Cell, Vol. 121, 761–772, June 3, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.cell.2005.03.029

A β-Catenin Identified by Functional Rather Than Sequence Criteria and Its Role in Wnt/MAPK Signaling

Ambrose R. Kidd III,[1] Jennifer A. Miskowski,[2,6] Kellee R. Siegfried,[3,7] Hitoshi Sawa,[5] and Judith Kimble[1,2,3,4,*]

[1]Program in Cellular and Molecular Biology
[2]Department of Biochemistry
[3]Laboratory of Genetics
[4]Howard Hughes Medical Institute
University of Wisconsin–Madison
433 Babcock Drive
Madison, Wisconsin 53706
[5]Laboratory for Cell Fate Decision
RIKEN Center for Developmental Biology
Kobe 650-0047
Japan

Summary

Wnt/MAPK signaling is a common variant of Wnt signaling in *C. elegans* and has been implicated in vertebrates. The *sys-1* gene works with Wnt/MAPK signaling to control cell fates during *C. elegans* development. We report that the SYS-1 amino acid sequence is novel but that SYS-1 functions as β-catenin: SYS-1 rescues a *bar-1*/β-catenin null mutant, binds the POP-1/TCF β-catenin binding domain, and coactivates POP-1-dependent transcription. Moreover, we provide genetic and molecular evidence that SYS-1 levels are crucial to POP-1 activity. Our results suggest that Wnt/MAPK signaling promotes POP-1 export from the nucleus to accommodate the limiting availability of its SYS-1/β-catenin transcriptional coactivator. Discovery of SYS-1/β-catenin extends our definition of β-catenins and brings together aspects of the canonical mechanism for Wnt signaling with the noncanonical Wnt/MAPK mechanism. We discuss the idea that a similar pathway may be employed broadly in animal development.

Introduction

β-catenins are key regulators of animal development (Logan and Nusse, 2004). These broadly conserved proteins can control transcriptional activation, cell adhesion, or establishment of a bipolar spindle (Kaplan et al., 2004; Nelson and Nusse, 2004; Wodarz and Nusse, 1998). In its guise as a transcriptional regulator, β-catenin functions as a coactivator of the TCF/LEF DNA binding protein (reviewed in van Noort and Clevers, 2002). Indeed, TCF/LEF and β-catenin are the terminal regulators of the canonical Wnt signaling pathway (Brantjes et al., 2002). Without β-catenin, TCF represses transcription (Brantjes et al., 2001; Cavallo et al., 1998; Roose et al., 1998), but with β-catenin, TCF is transformed to a transcriptional activator (reviewed in Bienz, 1998; van Noort and Clevers, 2002).

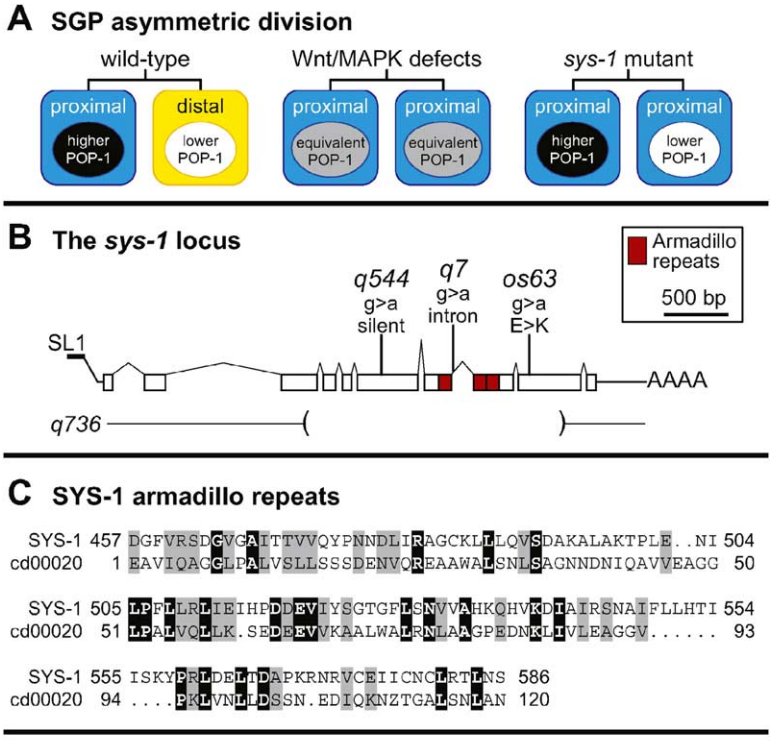
C. elegans possesses three β-catenins recognizable by amino acid sequence (Korswagen et al., 2000; Natarajan et al., 2001) and a single TCF/LEF homolog, called POP-1 (Lin et al., 1995). BAR-1/β-catenin is a transcriptional coactivator (Korswagen et al., 2000), HMP-2/β-catenin is specialized for cell adhesion (Costa et al., 1998; Korswagen et al., 2000; Natarajan et al., 2001), and WRM-1/β-catenin mediates Wnt signaling by a noncanonical pathway (reviewed in Thorpe et al., 2000). Therefore, in *C. elegans,* individual β-catenin paralogs assume largely discrete functions, including those typical of the single β-catenins in flies and vertebrates.

The *C. elegans* POP-1/TCF transcription factor controls multiple binary fate decisions associated with asymmetric divisions (Herman, 2001; Lin et al., 1998; Lin et al., 1995; Siegfried and Kimble, 2002). Nematode POP-1 has the hallmark molecular features of all TCF/LEF transcription factors. For example, POP-1 can repress transcription in the absence of a coactivator (Calvo et al., 2001), but POP-1 and BAR-1/β-catenin can activate transcription of a reporter transgene driven by a promoter with TCF binding sites (Korswagen et al., 2000). Therefore, POP-1 and TCF bind a common DNA sequence and can act as either transcriptional repressors or activators, depending on the presence of a β-catenin coactivator.

In *C. elegans,* POP-1 activity can be assessed in individual cells with well-defined fates. Indeed, POP-1 is differentially active in sister cells of an asymmetric division, which is accomplished, at least in part, by a noncanonical variant of Wnt signaling termed Wnt/MAPK signaling (reviewed in Behrens, 2000; Korswagen, 2002; Thorpe et al., 2000). Wnt/MAPK signaling relies on components of canonical Wnt signaling (e.g., frizzled receptor, disheveled, β-catenin, TCF) as well as components of a MAPK-related pathway (e.g., MOM-4/TAK-1, also called TGF-β-activated kinase, and LIT-1/NLK, also called nemo-like kinase). Because MOM-4/TAK-1 and LIT-1/NLK are critical for Wnt signaling in both vertebrates and invertebrates (Ishitani et al., 1999; Ishitani et al., 2003; Meneghini et al., 1999; Rocheleau et al., 1999; Shin et al., 1999; Thorpe and Moon, 2004; Zeng and Verheyen, 2004), an understanding of their control in *C. elegans* is likely to be of broad significance.

Wnt/MAPK activation has a counterintuitive effect on the subcellular localization of POP-1: in the activated daughter of an asymmetric division, nuclear POP-1 is reduced relative to its level in the inactivated daughter (Figure 1A, left diagram). This difference has been dubbed "POP-1 asymmetry" and occurs in daughters of the EMS blastomere (Lin et al., 1995; Maduro et al., 2002) as well as in daughters of many other asymmetric divisions, both in later embryos (Lin et al., 1998) and during larval development (Deshpande et al., 2005; Herman, 2001; Siegfried et al., 2004). Why is nuclear POP-1 reduced in daughters requiring its activity? Two main

*Correspondence: jekimble@wisc.edu
[6]Present address: Biology Department, University of Wisconsin–La Crosse, La Crosse, Wisconsin 54601.
[7]Present address: Max-Planck-Institut für Entwicklungsbiologie, Abt. Genetik, 72076 Tübingen, Germany.



D

Protein	Total aa	% id (% sim) to SYS-1	% id (% sim) to SYS-1(arm)
SYS-1 (Ce)	811	100 (100)	100 (100)
SYS-1 (Cb)	839	50.9 (71.1)	52.1 (67.9)
SYS-1 (Cr)	829	50.6 (68.7)	46.6 (74.8)
β-catenin (Hs)	781	11.3 (34.2)	10.9 (35.2)
plakoglobin (Hs)	745	12.4 (31.6)	12.7 (37.3)
p120 (Mm)	911	11.1 (31.6)	11.9 (30.7)
armadillo (Dm)	843	12.3 (29.7)	11.4 (34.3)
BAR-1 (Ce)	811	13.3 (31.9)	17.6 (37.4)
HMP-2 (Ce)	678	15.6 (29.2)	13.3 (32.0)
WRM-1 (Ce)	796	11.4 (37.5)	20.6 (45.8)
p120 (Ce)	1254	10.2 (24.2)	13.5 (37.4)
α-actinin (Hs)	894	10.3 (29.6)	12.3 (34.6)
AAP (Hs)	730	11.4 (28.9)	12.5 (32.0)

Figure 1. Molecular Identification of the *sys-1* Gene

(A) SGP asymmetric division and its control by *sys-1* and Wnt/MAPK signaling. Light blue, proximal fate; yellow, distal fate. See text for further explanation.

(B) The *sys-1* gene and mutations. The *sys-1* gene encodes a 2.91 kb transcript. Boxes, exons; lines, introns and UTRs; red boxes, armadillo repeats; black bar, SL1 *trans*-spliced leader. Point mutations are shown above; size of internal deletion mutation shown below. Surprisingly, *sys-1(q544)* is a silent mutation; since this allele is a strong loss-of-function mutation and its new codon remains a common one for *C. elegans*, we speculate that it may affect a tissue-specific exon splicing element.

