ELSEVIER

Seminars in Cell & Developmental Biology 17 (2006) 204–213

seminars in
CELL & DEVELOPMENTAL
BIOLOGY

www.elsevier.com/locate/semcdb

Review

Wnt-dependent spindle polarization in the early *C. elegans* embryo

Timothy D. Walston [a], Jeff Hardin [a,b,*]

[a] *Laboratory of Genetics, University of Wisconsin, Madison, WI 53706, United States*
[b] *Department of Zoology, University of Wisconsin, Madison, WI 53706, United States*

Available online 2 May 2006

Abstract

Correct orientation of the mitotic spindle is crucial for the proper segregation of localized determinants and the correct spatial organization of cells in early embryos. The cues dividing cells use to orient their mitotic spindles are currently the subject of intensive investigation in a number of model systems. One of the cues that cells use during spindle orientation is provided by components of the Wnt signaling pathway. Because of its stereotypical cleavage divisions, the availability of Wnt pathway mutants and the ability to perform RNAi, and because cell–cell interactions can be studied in vitro, the *C. elegans* embryo continues to be a useful system for identifying specific cell–cell interactions in which Wnt-dependent signals polarize the mitotic spindle. This review discusses the evidence for involvement of Wnt signaling during spindle orientation in several contexts in the early *C. elegans* embryo, a process that involves upstream Wnt effectors but does not involve downstream nuclear effectors of Wnt signaling, and places this Wnt spindle orientation pathway in the larger context of other known modulators of spindle orientation in animal embryos.

Keywords: Wnt signaling; *C. elegans*; Spindle polarity; Asymmetric cell division; Dishevelled

Contents

1. Orientation of the mitotic apparatus is a key regulatory event in many developmental processes

The correct orientation of mitotic divisions is crucial for many events in the early embryo. In many situations, blastomeres in the early embryo are highly polarized, and precise planes of division segregate components within these polarized cells into one cell versus another. The segregation of such determinants in turn leads to differences in fate of the resulting daughter cells (reviewed in [1–6]). Correctly oriented divisions also lead to precise spatial relationships between cells in the embryo. In many cases, failure of oriented divisions leads to wholesale disruption of this spatial arrangement, giving rise to widespread defects in the cell–cell interactions normally required for inductive signals to be transmitted in the early embryo. Understanding the cell–cell signals that regulate orientation of cell divisions in the early embryo is thus important for our understanding of how the fertilized egg, through its subsequent partitioning, becomes a multicellular embryo. A key regulator of oriented cell divi-

* Corresponding author at: 1117 W. Johnson St., Madison, WI 53706, United States. Tel.: +1 608 262 9634; fax: +1 608 262 7319.
E-mail address: jdhardin@wisc.edu (J. Hardin).

1084-9521/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.semcdb.2006.04.005

sion is the Wnt pathway (for an overview of the basic molecular components of canonical Wnt signaling, see [7–9]; for reviews of non-transcriptional Wnt effector pathways, especially planar cell polarity, see [10–15]). In this review, we examine insights into Wnt-dependent regulation of the mitotic spindle gleaned from experiments performed on the early *C. elegans* embryo. For extensive reviews of other mechanisms by which spindle positioning is regulated in the *C. elegans* embryo, especially the one-celled zygote, please see recent substantial reviews [16,17].

2. Introduction to the early *C. elegans* embryo

Its simple body plan makes *C. elegans* a unique model system for studying cell–cell interactions and the molecular pathways that drive those interactions. Because the lineage and position of every cell in the wild-type embryo has been identified, individual cells can be identified and the interactions between specific cells can be studied in detail in vivo and in vitro. Although the nomenclature of cell lineages can seem arcane to the uninitiated, naming conventions actually follow a few simple rules [18]. Until terminal differentiation, most names are based on the names of a few key founder cells in the early embryo, which are born between the two-cell and approximately 20-cell stages, depending on the particular founder lineage. Subsequently, most of the cells are named based on orientation of the prior cell divisions: “a” or “p” for anterior–posterior divisions, “l” or “r” for left–right divisions, and occasionally, “d” or “v” for dorsal–ventral divisions. For example, the blastomere named ABar is derived from the AB blastomere of the two-cell embryo, which divides in an anterior–posterior orientation to form ABa and ABp. Next, ABa divides in a left–right orientation to form two daughter cells, ABar and ABal. Such lineage information is useful when studying the role of signaling pathways in orienting cells divisions in the embryo because it provides a wealth of information about wild-type patterns of division that are typically disrupted in various mutant backgrounds. Here we review what is known about how Wnt signaling regulates three divisions in the *C. elegans* embryo: (1) the division of the EMS blastomere to generate the endodermal and mesodermal precursors, E and MS; (2) the division of a cell called ABar at the eight-cell stage, and (3) the divisions of selected AB progeny in the later embryo. Each has provided insights into how Wnt signals regulate highly stereotyped planes of cell division in early embryos.

3. Two separate Wnt signaling pathways regulate cell fate and spindle orientation in the EMS blastomere

The four-cell *C. elegans* embryo consists of four blastomeres that are already adopting individualized fates. One of the four cells, EMS, divides to generate a posterior daughter, E, and an anterior daughter, MS (Fig. 1A). The E lineage generates all of the endoderm in the embryo, while the MS cell lineage produces a significant fraction of the mesoderm, including the posterior pharynx and some muscle, as well as neurons.

Through a series of elegant embryological experiments, Bob Goldstein demonstrated that the P2 cell induces one of the daughters of EMS to produce gut. Using blastomere isolation and recombination, the EMS blastomere was removed from its endogenous contacts and placed next to either AB-derived cells or P2 [19]. EMS formed gut only when it was in contact with P2. Moreover, when P2 was removed and placed on the anterior side of EMS, the anterior cell then became E and the posterior cell became MS (i.e., the opposite arrangement from wildtype) [20]. This result demonstrates that P2 polarizes EMS, and hence the

