Developmental Cell, Vol. 7, 831–841, December, 2004, Copyright ©2004 by Cell Press

Multiple Wnt Signaling Pathways Converge to Orient the Mitotic Spindle in Early *C. elegans* Embryos

Timothy Walston,[1] Christina Tuskey,[2] Lois Edgar,[4] Nancy Hawkins,[5] Gregory Ellis,[6] Bruce Bowerman,[6] William Wood,[4] and Jeff Hardin[1,2,3,*]

[1]Laboratory of Genetics
[2]Program in Cellular and Molecular Biology
[3]Department of Zoology
University of Wisconsin-Madison
Madison, Wisconsin 53706
[4]Department of Molecular, Cellular, and Developmental Biology
University of Colorado-Boulder
Boulder, Colorado 80309
[5]Department of Molecular Biology and Biochemistry
Simon Fraser University
Burnaby, British Columbia V5A 1S6
Canada
[6]Institute of Molecular Biology
University of Oregon
Eugene, Oregon 97403

Summary

How cells integrate the input of multiple polarizing signals during division is poorly understood. We demonstrate that two distinct *Caenorhabditis elegans* Wnt pathways contribute to the polarization of the ABar blastomere by differentially regulating its duplicated centrosomes. Contact with the C blastomere orients the ABar spindle through a nontranscriptional Wnt spindle alignment pathway, while a Wnt/β-catenin pathway controls the timing of ABar spindle rotation. The three *C. elegans* Dishevelled homologs contribute to these processes in different ways, suggesting that functional distinctions may exist among them. We also find that CKI (KIN-19) plays a role not only in the Wnt/β-catenin pathway, but also in the Wnt spindle orientation pathway as well. Based on these findings, we establish a model for the coordination of cell-cell interactions and distinct Wnt signaling pathways that ensures the robust timing and orientation of spindle rotation during a developmentally regulated cell division event.

Introduction

During development, certain cell divisions must occur with a specific orientation to form complex structures and body plans. In many cases, the polarizing input for oriented divisions involves Wnt signaling (reviewed in Shulman et al., 1998). One example of such division involves neuroblasts in *Drosophila melanogaster*, in which the first division of the pI sensory organ precursor cell is under the control of Frizzled (Fz) and Dishevelled (Dsh) (Bellaiche et al., 2001; Gho and Schweisguth, 1998).

*Correspondence: jdhardin@wisc.edu

The orientation of blastomere divisions in the early *Caenorhabditis elegans* embryo (Figure 1A) has also been shown to require Wnt signaling (reviewed in Goldstein, 2000; Herman, 2002; Korswagen, 2002). In the 4-cell embryo, the EMS blastomere is induced by its posterior neighbor, the P2 blastomere (Goldstein, 1993, 1995). This induction has two consequences: it specifies the fates of EMS daughter cells (Goldstein, 1993, 1995) and properly positions the mitotic spindle of EMS (Schlesinger et al., 1999; Thorpe et al., 1997). Although both processes are under the control of Wnt signaling, they are controlled through divergent pathways (Rocheleau et al., 1997; Schlesinger et al., 1999). When EMS divides, the anterior daughter, MS, gives rise to progeny that are primarily mesodermal, and the posterior daughter, E, produces all of the endoderm (Sulston et al., 1983). The fates of MS and E are controlled in part by a Wnt signaling pathway that regulates the activity of the Tcf/Lef transcription factor, POP-1, in conjunction with the β-catenin WRM-1 (Lin et al., 1995; Rocheleau et al., 1997; Thorpe et al., 1997). WRM-1 interacts with POP-1 through a cofactor, LIT-1, a NEMO-like kinase that is activated through a parallel mitogen-activated protein kinase (MAPK) pathway (Kaletta et al., 1997; Meneghini et al., 1999; Rocheleau et al., 1999; Shin et al., 1999). We will refer to pathways that utilize a β-catenin to alter transcription as Wnt/β-catenin pathways. Removal of some components of the Wnt/β-catenin pathway alters the fates of the two EMS daughters (Lin et al., 1995, 1998; Rocheleau et al., 1997; Thorpe et al., 1997).

Although the fate of the EMS daughters is controlled by a Wnt/β-catenin pathway, the orientation of the EMS division is controlled by a different Wnt pathway. In wild-type embryos, the EMS spindle initially aligns along the left/right (L/R) axis and rotates to adopt an anterior/posterior (A/P) orientation during the initial stages of mitosis (Hyman and White, 1987). In embryos that lack the function of certain Wnt signaling components, the EMS spindle often sets up in the proper orientation but fails to rotate along the A/P axis until the onset of anaphase. In some cases, the delayed spindle rotates dorsoventrally (D/V) before it adopts the proper A/P alignment (Schlesinger et al., 1999; Thorpe et al., 1997). The Wnt spindle orientation pathway that controls EMS orientation involves a Wnt (MOM-2), Porcupine (Porc; MOM-1), and Fz (MOM-5) (Schlesinger et al., 1999; Thorpe et al., 1997). GSK-3, the *C. elegans* GSK-3β homolog, has been reported to act positively downstream of the Fz receptor to regulate EMS spindle positioning, rather than as a downregulator of β-catenin accumulation as observed with Wnt/β-catenin signaling (Schlesinger et al., 1999). Indeed, Wnt/β-catenin signaling components downstream of GSK-3 are not involved in controlling EMS spindle alignment, and EMS spindle alignment occurs independently of gene transcription (Edgar et al., 1994; Powell-Coffman et al., 1996; Schlesinger et al., 1999). We will refer to pathways such as the one that positions the spindle in EMS, which utilize

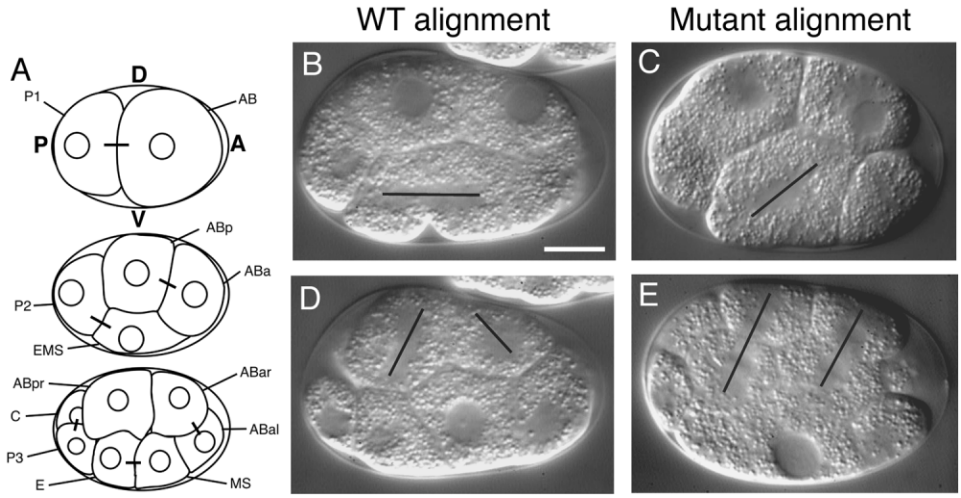

Figure 1. Defects in Alignment of the EMS and ABar Spindles

(A) A schematic diagram of blastomere positioning in the *C. elegans* embryo. Top, 2-cell stage; middle, 4-cell stage; bottom, 8-cell stage. Lines between blastomeres indicate sister cells resulting from previous cell division. All embryos are shown in lateral views with anterior to the right and dorsal at the top in order to visualize the right-hand side of the embryo. ABpl is located behind the plane of view on the left side of the 8-cell embryo.

(B–E) Nomarski time-lapse images of wild-type or mutant embryos. Black lines indicate spindle position.

(B) In wild-type embryos, the division of the EMS blastomere is in an anterior-posterior (A/P) orientation.

(C) In *dsh-2(or302)* embryos, the EMS spindle often orients such that it is not aligned along the A/P axis (shown dorsoventrally skewed). Prior to division, the spindle rotates into a wild-type, A/P orientation.

(D) In wild-type embryos, ABar divides perpendicularly and transversely to ABpr.

(E) In a *dsh-1(RNAi)*; *dsh-2(or302)*; *mig-5(RNAi)* embryo, ABar divides parallel to ABpr.

