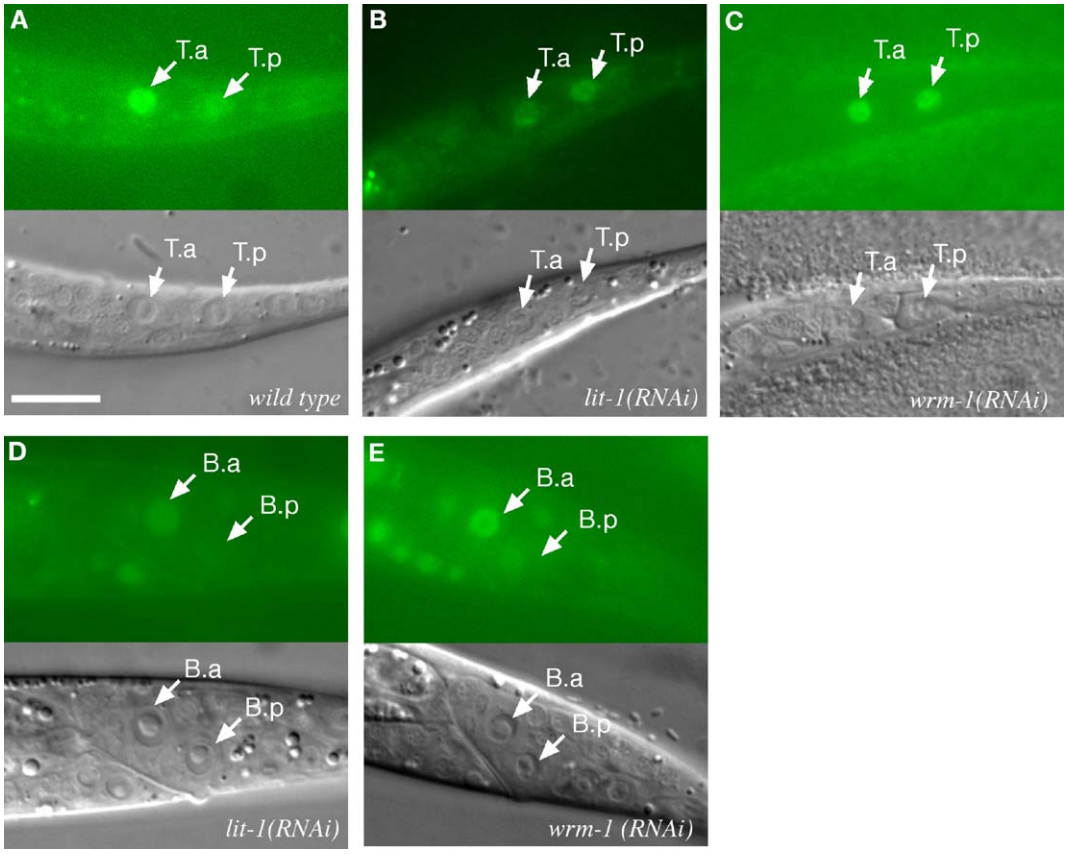

Fig. 4. *lit-1* and *wrm-1* play different roles in the regulation of T and B cell polarities. (A) POP-1 is asymmetrically distributed to the T.a and T.p nuclei in *qIs74* males (arrows). (B) POP-1 was distributed equally to the T.a and T.p nuclei in *qIs74; lit-1(RNAi)* (arrows). (C) POP-1 was distributed equally to the T.a and T.p nuclei in *qIs74; wrm-1(RNAi)* (arrows). POP-1 was distributed asymmetrically to the B.a and B.p nuclei in *qIs74; lit-1(RNAi)* (D) and *qIs74; wrm-1(RNAi)* males (E) and the polarity of the division was normal (arrows).

processes during *C. elegans* development (Korswagen et al., 2000) but not in the control of T cell polarity (Herman, 2001). As the pathways that control the polarities of B and T cells appeared to differ, we wanted to know whether other canonical Wnt signaling components might be involved in the control of B cell polarity. Neither *dsh-2*, *bar-1* nor *pry-1* mutants affected B cell polarity (Table 3). Since either mutation or RNAi of *hmp-2* and *apr-1* caused embryonic lethality, we used zygotic RNAi to determine that they were not involved in the regulation of B cell polarity. This was also true for *sgg-1* (Table 3). Since zygotic RNAi has been shown to be effective for genes involved in T cell polarity (Herman, 2001), these negative RNAi results are likely to be informative. Finally, since mutations in *bar-1*, the canonical *C. elegans* β-catenin (Eisenmann, 2005), did not cause a B cell polarity defect, we conclude that the canonical Wnt pathway does not appear to control B or T cell polarity.