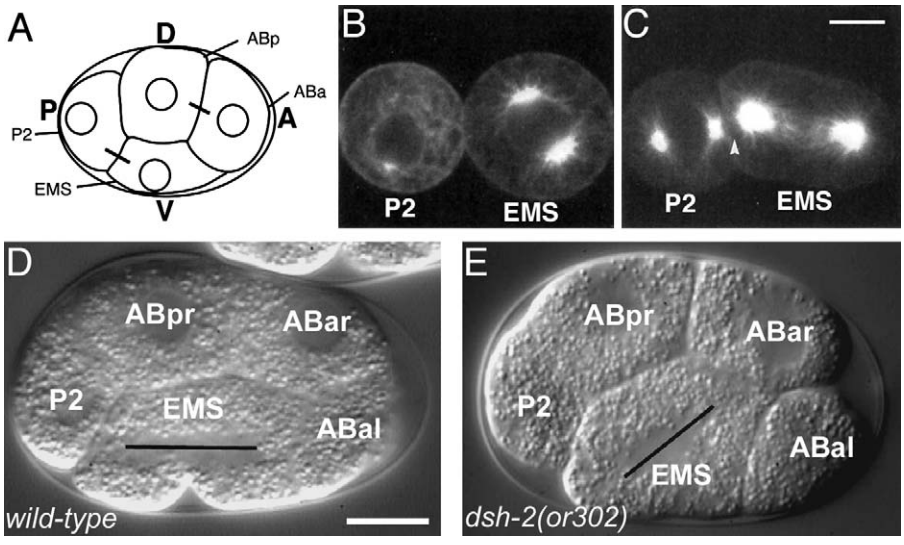

Fig. 1. The EMS spindle is oriented by Wnt-dependent signals from P2: (A) a schematic diagram of blastomere positioning in the *C. elegans* embryo at the four-cell stage. Lines between blastomeres indicate sister cells resulting from the previous cell division. All embryos are shown in lateral views with anterior to the right and dorsal at the top, as opposed to the normal convention, in order to visualize the right-hand side of the embryo; (B and C) anti-microtubule immunofluorescence in P2-EMS cell pairs, during centrosome migration and spindle rotation (B) and anaphase (C); (D and E) Nomarski time-lapse images of wild-type or mutant four-cell embryos. Black lines indicate spindle position. In wild-type embryos (D), the division of the EMS blastomere is in an anterior–posterior (A/P) orientation and in *dsh-2*(*or302*) embryos (E), 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. Scale bars = 10 μm. A, D, and E from [28], with permission of Cell Press; B, C from [22], with permission of Rockefeller Press.

differentiation of its daughter cells. Goldstein later demonstrated that the inducing signal from P2 must be present no later than 3 min before division of EMS in order to induce gut [21]. These experiments demonstrated that cell–cell interactions between the P2 and EMS blastomeres are essential for fate specification in the EMS blastomere.

In addition to its role in polarizing the differentiated progeny of EMS, P2 also polarizes the plane of division of EMS (Fig. 1B and C). In wild-type embryos, the centrosomes of the EMS blastomere initially set up in a left–right orientation. However, during the early stages of mitosis, the centrosomes migrate to an anterior–posterior position, thereby establishing the proper cell division orientation. In isolated and recombined blastomeres, Goldstein demonstrated that placing P2 next to EMS at least 9–10 min before the division of EMS resulted in a rotation of the EMS centrosomes, such that the spindle would divide in alignment with the site of contact with P2 [22]. Thus contact between P2 and EMS not only specifies the fates of the EMS daughters, but also specifies the alignment of the EMS spindle.

The next step was to determine what signaling pathways regulate these two events within EMS. The initial suggestion that Wnt signaling was involved in determining the cell fate of the EMS daughter cells came from the identification of a *pop-1/Tcf/Lef* (*pop* = posterior pharynx defective) allele that resulted in both EMS progeny adopting endodermal fate [23]. Curiously, no spindle alignment defects were identified, suggesting that another pathway independent of events in the nucleus of EMS regulates the polarity of the EMS spindle. To further understand the induction of EMS by P2, Thorpe et al. performed a forward genetic screen to identify genes that result in polarity defects in EMS [24]. They named the phenotype of the resulting maternal-effect mutants Mom, for *mo*re *m*esoderm, because the mutants failed to induce endoderm and thus had twice as much EMS-derived mesoderm as in wildtype. Cloning of the *mom* genes identified them as components of a Wnt signaling pathway, including *mom-1/Porcupine*, *mom-2/Wnt*, *mom-4/MAPKKK* and *mom-5/Frizzled* [24,25]. Removal of the function of *gsk-3/GSK-3β* demonstrated that GSK-3 is also required for endoderm induction [26]. These results demonstrated that induction of endoderm fate in one of the EMS daughters was the result of a transcriptionally dependent Wnt/β-catenin pathway. A recent, in-depth review of this process can be found elsewhere [27].

Besides regulating the fate of the EMS daughter cells, the *mom* genes also regulate the spindle orientation of EMS. In the *mom* mutants, except for *mom-2/Wnt*, the spindle of EMS sets up in either the wild-type (i.e., left–right) orientation or an orientation that is skewed along the dorsal–ventral axis. Contrary to wildtype, the spindle delays its rotation into the proper anterior–posterior orientation until late in mitosis. However, even in the *mom* mutants, EMS always eventually divides in the proper orientation [24,28]. Unlike wild-type blastomeres, when both P2 and EMS are isolated from *mom* mutants and recombined, the blastomeres display an initially random spindle orientation, indicating that that the normal P2/EMS interaction is Wnt-dependent. Similar results were obtained following removal of the function of *gsk*-3 by RNAi [26]. Significantly, Schlesinger et al. went on to demonstrate that the signal for blastomere orientation in EMS was due to a Wnt signal from P2 that must be received by EMS by placing *mom* mutant P2 or EMS blastomeres next to the corresponding wild-type blastomere. In recombination experiments, P2 cells lacking *mom-2*/Wnt function and EMS cells lacking *mom-5*/Frizzled resulted in a significant percentage of failures of EMS to correctly orient its spindle. It has been proposed that the penetrance of such defects in *mom-2* embryos is low because one of the other four Wnts in *C. elegans* may be acting redundantly with *mom-2* to orient the spindle of EMS [26].

More recent experiments have identified other molecular components involved in the Wnt-dependent orientation of the EMS spindle. Bei et al. demonstrated that two of the three Dishevelleds in *C. elegans*, *dsh-2* and *mig-5*, were involved in spindle positioning of EMS [29]; later work by our laboratory demonstrated that *dsh-2* has a stronger effect on spindle positioning in EMS than *mig-5* (Fig. 1D and E). We also demonstrated that *kin-19/CKIα* regulates spindle orientation through Wnt signaling in EMS [28].

Taken together, these experiments clearly implicate a Wnt signaling pathway in orienting the mitotic spindle in the EMS blastomere. Unexpectedly, however, Schlesinger et al. demonstrated that the Wnt pathway that regulates spindle positioning in EMS does not require transcription [26]. In *pop-1/Tcf/Lef* mutants, the EMS spindle orients properly in isolated blastomeres. Additionally, when transcription was blocked by removing the function of *ama-1*, the largest subunit of RNA polymerase II, by RNAi or by exposing isolated blastomeres to actinomycin D, the EMS spindle still aligned in a normal fashion relative to the contact site with P2. These results demonstrated that the Wnt pathways regulating cell fate and spindle orientation of the EMS blastomere are separable: a *pop-1*/Tcf-dependent transcriptional pathway ultimately leads to polarized differentiation of the EMS daughters, but the other pathway acts positively downstream of GSK-3 to position the mitotic spindle.

