Mechanisms of spindle positioning: focus on flies and worms

Pierre Gönczy

Accurate spindle positioning is crucial for spatial control of cell division. During metazoan development, coordination between polarity cues and spindle position also ensures correct segregation of cell fate determinants. Converging evidence indicates that spindle positioning is achieved through interactions between cortical anchors and the plus ends of microtubules, generating pulling forces acting on spindle poles. This article discusses recent findings that indicate how this mechanism might be used for spindle positioning during *Drosophila* and *Caenorhabditis elegans* development.

The position of the mitotic spindle plays a key role in spatial control of cell division. In animal cells, the position of the spindle at anaphase determines the placement of the cleavage furrow, which forms so as to bisect the mitotic spindle [1]. Therefore, spindle position dictates the relative size of daughter cells, because a centrally located spindle leads to equal cleavage, whereas an eccentrically located spindle results in unequal cleavage (Fig. 1a). Such asymmetric divisions are prevalent during metazoan development, when they contribute to the generation of cell fate diversity. Moreover, spindle position dictates the spatial relationship between daughter cells. For example, a spindle oriented parallel to the plane of an epithelial sheath leads to both daughter cells remaining in the epithelium, whereas a spindle oriented perpendicular to that plane results in one daughter departing from the epithelium (Fig. 1b). Such perpendicular divisions take place when, for instance, neuroblasts delaminate from the neurectodermal sheath during insect neurogenesis. Importantly, spindle position must be coordinated with cell polarity. Several components are unevenly distributed in polarized cells and spindle position dictates their inheritance by each daughter cell (Fig. 1b). Thus, polarity cues and spindle position together ensure the correct segregation of cell-fate determinants during development.

What are the mechanisms that ensure proper spindle positioning? Evidence from several systems indicates that interactions between microtubules emanating from the spindle poles and the cell cortex play a major role. Here, I focus on spindle positioning in the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans*. I begin, however, by considering a general mechanistic framework for spindle positioning that draws on findings from several organisms. I end by speculating that a spindle positioning checkpoint in metazoan organisms might ensure coordination between temporal and spatial cell division control.

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Mechanistic framework for spindle positioning

The position of the spindle follows from that of the two spindle poles, which nucleate astral and spindle microtubules of the mitotic apparatus. What are the mechanical forces that act on spindle poles to achieve proper spindle positioning? In principle, 'pushing' forces resulting from polymerization of astral microtubules could help to position spindle poles. When the plus end of a growing microtubule encounters a fixed object like the cortex, the addition of tubulin dimers displaces the microtubule in the opposite direction. As a result, the tethered spindle pole is also displaced away from the cortex. Although microtubule polymerization forces can position an aster *in vitro* or the interphase spindle-pole body in *Saccharomyces cerevisiae* [2,3], it remains to be established whether they contribute to the positioning of spindle poles during mitosis.

By contrast, the contribution of 'pulling' forces that bring spindle poles closer to the cell cortex has been well documented. For instance, when the meiotic spindle is displaced from the cortex of *Chaetopterus* oocytes using a microneedle and then released, one of the spindle poles is rapidly pulled back towards the cortex [4]. Pulling forces also act on spindle poles in somatic cells. For example, when the spindle of rat kangaroo Ptk2 cells is severed using a laser microbeam, spindle poles move towards the cell cortex with higher peak velocities than in untreated cells, demonstrating the existence of forces external to the spindle pulling on spindle poles [5]. Findings in several systems indicate that such pulling forces are microtubule dependent. For instance, astral microtubules connect the moving spindle pole and the cortex in *Chaetopterus* oocytes, whereas severing of astral microtubule connections between the spindle pole and the cortex abolishes spindle pole movement in Ptk2 cells [4,5].