Scale bar equals 10 μm.

GSK-3 but are independent of transcription, as Wnt spindle orientation pathways.

Although many Wnt signaling components have been identified that participate in spindle orientation, the role of the Dsh family has not been clearly characterized. The Dsh family proteins transmit Wnt signals received from Fz receptors. The Dshs use three domains (DIX, PDZ, and DEP) to interact with different downstream proteins and activate multiple Wnt pathways specifically (reviewed in Wharton, 2003). The *C. elegans* genome contains three Dsh family genes that possess the three conserved domains: *dsh-1, dsh-2,* and *mig-5*. Transcripts of *dsh-2* and *mig-5* are at similar, enriched levels in the 4- and 8-cell embryo based on microarray analysis, while *dsh-1* levels are low (Baugh et al., 2003).

Another molecule involved in Wnt signaling is Casein Kinase I (CKI) (reviewed in Polakis, 2002). CKI has been shown to prime β-catenin for degradation by phosphorylating it at a specific serine residue (Amit et al., 2002; Hagen and Vidal-Puig, 2002; Liu et al., 2002; Sakanaka, 2002; Yanagawa et al., 2002). Once primed, the β-catenin can be further phosphorylated and targeted for destruction by GSK-3β (Hagen et al., 2002). CKI has also been shown to bind and phosphorylate Dsh and may assist in inhibiting GSK-3β when Wnt signaling is active (Amit et al., 2002; Gao et al., 2002; Kishida et al., 2001; Lee et al., 2001; McKay et al., 2001; Peters et al., 1999). Loss of function of the CKIα homolog, *kin-19,* causes defects in the fate of EMS daughter cells (Peters et al., 1999). Although the role of CKI in spindle alignment has not been examined, CKIα localizes to centrosomes and mitotic spindles in vertebrate systems (Brockman et al., 1992).

A pathway involving MES-1, a receptor tyrosine kinase, and SRC-1, a Src family tyrosine kinase, acts redundantly with Wnt signaling with respect to the fate of EMS daughters and the orientation of the EMS spindle (Bei et al., 2002). When a Src pathway member and a member of the Wnt spindle orientation pathway are removed simultaneously, the EMS spindle fails to rotate into the proper A/P position prior to division and remains misaligned throughout division. Removal of Src pathway members also enhances endoderm fate specification defects observed following removal of Wnt/β-catenin pathway members. Spindle orientation defects in *dsh-2(RNAi);mig-5(RNAi)* embryos have not been reported unless the Src pathway is also removed; however, Bei et al. (2002) only reported defects in cell division orientation, as opposed to abnormalities in initial spindle positioning.

In addition to regulating the orientation of the EMS division, four of the *mom* (more mesoderm) genes, *mom-1* (Porc), *mom-2* (Wnt), *mom-5* (Fz), and *mom-3* (uncloned), cause spindle alignment defects in the ABar blastomere of the 8-cell embryo (Rocheleau et al., 1997; Thorpe et al., 1997). Three of the four AB granddaughters, ABal, ABpl, and ABpr, divide with spindle orientations that are parallel to one another. ABar divides in an orientation that is roughly perpendicular to the other three, an event best viewed from the right side of the embryo, placing anterior to the right (Figure 1). When the function of one of the above *mom* genes is removed, ABar divides parallel to the other AB granddaughters, resulting in mispositioning of its daughter cells, such that ABarp, the wild-type posterior daughter cell, adopts a position that is anterior to its sister, ABara. The source

of the polarizing cue(s) that orients the division of ABar is unclear. However, using blastomere isolations, Park and Priess (2003) demonstrated that C, MS, and E are all competent to align the spindle and generate asymmetric expression of POP-1 within unidentified, dividing AB granddaughters, suggesting that one or more of these cells could produce signals that orient the division of ABar in vitro.

In this study, we demonstrate the role of two Wnt signaling pathways involved in regulating the mitotic spindle. First, the nontranscriptional Wnt spindle alignment pathway requires contact from the C blastomere to align the spindle of ABar. We show that the three Dshs differentially participate in aligning the spindles of EMS and ABar and vary with respect to their interaction with the Src signaling pathway during spindle orientation. Moreover, while KIN-19 participates in endoderm induction through the Wnt/β-catenin pathway (Peters et al., 1999), we demonstrate that it also acts in the Wnt spindle orientation pathway. Second, a Wnt/β-catenin pathway regulates the timing of spindle rotation in ABar, presumably by specifying the fate of neighboring blastomeres. Taken together, these studies indicate that spindle orientation during early development is a tightly regulated event, influenced by multiple cues transmitted via redundant pathways.

Results

Multiple Dishevelleds Act Redundantly along with CKI to Orient the EMS Spindle

The spindle of EMS initially sets up along the L/R axis and then rotates to divide in an A/P orientation (Figure 1B). When certain Wnt signaling components, such as *mom-2*/Wnt, *mom-5*/Fz, and *gsk-3*, are removed, the EMS spindle often sets up with a D/V orientation or maintains the L/R orientation until immediately prior to division, when it belatedly rotates into the proper orientation (Figure 1C).

Removal of the function of each *dsh* individually results in spindle orientation defects in EMS. Progeny of *dsh-2(or302)* mothers display the highest penetrance (41%) of orientation defects of the EMS spindle, while both *mig-5(RNAi)* and *dsh-1(RNAi)* embryos show a lower penetrance (10% and 8%, respectively) (Table 1). This suggests that each Dsh participates in positioning the EMS spindle.

Redundancy between the three Dshs could explain why a Dsh has not been identified in screens targeting spindle alignment and cell fate mutants. To test for redundancy between the three Dshs, pairwise removal of gene function was performed. The most highly penetrant defects occur in *dsh-2(or302)*; *mig-5(RNAi)* embryos (48%) (Table 1). Simultaneous removal of *dsh-1* function via RNAi has essentially no effect on the frequency of defects in *dsh-2(or302)* embryos (35%) or *mig-5(RNAi)* embryos (14%). When all three Dshs are depleted, 45% of embryos have EMS spindles that set up incorrectly, which correlates well with reported data for the removal of other Wnt components (Schlesinger et al., 1999; Thorpe et al., 1997).

A Src family tyrosine kinase, *src-1*, acts in a pathway parallel to Wnt signaling during EMS spindle orientation (Bei et al., 2002). Bei et al. demonstrated that removal of the function of *dsh-2* and *mig-5* by RNAi in a *src-1* mutant resulted in nearly completely penetrant defects in both endoderm formation and EMS spindle orientation. To determine the genetic interaction of each *dsh* gene with *src-1*, the functions of the *dsh* genes were removed along with *src-1* (Table 1). When the function of either *mig-5* or *dsh-1* is removed by RNAi together with *src-1*, the percentage of embryos with EMS spindle defects remains essentially unchanged (65% for *mig-5* and 52% for *dsh-1*) compared with *src-1* alone (57%). Surprisingly, *src-1(RNAi)*; *dsh-2(or302)* embryos display 100% EMS spindle defects. In contrast, removal of *dsh-2* activity has no effect on endoderm formation in a *src-1(RNAi)* background; only *mig-5(RNAi); src-1(RNAi)* results in endoderm induction defects (22%). Thus, while MIG-5 and DSH-2 act in both processes, MIG-5 is more important for endoderm specification, while DSH-2 plays the primary role in alignment of the EMS spindle. Since these two proteins are expressed at similar levels throughout the embryo at this stage of development, these data suggest that they may have distinct activities.

kin-19/CKI acts in the Wnt/β-catenin pathway to control specification of the EMS daughter, E, which generates endoderm (Peters et al., 1999). We found that removal of the function of *kin-19* by RNAi also results in 40% of embryos with EMS spindle misalignment, suggesting a role for KIN-19 in the Wnt spindle orientation pathway as well (Table 1). Since KIN-19 is a downstream member of the Wnt spindle orientation pathway, we examined the localization of KIN-19 in order to determine where it may be acting in order to regulate spindle orientation. Anti-CKIα antibodies localize to centrosomes and P granules (Figures 2A–2C), both common locations for nonspecific binding in early embryos. However, in *kin-19(RNAi)* embryos, the centrosomal staining is absent (Figure 2D), suggesting that it localizes to centrosomes.