4. A Src tyrosine kinase pathway acts in parallel with the Wnt spindle alignment pathway to regulate the axis of cell division in the EMS blastomere

Although the foregoing experiments clearly demonstrated a role for a transcription-independent Wnt pathway in orienting the EMS spindle, subsequent experiments by Bei et al. demonstrated that a Src tyrosine kinase pathway acts on or in parallel with the Wnt pathway to regulate spindle positioning in EMS [29]. In some *src-1* mutant embryos, the spindle of EMS behaves just as it does in the Wnt spindle alignment pathway mutants, i.e., the spindle displays a delay before rotating into the proper orientation. However, in other *src-1* mutant embryos, the spindle of the EMS blastomere never rotates into the proper orientation, resulting in EMS dividing along the left–right axis (i.e., the axis along which the centrosomes originally align before rotation; Fig. 2A, B). Moreover, *mes-1*, a probable receptor tyrosine kinase, was also shown to act in the Src pathway. Blastomere isolation and recombination experiments demonstrated that *mes-1* is required in both P2 and EMS, but *src-1* is only necessary in EMS. Bei et al. further demonstrated that the improper

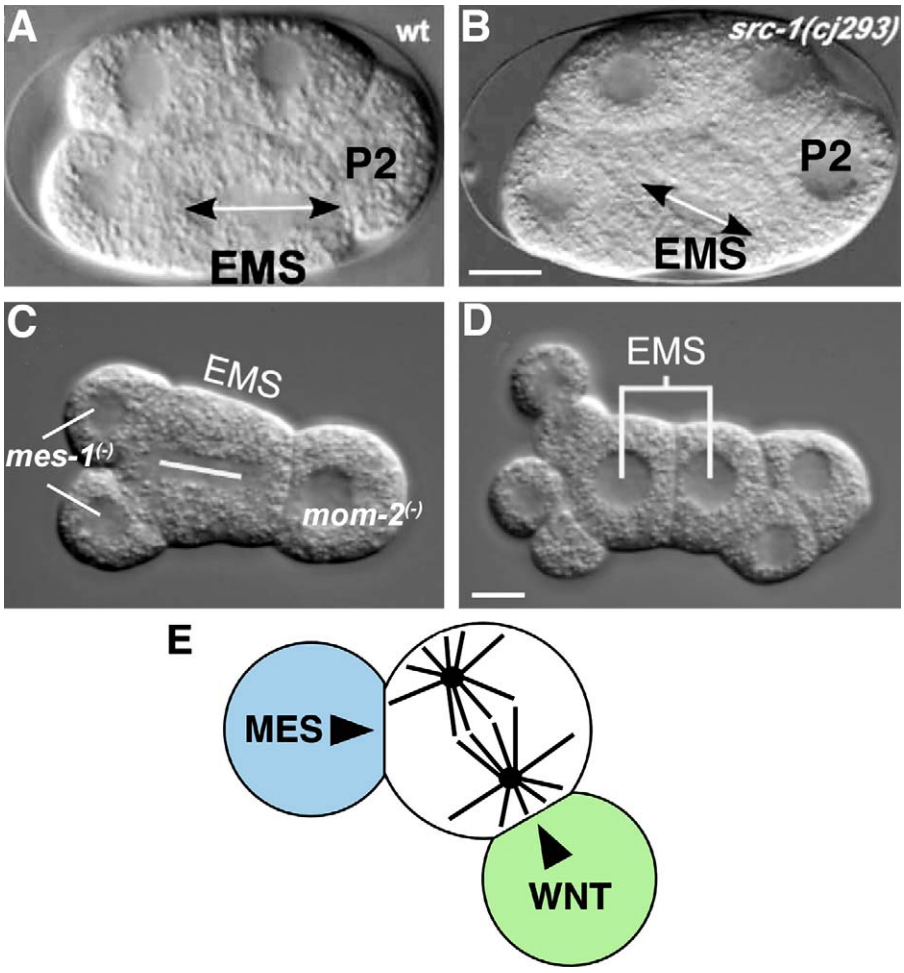

Fig. 2. Wnt- and Src-dependent signals act together to orient the division of EMS: (A and B) *src-1* is required for proper cell division polarity. Nomarski light micrographs showing a wild-type EMS cell division (A) and a skewed *src-1* mutant EMS cell division (B). (C and D) Using a blastomere "competition" experiment to assess polarizing cues for division of EMS. (C) A *mes-1* mutant signaling cell (left) and a *mom-2* mutant signaling cell (right) were each isolated and then placed on opposite sides of EMS. The orientation of the EMS spindle is indicated by the white line. In C, the *mes-1* mutant signaling cell has divided once. In the original experiment, *mom-2* mutant cells were identified by a GFP-histone fusion (data not shown). In D, EMS has divided; when EMS is placed in contact with a *mom-2* (+); mes-1 (−) (Wnt) signaling cell and a *mes-1* (+); mom-2 (−) cell in an orthogonal position, EMS can divide successfully, and orient its spindle to reflect the site of contact with the Wnt-presenting cell (E). Scale bars = 10 μm. A and B are from [29]; C and D are from [30], with permission of Cell Press.

orientation of division is enhanced to full penetrance when a member of the Wnt spindle alignment pathway is simultaneously removed. These results demonstrate that two parallel pathways, a Wnt spindle alignment pathway and a Src tyrosine kinase pathway, coordinate the spindle orientation and resulting axis of cell division of the EMS blastomere in four-cell *C. elegans* embryos.

Although both pathways have been shown to regulate the orientation of the EMS spindle, it is still poorly understood how the two pathways interact. However, blastomere isolation and recombination experiments are beginning to shed light on the requirements for the two pathways. Confirming previous studies [29], Goldstein et al. [30] placed single P2 blastomeres mutant for either Wnt signaling (e.g., from *mom-2* mutants) or Src signaling (e.g., from *mes-1* mutants) against isolated EMS cells; neither was able to orient the EMS spindle. Next, Goldstein et al. placed two P2 blastomeres, one mutant for the Src pathway and one mutant for the Wnt spindle alignment pathway, in contact with a single EMS cell to address whether the Wnt signal and/or the MES-1 mediated signal provide directional cues to the EMS spindle. These "competition" experiments showed that the EMS spindle consistently aligns to reflect the site of contact with the Wnt-positive blastomere; thus the Src pathway is a permissive requirement for spindle alignment. Similar results were obtained postembryonically: the division axis of the T blast cell is aligned by contact with a LIN-44/Wnt expressing cell, with a concomitant accumulation of LIN-17/Frizzled at the site of contact [30]. Whether similar localized accumulation of MOM-5/Frizzled occurs in the early embryo has not thus far been demonstrated, and published reports of MOM-5 localization have not suggested an obvious polarized accumulation of MOM-5 at the P2/EMS interface [31].