How are microtubule-dependent pulling forces generated? Two possibilities are suggested by an analogy between spindle positioning and chromosome segregation (Fig. 2) [6,7]. In both cases, the minus ends of microtubules are at the spindle pole, whereas the plus-ends are at either the cell cortex or the kinetochore. Therefore, microtubule-dependent pulling forces driving spindle positioning might be mechanistically related to those driving chromosome segregation. In the case of spindle positioning, these forces pull the spindle pole towards the cortex because the cortex is in a fixed

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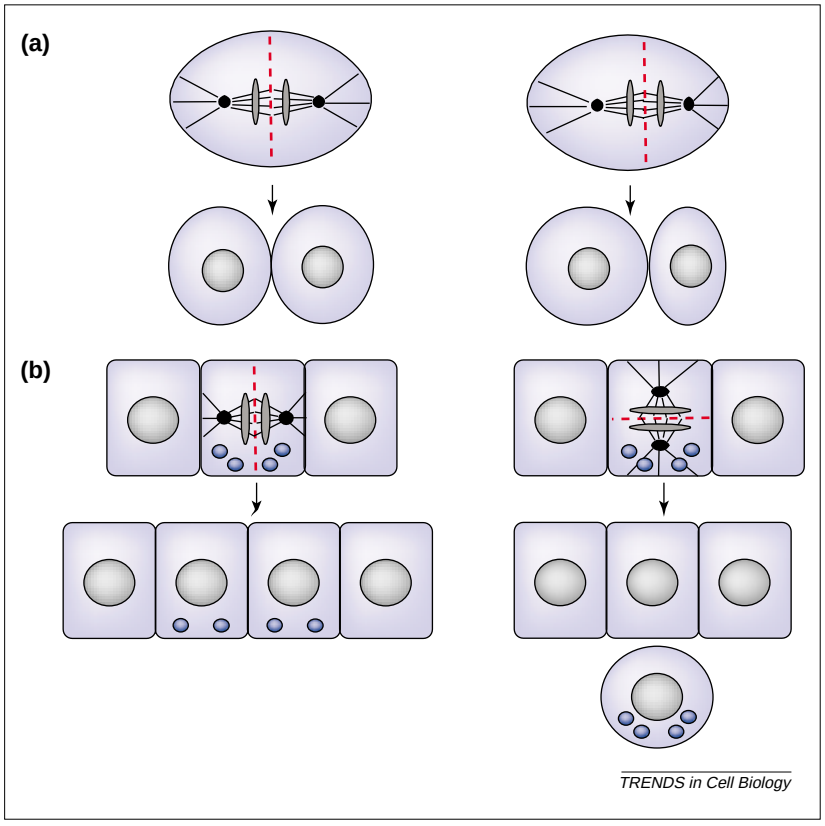

Fig. 1. The position of the spindle at anaphase dictates the relative sizes of and the spatial relationship between daughter cells. Spindle poles are shown as black disks, astral and spindle microtubules as black lines, chromosomes and nuclei as grey ovals and disks, the cleavage furrow as a dashed red line, and cell-fate determinants as blue disks. (a) A centrally located spindle results in two daughter cells of equal sizes (left), whereas an eccentrically positioned spindle results in two daughter of different sizes (right). (b) A spindle oriented parallel to the epithelial sheath results in both daughter cells remaining in the epithelium (left), whereas a spindle oriented perpendicular to the epithelial sheath results in one of the daughters leaving the epithelium (right). Spindle orientation dictates the inheritance of cell fate determinants by daughter cells. If cell fate determinants are present on the basal side of the mother cell then, when spindle orientation is parallel to the epithelium (left), both daughters inherit cell fate determinants. However, only one daughter cell inherits them when spindle orientation is perpendicular to the epithelium (right).