RHO-1/RhoA and LET-502/ROCK are involved in the control of B cell polarity and the asymmetric distribution of POP-1 to B cell daughters

RhoA and RhoA associated kinase (Rock) have been shown to be part of the PCP pathway that regulates vertebrate gastrulation and the actin cytoskeleton in *Drosophila* (Habas et al., 2001, 2003; Winter et al., 2001). The *C. elegans* RhoA ortholog, *rho-1*, has been shown to be involved in cytokinesis (Jantsch-Plunger et al., 2000) and P cell migration (Spencer et al., 2001). Since *rho-1(RNAi)* caused embryonic lethality, we used zygotic RNAi to determine that in 25% (n = 68) of *rho-1(RNAi)* males, the size of B.a nucleus was equal to that of B.p (Fig. 5A), while in 47% of males, B.a was slightly larger than B.p, however, the size difference was not as great as that observed in wild-type males (Fig. 5B). We also observed a cytokinesis defect in *rho-1(RNAi)* males. Using *lin-17::gfp* to visualize the B cell membrane, we observed two, three and ten B daughter nuclei within a common cytoplasm (Figs. 5C, D). Despite the lack of normal cytokinesis, 15 of 22 *rho-1 (RNAi)* B cells produced ten nuclei by the late L2 stage (as occurred in wild-type males), and seven of 22 animals produced six or seven nuclei, suggesting that B cell fate is somewhat normal. However, the orientation of the B daughter nuclei was abnormal. In wild-type males, the B.al/raa and B. al/rpp cells migrate to assume the Bα, Bβ, Bγ and Bδ fates, respectively (Sulston and Horvitz, 1977; Chamberlin and Sternberg, 1993). However, these cell migrations did not occur in 12 of the 15 *rho-1(RNAi)* males (Fig. 5D), probably because the nuclei were within the same cell membrane. RHO-1 is also involved in spindle orientation during the B cell division. In wild-type males, the B cell spindle is orientated almost parallel to the rectum, with the angle between the spindle axis and the rectum being less than 9° (n = 15), while in 82% of *rho-1(RNAi)* (n = 70) males, the angle between the B cell spindle and the rectum varied from 10° to 45° (Figs. 5E–G).

We performed *rho-1* zygotic RNAi in a *lin-44* mutant background to determine that RHO-1 functions downstream of LIN-44 (Table 2 and Fig. 5H). In addition, GFP::POP-1 was symmetrically distributed to the B.a and B.p nuclei in all *rho-1 (RNAi)* males in which B.a was equal to or slightly larger than B.p (n = 23) (Figs. 5A, B). That GFP::POP-1 was localized to the nuclei in *rho-1 (RNAi)* animals (Figs. 5A, B) indicated that *rho-1* does not affect the ability of POP-1 to localize to the nucleus but does not rule out the possibility that the symmetric distribution is caused by the cytokinesis defect.

The *C. elegans* Rock homolog, LET-502, has been shown to be involved in embryonic elongation (Wissmann et al., 1997) and P cell migration (Spencer et al., 2001) in a RHO-1 dependent manner. The *Drosophila* Rock homolog, Drok, links Frizzled-mediated PCP signaling to the actin cytoskeleton (Winter et al., 2001). In budding yeast, Pkc1p, which is structurally and functionally related to mammalian Rock, is localized to sites of polarized growth in a Rho1p dependent manner (Andrews and Stark, 2000). Thus, Rock functions downstream of RhoA and is involved in the regulation of polarization in several systems. *let-502(RNAi)* animals had severe body morphology and cytokinesis defects as well as B cell polarity defects (Table 2 and Fig. 5I). *lin-44(n1792) let-502(RNAi)* males also displayed loss of B cell polarity (Fig. 5J, Tables 1, 2), which indicated that LET-502/Rock functions downstream of LIN-44/Wnt. Like *rho-1(RNAi)*, *let-502(RNAi)* also caused GFP::POP-1 to be symmetrically distributed to the B.a and B.p cell nuclei (data not shown). *mlc-4/Sqh (RNAi)* males also displayed B cell cytokinesis defects and a loss of B cell polarity, although with a lower penetrance (Table 1), as well as symmetric distribution of GFP::POP-1 to B.a and B.p nuclei (Fig. 5L).