(C) SYS-1 armadillo repeats. Model cd00020 of the Conserved Domain Database (NCBI) identified three armadillo repeats in the SYS-1 amino acid sequence. Top line, SYS-1 arm repeats; bottom line, most common amino acids in cd00020. Black boxes, SYS-1 amino acids identical to most common amino acids in cd00020; gray boxes, SYS-1 amino acids included among acceptable amino acids in cd00020 but not most common. See Figure S1 for additional information.

(D) SYS-1 amino acid sequence comparisons. ClustalW (EBI) was used to align SYS-1 (accession number NP_492639) to homologs from *C. briggsae* and *C. remanei* (identified by BLAST), human β-catenin (accession number AAR18817), human plakoglobin (accession number AAA64895), mouse p120 (accession number NP_031641), armadillo (accession number NP_476666), BAR-1 (accession number NP_509206), HMP-2 (accession number NP_493566), WRM-1 (accession number NP_498236), worm p120 (accession number NP_502910), human α-actinin (accession number NP_001094), and human actin-associated protein (AAP) (accession number AAH32777). Hs, *Homo sapiens*; Mm, *Mus musculus*; Dm, *Drosophila melanogaster*; Ce, *C. elegans*; Cb, *C. briggsae*; Cr, *C. remanei*. Total aa, total number of amino acids in full-length protein; % id (% sim), percent identity (percent similarity); SYS-1(arm), SYS-1 armadillo repeat region (amino acids 457–586) and corresponding region of other proteins.

ideas have been put forward to explain the function of POP-1 asymmetry. In early embryos, POP-1 reduction from the nucleus results in derepression of target genes (Calvo et al., 2001; Maduro et al., 2002); in the postembryonic T cell, POP-1 reduction is thought to reflect a change in the activity state of POP-1 by an undetermined mechanism (Herman, 2001; Herman and Wu, 2004). The function of POP-1 asymmetry remains poorly understood.

Several Wnt/MAPK regulators are essential for POP-1 asymmetry. In animals depleted for these regulators (e.g., LIN-17/Fz, WRM-1/β-catenin, LIT-1/NLK), POP-1 asymmetry is abolished: daughter cells possess equivalent levels of nuclear POP-1 and adopt the Wnt/MAPK-independent fate (Figure 1A, central diagram) (Herman, 2001; Meneghini et al., 1999; Park and Priess, 2003; Rocheleau et al., 1997; Rocheleau et al., 1999; Shin et al., 1999; Siegfried et al., 2004; Thorpe et al., 1997). To establish POP-1 asymmetry, POP-1/TCF is phosphorylated by LIT-1/NLK (Rocheleau et al., 1999; Shin et al., 1999) and exported from the nucleus (Lo et al., 2004; Maduro et al., 2002). POP-1 phosphorylation occurs in a complex that includes WRM-1/β-catenin and LIT-1/NLK (Rocheleau et al., 1999). Therefore, WRM-1/β-catenin does not function as a canonical β-catenin but instead is part of a mechanism for reducing nuclear POP-1 in response to WNT/MAPK signaling.

The *sys-1* gene (for *sy*mmetrical *s*isters) was identified in a genetic screen for regulators of gonadogenesis (Miskowski et al., 2001). Normally, the somatic gonadal precursor (SGP) divides asymmetrically to generate one distal daughter and one proximal daughter (Kimble and Hirsh, 1979). These daughters exhibit POP-1 asymmetry with nuclear POP-1 lower in the dis-

tal cell (Figure 1A, left diagram). In animals depleted for Wnt/MAPK signaling, POP-1 asymmetry is abolished, and both SGP daughters adopt the proximal fate (Siegfried and Kimble, 2002; Sternberg and Horvitz, 1988) (Figure 1A, central diagram). Therefore, in distal SGP daughters, Wnt/MAPK signaling both reduces the nuclear level of POP-1 and promotes the distal fate. Unlike upstream regulators (e.g., LIN-17/Fz, WRM-1/β-catenin, LIT-1/NLK), POP-1 asymmetry is not altered in *sys-1* mutants (Siegfried et al., 2004) (Figure 1A, right diagram). Nonetheless, both SGP daughters adopt a proximal fate in *sys-1* mutants, suggesting that SYS-1 may function with POP-1 to specify the distal fate (Siegfried et al., 2004). In addition to its control of the SGP division, SYS-1 appears to have a broader role in *C. elegans* development as well (see Discussion).

In this paper, we report that SYS-1 is a novel protein containing three divergent armadillo repeats. Despite its uninformative amino acid sequence, we find that SYS-1 is a functional β-catenin, a discovery that suggests the existence of additional β-catenins not yet identified by sequence criteria. We propose a model in which Wnt/MAPK signaling reduces nuclear POP-1 to accommodate the limiting availability of its SYS-1/β-catenin coactivator. Both genetic and molecular lines of evidence support this model. We discuss the idea that the SYS mechanism may be used broadly in animal development.

Results

The *sys-1* Locus Encodes a Protein with Armadillo Repeats

We identified T23D8.9 as the *sys-1* locus by standard methods (see Experimental Procedures). The *sys-1* gene encodes one major SL1 *trans*-spliced mRNA corresponding to the predicted transcript, T23D8.9a (see Experimental Procedures and Figure 1B). Each of three *sys-1* point mutations is associated with a molecular lesion, but none is likely to be null (Figure 1B). Only *sys-1(os63)* is predicted to alter the amino acid sequence. We therefore generated *sys-1(q736)*, a 2.19 kb internal deletion (Figure 1B), which removes most of the coding region and shifts the reading frame to generate a premature termination codon. The *sys-1(q736)* deletion is embryonic lethal as a homozygote and failed to complement *sys-1(q544)* with respect to the Sys gonadal defect. We conclude that *sys-1* corresponds to T23D8.9.

The predicted SYS-1 protein is composed of 811 amino acids. Using the Conserved Domain Database (CDD) from NCBI (Marchler-Bauer and Bryant, 2004), we found three predicted armadillo repeats in SYS-1 (amino acids 457–586) (Figure 1C). A similar analysis of armadillo repeats in known β-catenins is provided for comparison (Figure S1). We next used BLAST to identify related proteins. The only homologs were in closely related nematodes, *C. briggsae* (CBG03820) and *C. remanei* (Figure 1D). Sequence comparisons between SYS-1 and known β-catenins or other armadillo-repeat-containing proteins revealed no convincing relationship (Figure 1D). Therefore, SYS-1 is a novel protein.

SYS-1 Can Substitute for BAR-1/β-Catenin

SYS-1 has been implicated in Wnt/MAPK signaling (Siegfried and Kimble, 2002). Because β-catenins possess armadillo repeats (Hatzfeld, 1999), we asked if SYS-1 might function as a β-catenin. To this end, we used a transgenic assay (Natarajan et al., 2001). The *bar-1* locus encodes a classical β-catenin, and *bar-1(ga80)* null mutants, hereafter called *bar-1(0)*, have a partially penetrant defect in vulval development (Figure 2A; Eisenmann et al., 1998). Natarajan et al. (2001) showed that a full-length *bar-1* cDNA flanked by 5′ and 3′ regulatory sequences, called *bar-1::BAR-1*, rescues *bar-1(0)* from 55% to 90% with a wild-type vulva; a similar transgene bearing only the *bar-1* armadillo repeat region *(bar-1::BAR-1ΔNΔC)* did not rescue (Figure 2A).

To ask if SYS-1 might have β-catenin function, we created *bar-1::SYS-1*, which contains the full-length *sys-1* cDNA cloned between *bar-1* 5′ and 3′ flanking regions (Figure 2B). We then generated transgenic *bar-1(0)* null mutants carrying *bar-1::SYS-1*. A wild-type vulva is easily recognizable (Figure 2C); about half the *bar-1(0)* mutants had a protruding vulva (Figures 2B and 2D; Gleason et al., 2002; Natarajan et al., 2001). By contrast, most *bar-1(0)* mutants carrying *bar-1::SYS-1* had a typical wild-type vulva (Figures 2B and 2E). We conclude that rescue by *bar-1::BAR-1* and *bar-1::SYS-1* transgenes is similar (Figures 2A and 2B) and that SYS-1 can substitute for BAR-1/β-catenin in vulva development.

SYS-1 Interacts Specifically with the POP-1 N-Terminal Domain

Canonical β-catenins bind to TCF transcription factors (Behrens et al., 1996; Brunner et al., 1997; Huber et al., 1996; Molenaar et al., 1996; van de Wetering et al., 1997). This binding is easily detected by yeast two-hybrid assay (Behrens et al., 1996; Korswagen et al., 2000; van de Wetering et al., 1997). Previous studies showed that BAR-1/β-catenin binds POP-1/TCF strongly and specifically but that WRM-1/β-catenin and HMP-2/β-catenin either do not bind POP-1/TCF or bind weakly (Korswagen et al., 2000; Natarajan et al., 2001; Rocheleau et al., 1999). SYS-1 interacted reproducibly with POP-1 (Figure 3A, line 1), but neither was positive on its own (Figure 3A, lines 2 and 3). We found no interaction between WRM-1/β-catenin and POP-1 (Figure 3A, line 4) but did see a strong interaction between BAR-1/β-catenin and POP-1 (Figure 3A, line 5).