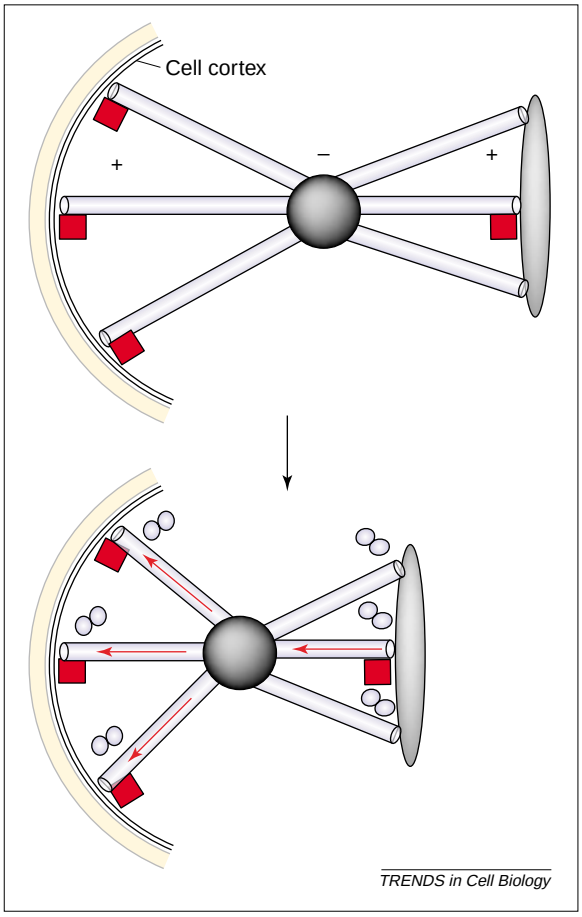

Fig. 2. Analogous mechanisms might be used to generate pulling forces during chromosome segregation and spindle positioning. The polarities of astral and spindle microtubules are indicated, and depolymerizing microtubules are indicated by the loss of tubulin dimers from the plus ends. For simplicity, only a limited cortical area and one set of sister chromatids is represented. The spindle pole is shown as a black disk, astral and spindle microtubules as black lines, and chromosomes as a gray oval. Orange squares indicate anchors that recognize the plus ends of microtubules or minus-end-directed motors such as cytoplasmic dynein. Orange arrows indicate direction of movement resulting from pulling forces acting along microtubules. Two types of mechanisms might generate pulling forces: microtubule-depolymerization-coupled movements and motor-protein-driven movements. In the first scenario, anchors at kinetochores or the cell cortex (orange squares) maintain interactions with the plus ends of depolymerizing microtubules, thus generating pulling forces (orange arrows). In the second scenario, minus-end-directed motors such as cytoplasmic dynein anchored at kinetochores or at the cell cortex (orange squares) exhibit minus-end-directed motility, thus generating pulling forces (orange arrows). Microtubules also shorten in the second scenario, although without resulting in force generation.

position. By contrast, in the case of chromosome segregation, these forces pull the kinetochore towards the spindle pole because the spindle pole and associated astral microtubules have a larger drag than the kinetochore.

Two types of mechanisms are thought to generate pulling forces during chromosome segregation: microtubule-depolymerization-coupled movements and minus-end-directed motor-driven movements [8]. Support for the first of these comes, for instance, from observations that kinesin coupled to beads remains attached to the plus ends of depolymerizing microtubules *in vitro* [9]. Support for the second comes from the presence of the minus-end-directed motor protein cytoplasmic dynein at kinetochores in several species, as well as from the requirement for dynein for proper chromosome segregation in *Drosophila* [10–13]. These two mechanisms are likely to be coupled, because cytoplasmic dynein could also maintain attachment with the plus ends of depolymerizing microtubules, whereas microtubules must shorten concomitant with dynein moving towards the spindle pole by virtue of its motor activity. Also, dynein might act by modulating microtubule dynamics, a possibility suggested by the observation that microtubules are more stable in dynein heavy-chain mutants in *S. cerevisiae* and *Aspergillus nidulans* [14,15]. By analogy to chromosome segregation, the two types of mechanism could also generate pulling forces during spindle positioning. Cortical anchors could maintain interactions with the plus ends of depolymerizing microtubules, whereas cortically bound cytoplasmic dynein could exhibit

Box 1. How else could minus-end-directed motors exert pulling forces on spindle poles?

The prevailing view for the generation of pulling forces acting on spindle poles invokes interactions between microtubules and the cell cortex. However, this need not be the case. For instance, minus-end-directed motors such as cytoplasmic dynein could be anchored to a peripheral structure some distance from the cortex. Alternatively, minus-end-directed motors anchored throughout the cell could generate pulling forces proportional to the length of microtubules. Such length-dependent forces have been revealed in newly fertilized sand dollar eggs [a] and could account for centrosome separation in vertebrate somatic cells [b]. In such scenarios, minus-end-directed motors must be anchored to a structure that has a higher drag than that of spindle poles; this structure might, for instance, be the actin cytoskeleton or the endoplasmic reticulum.

References

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minus-end-directed motility. In both types of mechanism, pulling forces would move the minus ends of microtubules and tethered spindle poles towards the cell cortex.