To determine whether the B cell polarity defect was secondary to the cytokinesis defect we observed in *rho-1 (RNAi)*, *let-502(RNAi)* and *mlc-4(RNAi)* males, we examined the polarity and cytokinesis of the B cell division in *cyk-4 (RNAi)* males. CYK-4 is a GTPase activating protein (GAP) and functions with RHO-1 in the completion of cytokinesis (Jantsch-Plunger et al., 2000). 55% (n = 58) of *cyk-4(RNAi)* males displayed a B cell cytokinesis defect, and the B.a. nucleus was larger than the B.p nucleus (Fig. 5K), suggesting that polarity was normal. However, 79% (n = 19) of *cyk-4(RNAi)* animals that displayed a cytokinesis defect showed an equal distribution of GFP::POP-1 to the B daughter nuclei (Fig. 5K), and 95% of these showed normal polarity. Another 21% showed a slightly asymmetric distribution (data not shown), but the difference in GFP::POP-1 levels between the B.a and B.p nuclei was not nearly as great as that in *wild type* or *lin-44* animals. This suggests that while the cytokinesis defect caused by *cyk-4 (RNAi)* does not affect the polarity of the B cell nuclear division, it may interfere with the differential nuclear distribution of the GFP::POP-1. Finally, *cyk-1* is also involved in cytokinesis but not B cell polarity (Table 1). Nine of 32 *cyk-1* males displayed a cytokinesis defect, but in all nine, B.a was larger than B.p indicating normal polarity. Thus, while the polarity of the B cell nuclear division may not be secondary to the cytokinesis defect in *rho-1* and *let-502* males, the asymmetric distribution of GFP::POP-1 may be.