DSH-2 Is Localized to the Cell Cortex in the Early Embryo

Because DSH protein localizes to the cell cortex in cells that are known to receive Wnt signals (Axelrod et al., 1998; Rothbacher et al., 2000), we examined DSH-2 localization in the early embryo via antibody staining. DSH-2 has a predominantly cytoplasmic localization through the early 4-cell stage. During the late 4-cell stage, it redistributes to the cortex of most blastomeres but is enriched at the boundary between P2 and EMS through the early 6-cell stage (Figure 2E). This localization is consistent with P2 signaling to EMS to specify endoderm fate and EMS spindle orientation. Between the late 6- and 32-cell stages, DSH-2 staining is evenly distributed at the cortex of all cells, including all AB granddaughters (data not shown). Staining for DSH-2 is absent in *dsh-2(or302)* embryos (data not shown). Cong et al. (2004) demonstrated that *Xenopus* CKIε regulates cortical localization of *Xenopus* Dvl-GFP in HEK 293 and NIH 3T3 cell lines. In contrast, we found that removal of the function of *kin-19* by RNAi does not result in a loss of DSH-2 enrichment between P2 and EMS (data not shown).

Table 1. Defects in EMS

Embryo	% EMS Spindle Defects	n	% Endoderm Specification Defects	n
dsh-1(RNAi)[a]	8	88	0	102
dsh-2[b]	41	22	0	57
mig-5(RNAi)	10	21	0	54
dsh-1(RNAi)[c]*;dsh-2*[b]	35	34	0	81
dsh-1(RNAi);mig-5(RNAi)[d]	14	35	0	100
dsh-2[b]*;mig-5(RNAi)*	48	25	0	109
dsh-1(RNAi)[d]*;mig-5(RNAi);dsh-2*[b]	45	22	0	67
kin-19(RNAi)	40	21	nd	
src-1(RNAi)	57	23	0	111
src-1(RNAi);dsh-1(RNAi)[d]	52	24	0	74
src-1(RNAi);dsh-2[b]	100	22	0	75
src-1(RNAi);mig-5(RNAi)	65	52	22	60
src-1(RNAi);dsh-2(RNAi);mig-5(RNAi)	nd		100	43
src-1(RNAi);gsk-3(RNAi)	92	25	nd	

nd = not determined
[a] Sum of results obtained with the following *dsh-1* clones: yk291a11, yk397b6, and yk653h6.
[b] Spindle defects examined using *dsh-2(or302)*; endoderm specification defects examined using *dsh-2(RNAi)*.
[c] Sum of results obtained with the following *dsh-1* clones: yk397b6 and yk653h6.
[d] Result only from the *dsh-1* clone yk397b6.

Multiple Dishevelleds Act Redundantly in Spindle Orientation of the ABar Blastomere

While the pathways that contribute to spindle orientation dynamics in EMS have been investigated, the pathways that orient the spindle of the ABar blastomere are less clear. In wild-type embryos, ABar divides perpendicular to the other AB granddaughters (Figure 1D; Supplemental Figure S1 at http://www.developmentalcell.com/cgi/content/full/7/6/831/DC1/). In some *mom* mutants, ABar divides parallel to the other AB granddaughters (Figure 1E; Supplemental Figure S1; Thorpe et al., 1997). Proteins downstream of Fz have not been shown to be involved in orienting the spindle of ABar.

We found that the three *C. elegans* Dsh proteins act redundantly during orientation of the ABar spindle. Individually, only *dsh-2(or302)* and *mig-5(RNAi)* embryos show misalignment of the ABar spindle, with 38% and 52% penetrance, respectively (Table 2). These results suggest that, contrary to EMS, in which DSH-2 appears to play the most important role, MIG-5 is most important in aligning the spindle of ABar. When the function of the *dsh* genes are removed pairwise, their redundant activity is evident. The misalignment defect is nearly completely penetrant (97%) in *dsh-1(RNAi)*; *mig-5(RNAi)* embryos and is completely penetrant in *dsh-2(or302)*; *mig-5(RNAi)* embryos. Curiously, there is no enhancement of the frequency of ABar spindle misalignment in *dsh-1(RNAi); dsh-2(or302)* embryos. Together, these data suggest that *mig-5* may function differently in ABar spindle orientation than the other two *dsh*s. When the function of all three *dsh* genes is removed, spindle orientation in ABar is defective in almost all cases (95%). Significantly, this result demonstrates that misalignment of the ABar spindle is not due to steric effects of a misaligned EMS spindle, since only 45% of these embryos had EMS spindle orientation defects (Table 1).

A Wnt Spindle Orientation Pathway Controls Alignment of the ABar Spindle

Because Dsh is a branch point for several Wnt signaling pathways, we examined components downstream of Dsh to determine the pathway(s) controlling alignment of the ABar spindle. RNAi of *jnk-1*, a component of the JNK/planar cell polarity pathway, does not result in any defects in the ABar spindle (Table 2). In other systems, a β-catenin destruction complex, composed of GSK3β, APC, and axin, is downstream of Dsh in Wnt/β-catenin signaling. Although this complex has not been shown to act during spindle alignment in the early *C. elegans* embryo, we examined homologs of each gene. When the function of *apr-1/APC* and *pry-1/axin* are removed by RNAi individually or pairwise, the alignment of the ABar spindle is unaffected (Table 2). Because Dsh inhibits the destruction complex, if ABar spindle alignment involves the Wnt/β-catenin pathway, removing any component of the complex might overcome defects due to loss of Dsh function. However, when *mig-5(RNAi)* or *dsh-2(or302)* are combined with either *pry-1(RNAi)* or *apr-1(RNAi)*, the frequency of ABar spindle misalignment is identical to that seen following removal of the function of either *dsh* gene alone. Therefore, it is unlikely that *apr-1* and *pry-1* function during alignment of the ABar spindle.

In the early *C. elegans* embryo, the β-catenin WRM-1 functions downstream of the destruction complex and interacts with the Tcf/Lef transcription factor, POP-1. This interaction is dependent on LIT-1, a MAPK pathway member, that complexes with WRM-1 to bind POP-1. Removal of any of these three proteins should give phenotypes similar to those seen in *dsh* mutants if the misalignment of ABar is transduced through Wnt/β-catenin signaling. However, following RNAi of either *wrm-1* or *lit-1*, the ABar spindle ultimately divides in the wild-type orientation (Table 2), demonstrating that the *dshs* do not act via a Wnt/β-catenin pathway to control spindle orientation.

GSK-3 could function in either the Wnt/β-catenin pathway or the Wnt spindle alignment pathway. When *gsk-3* function is removed by RNAi, the ABar spindle is misaligned in all cases. This suggests that GSK-3 does not act in the destruction complex, but rather as a positive regulator of spindle orientation, as in EMS (Schlesinger et

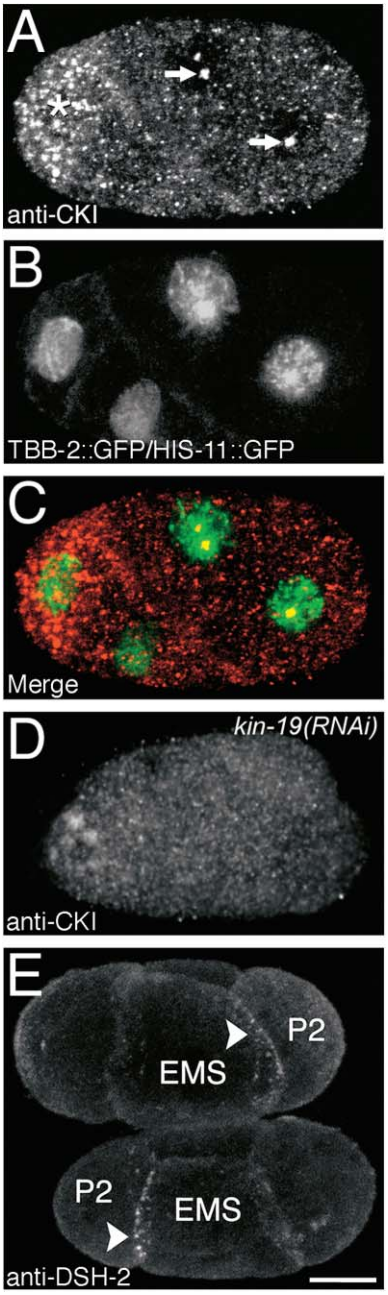

Figure 2. KIN-19/CKI Localizes to Centrosomes and DSH-2 Accumulates between P2 and EMS

(A–C) Localization of KIN-19 in the 4-cell embryo.