5. Two separable Wnt pathways regulate the alignment of the spindle of the ABar blastomere

In the eight-cell *C. elegans* embryo, ABar is one of four cells derived from the AB founder cell, along with ABal, ABpr, and

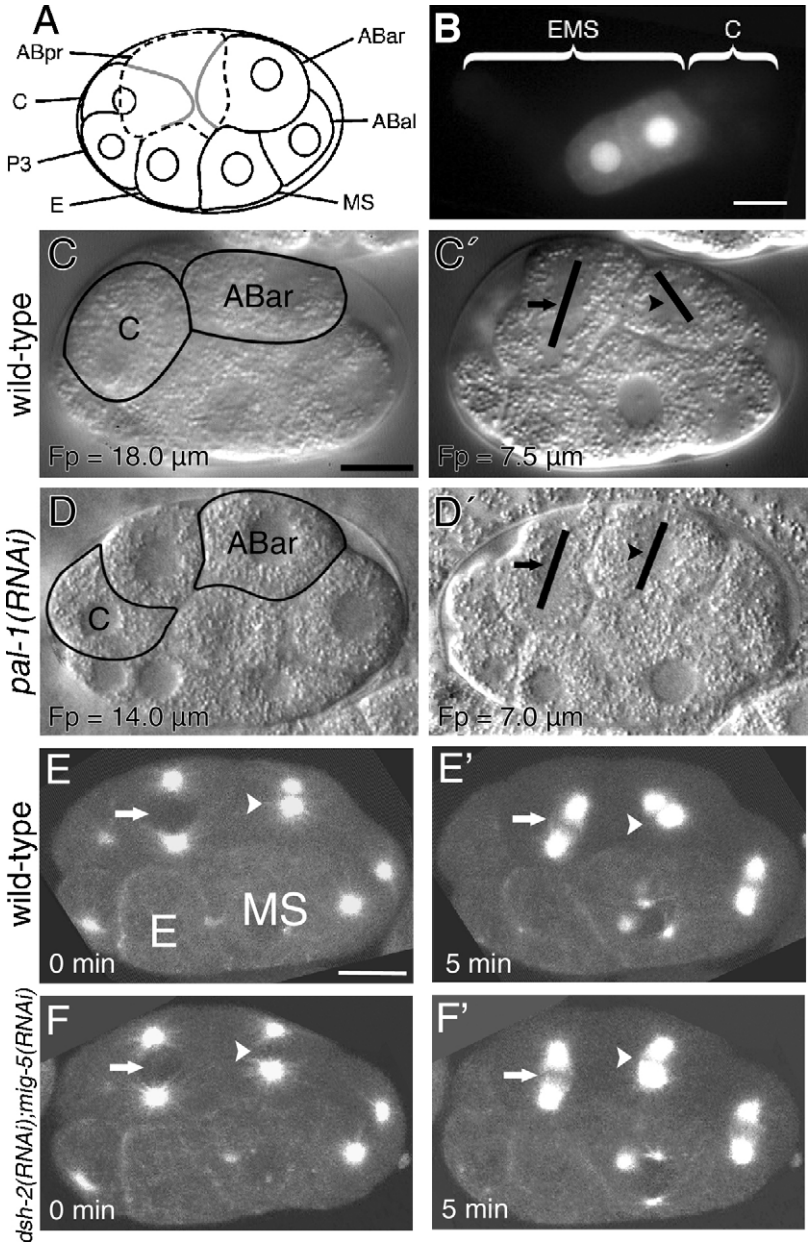

Fig. 3. The division of ABar is oriented by Wnt-dependent interactions with C: (A) a schematic diagram of blastomere positioning in the eight-cell *C. elegans* embryo, prior to the division of the AB granddaughter cells. As opposed to normal convention, in this and panels C–F′, embryos are shown in lateral views with anterior to the right and dorsal at the top in order to visualize the right-hand side of the embryo. 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) C can produce a signal, presumably MOM-2/Wnt, capable of inducing gut in EMS progeny. GFP expression of an endoderm-specific transgene was monitored after combining isolated EMS and C cells. The endodermal marker is expressed by the progeny of EMS that is in contact with C (the E-like cell has divided once in this image). Scale bar = 5 μm. (C and D′) Nomarski time-lapse images of wild-type or *pal-1* (*RNAi*) embryos. Black lines indicate spindle position. (C) In a wild-type embryo, ABar and C come into contact shortly after C is born. (C′) The ABar spindle (arrowhead) rotates toward the contact between ABar and C, which results in a division nearly perpendicular to ABpr (arrow). (D) In some *pal-1(RNAi)* embryos, there is no contact between ABar and C. In these embryos, ABar divides parallel to ABpr (D′). Scale bar = 10 μm. (E–F′) 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. (E and E′) In a wild-type embryo, the ABar spindle quickly rotates into the proper position. Arrows indicate ABpr and arrowheads indicate ABar. (F and F′) In a *dsh-2(RNAi)*; *mig-5(RNAi)* embryo, ABar remains parallel to ABpr. Scale bar = 10 μm. A and C–F′ are from [28], with permission of Cell Press; B is from [33], with permission of the Company of Biologists Ltd.

ABpl; these four granddaughters of AB are sometimes collectively referred to as AB4 cells (Fig. 3A). When the AB4 cells begin to divide, three of them set up their spindles in parallel along the anterior–posterior axis, such that the anterior end of the spindle is skewed slightly towards the dorsal and left-hand sides of the embryo. The remaining AB4 cell, ABar, sets up its spindle perpendicular to the others, such that the anterior end of the spindle is skewed slightly towards the right and ventral axes. After the division of the AB4 cells, the posterior daughter of ABar, ABarp, is positioned to the left of the anterior daughter of ABpr, ABpra. The anterior daughter of ABar, ABara, is positioned just anterior to ABpra. These differences in division

orientation are an indication of an important process in development: how clonally related cells become different. Investigation of the mechanisms that regulate the division axis of ABar has provided one example of how this process may occur.

As with EMS, genetic and embryonic manipulations have been used to determine the source of the signals that may be orienting the spindles of the AB4 cells, especially ABar. Hutter and Schnabel originally noted that the spindle orientation of the AB4 cells was aberrant when the P1 blastomere was removed from the two-cell embryo [32]. Moreover, they found that ABar divided with an orientation nearly parallel to other AB4 cells in embryos in which either P2 or ABp had been laser killed (or "ablated"; note, however, that the cell surface of the resulting carcass is still present in such experiments).