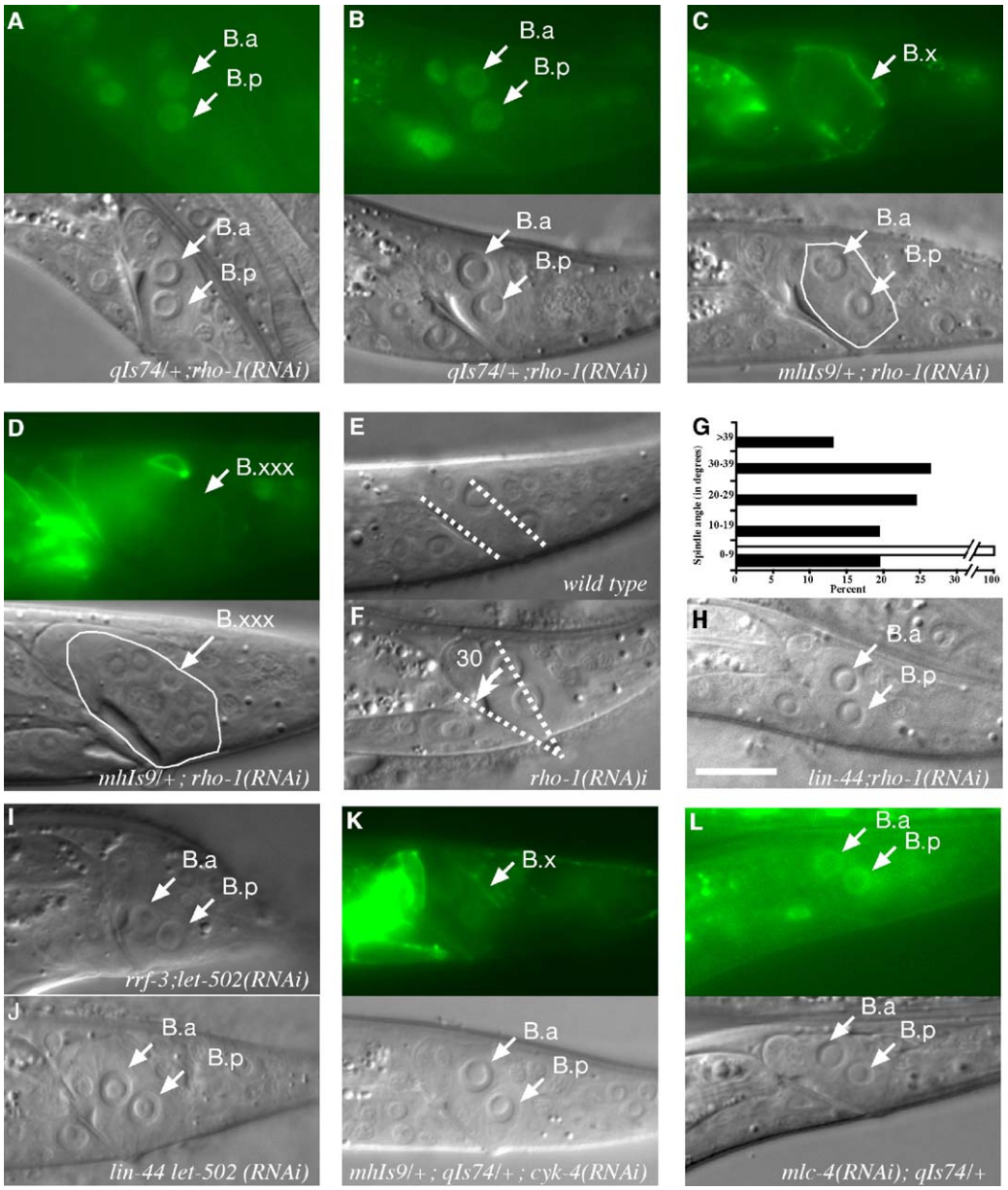

Fig. 5. RHO-1 and LET-502 function after LIN-44 in the control of B cell polarity and affect the asymmetric distribution of POP-1 to the daughter nuclei. Panels (A–D) and (K, L) show the fluorescent image above and corresponding DIC images below. (A, B) The size of B.a is equal to (A) or slightly larger (B) than B.p nuclei in *rho-1 (RNAi)* males, and GFP::POP-1 is equally distributed to each nucleus. (C, D) *rho-1(RNAi); mhIs9* males, (C) B.a and B.p nuclei or (D) ten descendant nuclei are contained within a common membrane. White line in lower panels indicates B cell membrane traced from *lin-17::gfp* localization in upper panel. (E) The spindle axis of B cell division (white dashed line) is almost parallel to the rectum (white dashed line) in wild-type males; the angle between the spindle axis and the rectum is less than 9° (n = 15). (F) The angle between the B cell division spindle and the rectum is 30° in *rho-1(RNAi)* males. (G) Summary of spindle orientation defects in *rho-1 (RNAi)* males (n = 70) and wild-type worms (n = 15). (H) A *lin-44(n1792); rho-1(RNAi)* male displayed a loss of B cell polarity. (I) A *let-502(RNAi); rrf-3* male displayed cytokinesis and polarity defects at the B cell division. (J) The B cell defect of *lin-44 let-502(RNAi)* male is similar to *let-502(RNAi)* alone. (K) A *cyk-4(RNAi)* male in which cytokinesis has failed at the B cell division, yet the B.a nucleus is larger than the B.p nucleus. (L) *mlc-4(zygotic RNAi)* male displayed a symmetric distribution of GFP::POP-1 to the B.a and B.p nuclei and B cell polarity defect.

Other PCP components weakly affect B cell polarity

We identified *C. elegans* homologs of several conserved PCP signaling components. It was sometimes difficult to determine the *C. elegans* ortholog by sequence analysis alone; thus, we investigated several candidate orthologs (Table 1).

In mutations of PCP core genes, *B0496.8/Pk* (Gubb et al., 1999), *B0410.2a/Stbm* and *cdh-6/Fmi*, each showed weak B cell polarity defects (Table 1). There did not appear to be a homolog of Dgo or Four-jointed in the *C. elegans* genome. Dachsous (Ds) and Fat (Ft), two other components that function upstream of the six PCP core genes in *Drosophila*, are cadherin-like proteins, and Ds is required for Wg-dependent pattern formation in the *Drosophila* wing disc. It was difficult to assign Ds and Ft orthologs in *C. elegans*, however, *hmr-1*, *cdh-1*, *cdh-3* and *cdh-4* are clear homologs, thus, we investigated the role of each in the

control of B cell polarity. *hmr-1(RNAi)*, *cdh-1*, *cdh-3* and *cdh-4 (RNAi)* also showed weak B cell polarity defects (Table 1), and CDH-3 was expressed in the B cell (Chamberlin et al., 1999). Downstream of the PCP core components, the molecules that are responsible for polarity are tissue specific (Tree et al., 2002). In *Drosophila*, c-Jun N-terminal kinase (Jnk) and c-Jun kinase kinase (Jkk) function downstream of PCP core components to control ommatidal polarity. Mutations in the *C. elegans JNK* and *JKK* orthologs, *jnk-1* and *jkk-1*, did not cause a B cell polarity defect. We also tested *C. elegans* homologs of the formin-homolog protein dishevelled associated activator of morphogenesis 1 (Daam1), reported to be involved in the regulation of gastrulation in *Xenopus* and to physically interact with both Dsh and RhoA (Habas et al., 2001). However, these *C. elegans* homologs as well as CYK-1 are also similar to Diaphanous (Dia), which is involved in cell division in *Drosophila* (Castrillon and Wasserman, 1994) and vertebrate homologs function with RhoA to regulate cell polarity (reviewed by Fukata et al., 2003). RNAi of the two conserved *C. elegans* Daam1 homologs, Y48G9A.4 and F56E10.2, weakly affected B cell polarity. Finally, the *C. elegans* ortholog of Ca^{2+}/calmodulin-dependent protein kinase II (CamKII), which has been shown to be involved in the Wnt/Ca^{2+}pathway, *unc-43*, did not affect B cell polarity (Table 1).