(A) Antibody staining using an anti-human CKI antibody reveals KIN-19 localization at centrosomes during mitosis (arrows) and P granules (asterisk).

(B) TBB-2/β-tubulin::GFP and HIS-11::GFP localize to microtubules and condensed chromosomes, respectively.

(C) Merged image of anti-CKI (red) and TBB-2::GFP/HIS-11::GFP (both green).

(D) In *kin-19(RNAi)* embryos, anti-CKI staining is not detectable at centrosomes and is reduced in P granules.

(E) DSH-2 localizes to the cell cortex and is enriched at the boundary between P2 and EMS in the four-cell embryo (arrowheads). Scale bar equals 10 μm.

al., 1999). Protein phosphatase 2A (PP2A) interacts with axin, APC, and β-catenin and can dephosphorylate GSK-3β targets (Ikeda et al., 2000; Seeling et al., 1999; Willert et al., 1999). Unfortunately, RNAi of the catalytic subunit of PP2A, *let-92*, is uninformative due to defects in the 1-cell embryo (data not shown).

As seen in EMS, removal of *kin-19* function by RNAi results in embryos with ABar spindle misalignment (96%; Table 2). This suggests that in ABar, spindle orientation is regulated by both KIN-19 and GSK-3 through the β-catenin-independent, Wnt spindle alignment pathway.

src-1 and Spindle Alignment in ABar

Since removal of *src-1* strongly enhances Wnt-dependent spindle alignment defects in EMS (Bei et al., 2002), we examined the role of *src-1* during the alignment of the ABar spindle. Removal of *src-1* by RNAi results in weakly penetrant ABar spindle misalignment phenotypes (19%) (Table 2). When the function of *src-1* and *mig-5* are removed together by RNAi, the frequency of ABar spindle misalignment (65%) is only slightly increased from the frequency of *mig-5(RNAi)* alone (52%). The frequency of embryos with spindle misalignment when both *src-1* and *dsh-1* function are removed by RNAi is only slightly higher (22%) than that seen following *src-1(RNAi)* alone. As with EMS, *src-1(RNAi)* in *dsh-2(or302)* mutants strongly enhances the frequency of misalignment of the ABar spindle (96%). Therefore, SRC-1 appears to act in parallel with the Dshs, especially DSH-2, to control orientation of the ABar spindle.

The Wnt Spindle Orientation Pathway in ABar Requires Interaction with C

Since ABar spindle orientation is presumably controlled by a localized Wnt signal, we sought to identify the source of this signal. One candidate is the C blastomere. Shortly after C is born, it establishes contact with ABar (Figures 3A–3B′). The fate of C is largely controlled by the Caudal homolog, PAL-1 (Hunter and Kenyon, 1996). Manipulation of the fate of C via *pal-1* RNAi was used to determine whether C contributes to ABar spindle alignment. The ABar spindle is misaligned in 15% of *pal-1(RNAi)* embryos (n = 13). Interestingly, in the cases of ABar misalignment, the C blastomere is positioned such that it never contacts ABar (Figures 3C and 3C′). A similar result is also observed by laser killing the ABp blastomere (Figures 3D and 3D′). In 100% of the embryos in which the ABp carcass prevents contact between ABar and C, the ABar spindle is misaligned. If C maintains the ability to touch ABar in the proper location, the ABar spindle aligns in the wild-type orientation (7/8; data not shown). Interestingly, in 100% of ABp laser-killed embryos in which C contacts ABar in an incorrect location, the ABar spindle aligns with the site of ectopic C contact (n = 5; data not shown). These data strongly suggest that the alignment of the ABar spindle is regulated by its contact with C.

The interaction of astral microtubules with the cortex is thought to influence spindle rotation in the early zygote (Hyman and White, 1987). We therefore used β-tubulin::GFP (Strome et al., 2001) to examine the positioning of centrosomes and mitotic spindles in AB

Table 2. Spindle Defects in ABar

Embryo	% Embryos with ABar Spindle Misalignment	% Embryos with Delayed Rotation of ABar Spindle	n
mom-5(RNAi)	100	nd	21
dsh-1(RNAi)[a]	0	0	88
dsh-2(or302)	38	0	21
mig-5(RNAi)	52	0	25
dsh-1(RNAi);dsh-2(or302)[b]	40	0	38
dsh-1(RNAi);mig-5(RNAi)[c]	97	nd	36
dsh-2(or302);mig-5(RNAi)	100	nd	29
dsh-1(RNAi);mig-5(RNAi);dsh-2(or302)[c]	95	nd	22
jnk-1(RNAi)	0	0	21
kin-19(RNAi)	96	0	22
apr-1(RNAi)	0	0	26
pry-1(RNAi)	0	0	29
gsk-3(RNAi)	100	nd	22
apr-1(RNAi);pry-1(RNAi)	0	0	33
apr-1(RNAi);gsk-3(RNAi)	100	nd	22
gsk-3(RNAi);pry-1(RNAi)	100	nd	28
pry-1(RNAi);mig-5(RNAi)	57	0	30
apr-1(RNAi);mig-5(RNAi)	50	0	36
apr-1(RNAi);dsh-2(or302)	41	0	17
wrm-1(RNAi)	0	30	20
lit-1(RNAi)	0	36	25
wrm-1(RNAi);dsh-2(or302)	40	30	20
wrm-1(RNAi);apr-1(RNAi)	0	30	20
wrm-1(RNAi);gsk-3(RNAi)	100	nd	29
pop-1(RNAi)	6	23	35
ama-1(RNAi)	0	30	22
src-1(RNAi)	19	0	28
src-1(RNAi);dsh-1(RNAi)[c]	22	0	27
src-1(RNAi);dsh-2(or302)	96	0	25
src-1(RNAi);mig-5(RNAi)	65	0	54
src-1(RNAi);gsk-3(RNAi)	90	0	23

nd = not determined, unable to score due to ~100% spindle misalignment in Abar.
[a] Sum of results obtained with the following *dsh-1* clones: yk291a11, yk397b6, and yk653h6.
[b] Sum of results obtained with the following *dsh-1* clones: yk397b6 and yk653h6.
[c] Results only from the *dsh-1* clone yk397b6.

granddaughters to see if similar events regulate ABar spindle positioning. In wild-type embryos, the duplicated centrosomes of ABar align parallel to the other AB granddaughters. The centrosome closest to the ABar-C contact quickly rotates into the proper orientation prior to mitosis (Figures 4A–4D; see Supplemental Movie S1). In contrast, in embryos in which the ABar spindle is misaligned, the centrosomes of ABar align parallel to the other AB granddaughters but fail to rotate to the proper perpendicular position (Figures 4E–4H; see Supplemental Movie S1).

Finally, we examined the role of right/left asymmetries in regulating the contact of C with a specific AB granddaughter, and hence its spindle orientation. Embryonic handedness is controlled by *gpa-16*, a Gα protein (Bergmann et al., 2003). Mutations in *gpa-16* result in randomization of the left-right axis. In embryos with a reversed left-right axis, ABal divided perpendicular to the other three AB granddaughters, rather than ABar (12/12). This suggests that both ABal and ABar are competent to receive polarizing signals from C, and their placement within the embryo determines which one contacts C.

A Wnt/β-Catenin Pathway Controls the Timing of Spindle Alignment in the ABar Blastomere

Although the Wnt/β-catenin pathway does not determine the final orientation of the ABar spindle, we found that it does control the timing of spindle rotation in ABar. In wild-type embryos, the spindle of ABar initially aligns parallel to the spindles of the other AB-derived cells but rotates to the proper perpendicular position prior to mitosis (Figures 4A–4D; see Supplemental Movie S1). In 30% of *wrm-1(RNAi)* embryos and 36% of *lit-1(RNAi)* embryos, the rotation of the ABar spindle is delayed until mid- to late anaphase (Figures 4I–4L; Table 2; Supplemental Figure S1). Analysis of spindle dynamics using β-tubulin::GFP in *wrm-1(RNAi)* embryos uncovers a variety of defects, including a "wandering centrosome" phenotype, in which the centrosomes do not rotate directly to their final positions. Instead, the centrosome that eventually aligns closest to MS displays random movements around the nucleus. This random movement delays the alignment of the ABar spindle, until it ultimately rotates into position at anaphase (compare Figure 4D to 4L). The timing of other mitotic events, including nuclear breakdown and cytokinesis, are not affected, suggesting that the phenotype does not represent a cell cycle defect.