More recently, Park and Priess have used elegant in vitro blastomere recombination experiments to examine the signals that polarize the fates of AB4 cells, as well as their spindle orientation [33]. Noting that various AB4 cells are in contact to different extents with three founder cells (E, MS, and C), they placed these cells in contact with AB4 cells in vitro. AB4 cells are competent to receive signals from all of these cells, such that they orient their spindles to align with the site of contact of the non-AB4 blastomere. Moreover, by placing pairs of blastomeres next to AB4 cells, they determined that the AB4 cells could differentially respond to signals when in contact with any of these cells, such that the AB4 cells preferentially divided in an orientation determined by the site of contact with E or C as opposed to MS. Taken together, such experiments indicate that multiple, contact-dependent cues may operate to orient the spindles of the AB4 cells, and that the signals emanating from E and C are stronger than those from MS.

Park and Priess next used mutant blastomeres to address the molecular cues that E, MS, and C use to orient AB4 cells. Significantly, they found that MS cells mutant for *mom-2*/Wnt could still orient AB4 cells, whereas *mom-2* mutant C cells could not. These results suggest that C uses a contact-dependent Wnt signal to orient AB4 cells in vitro. Further evidence that such a Wnt signal emanates from C comes from heterchronic explants: when C is placed in contact with EMS, it can induce gut [33]. The nature of the signal emanating from MS is currently unknown; it is possible that MS produces a redundant Wnt signal, or perhaps another pathway is involved that acts as a non-essential modulator of AB4 polarity. Further experiments will be required to distinguish these possibilities.

Although informative, a true test to determine which blastomeres are signaling to orient the spindle of ABar must be performed in the context of an intact embryo. Such experiments allow the geometry of the embryo to dictate which cells are in contact with each other, and hence which cells are actually capable, under normal circumstances, of receiving such signals from their neighbors. In wild-type embryos, the division of P2 to form P3 and C occurs several minutes prior to the start of mitosis of the AB4 cells. Once the C blastomere is born, it begins to extend towards the anterior of the embryo along the left-hand side of the embryo. Reminiscent of migrating cells in culture, C initially sends out a small extension, which touches ABar prior to the orientation of the centromeres in ABar; the body of C soon follows (Fig. 3C and C′). We demonstrated that the contact between C and ABar is primarily responsible for orienting the spindle of ABar in the intact embryo [28]. By laser killing ABpr to create a steric barrier to prevent C from accessing ABar, we showed that in those cases in which the carcass of ABpr prevented contact between C and ABar, the orientation of the spindle of ABar was parallel with the other AB4 cells. In some ABpr-killed embryos, C still managed to contact ABar. In those cases, the spindle of ABar was oriented towards the site of contact with the C blastomere, even when the C blastomere touched ABar in the incorrect location. In addition, we examined *pal-1*/caudal mutant embryos, because *pal-1* has been shown to regulate the fate of the C blastomere [34]. In some *pal-1* embryos, C is delayed in its contact with ABar, and does not contact ABar until after mitosis has begun. In these embryos, the ABar spindle, and hence the resulting cell division, is parallel with that in other AB4 cells (Fig. 3D and D′). Thus, the C blastomere seems to be primarily responsible for orienting the spindle of the ABar blastomere in vivo, such that it divides perpendicular to the other AB4 blastomeres.

Consistent with the in vitro experiments, in *mom* mutants, the orientation of the spindle of the ABar blastomere is misaligned. In these cases, ABar divides parallel to the other AB4 cells, resulting in ABarp adopting a position anterior to ABara (its anterior sister in wild-type embryos; Fig. 3C) Similar results are observed for *mom-1/Porc, mom-2/Wnt,* and *mom-5/Fz* [24,28]. Experiments by our laboratory [28] demonstrated that the other members of the Wnt spindle alignment pathway, already known to regulate EMS spindle orientation, also act during alignment of the ABar spindle, including *dsh-2/Dsh, mig-5/Dsh, kin-19/CKIα,* and *gsk-3/GSK3β* (Fig. 3E–F′). Additionally, as in EMS, when zygotic transcription is blocked by removal of the function of *ama-1/RNA pol II*, the spindle of ABar still divides in the wild-type orientation. This suggests that the pathway that controls the orientation of the spindle in ABar is a Wnt spindle alignment pathway that, as in EMS, acts directly upon the cytoskeleton, centrosomes, or other structural components of the mitotic machinery.

Removal of the function of *src-1* reveals that the Src tyrosine kinase pathway is also used during ABar spindle alignment: removal of Src pathway function enhances the penetrance of ABar division phenotypes when only one of the three redundant Dshs is removed [28]. However, unlike in EMS, when members of the Wnt spindle alignment pathway are removed, the ABar blastomere divides in the misaligned orientation, rather than belatedly rotating to the wild-type position. Thus, although both the Wnt spindle alignment pathway and the Src tyrosine kinase pathway are required and functionally redundant, there are subtle differences regarding how the two pathways regulate EMS and ABar. It remains to be determined whether the C blastomere is providing signals for both pathways or whether other blastomeres, such as MS, may be providing signals for one of the pathways as well.

In addition to a non-transcriptional Wnt pathway, a separate Wnt/β-catenin pathway regulates the spindle orientation of ABar. When the function of *wrm-1/β-catenin*, *lit-1/NLK*, *pop-1/Tcf/Lef*, or *ama-1/RNA pol II* is removed, the centrosomes

display a "wandering" phenotype, which results in a delay in alignment of the ABar spindle in the correct orientation. Ultimately however, the spindle sets up and divides in the proper wild-type orientation [28]. By Nomarski microscopy, this phenotype appears to be a delay in spindle rotation to the proper position, but fluorescent imaging of β-tubulin reveals that the centrosomes migrate around the nucleus, seemingly "lost". This stands in stark contrast to the wild-type centrosomes, which migrate directly to the proper position. The Wnt pathway components involved demonstrate that this pathway is a transcriptional Wnt/β-catenin pathway; however, the source of the Wnt ligand and the blastomere that this Wnt pathway acts upon has not been determined.

In summary, in the ABar blastomere, there is evidence that at least three signaling pathways regulate centrosome migration and spindle alignment during mitosis: (1) a Wnt/β-catenin pathway controls centrosome migration, while (2) a non-transcriptional Wnt spindle alignment pathway and (3) a Src tyrosine kinase pathway determine spindle alignment. Contact of ABar with the C blastomere has been shown to be required for proper spindle alignment and is most likely the source of a Wnt ligand, probably MOM-2, which acts in the Wnt spindle alignment pathway. C presumably also produces a ligand that acts upstream of the Src tyrosine kinase pathway.

6. Wnt signaling controls the divisions of cells in the later *C. elegans* embryo

Although the oriented divisions of blastomeres in the early *C. elegans* embryo have been the most intensively studied, recent evidence indicates that Wnt signaling components regulate later cell divisions in the embryo. One such example follows from the experiments performed by Park and Priess mentioned in the previous section. In their studies of AB progeny, they found that after the AB8 stage, polarized divisions can still occur without the function of *mom-2*/Wnt. Moreover, removal of *mom-1*/Porcupine function, which is presumably required for export of all Wnts from the cells that produce them, does not affect these divisions [33]. It is possible that spindle orientation is accomplished in these situations via a Frizzled (e.g., MOM-5), but in a Wnt-independent manner.