We next examined interactions between SYS-1 and POP-1 in tissue culture cells. To this end, HEK293 T cells were transfected with plasmids encoding HA-tagged SYS-1 and FLAG-tagged POP-1, either singly or in combination. Both proteins were present in whole-cell lysates (Figure 3B, top two panels). We then immunoprecipitated POP-1 from lysates using an α-FLAG antibody. In cells producing either POP-1 alone or both POP-1 and SYS-1 together, POP-1 was reproducibly immunoprecipitated (Figure 3B, third panel). However, SYS-1 was only immunoprecipitated when transfected with POP-1 (Figure 3B, bottom panel). Therefore, SYS-1 and POP-1 interact with each other both by yeast two-hybrid assay and by coimmunoprecipitation from tissue culture cells.

Canonical β-catenins bind to TCF via an N-terminal

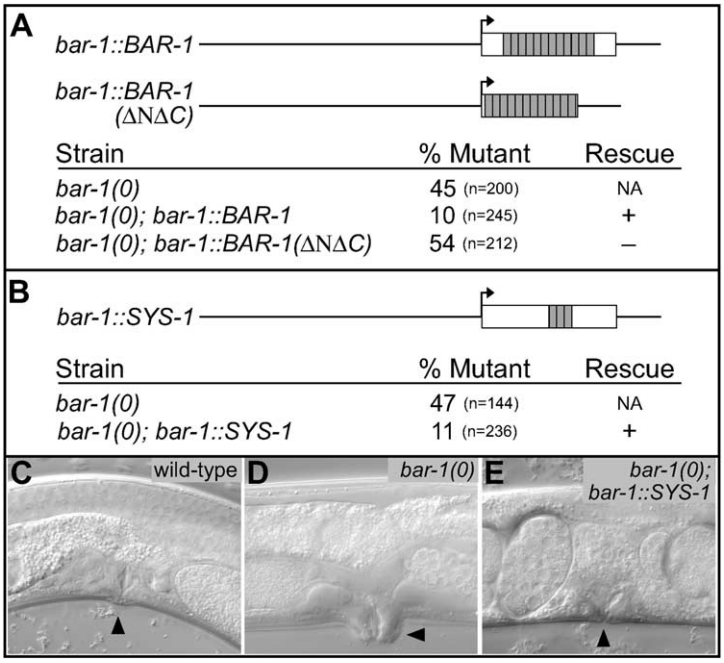


Figure 2. SYS-1 Can Substitute for BAR-1/β-Catenin

(A) Rescue results from Natarajan et al. (2001). Top, *bar-1::BAR-1* contains full-length BAR-1/β-catenin cDNA; *bar-1::BAR-1(ΔNΔC)* contains cDNA encoding all BAR-1 armadillo repeats but deleted for N- and C-terminal transactivation domains. Both include *bar-1* 5′ and 3′ flanking sequences (drawn to scale). Gray box, armadillo repeat. Bottom, summary of results from Natarajan et al. (2001). % Mutant, percent animals with protruding vulva; n, number animals scored.

(B–E) SYS-1 rescues *bar-1(0)* vulval defects. (B) Top, *bar-1::SYS-1* transgene contains cDNA encoding full-length SYS-1 inserted between 5′ and 3′ *bar-1* flanking sequences. Bottom, conventions as in (A). (C–E) Nomarski images; arrowheads mark vulva. (C) Wild-type. (D) *bar-1(0)* homozygote with protruding vulva. (E) *bar-1(0)* homozygote carrying *bar-1::SYS-1* transgene. Vulva has wild-type morphology.

region called the β-catenin binding domain (Behrens et al., 1996; Huber et al., 1996; Molenaar et al., 1996; van de Wetering et al., 1997). Similarly, BAR-1/β-catenin binds the N-terminal region of POP-1 (Korswagen et al., 2000; Natarajan et al., 2001), but the weak WRM-1 binding did not require the N-terminal region (Natarajan et al., 2001). To ask if SYS-1 interacts with the β-catenin binding domain, we again used the two-hybrid assay. Specifically, we tested SYS-1 interactions with a POP-1 missense mutant, called POP-1(q645), and truncated versions of POP-1. SYS-1 failed to interact with POP-1(q645) (Figure 3C, top line), which carries a single amino acid change in the β-catenin binding domain (Siegfried and Kimble, 2002). BAR-1 did interact with POP-1(q645), although at a reduced level compared to wild-type POP-1 (Figure 3C, bottom line). Furthermore, SYS-1 interacted with an N-terminal POP-1 fragment of 200 amino acids but not with a C-terminal fragment of 385 amino acids (Figure 3D). Finally, we assayed truncated versions of SYS-1 and found that most of the protein was required for its interaction with POP-1 (Figure 3E). These results correspond to similar findings for vertebrate β-catenin (Behrens et al., 1996; Graham et al., 2000) and *Drosophila* armadillo (van de Wetering et al., 1997). We conclude that SYS-1 interacts with POP-1 and depends on the specific amino acid sequence of the β-catenin binding domain.

SYS-1 Is a Transcriptional Coactivator of POP-1

Canonical β-catenins are transcriptional coactivators of TCF (reviewed in Willert and Nusse, 1998). To ask whether SYS-1 could act similarly, we employed a tissue culture assay designed to test TCF transcriptional activity (Molenaar et al., 1996; van de Wetering et al., 1997). This assay uses the 8×TOPFLASH reporter (Veeman et al., 2003), which harbors a minimal promoter with eight TCF binding sites and the luciferase-coding region. Previous work showed that this assay can be used to monitor POP-1 activity (Korswagen et al., 2000). We found luciferase expression to be low or undetectable in cells transfected with the reporter plasmid alone or with the reporter plus plasmids encoding either POP-1 or SYS-1 singly; however, robust expression occurred when the reporter was transfected together with both POP-1- and SYS-1-encoding plasmids (Figure 3F). The ability of POP-1 and SYS-1 to promote reporter expression required the TCF binding sites: no expression was seen using the 8×FOPFLASH promoter that has defective TCF binding sites (Figure 3F). We conclude that SYS-1 stimulates POP-1/TCF to activate transcription.

Model: SYS-1, a Limiting Coactivator of POP-1

Figure 4A diagrams a model to explain how SYS-1/β-catenin may control cell fates. This model is based on several lines of evidence. First, POP-1 can repress target genes in nuclei with high POP-1 (Calvo et al., 2001; Maduro et al., 2002); second, POP-1 can activate target genes together with BAR-1/β-catenin (Herman, 2001; Korswagen et al., 2000); third, SYS-1/β-catenin can function as a coactivator of POP-1 in tissue culture cells (Figure 3F); fourth, nuclear POP-1 decreases in daughters that require POP-1 (Herman, 2001; Siegfried et al., 2004); fifth, SYS-1 does not regulate POP-1 asymmetry but is required for directing the same cell fate as POP-1 and upstream Wnt/MAPK regulators (Miskowski et al., 2001; Siegfried and Kimble, 2002; Siegfried et al., 2004).

We suggest that POP-1 can exist in the nucleus as either of two major forms and that their relative abundance dictates expression of target genes. When nuclear POP-1 is abundant (blue proximal daughter cell), most POP-1 is not complexed with its SYS-1 coactivator, and target genes are repressed. By contrast,