To confirm that delayed spindle rotation is regulated transcriptionally through Wnt/β-catenin signaling, *pop-1* function was removed via RNAi. In 23% of *pop-1(RNAi)* embryos, rotation of the ABar spindle is delayed (Table 2). The ABar spindle fails to rotate in 6% of the *pop-1(RNAi)* embryos; in these cases, a slight mispositioning

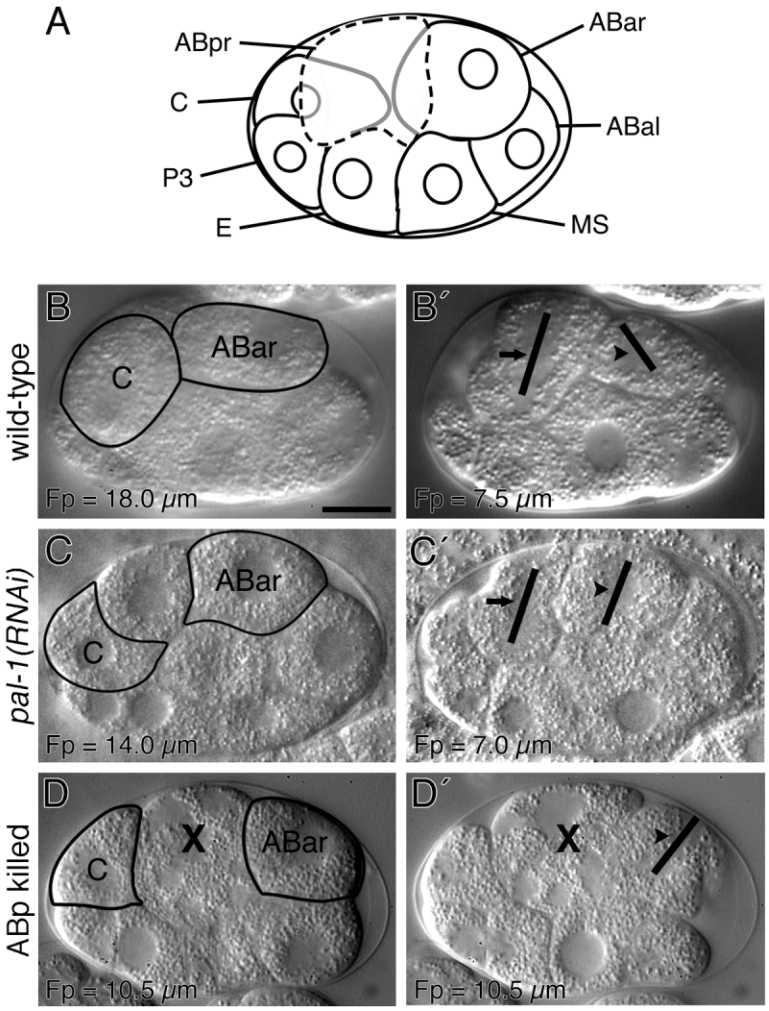

Figure 3. Contact with the C Blastomere Aligns the Spindle in ABar

(A) Schematic diagram of the embryo prior to the division of the AB granddaughter cells. ABpr (dotted outline) and ABar reside on the right side of the embryo. C and ABar make contact (light gray outline) medial to ABpr.

(B) In a wild-type embryo, ABar and C come into contact shortly after C is born.

(B′) The ABar spindle (arrowhead) rotates toward the contact between ABar and C, which results in a division nearly perpendicular to ABpr (arrow).

(C) In some *pal-1(RNAi)* embryos, there is no contact between ABar and C.

(C′) In these embryos, ABar divides parallel to ABpr.

(D) When ABp is laser killed, the ABp carcass (marked by the X) acts as a steric impediment that prevents contact between ABar and C.

(D′) This results in ABar dividing in a misaligned orientation (parallel to ABal, not shown).

(B–D) Focal plane showing closest C-ABar contact in each embryo, t = 0 min.

(B′–D′) Focal plane showing orientation of the ABar spindle, t = 5 min. Focal position (Fp) is relative to the coverslip.

Scale bar equals 10 μm.

of blastomeres occurs, such that C no longer contacts ABar (data not shown). If POP-1 activates transcription of genes required to direct the timing of spindle rotation, abolishing zygotic transcription should mimic results obtained with *pop-1(RNAi)*. RNAi against the gene *ama-1*, the largest subunit of RNA polymerase, abrogates gene transcription in the embryo (Powell-Coffman et al., 1996; Schlesinger et al., 1999). In 30% of *ama-1(RNAi)* embryos, rotation of the ABar spindle is delayed. These results suggest that a Wnt/β-catenin pathway transcriptionally controls the timing of ABar spindle rotation. Upstream components cannot be examined directly because removal of these components results in ABar spindle misalignment, making it impossible to score for delayed rotation phenotypes.

To determine if the spindle misalignment and delayed rotation are separable phenotypes or represent different expressivity of the same phenotype, *wrm-1* function was removed by RNAi along with the function of genes required for proper spindle alignment. *dsh-2(or302)*; *wrm-1(RNAi)* embryos display both phenotypes at the expected frequencies, assuming that the delayed rotation phenotype is epistatic to the misalignment phenotype (Table 2). When both *gsk-3* and *wrm-1* function are removed by RNAi, the ABar spindle is always misaligned. These results suggest that spindle misalignment and delayed rotation are two separable phenotypes controlled by two divergent Wnt pathways.

Discussion

Previous studies have identified several downstream effectors of Wnt signaling that are required to orient the spindle of the EMS blastomere (Schlesinger et al., 1999; Thorpe et al., 1997). Our analysis identifies a role for each of the three *dsh* genes and *kin-19/CKI* in the orientation of the spindles of both the EMS and ABar blastomeres. We have also clarified the roles of both the Wnt/β-catenin and Wnt spindle orientation signaling pathways in the control of ABar spindle positioning. Our results provide new insights into how multiple signals contribute to cell division.

The Dshs and KIN-19 Regulate Orientation of the EMS Spindle

Wnt signals in the early embryo are transmitted from P2 to EMS to orient its spindle and to specify the fate of the EMS daughters (Figure 5). The orientation of the spindle relies on Wnt ligands, including MOM-2, which are secreted from P2 and activate MOM-5/Fz on the

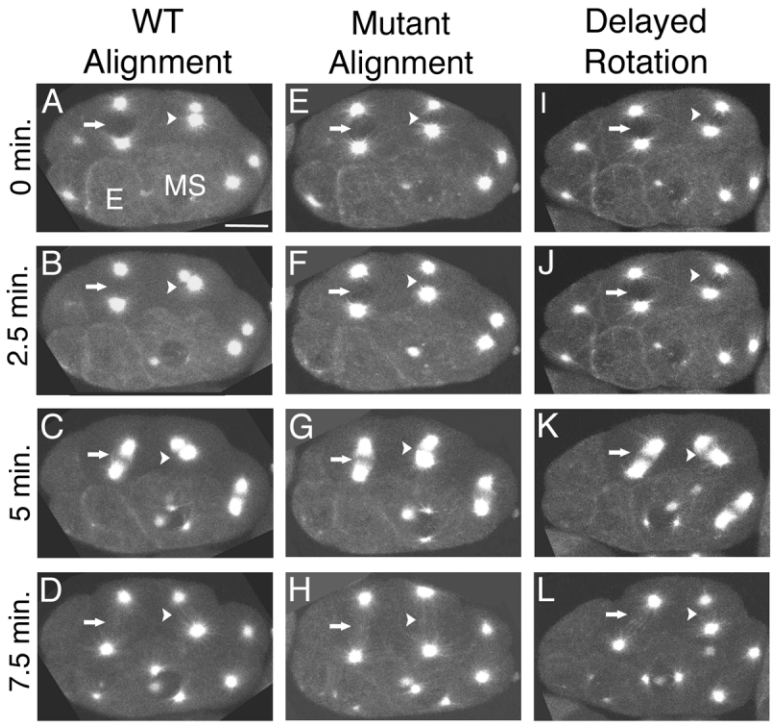

Figure 4. ABar Spindle Defects Visualized by β-Tubulin::GFP

Time course of confocal images of wild-type and mutant embryos expressing TBB-2/β-tubulin::GFP.