Although alternative mechanisms can be envisaged for the involvement of minus-end-directed motors in generating pulling forces on spindle poles (Box 1), analysis of spindle positioning in *S. cerevisiae* confirms that interactions between astral microtubules and cortical anchors play a major role. The mechanisms of spindle positioning in budding yeast have recently been reviewed elsewhere [16,17] and only principal findings that help in the discussion of spindle positioning in *Drosophila* and *C. elegans* are mentioned here.

In *S. cerevisiae*, the intranuclear spindle must be correctly positioned at the bud neck to segregate chromosomes faithfully to daughter cells. Spindle positioning is achieved in two steps. First, the nucleus is positioned along the mother–bud axis in a step that requires the function of Kar9p. Kar9p is located primarily at the bud cortex and interacts with Bim1p, a protein present at the plus ends of microtubules [18–21]. Cells with a *kar9*Δ or a *bim1*Δ mutation exhibit decreased pulling of the spindle pole body towards the cell cortex and inefficient nuclear positioning [19–21]. In a second step, the nucleus moves through the bud neck in a dynein-dependent manner. The cortical protein Num1p also plays a key role in this step: components of the dynein complex and Num1p immunoprecipitate together, and *num1* mutants resemble cells lacking dynein function [22–24].

Taken together, these considerations and findings support a mechanistic framework in which spindle positioning is achieved through interactions between cortical anchors and the plus ends of microtubules that result in the generation of pulling forces acting on spindle poles. Below, I discuss spindle positioning during *Drosophila* and *C. elegans* development in the light of this mechanistic framework.

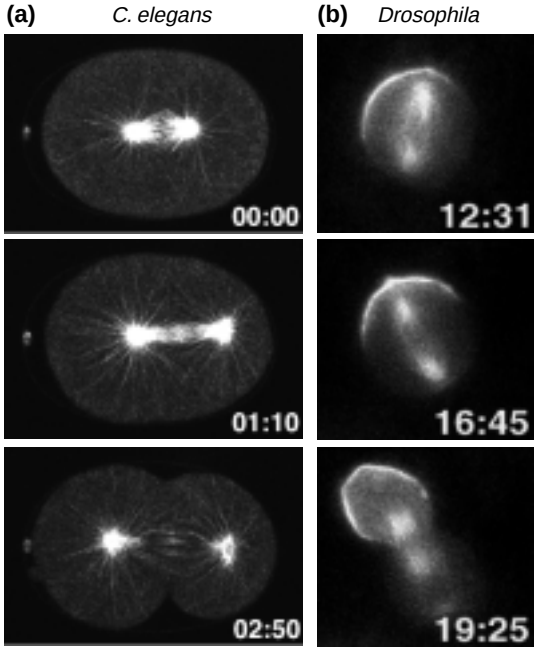

Fig. 3. Live imaging of spindle positioning in one-cell-stage *Caenorhabditis elegans* embryos and *Drosophila* sensory organ precursors. Elapsed time is indicated for each image in minutes and seconds. (a) Time-lapse fluorescence microscopy of one-cell-stage *C. elegans* embryo carrying a fusion between green fluorescent protein (GFP) and β-tubulin. The embryo is ~50 μm long, and the spindle poles and spindle microtubules are clearly visible, whereas astral microtubules are less apparent in these confocal slices. The spindle is initially in the cell centre (top panel) but is displaced towards the posterior during anaphase (middle), resulting in asymmetric cleavage (bottom). (b) Time-lapse fluorescence microscopy of *Drosophila* sensory organ precursor cell expressing a GFP–tau fusion to mark microtubules and GFP–Pon to mark the axis of polarity. The cell diameter is ~10 μm, and spindle poles are visible, whereas individual microtubules cannot be distinguished. The spindle is initially at a slight angle to the cortical Pon crescent (top panel) but is reoriented so that the spindle is lined up with the polarity axis at cell division (bottom). Photographs from panel (b) reproduced, with permission, from Ref. [50].