A second example of Wnt-dependent modulation of cell division in the later embryo involves embryonic neuroblasts. The 302 neuroblasts in the *C. elegans* embryo are all generated via asymmetric cell divisions [18], raising the possibility that Wnt signaling may also control the orientation of divisions that generate these cells. Indeed, several papers have identified a role for Wnt signaling in the divisions of postembryonic blast cells that generate neuronal cells in *C. elegans* (reviewed in [27]). Moreover, it is well known that the division of sensory organ precursors in *Drosophila* is regulated at least in part by Frizzled signaling [35,36]. Taken together, these results suggested that Wnt/Frizzled signaling might regulate embryonic neuroblast divisions in *C. elegans* as well. Recently, Hawkins et al. have shown that a chemosensory neuronal lineage in the tail, which generates the PHA neuron, requires Wnt signaling components [31]. The PHA cells, and a similar cell, PHB, are born during embryogenesis, and generate similar types of neurons, but via different lineages. PHB is generated from a precursor (ABpl/rapppap) that divides asymmetrically to produce a smaller anterior daughter that dies via apoptosis, and a larger posterior daughter that generates two neurons (HSN and PHB). This event is regulated by the novel protein, HAM-1, which localizes in a crescent at the posterior of many cells in the early embryo [37]. In contrast, the PHA division is not adversely affected by loss of *ham-1* function. However, loss of *dsh-2* function results in fate transformations that suggest defects in the polarity of the division of a cell (ABpl/rpppa) within the lineage that produces PHA. Loss of *mom-5/Frizzled* function also causes such lineage transformations, and causes DSH-2 to lose its normal cortical localization. Loss of *dsh-2* function has no obvious effects on the lineage that produces PHB. Taken together, these results indicate that DSH-2 activation downstream of MOM-5 regulates division of ABpl/rpppa. Significantly, loss of *pop-1* function does not affect these divisions, suggesting, as with EMS and ABar, that a transcription-independent pathway may be at work within ABpl/rpppa. Overexpression of *dsh-2* in transgenic lines causes a weakly penetrant phasmid neuron defect; since phenotypic defects resulting from Dishevelled overexpression have been most often associated with the planar cell polarity (PCP) pathway in other systems [38], these results are consistent with non-POP-1-mediated regulation of cell division orientation in ABpl/rpppa.

7. The future: identifying how Wnt pathways interact with other molecular pathways to orient the mitotic apparatus

This brief review has focused on the role of Wnt signaling in orienting the mitotic apparatus in the early *C. elegans* embryo, where multiple Wnt signaling pathways act to regulate cell fate and cell polarity. A non-canonical Wnt/β-catenin pathway also regulates the migration of the centrosomes during the division of ABar, through an unknown mechanism [28]. In both EMS and ABar, a non-transcriptional Wnt pathway acts positively through GSK-3/GSK3β to regulate the positioning of the EMS and ABar spindles, presumably by affecting structural components of the mitotic machinery [26,28]. The challenge for the future will be to determine how these Wnt signaling pathways interact with upstream components at the cell surface and with downstream effectors that act to align the spindle.

Although the effects of GSK-3 are exerted without the need for transcription, the means by which GSK-3/GSK3β acts during spindle alignment in EMS and ABar is still mysterious. Recent evidence that Wnt pathway components, such as GSK3β, APC, and Dishevelleds, may modulate microtubule function [39–42], suggests that this may be how GSK-3 acts in *C. elegans* as well, but further studies are needed to confirm this. Abundant evidence links the MES-1/SRC-1 pathway to Wnt signaling, in both EMS and ABar [28,29]. In EMS, the Src pathway, acting downstream of MES-1, has been shown to be a permissive, rather than directional, cue for spindle alignment [30]. Presumably SRC-1, through phosphorylation of its targets, somehow enables the spindle orientation machinery to respond

to a spatially localized Wnt signal. The nature of such permissive activation remains unknown. Besides *mes-1*, no other components of the Src pathway, either up- or downstream, have been identified. In order to understand more clearly how the non-transcriptional Wnt spindle alignment pathway and the Src tyrosine kinase pathway contribute to spindle orientation, it will be essential to determine how these signaling pathways interact with the structural components of the cell that contribute to mitosis.

What other pathways might interact with Wnt signaling during spindle orientation? Based on work in other systems and other work in *C. elegans*, it is possible to make several suggestions regarding fruitful avenues for future investigation. First, *C. elegans* may be a system in which to investigate the role of cell–cell adhesion in regulating Wnt-dependent signaling. Adhesion is thought to act cooperatively with cell signaling in several other contexts, including the neuronal synapse [43] and the "immunological synapse" during T cell signaling [44]. Might there be a similar "inductive synapse" that allows for efficient activation of Frizzleds at sites of cell–cell contact in *C. elegans*? Cadherin-based adhesion is known to affect asymmetric cell division in *Drosophila* [45,46], making this a possibility. Our own experiments involving contact between C and ABar suggest that adhesion between these two cells may rapidly reinforce their site of contact, which in turn may facilitate passage of a Wnt signal between these cells that orients the ABar spindle. Because the function of multiple adhesion molecules can be removed easily via RNAi in *C. elegans* embryos, and because blastomeres of different genetic constitution can be placed in direct contact in vitro, the early *C. elegans* embryo may be a place in which to study how adhesion stabilizes contact sites between cells engaged in contact-dependent Wnt signaling. Such studies will no doubt be complicated by the likelihood of extensive crosstalk between Wnt signaling and cadherin-based adhesion [9,47]. Nevertheless, *C. elegans*, with its specialized β-catenins, may be a good system in which to unravel the complexity of such crosstalk.

Second, there is abundant evidence in the one-celled *C. elegans* zygote indicating that two major molecular complexes regulate positioning of the first mitotic spindle along the anterior–posterior axis: the PAR/aPKC complex and a heterotrimeric G protein signaling complex. The PAR/aPKC complex is involved in regulating the localization of cell fate determinants in the *C. elegans* zygote, as well as in the initial placement of the spindle along the anterior–posterior axis, in part by regulating microtubule-dynamics and pulling forces at the cortex [16,48]. Heterotrimeric G protein signaling appears to act during spindle orientation as well, via the redundant Gα subunits, GOA-1 and GPA-16, their regulators RGS-7 and RIC-8, and the GoLoco motif proteins GPR-1/GPR-2, the latter probably regulated by the LET-99 protein (reviewed in [17]). Although some details differ, the PAR/aPKC and heterotrimeric G protein pathways operate during oriented division of neuronal precursors in the nervous system of *Drosophila* (reviewed in [5,6]). In the case of sensory organ precursors, PCP signals downstream of *frizzled* lead to an anterior crescent of proteins including the GoLoco protein Pins and Gαi [49], and there is evidence that Frizzled may act in canonical and PCP signaling via the Gα protein, Go [50].