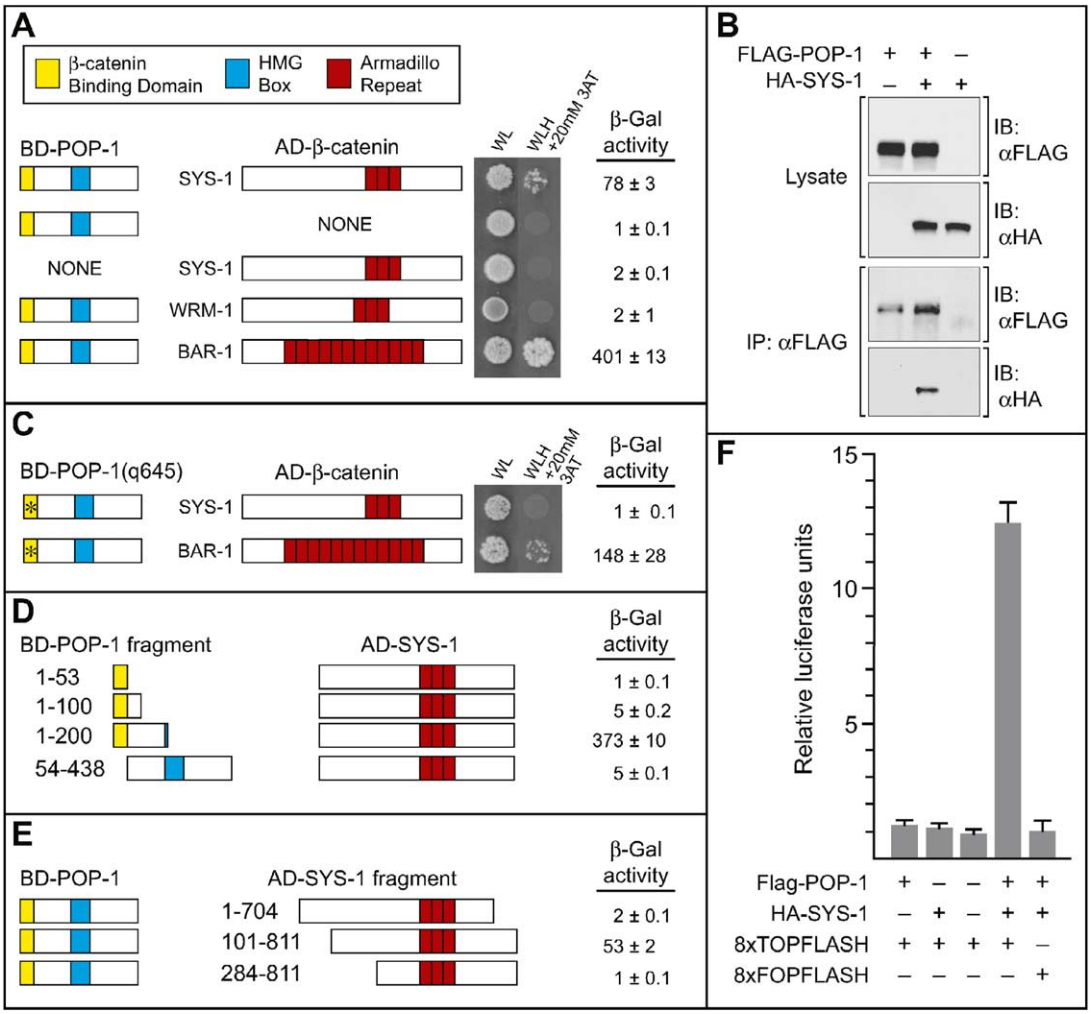


Figure 3. SYS-1 and POP-1 Interact Physically and Activate Transcription

(A) POP-1 interacts with SYS-1. POP-1 was fused to LexA DNA binding domain (BD) and SYS-1, WRM-1, and BAR-1 were fused to Gal4 activation domain (AD). Interactions were tested using growth assay and β-galactosidase assay. SYS-1 and POP-1 together activate reporters, but neither does so alone. POP-1 interacts with BAR-1 but not WRM-1. Protein motifs: yellow, β-catenin binding domain; blue, HMG box; red, armadillo repeat.

(B) SYS-1 CoIPs with POP-1. Cells were transfected with epitope-tagged versions of POP-1 and SYS-1, as indicated. Top two panels, POP-1 and SYS-1 are present in lysates; bottom two panels, lysates were incubated with α-FLAG antibody, proteins immunoprecipitated (IP), and POP-1 and SYS-1 analyzed by immunoblot (IB) using antibodies to respective epitope tags.

(C) SYS-1/POP-1 interaction abolished by mutation in β-catenin binding domain. *pop-1(q645)* changes a conserved aspartate to glutamate in the β-catenin binding domain and reduces POP-1 function (Siegfried and Kimble, 2002). POP-1(q645) does not interact with SYS-1 but does interact, albeit weakly, with BAR-1. Asterisk denotes *pop-1(q645)* mutation.

(D) SYS-1 interacts with N-terminal region of POP-1.

(E) Most of SYS-1 is required for POP-1 interaction.

(F) SYS-1 is a POP-1 transcriptional coactivator. NCI-H28 cells were transfected with constructs encoding epitope-tagged versions of POP-1 and SYS-1 plus 8×TOPFLASH reporter, with eight consensus TCF binding sites, or 8×FOPFLASH, with mutant TCF binding sites. Luciferase readings were normalized using control for transfection efficiency (see Experimental Procedures). Error bars represent the standard deviation.

when nuclear POP-1 is reduced (yellow distal cells), most is complexed with SYS-1, and target genes are activated. A key aspect of the model is that availability of SYS-1 is limiting for POP-1 transcriptional activation. We depict this as a low SYS-1 concentration. To address how much SYS-1 is present, we generated SYS-1 antibodies and *sys-1::GFP* transgenes but have not yet been able to detect SYS-1 (A.R.K., unpublished data). Other mechanisms for limiting SYS-1 are clearly possible (see Discussion).

Figures 4B and 4C diagram predictions of the model. One prediction is that a decrease in already limiting amounts of wild-type SYS-1/β-catenin will decrease transcriptional activation by POP-1 (Figure 4B). A second prediction is that an increase in wild-type SYS-1/β-catenin will ectopically transform POP-1 into its activated state (Figure 4C). We have tested both predictions in nematodes and by the TOPFLASH reporter assay in tissue culture cells.

SYS-1 Dosage Controls Cell Fate

To test the idea that SYS-1/β-catenin may be a limiting coactivator of POP-1/TCF, we examined the effect of changing *sys-1* gene dosage on cell fate. The wild-type

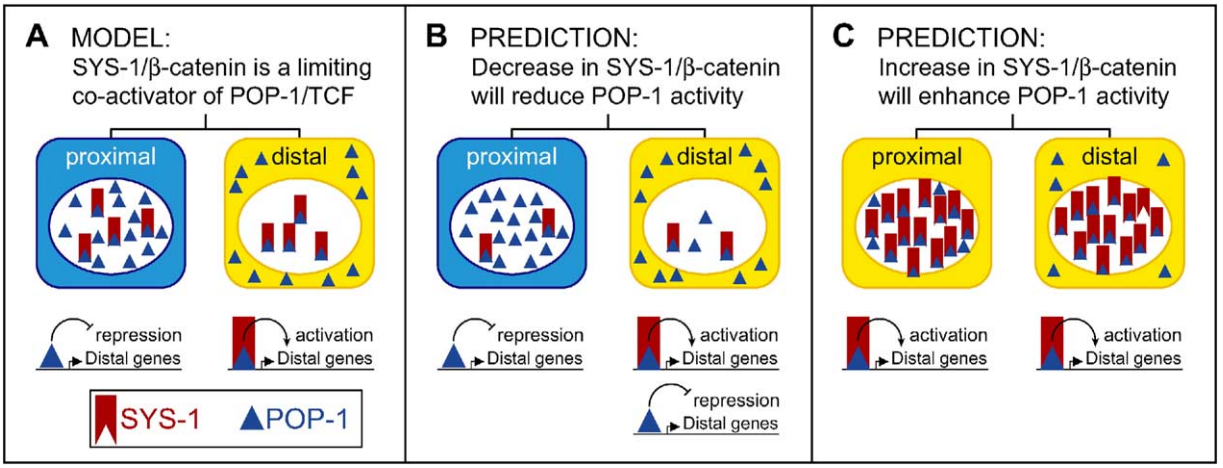


Figure 4. Model and Predictions

(A–C) Each diagram depicts SGP asymmetric division with nucleus as large white circle; SYS-1, red tooth; POP-1, blue triangle. Proposed effect on putative target gene is depicted at bottom.

(A) Model: SYS-1/β-catenin is a limiting coactivator of POP-1/TCF. In this model, we suggest that SYS-1 is expressed at a low level in both daughter cells and that the amount of available SYS-1 determines whether POP-1 functions as a transcriptional activator or repressor. In the proximal daughter (light blue), most POP-1 (dark-blue triangles) is not in a complex with SYS-1, and therefore most POP-1 cannot activate transcription. In distal daughter (yellow), most nuclear POP-1 exists in complex with SYS-1 and activates transcription. See text for additional explanation.

(B) Prediction: decrease in SYS-1 reduces transcriptional activation. In distal daughter, a SYS-1 decrease results in fewer complexes that contain both POP-1 and SYS-1, which in turn leads to decreased activation of genes required for distal fate. Proximal daughter remains unaffected.

(C) Prediction: increase in SYS-1 elevates transcriptional activation. In proximal cell, an increase in SYS-1 results in more complexes with both POP-1 and SYS-1, which leads to ectopic activation of distal-specific target genes. Distal cell remains unaffected.

gonadal primordium possesses two somatic gonadal precursors (SGPs) (Figure 5A). Each SGP divides asymmetrically to generate distinct daughter cells. The distal SGP daughter produces a distal tip cell (DTC), and the proximal daughter produces a cell with potential to become an anchor cell (AC). However, lateral signaling selects one of two potential ACs as the AC (Kimble, 1981). Therefore, the wild-type gonad possesses two DTCs and one AC, which can be visualized with DTC- and AC-specific reporter transgenes (Figures 5A and 5C). By contrast, animals depleted for *sys-1*—either *sys-1(q544)* homozygotes or *sys-1(RNAi)* animals—possess no DTCs and can have extra ACs (Figures 5B and 5D; Miskowski et al., 2001; data not shown).

To decrease *sys-1* dosage, we examined heterozygous animals that possessed one wild-type *sys-1* gene and one putative *sys-1* null mutation. The mutation employed was the *sys-1(q736)* internal deletion, hereafter called *sys-1(0)* (Figure 1B). A significant fraction of *sys-1(0)*/+ heterozygotes made only a single DTC instead of the normal two DTCs (10%, n = 82) (Figure 5B). This result confirmed previous results with *sys-1(q544)* or *qDf14*, a deficiency that removes multiple genes, including *sys-1* (8% and 10%, respectively) (Siegfried et al., 2004). Therefore, *sys-1* is a haploinsufficient locus, consistent with the idea that SYS-1 is present at a limiting concentration.