Spindle positioning in one-cell-stage *C. elegans* embryos

Recent experiments demonstrate that unbalanced pulling forces establish proper spindle position along the anteroposterior (AP) axis of one-cell-stage *C. elegans* embryos. In this organism, a sperm component provides an initial polarizing cue, which probably corresponds to astral microtubules nucleated from the paternally contributed centrosome [25,26]. The sperm component directs six PAR proteins (for 'partitioning-defective'), as well as the small G protein CDC-42 and the atypical protein kinase C PKC-3, to establish AP polarity [27,28]. In response to polarity cues, the spindle is displaced towards the posterior during anaphase (Fig. 3a). As a result, the first division is unequal, giving rise to a larger anterior blastomere (AB) and a smaller posterior blastomere (P_1). In most *par* mutant embryos, AP polarity cues are not established and posterior spindle displacement does not take place. As a result, the first division is equal.

Box 2. Distinct requirements for Gα subunits in *Drosophila* and *C. elegans*

In the fruit fly *Drosophila melanogaster*, the heterotrimeric G protein subunit $G\alpha_i$ was identified biochemically as a binding partner for Pins (Partner of Inscuteable), a GoLoco-domain-containing protein required for spindle orientation and asymmetric distribution of cell-fate determinants in neuroblasts [a,b]. Inactivation of $G\alpha_i$, which can be achieved using a mutation in the interacting Gβ subunit Gβ13F, results in spindle positioning defects in both neuroblasts and sensory organ precursors [c]. However, these defects are accompanied by dramatic alterations in the distribution of polarity cues. For instance, instead of being localized in a basal cortical crescent as in wild-type neuroblasts, Miranda is delocalized to the cytoplasm or present in a crescent that is not basal. Similarly, instead of being localized in a cortical crescent as in wild-type sensory organ precursors, Numb is distributed around the cell cortex.

In the nematode *Caenorhabditis elegans*, by contrast, the Gα subunits GOA-1 and GPA-16, and the GoLoco-domain-containing proteins AGS-3.2 and AGS-3.3 appear to act downstream of, or in parallel to, polarity cues (main text). Polarity defects might become apparent in *C. elegans* embryos that lack *goa-1/gpa-16* or *ags-3.2/ags-3.3* function when additional markers are examined and other cell types investigated. Alternatively, the difference might stem from the different nature of the proteins involved. Indeed, *C. elegans* GOA-1 and GPA-16 are more closely related to *Drosophila* $G\alpha_o$, a Gα subunit that does not bind to the Pins complex [c], than they are to *Drosophila* $G\alpha_i$. Moreover, *C. elegans* AGS-3.2 and AGS-3.3 shows little homology to *Drosophila* Pins outside the GoLoco domain. Because of these differences, the targets of Gα signaling might be distinct in different organisms and include polarity cues only in *Drosophila*.

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Spindle severing experiments demonstrate that extra-spindle pulling forces act on spindle poles in wild-type one-cell-stage embryos [29]. Interestingly, the posterior spindle pole moves faster than the anterior one after spindle severing, indicating that a larger net pulling force acts on the posterior spindle pole. Additional spindle severing experiments conducted in *par-2* and *par-3* mutant embryos establish that the extent of the pulling force is regulated by AP polarity cues. Therefore, polarity translates into asymmetric spindle positioning by modulating the net pulling force acting on each spindle pole. The mechanical basis of these pulling forces remains to be elucidated. It will be interesting to determine whether they are coupled to microtubule depolymerization or driven by cytoplasmic dynein-mediated minus-end-directed motility. Equally interesting will be to discover how polarity cues modulate force-generation mechanisms to result in unbalanced pulling forces. Mathematical modeling suggests that increased pulling forces on the posterior spindle pole might result from the destabilization of interactions between astral microtubules and cortical anchors specifically at the posterior cortex [29].

Two α subunits of heterotrimeric G proteins are important for generating the pulling forces that act on both anterior and posterior spindle poles. The Gα subunits GOA-1 and GPA-16 are required together to promote posterior spindle displacement in one-cell-stage *C. elegans* embryos [30]. There is a similar requirement for two essentially identical proteins tentatively named AGS-3.2 and AGS-3.3 (after the distantly related mammalian activator of G-protein signaling 3), which harbour a GoLoco domain characteristic of molecules that interact with Gα subunits [31,32]. The distributions of PAR proteins and of P granules, which are normally segregated to the posterior of one-cell-stage embryos in response to AP polarity [33,34], are not affected in *goa-1/gpa-16* (*RNAi*) or *ags-3.2/ags-3.3* (*RNAi*) embryos, indicating that polarity cues are intact [30] (K. Colombo and P. Gönczy, unpublished). Strikingly, spindle severing experiments reveal that pulling forces acting on both spindle poles are dramatically decreased in such embryos (P. Gönczy and S. Grill, unpublished). Therefore, GOA-1/GPA-16 and AGS-3.2/AGS-3.3 are essential for force generation in one-cell-stage *C. elegans* embryos. How this is achieved remains to be elucidated. Mammalian Gα subunits can promote microtubule catastrophes *in vitro* [35], raising the possibility that Gα subunits in one-cell-stage *C. elegans* embryos promote depolymerization-coupled movements and the generation of pulling forces.