Although Wnt signaling does not regulate the PAR and Gα complexes in the one-celled *C. elegans* zygote, it is possible that these pathways also act in EMS and ABar, either in parallel to or under the control of Wnt signaling. Park and Priess examined the effects of *goa-1* and *gpa-16* loss of function on cell division of AB daughters in intact embryos [33]. They found that the loss of function of these two genes results in seemingly randomized division planes, but that when division occurred in an anterior–posterior fashion (i.e., the normal orientation), asymmetries in POP-1 nuclear accumulation still occurred. Significantly, when the functions of *goa-1* and *gpa-16* are inhibited in *mom-2* mutant embryos, POP-1 asymmetry is abolished as well. These results suggest that G protein signaling is required for orientation of division in the later embryo, but is functionally separable from spatial asymmetries that act via non-canonical Wnt signaling in the nucleus. How or whether non-transcriptional Wnt pathways (i.e., such as those that are known to operate in EMS and ABar) interact with G protein signaling remains to be determined.

A third area for future investigation is suggested by the preceding discussion of the role of Frizzleds and G proteins, which raises a larger question regarding the planar cell polarity pathway. In *Drosophila*, it is well known that PCP signaling, acting downstream of the cadherin family members Flamingo, Dachsous, and Fat, leads to Wnt-dependent orientation of epithelial cells in imaginal tissues (reviewed in [10,11,51,52]), providing a mechanism whereby the cytoskeleton could be regulated during oriented divisions. This possibility is bolstered by experiments in zebrafish, in which overexpression of fragments of Dishevelled has been implicated in regulating oriented division during gastrulation and neurulation [53,54]. As mentioned previously, DSH-2 overexpression experiments in *C. elegans* yield similar disruptions of at least some oriented divisions [31], and MOM-5 may act independently of an upstream Wnt ligand in the later embryo [55]. Whether standard PCP-mediated mechanisms underlie these effects is unclear. The Strabismus homologue, *vang-1*, may modulate cell divisions in the intestinal lineage (O. Bossinger, personal communication), but efforts by many *C. elegans* laboratories have failed to uncover a widespread role for canonical PCP effectors and/or regulators ([28,31,55]; our unpublished observations). RHO-1/Rho may play a role in regulating the size of nuclei, and hence possibly the placement of the division plane, of the B cell in the *C. elegans* larva [56], although roles for RHO-1 have been difficult to assess, due to its ubiquitous role in regulating cytokinesis ([57,58]; our unpublished observations). An irenic view of this data suggests that a PCP-like pathway, but not all of the standard list of upstream PCP regulators or downstream effectors, may be involved in regulating spindle orientation downstream of Wnt signals in *C. elegans*. One possible reason for this difference is the rapidity of division in *C. elegans* (minutes) versus those in *Drosophila* (hours; see [55] for discussion). If this view turns out to be correct, the *C. elegans* embryo may be a place where other components that contribute to rapid Wnt-dependent orientation of the mitotic spindle can be identified.

Note added in proof

Bänziger et al. [59] recently reported the molecular identification of mom-3, which encodes the homologue of *Drosophila* Wntless, a protein required for secretion of Wnts. RNAi of the Wntless homologue phenocopies ABar defects associated with *mom-3* mutant progeny.

Acknowledgements

We wish to thank the editors for their incredible patience while this work was being prepared. We also wish to thank Mike Herman and Olaf Bossinger for sharing unpublished data. This work was supported by NSF grant IOB0518081 and NIH grant GM058038 to J.H.