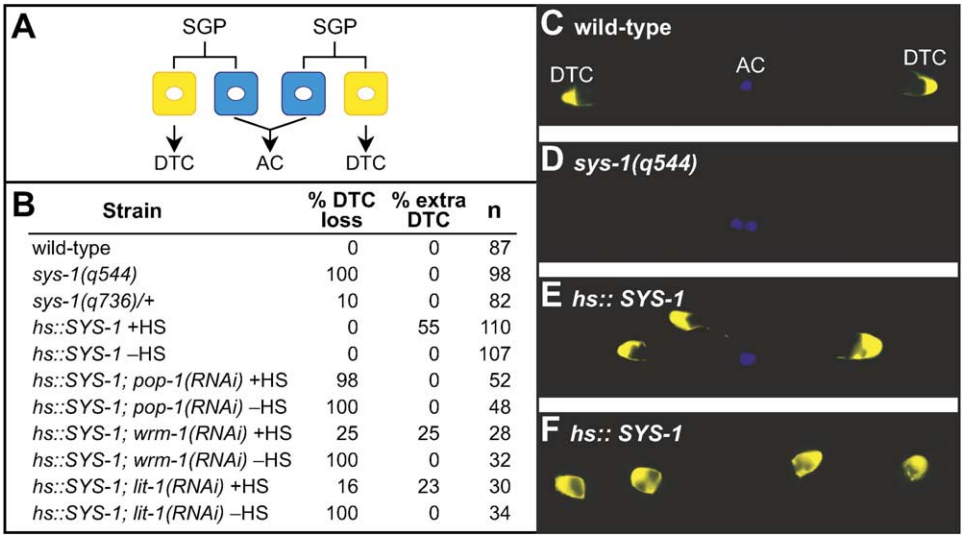
To increase *sys-1* expression, we generated transgenic animals carrying a *hs::SYS-1* transgene (*sys-1* cDNA driven by a heat shock promoter). Without heat shock, animals carrying *hs::SYS-1* had no extra DTCs (0%, n = 107), but after heat shock, many had extra DTCs (55%, n = 110) (Figures 5B, 5E, and 5F). The production of two or more extra DTCs correlated with loss of the anchor cell (Figure 5F). Therefore, an increase in *sys-1* expression leads to an increased ability of SGPs to generate daughters with distal fates and a decreased ability to generate daughters with proximal fates.

We also analyzed the ability of *hs::SYS-1* to drive extra DTCs in animals treated with RNAi to deplete POP-1, WRM-1, or LIT-1 (Figure 5B). After heat shock, the *hs::SYS-1* transgene specified virtually no DTCs in *pop-1(RNAi)* animals, but it generated more DTCs than normal in *wrm-1(RNAi)* and *lit-1(RNAi)* animals (Figure 5B). Control experiments confirmed RNAi treatment to be effective: in the absence of heat shock, the animals made virtually no DTCs (Figure 5B). We conclude that the ability of *hs::SYS-1* to drive extra DTCs does not require WRM-1 or LIT-1 but does require POP-1.

In summary, a decrease in *sys-1* gene dosage drives distal cells toward the proximal fate (consistent with the prediction in Figure 4B), and ectopic SYS-1/β-catenin expression drives proximal cells toward the distal fate (consistent with the prediction in Figure 4C).

SYS-1/β-Catenin Abundance Is Critical for POP-1 Activity

As a complementary test of the model, we changed the levels of SYS-1 and POP-1 in tissue culture cells. Robust reporter expression was obtained by transfecting cells with equal molar amounts of plasmids encoding POP-1 and SYS-1 (Figure 3F). We first transfected cells with four different levels of the two plasmids but maintained their relative ratios at 1:1. Activation was simi-



B

Strain	% DTC loss	% extra DTC	n
wild-type	0	0	87
sys-1(q544)	100	0	98
sys-1(q736)/+	10	0	82
hs::SYS-1 +HS	0	55	110
hs::SYS-1 –HS	0	0	107
hs::SYS-1; pop-1(RNAi) +HS	98	0	52
hs::SYS-1; pop-1(RNAi) –HS	100	0	48
hs::SYS-1; wrm-1(RNAi) +HS	25	25	28
hs::SYS-1; wrm-1(RNAi) –HS	100	0	32
hs::SYS-1; lit-1(RNAi) +HS	16	23	30
hs::SYS-1; lit-1(RNAi) –HS	100	0	34

Figure 5. Specification of Cell Fate Depends on Dosage of SYS-1/β-Catenin

(A) SGP division and subsequent fates. Distal daughters (yellow) both generate distal tip cells (DTC). Proximal daughters (light blue) both generate an anchor cell (AC) precursor, but only one AC is produced due to lateral signaling.

(B) Summary of tests for SYS-1 dose sensitivity. % DTC loss, percentage of animals with fewer than two DTCs; % extra DTC, percentage of animals with more than two DTCs; n, number of animals scored. +HS, heat-shocked; –HS, not heat-shocked.

(C–F) DTCs and ACs in L3 hermaphrodites. DTCs were marked with *lag-2::YFP* and ACs with *cdh-3::CFP*. Some images represent multiple focal planes fused into a single image. (C) Wild-type. One DTC is located at distal end of each gonadal arm, one extending anteriorly and the other posteriorly. The AC resides midventrally. (D) *sys-1(q544)* homozygote. No DTCs are present, but two ACs reside midventrally, as reported previously (Miskowski et al., 2001). (E) *hs::SYS-1*. Three DTCs (two anterior, one posterior) and one AC. (F) *hs::SYS-1*. Four DTCs (two anterior, two posterior) and no ACs.

larly high after transfection using 125, 250, or 500 ng of each plasmid but was not seen at 50 ng (Figure 6A). This experiment defined the working range that we used to vary relative abundance.

We then increased POP-1 relative to SYS-1. The SYS-1-encoding plasmid was transfected at a limiting concentration (either 125 or 250 ng) together with an excess of the POP-1-encoding plasmid (500 ng). In each case, excess POP-1 lowered expression when compared to equally transfected controls (Figure 6B). We finally compared reporter activation with transfections maintained at a high level of POP-1 (500 ng) but increasing levels of SYS-1 (125, 250, and 500 ng). When SYS-1 was transfected at a low level, reporter expression was low; when transfected at an intermediate level, expression was intermediate; and when transfected at a high level, expression was high (Figure 6C). We conclude that the relative levels of the POP-1- and SYS-1-encoding plasmids are crucial for TOPFLASH reporter expression. The simplest explanation is that more POP-1 than SYS-1 lowers expression and that increasing the number of POP-1/SYS-1 complexes leads to a corresponding increase in transcriptional activation.

Discussion

This work reports the existence of a new β-catenin in *C. elegans* and provides evidence that SYS-1/β-catenin may function as a limiting coactivator of POP-1/TCF. The significance of our findings is 2-fold. First, identification of SYS-1 as a β-catenin opens the door for identification of additional β-catenins by functional rather than sequence criteria. Second, our work suggests a mechanism that unifies the canonical model of Wnt signaling (reviewed in Logan and Nusse, 2004; van Es et al., 2003) with the noncanonical model of Wnt/MAPK signaling (reviewed in Herman and Wu, 2004; Korswagen, 2002). We suggest that this unified mechanism may be broadly used in the control of animal development.

SYS-1 Is a Functional β-Catenin

Several clues suggested that SYS-1 might function as a β-catenin despite having a novel amino acid sequence. SYS-1 is required for Wnt/MAPK signaling (Siegfried et al., 2004); *sys-1* and *pop-1* mutations interact genetically, suggesting a physical relationship (Siegfried et al., 2004); and the SYS-1 sequence possesses three divergent armadillo repeats (this work). We therefore asked if SYS-1 might function as a β-catenin and found it to have both molecular and functional properties of a classical β-catenin. SYS-1 can rescue a *bar-1* null mutant, it binds POP-1 via its N-terminal β-catenin binding domain, and it coactivates POP-1 in tissue culture reporter assays. At the current time, we do not know if SYS-1 represents a nematode-specific class of β-catenins or if it is a pioneer that will lead to the discovery of a new class of functional β-catenins. Database searches using the SYS-1 amino acid sequence identify convincing homologs in two other nematodes, *C. briggsae* and *C. remanei*, but not in other phyla. However, it remains plausible, and perhaps even likely, that identification of additional proteins with SYS-1/

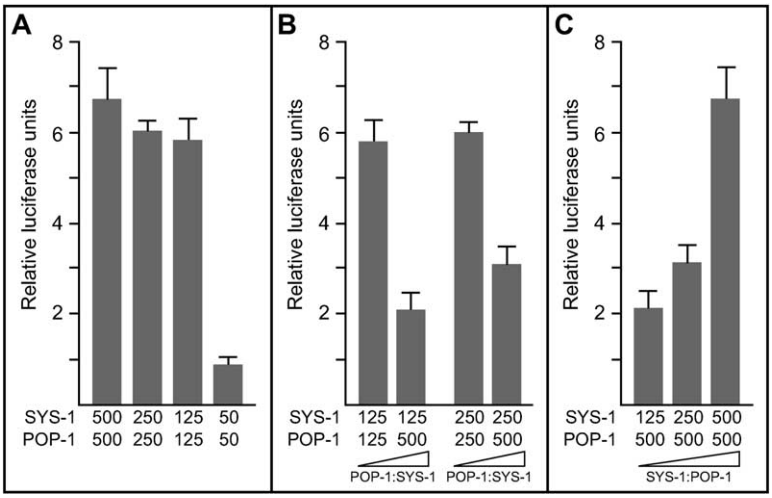


Figure 6. Relative Abundance of SYS-1/β-Catenin and POP-1 Controls Transcriptional Activity

NCI-H28 cells transfected with constructs encoding epitope-tagged versions of POP-1, SYS-1, and the 8×TOPFLASH reporter construct. Construct amounts are indicated below graph; all numbers refer to ng DNA. Luciferase readings were normalized using control for transfection efficiency (see Experimental Procedures). Error bars represent the standard deviation.

(A) POP-1/SYS-1 working range. POP-1 and SYS-1 constructs were transfected at 1:1 ratio to determine working range for further experiments. A minimum of 125 ng of each construct was necessary for reporter activation.