Interestingly, although a Gα subunit and a GoLoco-domain-containing protein are also required for proper spindle positioning in *Drosophila*, they act at the level of polarity cues in that organism (Box 2) [36–38]. Thus, signaling via α subunits of heterotrimeric G proteins appears to play distinct roles in governing asymmetric cell division in *C. elegans* and in *Drosophila*.

Rotation events that orient the spindle in *C. elegans* and in *Drosophila*

Interactions between a spatially restricted cortical site and astral microtubules ensure proper spindle orientation in the P_1 blastomere of two-cell-stage *C. elegans* embryos. In this cell, the centrosome pair lies initially perpendicular to the AP axis. During prophase, however, one centrosome moves towards a site in the anterior cortex, thus inducing a 90° rotation of the associated nucleus and other centrosome. As a result, the spindle sets up along the AP axis, and factors such as P granules that are segregated to the posterior of P_1 are inherited strictly by its posterior daughter [39]. Local laser irradiation experiments indicate that the 90° rotation is driven by interactions between astral microtubules emanating from the leading centrosome and a spatially restricted cortical site [40]. What is the nature of this cortical site? Components of the dynactin complex and the dynein heavy chain are enriched at that location [41–43], which suggests a model in which cortically anchored dynein interacts with astral microtubules and pulls the associated centrosome by minus-end-directed motility, thus inducing rotation. Although it is attractive, this model has not been directly tested, because embryos lacking dynein or dynactin function after inactivation by RNA-mediated interference have defects in the one-cell stage that preclude assessment of a potential requirement in P_1 [41,42].

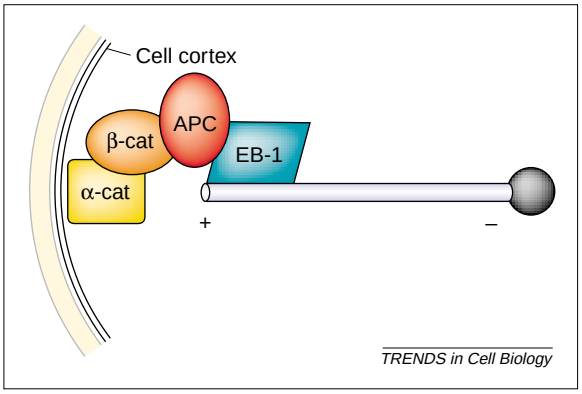

Fig. 4. Hypothetical model of interaction between cortical anchors and astral microtubules. α-Catenin (α-cat), β-catenin (β-cat) and the adenomatous polyposis coli protein (APC) form a complex that binds to cortical F-actin through the α-catenin moiety. The complex is linked to astral microtubules through the interaction between APC and EB-1, which is located at the plus ends of microtubules.

One crucial component of cortical anchor function in *C. elegans* might be *let-99* (for 'lethal') [44]. *let-99* mutant embryos exhibit spindle positioning defects in many embryonic blastomeres. For instance, the characteristic 90° rotation does not take place in P_1. Importantly, polarity cues are not affected in *let-99* mutant embryos, because asymmetric distribution of PAR-3, PAR-2, PAR-1 and P granules is the same as in the wild type. Therefore, *let-99* is required downstream of, or in parallel to, polarity cues to mediate proper spindle positioning. How does *let-99* achieve its function? Time-lapse microscopy shows that nucleus–centrosome complexes in *let-99* mutant embryos undergo abnormal vigorous movements towards and away from the cell cortex, suggesting that interactions between astral microtubules and cortical anchors are established but not properly maintained. The *let-99* gene encodes a protein with no clear ortholog in other organisms, and it will be particularly interesting to elucidate how it might modulate pulling forces that act on centrosomes and spindle poles.