Discussion

The Wnt pathway that regulates B cell polarity is different from the other known C. elegans Wnt pathways

Wnt signaling controls the polarities of several cell divisions during *C. elegans* development including the EMS, T, Z1, Z4, B, U and F cells. LIN-44/Wnt (Herman and Horvitz, 1994; Herman et al., 1995) and LIN-17/Fz (Sawa et al., 1996) are involved in the regulation of T and B cell polarities. The pathway that regulates T cell polarity also includes WRM-1, LIT-1 and POP-1, which is asymmetrically distributed to T cell daughters (Herman, 2001; Herman and Wu, 2004). Our results confirmed that mutations in *lit-1* and *wrm-1* caused defects in T cell polarity and the asymmetric distribution of GFP::POP-1 to the nuclei of T daughter cells. WRM-1 and LIT-1 are also involved in a branched Wnt/MAPK pathway that controls the polarity of the EMS division, with one branch controlling spindle orientation and the other specifying endodermal cell fates (Rocheleau et al., 1997; Thorpe et al., 1997; Schlesinger et al., 1999). In addition, a *C. elegans* Src kinase homolog, SRC-1, and MES-1 also function in the control of EMS polarity (Bei et al., 2002). All these pathways interact to control the nuclear levels of POP-1/Tcf in the EMS daughter nuclei and repress POP-1 function in the posterior E cell. Although WRM-1, LIT-1 and POP-1 all are involved in the control of T, Z1 and Z4 cell polarities, *pop-1* might play a positive role in specifying the posterior neural T.p and the distal Z1.a and Z4.p cell fates likely achieved by an interaction with SYS-1 (Herman, 2001; Herman and Wu, 2004; Siegfried et al., 2004; Kidd et al., 2005), which is different from its role in the specification of posterior EMS fate. Interestingly, we observed that mutations in *lit-1* and *sys-1* have a minor effect, and *wrm-1* has little or no effect on either the asymmetry of the B cell division or the asymmetric distribution of GFP::POP-1. Thus, the pathway that controls B cell polarity is different from those that control EMS, SGP and T cell polarities. We also determined that mutations of components of the canonical Wnt pathway, which regulates the migration of QL descendants (Korswagen et al., 2000), did not affect B cell polarity. This suggests that the Wnt pathway that regulates B cell polarity is different from the known *C. elegans* Wnt pathways.

Rho-1/RhoA and LET-502/Rock links Wnt/Fz signaling to the actin cytoskeleton to control B cell polarity

How Wnt/PCP signaling pathways relay information to the cytoskeleton and lead to cytoskeleton reorganization is still not clear. Recent findings suggest that the small GTPase RhoA may function as a critical link. RhoA and Rock are important regulators of cytoskeletal architecture. Expression of either the *Xenopus* Fz7, rat Fz1 or *Drosophila* Wnt1 activated RhoA in a Dsh-dependent, but β-catenin-independent manner in both human 293T cells and *Xenopus* embryos, suggesting that Wnt activation of RhoA may be a mechanism by which the cytoskeleton is regulated (Habas et al., 2001, 2003). During *Drosophila* eye development, RhoA functions within Fz/PCP signaling to regulate transcription and ommatidial polarity (Strutt et al., 1997). In *Drosophila*, *Drok/Rock* mutations cause the formation of multiple hairs in one cell and ommatidial orientation defects (Winter et al., 2001). Thus, RhoA might function downstream of Wnt/PCP to regulate the actin cytoskeleton in many systems. Homologs of RhoA and Rock also function in yeast to regulate polarization (Andrews and Stark, 2000). In *C. elegans*, Wnt signaling is involved in alignment of the mitotic spindle in the EMS cell along the anterior–posterior body axis, indicating that Wnt signaling can polarize the cytoskeleton (Thorpe et al., 2000). Thus, Wnt signaling in *C. elegans* may function through RhoA and Rock to regulate aspects of cell polarity. We demonstrated that *rho-1*/*RhoA* and *let-502*/*Rock* affected the sizes of the B.a and B.p nuclei, the orientation of the spindle during the B cell division and function downstream of *lin-44*.

In addition to the loss of polarity, we also observed that *rho-1*, *let-502* and *mlc-4* mutations blocked cytokinesis at the B cell division. This raised the question as to whether the cytokinesis defects were the primary cause of the polarity defects. RHO-1 is required for cytokinesis in the first and/or second cell cycle as RHO-1 is likely to be the critical target for CYK-4, the Rho GAP that functions in central spindle formation and cytokinesis (Jantsch-Plunger et al., 2000), and 90% of *rho-1(RNAi)* embryos displayed a cytokinesis defect. It was possible that the effect of *rho-1* on the relative sizes of the B daughter nuclei was nonspecific, and that RHO-1 was either required for all the cell divisions that occur during *C. elegans* development or involved in cytokinesis of several cells, including the B cell. We do not believe this to be the case because both *cyk-4(RNAi)* and *rho-1 (RNAi)* animals displayed B cell cytokinesis defects, but *cyk-4 (RNAi)* males still displayed an asymmetric nuclear division. Also, inactivation of RhoA GTPase disrupted the formation of