(B) Excess POP-1 inhibits SYS-1. A limiting amount of SYS-1 plasmid was transfected with an equal or excess amount of POP-1 plasmid. Excess POP-1 resulted in decreased reporter activation (bar 1 versus bar 2; bar 3 versus bar 4).

(C) Results with constant POP-1 plasmid and increasing SYS-1 plasmid, from limiting to equal amounts. SYS-1 increase results in a corresponding increase in reporter expression.

β-catenin-like properties will rely on functional assays rather than amino acid sequence.

Wnt Signaling Variants in *C. elegans*

Wnt signaling controls diverse events in *C. elegans*, including neuroblast migration, early embryonic fate, and gonadal axis formation (reviewed in Korswagen et al., 2002). To control these events, both canonical and noncanonical variants of the Wnt pathway are used (Figure 7A). A canonical pathway governs Q neuroblast migration (Maloof et al., 1999), and a noncanonical Wnt/MAPK pathway regulates early embryogenesis (Meneghini et al., 1999; Rocheleau et al., 1997; Rocheleau et al., 1999; Shin et al., 1999; Thorpe et al., 1997). In this latter case, WRM-1/β-catenin acts with LIT-1/nemo-like kinase (NLK) to downregulate POP-1/TCF, and as a result, target genes are relieved from POP-1 repression (Calvo et al., 2001; Maduro et al., 2002). In this paper, we report a variant of the pathway, which we tentatively call the SYS pathway. This variant brings together elements of the canonical and Wnt/MAPK pathways with our current understanding of SYS-1 (Figure 7, see below). The SYS pathway therefore unifies disparate views of Wnt signaling.

Figure 7B depicts the SYS pathway as it relates to cell-fate specification after an asymmetric division, and Figure 7C presents the SYS pathway in more generic form. In the context of the asymmetric division, one daughter, the left one in Figure 7B, does not receive the Wnt/MAPK signal, and POP-1/TCF therefore remains at a high level in the nucleus. In this situation, we suggest that the majority of POP-1 is not complexed with SYS-1. As a result, POP-1 can either actively repress its target genes, as shown, or they can fail to be activated. The other daughter receives the Wnt/MAPK signal, and therefore POP-1 is exported from the nucleus after phosphorylation by LIT-1/NLK (Lo et al., 2004; Meneghini et al., 1999; Rocheleau et al., 1999; Shin et al., 1999). In this case, we suggest that POP-1/SYS-1 complexes are now in the majority inside the nucleus and that they activate target genes. Consistent with this reasoning, SYS-1 overexpression requires POP-1 activity, but not WRM-1 or LIT-1 activity, to drive ectopic fates. Such a SYS pathway can be used either in asymmetric divisions, as shown in Figure 7B, or in populations of cells, depending on how the pathway is triggered.

The SYS pathway of *C. elegans* employs two β-catenins with distinct molecular functions that work together to activate transcription (Figures 7B and 7C). Both WRM-1 and SYS-1 β-catenins specify the same biological outcome, the distal fate in early gonads (Miskowski et al., 2001; Siegfried and Kimble, 2002). WRM-1 promotes the exit of POP-1/TCF from the nucleus in both the SGP distal daughter and daughters of many other asymmetric divisions (Rocheleau et al., 1999; Siegfried et al., 2004), while SYS-1 is a POP-1/TCF coactivator and is not required for POP-1/TCF exit from the nucleus (this work; Siegfried et al., 2004).

Our model suggests that SYS-1 availability is limiting. Several lines of evidence support this notion. First, *sys-1* is a haploinsufficient locus (this work; Siegfried et al., 2004). In *sys-1* heterozygotes, both SGP daughters can adopt the same fate—the fate not dependent on Wnt/MAPK signaling. By contrast, *sys-1* overexpression drives specification of the opposite fate. The simplest explanation is that SYS-1 protein abundance is a key factor in the cell-fate decision, with a low level resulting in one fate and a high level driving the alternative fate. Second, the relative abundance of SYS-1/β-catenin and POP-1 is crucial for expression of a transgene driven by a promoter with TCF binding sites (Figure 6). If POP-1 and SYS-1 were transfected at equal levels, expression was activated, even at widely different concentrations. However, when SYS-1 was transfected at a limiting level, increases in POP-1 led to decreases in reporter expression. By contrast, when POP-1 was transfected at a high level so that POP-1 was not limiting, increases in SYS-1 caused corresponding increases in reporter expression. Therefore, both genetic and molecular results support the idea

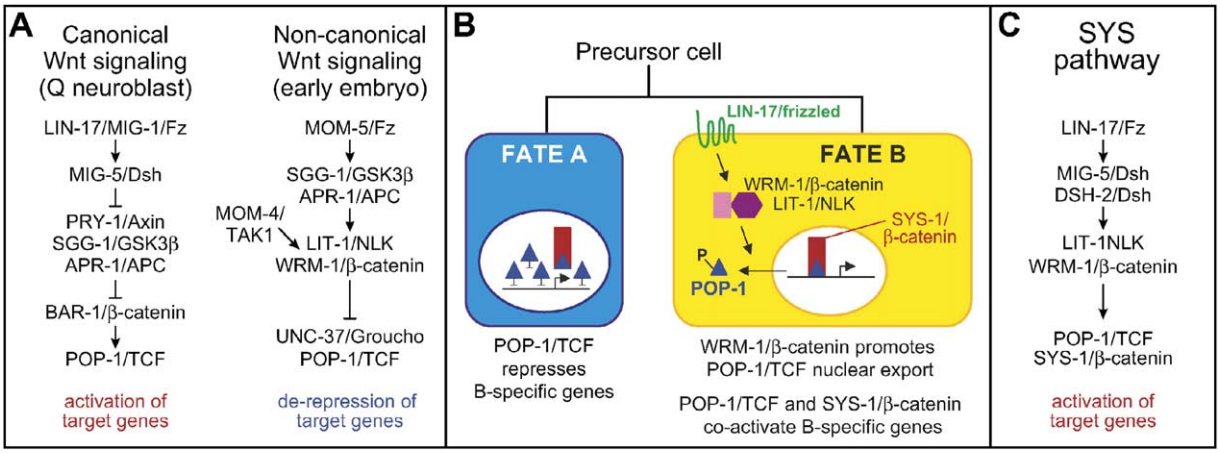


Figure 7. SYS Pathway, a Variant of Wnt Signaling

(A) Two Wnt signaling pathways in *C. elegans*. Left, canonical Wnt pathway. Right, Wnt/MAPK pathway. Q neuroblasts employ the classical β-catenin, BAR-1, to coactivate POP-1/TCF. The early embryo employs a divergent β-catenin, WRM-1, to relieve POP-1/TCF-mediated repression. See text for explanation and references.

(B) SYS variant of Wnt signaling. The precursor cell divides asymmetrically to generate two distinct daughters. Without Wnt signaling, Fate A is specified by either POP-1 repression of B-specific target genes as shown or by lack of POP-1 activation of B-specific genes. With Wnt signaling, Fate B is specified by SYS pathway. WRM-1/β-catenin and LIT-1/NLK promote POP-1 exit from the nucleus, and SYS-1/β-catenin and POP-1 coactivate B-specific target genes. SYS-1/β-catenin, red tooth; POP-1, blue triangle; WRM-1/β-catenin, pink rectangle; LIT-1, purple hexagon; LIN-17, green membrane protein.

(C) SYS signaling unifies the canonical and Wnt/MAPK forms of Wnt signaling (see text for explanation and references).

that SYS-1 abundance is critical for POP-1 activity. We emphasize that the model put forward is only one relatively simple possibility. The amount of available active SYS-1/β-catenin may be governed by some other mechanism, including sequestration or posttranslational modification.

The classical view of the Wnt pathway is that signaling facilitates formation of a β-catenin/TCF complex, which then activates transcription (reviewed in Waltzer and Bienz, 1999; Willert and Nusse, 1998). And the classical mechanism for facilitating formation of that complex is the regulated stabilization of β-catenin (reviewed in Waltzer and Bienz, 1999; Willert and Nusse, 1998). This work provides a different mechanism by which Wnt signaling can ensure transcriptional activation by a β-catenin/TCF complex. By this different mechanism, the amount of TCF transcription factor is reduced in the nucleus to accommodate the limiting availability of its β-catenin coactivator.

Is the SYS Pathway of Broad Significance?

The SYS pathway controls the *C. elegans* SGP asymmetric division and formation of the gonadal proximal-distal axis (this work; Miskowski et al., 2001; Siegfried and Kimble, 2002; Siegfried et al., 2004). But does it have broader significance? In *C. elegans,* SYS-1/β-catenin also governs the T cell asymmetric division (Siegfried et al., 2004; H.S., unpublished data) as well as poorly defined events in larval development and embryogenesis (Fraser et al., 2000; Simmer et al., 2003). Furthermore, the *sys-1* null phenotype is embryonic lethal (this work): *sys-1(0)* embryos make hundreds of cells that fail to undergo morphogenesis, and the *sys-1* null phenotype is similar to that of a *pop-1* deletion (A.R.K., P. Kroll-Conner, and J.K., unpublished data). These data suggest that the SYS pathway or modified forms of that pathway may govern many asymmetric divisions in *C. elegans*. Therefore, Wnt/MAPK signaling can act by either relieving POP-1 repression, as in EMS (Calvo et al., 2001; Maduro et al., 2002), or facilitating formation of a POP-1/SYS-1 complex, as in SGPs, the T cell, and perhaps other asymmetric divisions (this work).