References

[1] Kaltschmidt JA, Brand AH. Asymmetric cell division: microtubule dynamics and spindle asymmetry. J Cell Sci 2002;115(Part 11):2257–64.
[2] Betschinger J, Knoblich JA. Dare to be different: asymmetric cell division in *Drosophila*, *C. elegans* and vertebrates. Curr Biol 2004;14(16):R674–85.
[3] McCarthy EK, Goldstein B. Asymmetric spindle positioning. Curr Opin Cell Biol 2006;18(1):79–85.
[4] Roegiers F, Jan YN. Asymmetric cell division. Curr Opin Cell Biol 2004;16(2):195–205.
[5] Wodarz A. Molecular control of cell polarity and asymmetric cell division in *Drosophila* neuroblasts. Curr Opin Cell Biol 2005;17(5):475–81.
[6] Bellaiche Y, Gotta M. Heterotrimeric G proteins and regulation of size asymmetry during cell division. Curr Opin Cell Biol 2005;17(6):658–63.
[7] Cadigan KM, Liu YI. Wnt signaling: complexity at the surface. J Cell Sci 2006;119(Part 3):395–402.
[8] Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004;20:781–810.
[9] Nelson WJ, Nusse R. Convergence of Wnt, beta-catenin, and cadherin pathways. Science 2004;303(5663):1483–7.
[10] Eaton S. Cell biology of planar polarity transmission in the *Drosophila* wing. Mech Dev 2003;120(11):1257–64.
[11] Fanto M, McNeill H. Planar polarity from flies to vertebrates. J Cell Sci 2004;117(Part 4):527–33.
[12] Kiefer JC. Planar cell polarity: heading in the right direction. Dev Dyn 2005;233(2):695–700.
[13] Klein TJ, Mlodzik M. Planar cell polarization: an emerging model points in the right direction. Annu Rev Cell Dev Biol 2005;21:155–76.
[14] Strutt H, Strutt D. Long-range coordination of planar polarity in *Drosophila*. Bioessays 2005;27(12):1218–27.
[15] Veeman MT, Axelrod JD, Moon RT. A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. Dev Cell 2003;5(3):367–77.
[16] Cowan CR, Hyman AA. Asymmetric cell division in *C. elegans*: cortical polarity and spindle positioning. Annu Rev Cell Dev Biol 2004;20:427–53.
[17] Gönczy P, Rose LS, editors. Asymmetric cell division and axis formation in the embryo. WormBook, ed. T.C.e.R. Community. Vol. WormBook, doi:10.1895/wormbook.1.30.1, http://www.wormbook.org; 2005.
[18] Sulston JE, et al. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev Biol 1983;100(1):64–119.
[19] Goldstein B. Induction of gut in *Caenorhabditis elegans* embryos. Nature 1992;357(6375):255–7.
[20] Goldstein B. Establishment of gut fate in the E lineage of *C. elegans*: the roles of lineage-dependent mechanisms and cell interactions. Development 1993;118(4):1267–77.
[21] Goldstein B. An analysis of the response to gut induction in the *C. elegans* embryo. Development 1995;121(4):1227–36.
[22] Goldstein B. Cell contacts orient some cell division axes in the *Caenorhabditis elegans* embryo. J Cell Biol 1995;129(4):1071–80.
[23] Lin R, Thompson S, Priess JR. pop-1 encodes an HMG box protein required for the specification of a mesoderm precursor in early *C. elegans* embryos. Cell 1995;83(4):599–609.
[24] Thorpe CJ, et al. Wnt signaling polarizes an early *C. elegans* blastomere to distinguish endoderm from mesoderm. Cell 1997;90(4):695–705.
[25] Rocheleau CE, et al. Wnt signaling and an APC-related gene specify endoderm in early *C. elegans* embryos. Cell 1997;90(4):707–16.
[26] Schlesinger A, et al. Wnt pathway components orient a mitotic spindle in the early *Caenorhabditis elegans* embryo without requiring gene transcription in the responding cell. Genes Dev 1999;13(15):2028–38.
[27] Herman MA, Wu M. Noncanonical Wnt signaling pathways in *C. elegans* converge on POP-1/TCF and control cell polarity. Front Biosci 2004;9:1530–2159.
[28] Walston T, et al. Multiple Wnt signaling pathways converge to orient the mitotic spindle in early *C. elegans* embryos. Dev Cell 2004;7(6):831–41.
[29] Bei Y, et al. SRC-1 and Wnt signaling act together to specify endoderm and to control cleavage orientation in early *C. elegans* embryos. Dev Cell 2002;3(1):113–25.
[30] Goldstein B, et al. Wnt signals can function as positional cues in establishing cell polarity. Dev Cell 2006;10(3):391–6.
[31] Hawkins NC, et al. MOM-5 frizzled regulates the distribution of DSH-2 to control *C. elegans* asymmetric neuroblast divisions. Dev Biol 2005;284(1):246–59.
[32] Hutter H, Schnabel R. Specification of anterior–posterior differences within the AB lineage in the *C. elegans* embryo: a polarising induction. Development 1995;121(5):1559–68.
[33] Park FD, Priess JR. Establishment of POP-1 asymmetry in early *C. elegans* embryos. Development 2003;130(15):3547–56.
[34] Hunter CP, Kenyon C. Spatial and temporal controls target pal-1 blastomere-specification activity to a single blastomere lineage in *C. elegans* embryos. Cell 1996;87(2):217–26.
[35] Gho M, Schweisguth F. Frizzled signalling controls orientation of asymmetric sense organ precursor cell divisions in *Drosophila*. Nature 1998;393(6681):178–81.
[36] Bellaiche Y, et al. Frizzled regulates localization of cell-fate determinants and mitotic spindle rotation during asymmetric cell division. Nat Cell Biol 2001;3(1):50–7.
[37] Frank CA, et al. *C. elegans* HAM-1 positions the cleavage plane and regulates apoptosis in asymmetric neuroblast divisions. Dev Biol 2005;284(2):301–10.
[38] Axelrod JD, et al. Differential recruitment of Dishevelled provides signaling specificity in the planar cell polarity and Wingless signaling pathways. Genes Dev 1998;12(16):2610–22.
[39] McCartney BM, et al. *Drosophila* APC2 and Armadillo participate in tethering mitotic spindles to cortical actin. Nat Cell Biol 2001;3(10):933–8.
[40] Wen Y, et al. EB1 and APC bind to mDia to stabilize microtubules downstream of Rho and promote cell migration. Nat Cell Biol 2004;6(9):820–30.
[41] Wakefield JG, Stephens DJ, Tavare JM. A role for glycogen synthase kinase-3 in mitotic spindle dynamics and chromosome alignment. J Cell Sci 2003;116(Part 4):637–46.
[42] Krylova O, Messenger MJ, Salinas PC. Dishevelled-1 regulates microtubule stability: a new function mediated by glycogen synthase kinase-3beta. J Cell Biol 2000;151(1):83–94.
[43] Washbourne P, et al. Cell adhesion molecules in synapse formation. J Neurosci 2004;24(42):9244–9.
[44] Friedl P, Storim J. Diversity in immune-cell interactions: states and functions of the immunological synapse. Trends Cell Biol 2004;14(10):557–67.
[45] Le Borgne R, Bellaiche Y, Schweisguth F. *Drosophila* E-cadherin regulates the orientation of asymmetric cell division in the sensory organ lineage. Curr Biol 2002;12(2):95–104.

[46] Lu B, et al. Adherens junctions inhibit asymmetric division in the *Drosophila* epithelium. Nature 2001;409(6819):522–5.
[47] Jaiswal M, Agrawal N, Sinha P. Fat and Wingless signaling oppositely regulate epithelial cell–cell adhesion and distal wing development in *Drosophila*. Development 2006.
[48] Labbe JC, et al. PAR proteins regulate microtubule dynamics at the cell cortex in *C. elegans*. Curr Biol 2003;13(9):707–14.
[49] Bellaiche Y, et al. The Partner of inscuteable/discs-large complex is required to establish planar polarity during asymmetric cell division in *Drosophila*. Cell 2001;106(3):355–66.
[50] Katanaev VL, et al. Trimeric G protein-dependent frizzled signaling in *Drosophila*. Cell 2005;120(1):111–22.
[51] Adler PN. Planar signaling and morphogenesis in *Drosophila*. Dev Cell 2002;2(5):525–35.
[52] Saburi S, McNeill H. Organising cells into tissues: new roles for cell adhesion molecules in planar cell polarity. Curr Opin Cell Biol 2005;17(5):482–8.
[53] Ciruna B, et al. Planar cell polarity signalling couples cell division and morphogenesis during neurulation. Nature 2006;439(7073):220–4.
[54] Gong Y, Mo C, Fraser SE. Planar cell polarity signalling controls cell division orientation during zebrafish gastrulation. Nature 2004;430(7000):689–93.
[55] Park FD, Tenlen JR, Priess JR. *C. elegans* MOM-5/frizzled functions in MOM-2/Wnt-independent cell polarity and is localized asymmetrically prior to cell division. Curr Biol 2004;14(24):2252–8.
[56] Wu M, Herman MA. A novel noncanonical Wnt pathway is involved in the regulation of the asymmetric B cell division in *C. elegans*. Dev Biol 2006;293(2):316–29.
[57] Piekny AJ, Mains PE. Rho-binding kinase (LET-502) and myosin phosphatase (MEL-11) regulate cytokinesis in the early *Caenorhabditis elegans* embryo. J Cell Sci 2002;115(Part 11):2271–82.
[58] Spencer AG, et al. A RHO GTPase-mediated pathway is required during P cell migration in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 2001;98(23):13132–7.
[59] Bänziger C, et al. Wntless, a conserved membrane protein dedicated to the secretion of Wnt proteins from signaling cells. Cell 2006;125(3):509–22.