cortical actin structures and the contractile ring but not chromosomal separation or nuclear envelope reformation (Kishi et al., 1993; O'Connell et al., 1999; Jantsch-Plunger et al., 2000), suggesting that the symmetric nuclear division in *rho-1(RNAi)* mutants occurred before the cytokinesis defect. This and other considerations suggest that cytokinesis is not coupled to cell polarity. Specifically, cytokinesis occurs after formation of the daughter nuclei (reviewed by Straight and Field, 2000). In *C. elegans*, ZEN-4, CYK-4 and RHO-1 are involved in the assembly of the central spindle (Mishima et al., 2002). LET-502 (Piekny and Mains, 2002) and MLC-4 (Shelton et al., 1999) are also required for cytokinesis. We showed that *cyk-1*, *cyk-4*, *rho-1*, *let-502* and *mlc-4* males displayed B cell division cytokinesis defects. However, in *rho-1*, *let-502* and *mlc-4*, but not *cyk-1* and *cyk-4* males, the B cell nucleus divided symmetrically. Thus, we suggest that RHO-1, LET-502 and MLC-4 play roles in cytokinesis and the control of cell polarity. For example, while both *mlc-4* and *cyk-4* affected the division of the *C. elegans* zygote, the daughter nuclei were positioned symmetrically within the same cytoplasm after the first division in *mlc-4* embryos, while the two nuclei were in the same cytoplasm but displayed normal asymmetric localization after the first cell division in *cyk-4* embryos (Jantsch-Plunger et al., 2000). Thus, *mlc-4* affects polarity and cytokinesis, while *cyk-4* only affects cytokinesis. Also, RHO-1 and LET-502 have not been reported to be involved in the establishment of anterior–posterior polarity of the zygote, however, it appears to us that *rho-1* embryos displayed cytokinesis defects, and the two nuclei were symmetrically localized after the first cell division (Fig. 6, Jantsch-Plunger et al., 2000), indicating that *rho-1* might also be involved in the control of cell polarity. Finally, ZEN-4, CYK-4 and RHO-1 are involved in P cell cytokinesis, but only RHO-1 and LET-502 are also involved in P cell nuclear migration, indicating that the pathways that regulate P cell nuclear migration and P cell cytokinesis are decoupled, although they share some components, similar to what we observed for B cell polarity. Thus, although CYK-1, CYK-4, MLC-4, LET-502 and RHO-1 are involved in cytokinesis, MLC-4, LET-502 and RHO-1 may be also involved in the asymmetric B cell nuclear division. In addition, *rho-1(RNAi)* animals only displayed weak T cell polarity defects, demonstrating the difference between the control of B and T cell polarities and suggesting that the *rho-1* B cell polarity defect is specific.

A PCP-like pathway might regulate B cell polarity

Disrupting the functions of *C. elegans* homologs of *Drosophila* PCP genes caused defects of B cell polarity. Although it was not possible to identify *C. elegans* orthologs in all cases, such as Ft, Ds and Pk, several components had clear orthologs, such as RhoA, Rock, Fmi and Stbm. The PCP pathway functions to control the polarities of epithelial cells that lie in a tissue. Are there any analogous tissues in *C. elegans*? We think that there might be. LIN-44/Wnt is expressed in the tail hypodermal cells and regulates cell polarities of the T, B, U and F cells in the *C. elegans* male tail. These are adjacent ectodermal blast cells that have an epithelial character. For an animal that has only about one thousand cells, these cells might comprise a kind of epithelia sheet. Killing the B cell with a laser microbeam caused polarity defects in the F and U cells, indicating that F and U cells polarities are dependent upon the B cell (Herman and Horvitz, 1994). This behavior is somewhat reminiscent of the directional nonautonomous effect caused by *Frizzled* mutant clones in *Drosophila* (Strutt and Strutt, 2002). Thus, it is possible that the pathway that regulates B cell polarity could be similar to the *Drosophila* PCP pathway. Different components function downstream of the six core PCP genes in the *Drosophila* wing and eye. In the eye, Jnk and Jkk mediate ommatidial polarity, whereas RhoA and Drok/Rock function in the wing hair cells. We did not observe a B cell polarity defect when we interfered with the functions of *jnk-1* and *jkk-1*, but did when we interfered with the functions of *rho-1/RhoA*, *let-502/Rock* and *mlc-4/Sqh*. This suggests that the pathway that regulates B cell polarity is more similar to the PCP pathway that regulates polarity in the *Drosophila* wing.