(A–D) In a wild-type embryo, the ABar spindle quickly rotates into the proper position. Arrows indicate ABpr and arrowheads indicate ABar.

(E–H) In a *dsh-2(RNAi)*; *mig-5(RNAi)* embryo, ABar remains parallel to ABpr.

(I–L) Delayed rotation of the ABar spindle in a *wrm-1(RNAi)* embryo, in which E has adopted an MS-like fate.

Scale bar equals 10 μm.

surface of EMS. This ultimately activates GSK-3, resulting in spindle alignment irrespective of gene transcription or other downstream Wnt/β-catenin components (Schlesinger et al., 1999). Our analysis suggests that all three Dsh proteins are upstream of GSK-3 activation. Removal of the function of any of the *dshs* results in an incorrectly positioned EMS spindle, with varying penetrance. The strongest effect is seen in offspring of *dsh-2* mutant mothers, suggesting that DSH-2 is primarily responsible for transducing the signal from MOM-5 to GSK-3 in EMS. Antibody staining shows an enrichment of DSH-2 at the area of cell-cell contact between EMS and P2, consistent with a MOM-2/Wnt signal activating DSH-2 at the cell cortex through the MOM-5/Fz receptor.

Our analysis also shows that *kin-19* contributes to the Wnt spindle orientation pathway in both EMS and ABar. Although KIN-19 participates in EMS fate specification, it has not been demonstrated to influence the orientation of the EMS spindle (Peters et al., 1999). We found that

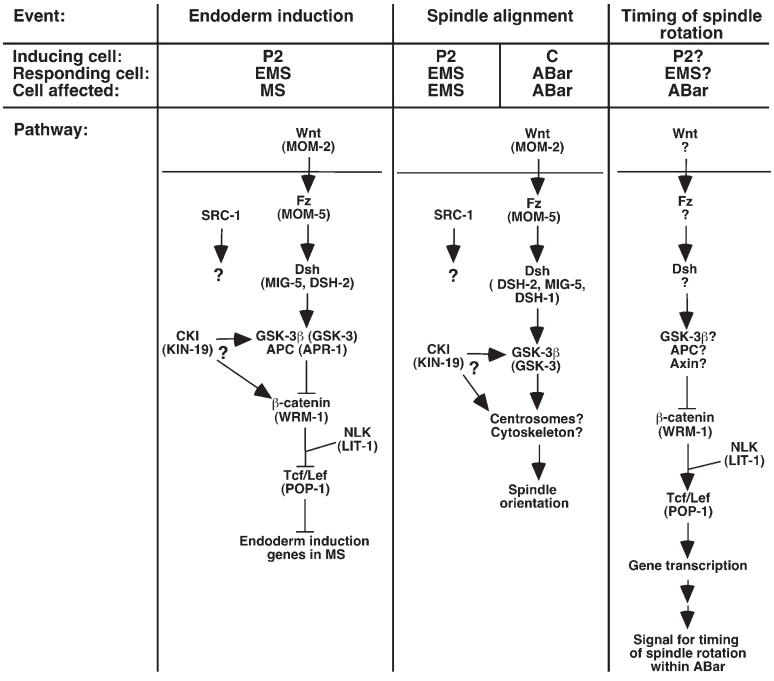

Figure 5. Three Wnt Signaling Pathways Operate in the Early *C. elegans* Embryo

Left: A Wnt/β-catenin pathway regulates the cell fate of the daughters of the EMS blastomere by inhibiting transcription of endoderm genes in the MS blastomere.

Center: The Wnt spindle orientation pathway controls spindle alignment in the EMS and ABar blastomeres. The pathway branches from Wnt/β-catenin signaling at KIN-19 and GSK-3 and presumably alters the cytoskeleton to polarize the division of these blastomeres.

Right: A Wnt/β-catenin pathway controls the timing of spindle rotation in the ABar blastomere. This pathway requires transcriptional activation, controlled by POP-1, presumably by altering the fate of the EMS daughters to regulate the timing of spindle rotation in ABar. Due to the misalignment phenotype, components upstream of WRM-1 cannot be assessed.

depletion of KIN-19 resulted in spindle misalignment in EMS and ABar. Additionally, we observed that KIN-19 localizes to centrosomes during mitosis, which have recently been shown to be important in establishing the initial polarization axis in the 1-cell embryo (Cowan and Hyman, 2004). How *kin-19* operates within the pathway remains unclear. Because CKI family members have the ability to prime β-catenin for further phosphorylation by GSK-3 (Amit et al., 2002; Hagen and Vidal-Puig, 2002; Liu et al., 2002; Sakanaka, 2002; Yanagawa et al., 2002), KIN-19 may act as a priming kinase for GSK-3-mediated phosphorylation of other unidentified target proteins. Based on the localization of KIN-19, these targets may be linked to the cytoskeleton, thereby affecting the physical alignment of the spindles of EMS and ABar.

General Spindle Polarity Pathways Are Similar in EMS and ABar

Our analysis shows that the same Wnt spindle orientation pathway that orients the EMS blastomere also aligns the spindle of the ABar blastomere (Figure 5). Our results indicate that, as in EMS, this pathway does not require gene transcription to align the ABar spindle and that GSK-3 could be interacting directly or indirectly with the cytoskeleton (Schlesinger et al., 1999).

We found that all three *dsh* genes also act redundantly during ABar spindle orientation as well. Surprisingly, our data show that MIG-5 is the Dsh that is most important during ABar spindle orientation, contrary to the case for EMS spindle alignment, where DSH-2 is most important. The ABar spindle defects seen in *dsh-2(or302)* embryos suggest that DSH-2 also contributes significantly to ABar spindle orientation. DSH-1 seems to play only a minor role, since *dsh-1(RNAi)* does not result in ABar spindle defects unless performed along with *mig-5(RNAi)*. This combination may remove enough total Dsh protein to prevent ABar from dividing correctly. In contrast, when *dsh-1* function is removed in combination with that of *dsh-2*, the amount of MIG-5 present may be sufficient to maintain the total Dsh protein at a high enough level that the removal of *dsh-1* function has no effect. Alternatively, the Dshs may have slightly different functions in regulating spindle orientation.

In Wnt signaling mutants, defective EMS spindle orientation is eventually corrected to the proper orientation, which is presumably due to the activity of the parallel *src-1* pathway (Bei et al., 2002). In contrast, the Src pathway does not rescue spindle defects in ABar, although we have shown that the *src-1* pathway does influence ABar division. At this time, targets of SRC-1 in spindle orientation are unknown. It is possible that one or more of the Dshs are SRC-1 targets; however, the more severe phenotype of *src-1* mutants in EMS suggests that other targets are also affected. Interestingly, in EMS and ABar, removal of *src-1* function along with the function of either *dsh-1* or *mig-5* has very little additional effect on spindle polarity; however, when *src-1* function is removed in *dsh-2(or302)* mutants, spindle misalignment is enhanced to nearly complete penetrance in EMS and ABar. Thus, while the three Dsh proteins act partially redundantly, there may be differences in how they impinge on other pathways.

The C Blastomere Orients the Spindle of ABar

In the 8-cell embryo, ABar contacts the C and MS blastomeres. Park and Priess (2003) used blastomere isolations to demonstrate that C and MS can orient the spindle of unidentified AB granddaughters. They also demonstrated that AB granddaughters have random spindle orientation when presented with a *mom-2* mutant C blastomere, but not with a *mom-2* mutant MS blastomere. Using *pal-1(RNAi)* to alter the fate of C and laser killing of blastomeres to create steric hindrance within the embryo, we are able to unambiguously identify ABar. Our results show that a loss of contact between C and ABar results in misalignment of its spindle in virtually all cases. Thus, contact with C is not only sufficient to align the spindle of an AB granddaughter (as demonstrated by Park and Priess) but is also necessary to properly orient the ABar spindle through the Wnt spindle alignment pathway. Our results and those of Park and Priess (2003) further suggest that the polarizing activity of C is mediated by MOM-2/Wnt.