Rotations events similar to the one observed in the P_1 blastomere of *C. elegans* embryos are crucial for achieving proper spindle orientation in *Drosophila* neuroblasts and sensory organ precursor cells. During development of the fly's central nervous system, neuroblasts divide asymmetrically in a stem-cell lineage, generating a neuroblast and a smaller ganglion mother cell at each cell division [45]. Dedicated proteins destined to the ganglion mother cell, including Numb, Partner of Numb (Pon), Prospero and Miranda, become localized during mitosis in a crescent on the basal side of neuroblasts [46,47]. For these components to be correctly partitioned at cell division, the spindle must be positioned along the apico-basal axis. Live imaging of a fusion protein between green fluorescent protein (GFP) and the microtubule-associated protein tau reveals that the centrosome pair initially lies perpendicular to this axis [48]. At the onset of mitosis, however, the spindle poles undergo a 90° rotation, thus achieving proper spindle orientation by anaphase. Rotation also positions the spindle along an axis of polarity in the *Drosophila* sensory organ precursor cell, which similarly undergoes asymmetric division [49]. In this case, live imaging using GFP–tau and GFP–Pon demonstrates that spindle orientation is achieved so as to align the spindle with the asymmetrically distributed Pon crescent (Fig. 3b) [50].

Cortical anchors in metazoan organisms

Although the molecular nature of cortical anchors underlying rotation events in *C. elegans* and *Drosophila* remains to be elucidated, recent findings suggest that an evolutionarily conserved complex containing a protein related to adenomatous polyposis coli (APC) can link cortical actin to astral microtubules (Fig. 4). In the syncytial *Drosophila* embryo, the β-catenin homolog Armadillo (Arm) is required to tether the spindle to cortical actin [51]. Mutant analysis indicates that a complex composed of Arm, α-catenin and the APC-related protein dAPC2 interacts with cortical actin through the α-catenin moiety. Interference with dAPC2 function later in development results in spindle positioning defects in neurectodermal cells, and similar defects are observed following inactivation of dEB1, a *Drosophila* homolog of EB1 [52]. Interestingly, human EB1 is a microtubule-associated protein that localizes to the plus ends of growing microtubules and interacts with APC [53,54]. Although dEB1 does not appear to interact with dAPC2 [52], the presence of two EB1-related genes and two APC-related genes in *Drosophila* leaves open the possibility that the molecular interaction between an α-catenin–β-catenin–APC complex and EB1 is conserved across metazoan evolution. In fact, the functional conservation might extend in part to unicellular eukaryotes, because *S. cerevisiae* Kar9p has a small stretch of homology with APC and interacts with the EB1 homolog Bim1p [19–21,55]. Although a complex containing APC/Kar9p appears to play an important role in some instances of spindle positioning, distinct cortical anchors for astral microtubules probably remain to be discovered.

Relationships between polarity cues and spindle positioning

Spindle position must be coordinated with cell polarity to ensure the correct segregation of cell fate determinants during development (Fig. 1b). Such coordination is achieved chiefly because polarity cues control spindle positioning. Thus, the spindle is not displaced towards the posterior of one-cell-stage *C. elegans* embryos in most *par* mutants [34]. Likewise, spindle orientation is impaired in *Drosophila inscuteable* mutant neuroblasts, which lack proper apico-basal polarity [56]. In some cases, polarity cues might control spindle positioning quite directly. For example, *par-1* encodes a Ser/Thr protein