Although there appear to be many differences between the pathway that regulates B cell polarity and the pathway that regulates *Drosophila* wing cell polarity, much is conserved. Based upon our results and the PCP pathway that regulates *Drosophila* wing hair polarity, we propose a model for a PCP-like pathway that might function to regulate B cell polarity (Fig. 6). A major difference was that mutation or RNAi of the *C. elegans* homologs of PCP core proteins Pk, Stbm and Fmi only cause a minor B cell polarity defect. Similarly, homologs of the two global proteins Ds and Fat only play a minor role, suggesting that these proteins might function redundantly in the control of B cell polarity. Another difference is the involvement of LIN-44/Wnt in B cell polarity, whereas no Wnt has been shown to be involved in *Drosophila* PCP. Some of our results are based on RNAi experiments, and whether null mutants might have higher penetrance is unknown. However, based upon results from mutants alone, it is clear that *lin-44/Wnt*, *lin-17/Fz*, *mig-5/Dsh*, *rho-1/RhoA* and *let-502/Drok* play large roles; Ds or Fat homologs *cdh-3* and *cdh-4*, *cdh-6/Fmi* and *tag-15/Pk* play lesser roles; and *B04102.a/Stbm*, *jnk-1/Jnk* and *jkk-1/Jkk* do not appear to be involved in the control of B cell polarity. It is also possible that the cadherin-like proteins Ds and Fat and trans-membrane proteins Fmi and Stbm function nonspecifically and are involved in many cell–cell interactions. We also cannot exclude the possibility that all the homologs may not be orthologs. It is possible that multiple Wnt pathways may function downstream of the LIN-44 signal to control B cell polarity, for example, the WRM-1/LIT-1 pathway that controls the polarities of the EMS and T cells may function redundantly with PCP components to control B cell polarity (Fig. 6).

Function of POP-1 in the control of B cell polarity

There are at least three aspects of *pop-1* function during the B cell division that need to be explained: first, the relative sizes of the B cell daughters are equalized in *pop-1* mutants, which we interpret as a loss of B cell polarity; second, *pop-1* mutations cause a small, but significant B.p cell fate defect, which can also be explained by a loss of B

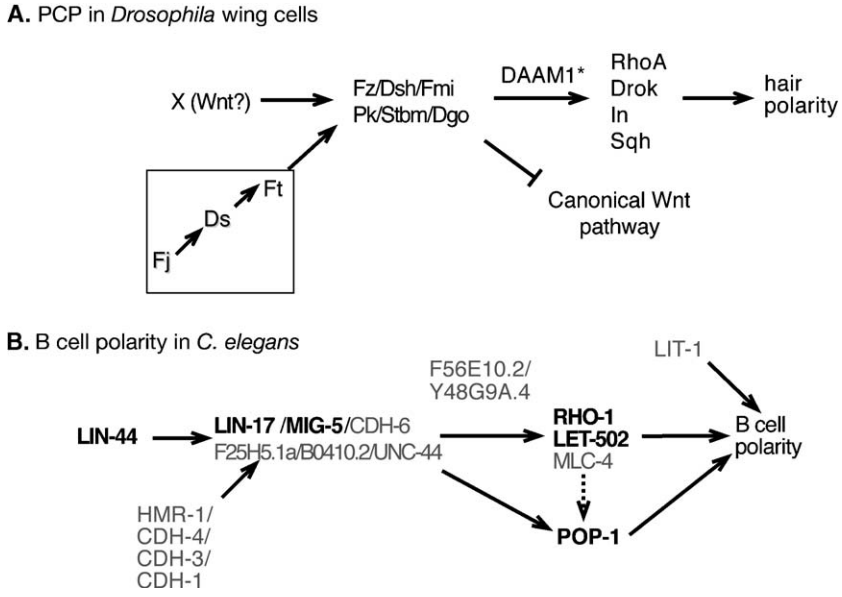

Fig. 6. A PCP-like pathway might regulate B cell polarity. (A). PCP pathway that regulates wing hair polarity modified from Tree et al. (2002). *The function of *Drosophila* DAAM1 is unclear. (B). A PCP-like pathway might regulate B cell asymmetric division in *C. elegans*. Black: components that play a major role in the control of B cell polarity; Gray: components that play a minor role. Pathway arrangement is based on the *Drosophila* PCP pathway shown in panel (A). Except for *cdh-3*, *cdh-4* and *hmr-1*, each gene functions after *lin-44*. The functional order among the genes downstream of *lin-44* was not able to be determined as they have a similar phenotype. However, *lin-44*, *lin-17*, *mig-5*, *rho-1* and *mlc-4* affected POP-1 asymmetric localization, thus might function upstream of *pop-1* (dashed line).

cell polarity; third, the asymmetric distribution of POP-1 to the B daughter nuclei is controlled by LIN-44 and LIN-17, although the roles of RHO-1 and LET-502 are less clear. Let us consider each of these in turn.