A Wnt/β-Catenin Signal Regulates the Timing of Spindle Rotation in the ABar Blastomere

The orientation of the EMS spindle is not affected when Wnt/β-catenin signaling is abrogated through disruption of transcription or removal of WRM-1/β-catenin or POP-1/Tcf/Lef (Schlesinger et al., 1999). In contrast, when *wrm-1, lit-1, pop-1,* or *ama-1* function is removed, the ABar spindle is delayed in rotating into position (Figure 5). All of these treatments are known to affect the differentiation of the progeny of EMS (Kaletta et al., 1997; Lin et al., 1995; Rocheleau et al., 1997). Moreover, Park and Priess (2003) showed that MS is capable of orienting the spindle of AB granddaughters in isolated blastomeres independent of MOM-2 function. Given the physical proximity of the blastomeres to ABar in the wild-type embryo, MS may produce a MOM-2-independent signal that ultimately affects positioning of the ABar centrosome further from C. Our data further suggest that abnormalities in the fate of EMS daughters result in rotation defects. In *wrm-1(RNAi)* embryos, both EMS daughters become MS-like, and β-tubulin::GFP analysis reveals that the centrosomes of ABar do not rotate properly in many cases. If a signal that aids orientation of the spindle of ABar is normally secreted by MS, the two MS-like daughter cells specified in *wrm-1(RNAi)* embryos could produce competing signals that result in spindle rotation defects in ABar. Similarly, when both of the EMS daughters adopt an E-like fate, as in *pop-1(RNAi)*, altered signaling from EMS daughters could again lead to a similar phenotype. In these cases, the centrosomal positioning presumably relies solely on the Wnt signal from C to eventually position the spindle in the correct orientation.

Conclusions

In conclusion, spindle orientation in the early *C. elegans* embryo is regulated through a Wnt spindle alignment pathway involving the Dshs and KIN-19 but independent of gene transcription. In addition, in ABar, the Wnt/β-catenin pathway regulates the timing of spindle rotation in a transcription-dependent manner, presumably indirectly by altering the fates of E and MS. The components of the Wnt spindle orientation pathway downstream of KIN-19 and GSK-3 are unknown; future work

should be aimed at identifying these components and determining which Wnts are involved in specific inductive events.

Experimental Procedures

Strains and Alleles

The Bristol strain N2 was used as wild-type. Strains were maintained and cultured as described (Brenner, 1974). Other strains used included NG3124 (*dsh-2(or302)/mIn1(dpy-10(e128)mIs14)*II) (G.E., unpublished), WH204 (*ojIs1(pie-1*::GFP*::tbb-2*) (Strome et al., 2001), TY3558 (*ruIs32(pie-1::his-11::GFP)*; *ojIs1*) (Strome et al., 2001), JR2274 (*wIs137 (rol-6, end-3::*END-3[P202L]::GFP)) (M. Maduro and J. Rothman, personal communication; Maduro and Rothman, 2002), and BW1809 (*gpa-16(it143)*I; *him-5(e1490)*V) (Bergmann et al., 2003).

RNAi

RNA templates were produced by PCR amplification of either cDNAs using T3 and T7 primers or genomic DNA using nested gene-specific primers, in which the inner primer pair had the T3 or T7 sequences added to the 5′ end. Ambion Megascript T3 and T7 kits (Ambion, Austin, TX) were used for in vitro transcription. Transcription reactions were purified, mixed in equimolar concentrations, and double-stranded. cDNA clones obtained from Y. Kohara (Gene Network Lab, NIG, Japan) included yk40c12 (*apr-1*), yk291a11, yk397b6 and yk653h6 (*dsh-1*), yk55h11 (*dsh-2*), yk1092h04 (*jnk-1*), yk216a12 (*mig-5*), yk471e5 (*mom-5*), yk1084e03 (*pry-1*), yk233b4 (*gsk-3*), yk117f2 (*src-1*), and yk213d6 (*wrm-1*). *ama-1, kin-19, lit-1, let-92,* and *pop-1* RNA templates were made from genomic DNA. The feeding clone corresponding to C38D4.6 was used for *pal-1* RNAi.

Double-stranded RNA was injected at concentrations of 1–2 μg/μl into L4 larvae or young adult hermaphrodites. Embryos were collected and imaged 20–40 hr postinjection. Efficiency of RNAi was verified by observation of published RNAi phenotypes, when available.

Nomarski and Fluorescence Imaging

Nomarski images were collected as described previously (Raich et al., 1998), with the following exception. 45 optical sections with 0.75 μm spacing were collected every 45 s. Fluorescent images were collected with a Bio-Rad MRC 1024 laser scanning confocal microscope. 15 optical sections 2–3 μm apart were captured at 1 min intervals for 4D analysis. For antibody staining, 45 optical sections 0.5–1.0 μm apart were collected. Nomarski and fluorescent images were analyzed using modified versions of the public domain applications NIH Image and ImageJ (available on request from J.H.).

Antibody Staining

To obtain embryos for staining, gravid hermaphrodites were washed off of plates and rinsed with water. The worms were then incubated in a 0.71 M NaOH/4.4% $NaOCl_3$ solution until they dissolved and were then rinsed in water. For DSH-2 staining, the embryos were fixed and permeabilized using a procedure described by Guenther and Garriga (1996) (modified from Finney and Ruvkun, 1990). An exception was that overnight antibody incubations were at 4°C. The primary antibody was a 1:100 dilution of rat anti-DSH-2 antiserum. The secondary antibody (goat FITC-conjugated anti-rat antibody) was used at a 1:50 dilution. Staining for CKI was performed using the freeze-cracking method as previously described (Williams-Masson et al., 1997). An exception was that the primary antibody was incubated overnight at 4°C. The primary antibody was a 1:25 dilution of rabbit anti-human CKI antiserum (a gift from R. Anderson). The secondary antibody (goat Texas red-conjugated anti-rabbit antibody) was used at a 1:50 dilution.

Laser Killing of Blastomeres

Laser killing was performed using a Micropoint tunable dye laser (Photonic Instruments) as previously described (Williams-Masson et al., 1997). Individual nuclei were identified and lased with approximately 10 pulses per s for approximately 10–15 s. Immediately following blastomere killing, embryos were imaged using 4D Nomarski microscopy.

Acknowledgments

We are grateful to G. Garriga for support and discussion and to John White for helpful discussion in the early phases of this project. We thank J. Rothman for making the *end-3::GFP* available to us, R. Anderson for the CKI antiserum, and J. Pawley for access to the confocal microscope (supported by NSF Grant 9724515). This work was supported by NSF Grant IBN-0112803 to J.H., NIH Grant GM49869 to B.B., and NIH Grant HD-14958 to L.E. and W.W. Some nematode strains used in this work were provided by the *Caenorhabditis* Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR).

Received: July 9, 2004
Revised: September 24, 2004
Accepted: October 5, 2004
Published: December 6, 2004

References

Amit, S., Hatzubai, A., Birman, Y., Andersen, J.S., Ben-Shushan, E., Mann, M., Ben-Neriah, Y., and Alkalay, I. (2002). Axin-mediated CKI phosphorylation of beta-catenin at Ser 45: a molecular switch for the Wnt pathway. Genes Dev. *16*, 1066–1076.

Axelrod, J.D., Miller, J.R., Shulman, J.M., Moon, R.T., and Perrimon, N. (1998). Differential recruitment of Dishevelled provides signaling specificity in the planar cell polarity and Wingless signaling pathways. Genes Dev. *12*, 2610–2622.

Baugh, L.R., Hill, A.A., Slonim, D.K., Brown, E.L., and Hunter, C.P. (2003). Composition and dynamics of the *Caenorhabditis elegans* early embryonic transcriptome. Development *130*, 889–900.

Bei, Y., Hogan, J., Berkowitz, L.A., Soto, M., Rocheleau, C.E., Pang, K.M., Collins, J., and Mello, C.C. (2002). SRC-1 and Wnt signaling act together to specify endoderm and to control cleavage orientation in early *C. elegans* embryos. Dev. Cell *3*, 113–125.