The importance of the SYS pathway for vertebrate Wnt/MAPK signaling remains unknown. In *Xenopus* embryos and mammalian tissue culture systems, NLK can phosphorylate TCF and downregulate its activity (Ishitani et al., 2003; Ishitani et al., 1999). In zebrafish, depletion of NLK using morpholinos supports the opposite idea—that NLK acts as a positive regulator of Wnt signaling (Thorpe and Moon, 2004). These two apparently contradictory results may be resolved by consideration of Wnt/MAPK signaling in *C. elegans*, which can either relieve POP-1 repression or promote POP-1 activation with SYS-1. A similar plasticity may be used in other organisms, including vertebrates. If true, what β-catenin might serve as the coactivator in vertebrates? Two simple possibilities exist. Perhaps a novel β-catenin similar to SYS-1 awaits identification. Alternatively, perhaps the canonical β-catenin is present at a vanishingly low concentration that can only be used for TCF coactivation when the nuclear TCF concentration is lowered.

Experimental Procedures

Nematode Analyses

The *sys-1(q736)* deletion was made by standard methods (Kraemer et al., 1999). The *sys-1(os63)* mutation was isolated in a screen for mutants that do not have phasmid socket cells (Sawa et al., 2000). Strains were derivatives of wild-type Bristol strain N2 and were

maintained at 20°C. We used many mutant strains to fine map and clone *sys-1* (details are available upon request) and the following transgenes: *qIs70 [lag-2::YFP insertion]* (this work), *syIs57 [cdh-3::CFP insertion]* (Inoue et al., 2002a; Inoue et al., 2002b), *qEx500 [hs::SYS-1]* (this work), *qEx502 [bar-1::SYS-1]* (this work). For heat shock experiments, N2 (wild-type) or JK3426 (*qEx500; qIs70; syIs57*) L1 larvae were subjected to two intervals of heat treatment (1 hr at 33°C, 2 hr recovery at 20°C, and 30 min at 33°); in parallel, animals were maintained at 20°C as controls.

sys-1 Cloning and Transcript Analysis

sys-1(q7) was mapped by standard methods; transgenic rescue identified T23D8.9, and zygotic RNAi (Herman, 2001) to T23D8.9 generated Sys progeny. To identify *sys-1* molecular lesions, T23D8.9 DNA was amplified from *sys-1* homozygotes and sequenced from at least three independent PCR products for each allele. To confirm identification of *sys-1(q544)* as a silent mutation, we sequenced DNA from the strain mutagenized to generate *q544* and from a *fog-3* mutant obtained in the same mutagenesis; both were wild-type. Based on standard cDNA analyses and Northern blots, *sys-1* encodes one major SL1 *trans*-spliced transcript corresponding to T23D8.9a.

Plasmids and Other Molecular Methods

Plasmids include pJK719 (*sys-1* cDNA), pJK932 (SYS-1-AD), pJK1004 (POP-1-BD), pJK1005 (BAR-1-AD), pJK1006 (*bar-1::SYS-1*), and pJK1013 (HA-SYS-1); details available upon request. Other constructs were available commercially or described previously: pRL-TK (Promega); pCDNA3 (Invitrogen); p1995, a modified version of pCDNA3, was a gift of B. Sugden, University of Wisconsin–Madison; pCDNA3-FLAG-POP-1 and pCDNA3-MYC-POP-1 (Korswagen et al., 2000) were gifts of H. Korswagen (Netherlands Institute for Developmental Biology); pSUPER8×TOPFLASH and pSUPER8×FOPFLASH (Veeman et al., 2003) were gifts of R. Moon (University of Washington); pDE280, pDE290, pDE204, and pJBW1 (Natarajan et al., 2001) were gifts of D. Eisenmann (University of Maryland, Baltimore County).

In yeast two-hybrid assays, we used PJ69-4a for pDE290 and L40 for others. After transformation, multiple independent TRP^+ LEU^+ colonies were transferred to SC –TRP –LEU media and grown at 30°C until OD600 reached 0.5. Five microliters of a 1:5 dilution of each culture were then seeded onto SC –TRP –LEU and SC –TRP –LEU –HIS +20 mM 3AT (3-amino-1,2,4-triazole) plates to select for HIS reporter expression. *lacZ* expression was assayed using Beta-glo system (Promega). At least four independent transformants were tested for each strain. Luciferase was measured using the Wallac MicroBeta Trilux liquid scintillation counter (Perkin Elmer).

For coimmunoprecipitations, we used HEK293 T cells (provided by T. Compton, University of Wisconsin–Madison); details available upon request. To assess POP-1 and SYS-1 for transcriptional activity, we used NCI-H28 cells (ATCC) and standard procedures (Usami et al., 2003). Briefly, transfected cells were harvested after a 48 hr incubation, and samples were analyzed using Dual Luciferase Reporter Assay System (Promega). Raw data for luciferase were normalized using data obtained from transfection control renilla luciferase.

Supplemental Data

Supplemental Data include one figure and are available with this article online at http://www.cell.com/cgi/content/full/121/5/761/DC1/.

Acknowledgments

We are grateful to R. Barstead, T. Compton, A. Coulson, D. Eisenmann, A. Fire, H. Korswagen, R. Moon, B. Sugden, M. Wickens, and the *Caenorhabditis* Genetics Center (CGC) for reagents. Kimble lab members gave helpful comments on the manuscript. We thank P. Kroll-Conner and H. Takeshita for technical assistance and A. Helsley-Marchbanks and L. Vanderploeg for manuscript and figure preparation. A.R.K. was supported by National Institutes of Health Predoctoral Training Grant T32 GM07215 in Molecular Biosciences and K.R.S. by T32 GM07133 to the Laboratory of Genetics. J.K. is an investigator with the Howard Hughes Medical Institute (HHMI).

Received: February 4, 2005
Revised: March 15, 2005
Accepted: March 30, 2005
Published: June 2, 2005

References

Behrens, J. (2000). Cross-regulation of the Wnt signalling pathway: a role of MAP kinases. J. Cell Sci. *113*, 911–919.

Behrens, J., von Kries, J.P., Kuhl, M., Bruhn, L., Wedlich, D., Grosschedl, R., and Birchmeier, W. (1996). Functional interaction of beta-catenin with the transcription factor LEF-1. Nature *382*, 638–642.

Bienz, M. (1998). TCF: transcriptional activator or repressor? Curr. Opin. Cell Biol. *10*, 366–372.

Brantjes, H., Roose, J., van De Wetering, M., and Clevers, H. (2001). All Tcf HMG box transcription factors interact with Groucho-related co-repressors. Nucleic Acids Res. *29*, 1410–1419.

Brantjes, H., Barker, N., van Es, J., and Clevers, H. (2002). TCF: Lady Justice casting the final verdict on the outcome of Wnt signalling. Biol. Chem. *383*, 255–261.

Brunner, E., Peter, O., Schweizer, L., and Basler, K. (1997). *pangolin* encodes a Lef-1 homologue that acts downstream of Armadillo to transduce the Wingless signal in *Drosophila*. Nature *385*, 829–833.

Calvo, D., Victor, M., Gay, F., Sui, G., Luke, M.P.-S., Dufourcq, P., Wen, G., Maduro, M., Rothman, J., and Shi, Y. (2001). A POP-1 repressor complex restricts inappropriate cell type-specific gene transcription during *Caenorhabditis elegans* embryogenesis. EMBO J. *20*, 7197–7208.

Cavallo, R.A., Cox, R.T., Moline, M.M., Roose, J., Polevoy, G.A., Clevers, H., Peifer, M., and Bejsovec, A. (1998). *Drosophila* Tcf and Groucho interact to repress Wingless signalling activity. Nature *395*, 604–608.

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.

Deshpande, R., Inoue, T., Priess, J.R., and Hill, R.J. (2005). *lin-17*/Frizzled and *lin-18* regulate POP-1/TCF-1 localization and cell type specification during *C. elegans* vulval development. Dev. Biol. *278*, 118–129.

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.

Fraser, A.G., Kamath, R.S., Zipperlen, P., Martinez-Campos, M., Sohrmann, M., and Ahringer, J. (2000). Functional genomic analysis of *C. elegans* chromosome I by systematic RNA interference. Nature *408*, 325–330.

Gleason, J.E., Korswagen, H.C., and Eisenmann, D.M. (2002). Activation of Wnt signaling bypasses the requirement for RTK/Ras signaling during *C. elegans* vulval induction. Genes Dev. *16*, 1281–1290.

Graham, T.A., Weaver, C., Mao, F., Kimelman, D., and Xu, W. (2000). Crystal structure of a β-catenin/Tcf complex. Cell *103*, 885–896.

Hatzfeld, M. (1999). The armadillo family of structural proteins. Int. Rev. Cytol. *186*, 179–224.

Herman, M.A. (2001). *C. elegans* POP-1/TCF functions in a canonical Wnt pathway that controls cell migration and in a noncanonical Wnt pathway that controls cell polarity. Development *128*, 581–590.

Herman, M.A., and Wu, M. (2004). Noncanonical Wnt signaling pathways in C. elegans converge on POP-1/TCF and control cell polarity. Front. Biosci. *9*, 1530–1539.

Huber, O., Korn, R., McLaughlin, J., Ohsugi, M., Herrmann, B.G., and Kemler, R. (1996). Nuclear localization of β-catenin by interaction with transcription factor LEF-1. Mech. Dev. *59*, 3–10.

Inoue, T., Sherwood, D.R., Aspock, G., Butler, J.A., Gupta, B.P., Kirouac, M., Wang, M., Lee, P.Y., Kramer, J.M., Hope, I., et al. (2002a). Gene expression markers for Caenorhabditis elegans vulval cells. Gene Expr. Patterns *2*, 235–241.