It is curious that POP-1, a member of the TCF/LEF transcription factor family, is involved in the control of cell size during an asymmetric cell division. The difference in relative sizes of the B cell daughters is apparent during and immediately after the B cell division. Thus, the processes that function to control the cell size difference must function prior to the completion of cytokinesis and separation of the B cell daughters. These processes are controlled by *lin-44*, as mutations in *lin-44* cause reversals of the relative sizes of the B daughters. We have shown that *lin-17*, *mig-5*, *rho-1*, *let-502* and even *pop-1* function downstream of *lin-44* in this process. The speed of the establishment of B daughter cells of different sizes suggests that the process occurs without new gene transcription. How can a transcription factor control such an early event? One possibility is that *pop-1* mutations cause a change in B cell fate prior to its division, such that it often does not undergo an asymmetric division and cannot respond to *lin-44*. However, our data suggest that B cell fate is normal in *pop-1* mutants: LIN-17::GFP is expressed in the membrane of the B cell, and B.a divides with a normal pattern. While it is possible that the B cell could be transformed to another *lin-17*-expressing cell in *pop-1* animals, the lack of effect on B.a fate suggests that B cell fate is fairly normal. It is likely that the establishment of cells of different sizes involves regulation of cytoskeletal elements, including the asymmetric positioning of the mitotic spindle during the B cell division, perhaps by small GTPases of the Rac or Rho family. POP-1 might interact with some of these cytoskeletal regulators, such as RHO-1 and LET-502. In support of this idea, Esufali and Bapat (2004) recently demonstrated that Rac1 GTPase binds to ß-catenin and TCF and affected the intracellular distribution of ß-catenin, leading to changes in target gene expression. Perhaps interaction of POP-1 with RHO-1, LET-502 and other cytoskeletal regulators is required for the asymmetric B cell division.

The role that POP-1 plays in the control of B cell fate and how this relates to the control of cell polarity is also unclear. Our cell lineage analysis of *pop-1* males with a loss of B cell polarity showed no obvious defects in B.a cell fate and minimal, but clear, defects in B.p cell fate. In addition, we observed crumpled spicules consistent with cell fate defects among the B cell descendants. The effect of *pop-1* mutations on fates of the B cell descendants could be caused by defects in B cell polarity. One possibility is that *pop-1* plays a minor role in the control of B cell polarity and subsequent fates of the B cell descendants and may function in parallel with another pathway, such as the PCP pathway (Fig. 6).

The asymmetric distribution of POP-1 to the B daughter nuclei is controlled by *lin-44* and *lin-17*. Recent work has shown that the asymmetric distribution of POP-1 to nuclei in the early embryo is controlled by differential nuclear export mediated by the 14-3-3 protein PAR-5 and nuclear exportin homolog IMB-4/CRM-1 (Lo et al., 2004; Nakamura et al., 2005). Furthermore, LIT-1 modification of POP-1 was shown to be required for its asymmetric nuclear distribution (Lo et al., 2004). Along with LIT-1, WRM-1, whose function is also required for asymmetric nuclear distribution of POP-1, was also localized differentially, with a higher level in the posterior E cell nucleus (Lo et al., 2004; Nakamura et al., 2005). WRM-1 was also localized to the anterior cortex of the anterior MS cell in a process that required MOM-5/Fz.

Nakamura et al. (2005) proposed that Wnt and Src signaling leads to the phosphorylation and retention of WRM-1 in the posterior E nucleus, where it phosphorylates POP-1 in a LIT-1-dependent manner. Thus, asymmetric nuclear retention of WRM-1 appears to drive the control of cell polarity during the EMS division. A similar process may also occur during the T cell division (Takeshita and Sawa, 2005). In this work, we have shown that neither *lit-1* nor *wrm-1* mutations affected the asymmetric nuclear accumulation of POP-1 in the B cell daughters (Fig. 4). This suggests that another mechanism may function to control asymmetric POP-1 nuclear accumulation in the B cell daughters. We have also shown that *rho-1*, *let-502* and *mlc-4* function specifically in the control of B cell polarity. However, their role in the asymmetric nuclear accumulation of POP-1 is less clear. While POP-1 was symmetrically distributed to the B daughter nuclei in all *rho-1(RNAi)* males that displayed B cell polarity defect (n = 50), cytokinesis was also blocked, so that the nuclei were in a common cytoplasm. Thus, it appears that the effect of *rho-1* and *let-502* on POP-1 distribution is a secondary consequence of the cytokinesis defect. This may be due to a single mixed pool of modified and nonmodified POP-1 in the binucleate B cell following the cytokinesis block. In support of this, POP-1 was symmetrically or nearly symmetrically distributed to the B daughter nuclei in all the *cyk-4(RNAi)* males, that displayed a cytokinesis defect, despite the fact that almost all displayed normal B cell polarities. However, a role for RHO-1, LET-502 and MLC-4 in POP-1 distribution cannot be completely ruled out. Finally, it should be noted that after the 28-cell stage, POP-1 nuclear asymmetry becomes independent of Wnt/MAPK signaling, yet still requires MOM-5/Fz (Park and Priess, 2003). The mechanism that regulates these POP-1 nuclear asymmetries has not been elucidated, however, it has been suggested that it may be similar to PCP signaling (Herman and Wu, 2004; Lo et al., 2004), and it may be this sort of mechanism that functions in the B cell.

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