Bellaiche, Y., Gho, M., Kaltschmidt, J.A., Brand, A.H., and Schweisguth, F. (2001). Frizzled regulates localization of cell-fate determinants and mitotic spindle rotation during asymmetric cell division. Nat. Cell Biol. *3*, 50–57.

Bergmann, D.C., Lee, M., Robertson, B., Tsou, M.F., Rose, L.S., and Wood, W.B. (2003). Embryonic handedness choice in *C. elegans* involves the Galpha protein GPA-16. Development *130*, 5731–5740.

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

Brockman, J.L., Gross, S.D., Sussman, M.R., and Anderson, R.A. (1992). Cell cycle-dependent localization of casein kinase I to mitotic spindles. Proc. Natl. Acad. Sci. USA *89*, 9454–9458.

Cong, F., Schweizer, L., and Varmus, H. (2004). Casein kinase Iepsilon modulates the signaling specificities of dishevelled. Mol. Cell. Biol. *24*, 2000–2011.

Cowan, C.R., and Hyman, A.A. (2004). Centrosomes direct cell polarity independently of microtubule assembly in *C. elegans* embryos. Nature *431*, 92–96.

Edgar, L.G., Wolf, N., and Wood, W.B. (1994). Early transcription in *Caenorhabditis elegans* embryos. Development *120*, 443–451.

Finney, M., and Ruvkun, G. (1990). The unc-86 gene product couples cell lineage and cell identity in *C. elegans*. Cell *63*, 895–905.

Gao, Z.H., Seeling, J.M., Hill, V., Yochum, A., and Virshup, D.M. (2002). Casein kinase I phosphorylates and destabilizes the beta-catenin degradation complex. Proc. Natl. Acad. Sci. USA *99*, 1182–1187.

Gho, M., and Schweisguth, F. (1998). Frizzled signalling controls orientation of asymmetric sense organ precursor cell divisions in *Drosophila*. Nature *393*, 178–181.

Goldstein, B. (1993). Establishment of gut fate in the E lineage of *C. elegans*: the roles of lineage-dependent mechanisms and cell interactions. Development *118*, 1267–1277.

Goldstein, B. (1995). An analysis of the response to gut induction in the *C. elegans* embryo. Development *121*, 1227–1236.

Goldstein, B. (2000). When cells tell their neighbors which direction to divide. Dev. Dyn. *218*, 23–29.

Guenther, C., and Garriga, G. (1996). Asymmetric distribution of the *C. elegans* HAM-1 protein in neuroblasts enables daughter cells to adopt distinct fates. Development *122*, 3509–3518.

Hagen, T., and Vidal-Puig, A. (2002). Characterisation of the phosphorylation of beta-catenin at the GSK-3 priming site Ser45. Biochem. Biophys. Res. Commun. *294*, 324–328.

Hagen, T., Di Daniel, E., Culbert, A.A., and Reith, A.D. (2002). Expression and characterization of GSK-3 mutants and their effect on beta-catenin phosphorylation in intact cells. J. Biol. Chem. *277*, 23330–23335.

Herman, M.A. (2002). Control of cell polarity by noncanonical Wnt signaling in *C. elegans*. Semin. Cell Dev. Biol. *13*, 233–241.

Hunter, C.P., and Kenyon, C. (1996). Spatial and temporal controls target pal-1 blastomere-specification activity to a single blastomere lineage in *C. elegans* embryos. Cell *87*, 217–226.

Hyman, A.A., and White, J.G. (1987). Determination of cell division axes in the early embryogenesis of *Caenorhabditis elegans*. J. Cell Biol. *105*, 2123–2135.

Ikeda, S., Kishida, M., Matsuura, Y., Usui, H., and Kikuchi, A. (2000). GSK-3beta-dependent phosphorylation of adenomatous polyposis coli gene product can be modulated by beta-catenin and protein phosphatase 2A complexed with Axin. Oncogene *19*, 537–545.

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

Kishida, M., Hino, S., Michiue, T., Yamamoto, H., Kishida, S., Fukui, A., Asashima, M., and Kikuchi, A. (2001). Synergistic activation of the Wnt signaling pathway by Dvl and casein kinase Iepsilon. J. Biol. Chem. *276*, 33147–33155.

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

Lee, E., Salic, A., and Kirschner, M.W. (2001). Physiological regulation of [beta]-catenin stability by Tcf3 and CK1epsilon. J. Cell Biol. *154*, 983–993.

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

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

Liu, C., Li, Y., Semenov, M., Han, C., Baeg, G.H., Tan, Y., Zhang, Z., Lin, X., and He, X. (2002). Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell *108*, 837–847.

Maduro, M.F., and Rothman, J.H. (2002). Making worm guts: the gene regulatory network of the *Caenorhabditis elegans* endoderm. Dev. Biol. *246*, 68–85.

McKay, R.M., Peters, J.M., and Graff, J.M. (2001). The casein kinase I family in Wnt signaling. Dev. Biol. *235*, 388–396.

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.

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

Peters, J.M., McKay, R.M., McKay, J.P., and Graff, J.M. (1999). Casein kinase I transduces Wnt signals. Nature *401*, 345–350.

Polakis, P. (2002). Casein kinase 1: a Wnt'er of disconnect. Curr. Biol. *12*, R499–R501.

Powell-Coffman, J.A., Knight, J., and Wood, W.B. (1996). Onset of *C. elegans* gastrulation is blocked by inhibition of embryonic transcription with an RNA polymerase antisense RNA. Dev. Biol. *178*, 472–483.

Raich, W.B., Moran, A.N., Rothman, J.H., and Hardin, J. (1998). Cytokinesis and midzone microtubule organization in *Caenorhabditis elegans* require the kinesin-like protein ZEN-4. Mol. Biol. Cell *9*, 2037–2049.

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.

Rothbacher, U., Laurent, M.N., Deardorff, M.A., Klein, P.S., Cho, K.W., and Fraser, S.E. (2000). Dishevelled phosphorylation, subcellular localization and multimerization regulate its role in early embryogenesis. EMBO J. *19*, 1010–1022.

Sakanaka, C. (2002). Phosphorylation and regulation of beta-catenin by casein kinase Iepsilon. J. Biochem. (Tokyo) *132*, 697–703.

Schlesinger, A., Shelton, C.A., Maloof, J.N., Meneghini, M., and Bowerman, B. (1999). Wnt pathway components orient a mitotic spindle in the early *Caenorhabditis elegans* embryo without requiring gene transcription in the responding cell. Genes Dev. *13*, 2028–2038.

Seeling, J.M., Miller, J.R., Gil, R., Moon, R.T., White, R., and Virshup, D.M. (1999). Regulation of beta-catenin signaling by the B56 subunit of protein phosphatase 2A. Science *283*, 2089–2091.

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.

Shulman, J.M., Perrimon, N., and Axelrod, J.D. (1998). Frizzled signaling and the developmental control of cell polarity. Trends Genet. *14*, 452–458.

Strome, S., Powers, J., Dunn, M., Reese, K., Malone, C.J., White, J., Seydoux, G., and Saxton, W. (2001). Spindle dynamics and the role of gamma-tubulin in early *Caenorhabditis elegans* embryos. Mol. Biol. Cell *12*, 1751–1764.

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

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

Wharton, K.A., Jr. (2003). Runnin' with the Dvl: proteins that associate with Dsh/Dvl and their significance to Wnt signal transduction. Dev. Biol. *253*, 1–17.

Willert, K., Shibamoto, S., and Nusse, R. (1999). Wnt-induced dephosphorylation of axin releases beta-catenin from the axin complex. Genes Dev. *13*, 1768–1773.

Williams-Masson, E.M., Malik, A.N., and Hardin, J. (1997). An actin-mediated two-step mechanism is required for ventral enclosure of the *C. elegans* hypodermis. Development *124*, 2889–2901.

Yanagawa, S., Matsuda, Y., Lee, J.S., Matsubayashi, H., Sese, S., Kadowaki, T., and Ishimoto, A. (2002). Casein kinase I phosphorylates the Armadillo protein and induces its degradation in *Drosophila*. EMBO J. *21*, 1733–1742.