Inoue, T., Sherwood, D.R., Aspock, G., Butler, J.A., Gupta, B.P., Kirouac, M., Wang, M., Lee, P.Y., Kramer, J.M., Hope, I., et al. (2002b). Gene expression markers for Caenorhabditis elegans vulval cells. Mech. Dev. *119* (Suppl. 1), S203–S209.

Ishitani, T., Ninomiya-Tsuji, J., Nagai, S., Nishita, M., Meneghini, M., Barker, N., Waterman, M., Bowerman, B., Clevers, H., Shibuya, H., and Matsumoto, K. (1999). The TAK1-NLK-MAPK-related pathway antagonizes signalling between β-catenin and transcription factor TCF. Nature *399*, 798–802.

Ishitani, T., Ninomiya-Tsuji, J., and Matsumoto, K. (2003). Regulation of lymphoid enhancer factor 1/T-cell factor by mitogen-activated protein kinase-related Nemo-like kinase-dependent phosphorylation in Wnt/β-catenin signaling. Mol. Cell. Biol. *23*, 1379–1389.

Kaplan, D.D., Meigs, T.E., Kelly, P., and Casey, P.J. (2004). Identification of a role for beta-catenin in the establishment of a bipolar mitotic spindle. J. Biol. Chem. *279*, 10829–10832.

Kimble, J. (1981). Alterations in cell lineage following laser ablation of cells in the somatic gonad of *Caenorhabditis elegans*. Dev. Biol. *87*, 286–300.

Kimble, J., and Hirsh, D. (1979). The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. Dev. Biol. *70*, 396–417.

Korswagen, H.C. (2002). Canonical and non-canonical Wnt signaling pathways in Caenorhabditis elegans: variations on a common signaling theme. Bioessays *24*, 801–810.

Korswagen, H.C., Herman, M.A., and Clevers, H.C. (2000). Distinct β-catenins mediate adhesion and signalling functions in *C. elegans*. Nature *406*, 527–532.

Korswagen, H.C., Coudreuse, D.Y.M., Betist, M.C., van de Water, S., Zivkovic, D., and Clevers, H.C. (2002). The Axin-like protein PRY-1 is a negative regulator of a canonical Wnt pathway in *C. elegans*. Genes Dev. *16*, 1291–1302.

Kraemer, B., Crittenden, S., Gallegos, M., Moulder, G., Barstead, R., Kimble, J., and Wickens, M. (1999). NANOS-3 and FBF proteins physically interact to control the sperm-oocyte switch in *Caenorhabditis elegans*. Curr. Biol. *9*, 1009–1018.

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 decision in *C. elegans* embryos. Cell *92*, 229–239.

Lo, M.-C., Gay, F., Odom, R., Shi, Y., and Lin, R. (2004). Phosphorylation by the β-catenin/MAPK complex promotes 14–3-3-mediated nuclear export of TCF/POP-1 in signal-responsive cells in *C. elegans*. Cell *117*, 95–106.

Logan, C.Y., and Nusse, R. (2004). The Wnt signaling pathway in development and disease. Annu. Rev. Cell Dev. Biol. *20*, 781–810.

Maduro, M.F., Lin, R., and Rothman, J.H. (2002). Dynamics of a developmental switch: recursive intracellular and intranuclear redistribution of *Caenorhabditis elegans* POP-1 parallels Wnt-inhibited transcriptional repression. Dev. Biol. *248*, 128–142.

Maloof, J.N., Shangbo, J., Harris, J.M., Jongeward, G.D., and Kenyon, C. (1999). A Wnt signaling pathway controls Hox gene expression and neuroblast migration in *C. elegans*. Development *126*, 37–49.

Marchler-Bauer, A., and Bryant, S.H. (2004). CD-Search: protein domain annotations on the fly. Nucleic Acids Res. *32*, W327–W331.

Meneghini, M.D., Ishitani, T., Carter, J.C., Hisamoto, N., Ninomiya-Tsuji, J., Thorpe, C.J., Hamill, D.R., Matsumoto, K., and Bowerman, B. (1999). MAP kinase and Wnt pathways converge to downregulate an HMG-domain repressor in *Caenorhabditis elegans*. Nature *399*, 793–797.

Miskowski, J., Li, Y., and Kimble, J. (2001). The *sys-1* gene and sexual dimorphism during gonadogenesis in *Caenorhabditis elegans*. Dev. Biol. *230*, 61–73.

Molenaar, M., van de Wetering, M., Oosterwegel, M., Peterson-Maduro, J., Godsave, S., Korinek, V., Roose, J., Destrée, O., and Clevers, H. (1996). XTcf-3 transcription factor mediates β-catenin-induced axis formation in Xenopus embryos. Cell *86*, 391–399.

Natarajan, L., Witwer, N.E., and Eisenmann, D.M. (2001). The divergent *Caenorhabditis elegans* β-catenin proteins BAR-1, WRM-1 and HMP-2 make distinct protein interactions but retain functional redundancy *in vivo*. Genetics *159*, 159–172.

Nelson, W.J., and Nusse, R. (2004). Convergence of Wnt, beta-catenin, and cadherin pathways. Science *303*, 1483–1487.

Park, F.D., and Priess, J.R. (2003). Establishment of POP-1 asymmetry in early *C. elegans* embryos. Development *130*, 3547–3556.

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.

Rocheleau, C.E., Yasuda, J., Shin, T.H., Lin, R., Sawa, H., Okano, H., Priess, J.R., Davis, R.J., and Mello, C.C. (1999). WRM-1 activates the LIT-1 protein kinase to transduce anterior/posterior polarity signals in *C. elegans*. Cell *97*, 717–726.

Roose, J., Molenaar, M., Peterson, J., Hurenkamp, J., Brantjes, H., Moerer, P., van de Wetering, M., Destree, O., and Clevers, H. (1998). The Xenopus Wnt effector XTcf-3 interacts with Groucho-related transcriptional repressors. Nature *395*, 608–612.

Sawa, H., Kouike, H., and Okano, H. (2000). Components of the SWI/SNF complex are required for asymmetric cell division in *C. elegans*. Mol. Cell *6*, 617–624.

Shin, T.H., Yasuda, J., Rocheleau, C.E., Lin, R., Soto, M., Bei, Y., Davis, R.J., and Mello, C.C. (1999). MOM-4, a MAP kinase kinase kinase-related protein, activates WRM-1/LIT-1 kinase to transduce anterior/posterior polarity signals in *C. elegans*. Mol. Cell *4*, 275–280.

Siegfried, K., and Kimble, J. (2002). POP-1 controls axis formation during early gonadogenesis in *C. elegans*. Development *129*, 443–453.

Siegfried, K.R., Kidd, A.R., III, Chesney, M.A., and Kimble, J. (2004). The *sys-1* and *sys-3* genes cooperate with Wnt signaling to establish the proximal-distal axis of the *C. elegans* gonad. Genetics *166*, 171–186.

Simmer, F., Moorman, C., van der Linden, A.M., Kuijk, E., van den Berghe, P.V.E., Kamath, R.S., Fraser, A.G., Ahringer, J., and Plasterk, R.H.A. (2003). Genome-wide RNAi of C. elegans using the hypersensitive rrf-3 strain reveals novel gene functions. PLoS Biol. *1*, e12. 10.1371/journal.pbio.0000012

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

Thorpe, C.J., and Moon, R.T. (2004). *nemo-like kinase* is an essential co-activator of Wnt signaling during early zebrafish development. Development *131*, 2899–2909.

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.

Thorpe, C.J., Schlesinger, A., and Bowerman, B. (2000). Wnt signalling in Caenorhabditis elegans: regulating repressors and polarizing the cytoskeleton. Trends Cell Biol. *10*, 10–17.

Usami, N., Sekido, Y., Maeda, O., Yamamoto, K., Minna, J.D., Hasegawa, Y., Yoshioka, H., Imaizumi, M., Ueda, Y., Takahashi, M., and Shimokata, K. (2003). β-catenin inhibits cell growth of a malignant mesothelioma cell line, NCI-H28, with a 3p21.3 homozygous deletion. Oncogene *22*, 7923–7930.

van de Wetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Loureiro, J., Ypma, A., Hursh, D., Jones, T., Bejsovec, A., et al. (1997). Armadillo coactivates transcription driven by the product of the Drosophila segment polarity gene dTCF. Cell *88*, 789–799.

van Es, J.H., Barker, N., and Clevers, H. (2003). You Wnt some, you

lose some: oncogenes in the Wnt signaling pathway. Curr. Opin. Genet. Dev. *13*, 28–33.

van Noort, M., and Clevers, H. (2002). TCF transcription factors, mediators of Wnt-signaling in development and cancer. Dev. Biol. *244*, 1–8.

Veeman, M.T., Slusarski, D.C., Kaykas, A., Louie, S.H., and Moon, R.T. (2003). Zebrafish prickle, a modulator of noncanonical Wnt/Fz signaling, regulates gastrulation movements. Curr. Biol. *13*, 680–685.

Waltzer, L., and Bienz, M. (1999). The control of β-catenin and TCF during embryonic development and cancer. Cancer Metastasis Rev. *18*, 231–246.

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

Wodarz, A., and Nusse, R. (1998). Mechanisms of Wnt signaling in development. Annu. Rev. Cell Dev. Biol. *14*, 59–88.

Zeng, Y.A., and Verheyen, E.M. (2004). Nemo is an inducible antagonist of Wingless signaling during Drosophila wing development. Development *131*, 2911–2920.