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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%