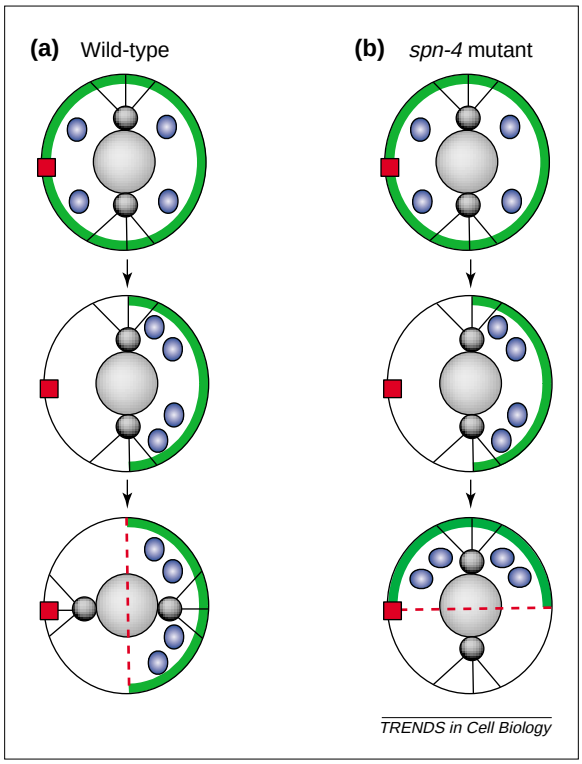

Fig. 5. Relationship between polarity cues and spindle positioning in the P_1 blastomere of wild-type and *spn-4* mutant *Caenorhabditis elegans* embryos. Spindle poles are shown as black disks, astral and spindle microtubules as black lines, nuclei as gray disks, and the cleavage furrow as a dashed red line. PAR-2 is shown as a thick green line, P granules as blue disks and the cortical anchor as an orange square. (a) In wild-type embryos, PAR-2 becomes restricted to the posterior cortex and P granules are segregated to the posterior of the P_1 blastomere. The characteristic 90° rotation of the centrosome–nucleus complex takes place after this. Therefore, the spindle is established along the longitudinal axis. As a result, PAR-2 and P granules will be present only in the posterior daughter cell P_2. (b) In *spn-4* mutant embryos, as in wild-type, PAR-2 becomes restricted to the posterior cortex, whereas P granules are segregated to the posterior of the P_1 blastomere. However, the 90° rotation of the centrosome–nucleus complex does not take place and so the spindle is established perpendicular to the longitudinal axis. PAR-2 and P granules relocalize before mitosis so as to lie above one of the spindle poles, thus restoring their spatial relationship to the spindle. As a result, PAR-2 and P granules will be present in only one daughter cell, as in the wild-type.

kinase whose mammalian ortholog can phosphorylate microtubule-associated proteins, leading to microtubule destabilization [57,58]. Because PAR-1 is restricted to the posterior cortex of one-cell-stage *C. elegans* embryos, it is tempting to speculate that its activity leads to local destabilization of microtubules, thus generating depolymerization-coupled movements and increased pulling forces. Compatible with a role in modulating microtubule dynamics, reduction of *par-1* function in *Drosophila* oocytes results in a disorganized microtubule network, as well as in defective AP axis formation [59,60]. In other cases, polarity cues might control spindle positioning less directly, perhaps by modulating components of the force generation machinery or cortical anchors.

Coordination between spindle position and cell polarity might, in some cases, be also achieved by altering the localization of polarity cues or cell-fate determinants to align them with a mispositioned spindle. Such feedback alterations might take place in *spn-4* (for 'spindle orientation defective') mutant embryos of *C. elegans* (Fig. 5) [61]. The *spn-4* gene encodes a predicted RNA-binding protein of the RRM family and is required for the characteristic 90° rotation in P_1. In *spn-4* mutant embryos, PAR-2 and P granules are initially restricted to the posterior of P_1, as in the wild type, indicating that the lack of rotation does not result from general polarity defects. Surprisingly, however, PAR-2 and P granules relocalize above one of the presumptive spindle poles before mitosis. In this manner, PAR-2 and P granules are segregated to only one daughter cell, as in the wild type, despite the absence of rotation. Although it is possible that the lack of *spn-4* function affects polarity cues strictly before mitosis, an attractive alternative is that PAR-2 and P granules relocalize in response to the spindle-positioning defect.
A potentially similar situation is encountered in *Drosophila* neuroblasts with the *asterless* mutation [62]. In these mutants, Miranda cortical crescents are incorrectly localized in 52% of cells in metaphase, but only in 11% of cells in telophase.

Although polarity cues and cell-fate determinants are correctly localized in the complete absence of spindle in *C. elegans* embryos and *Drosophila* neuroblasts [33,41,63], the above observations suggest that the distribution of polarity cues and cell fate determinants might be altered in some cases to align them with a mispositioned mitotic spindle.

Spindle positioning checkpoint in metazoan organisms?

As an extension to the observations discussed above, it is worth considering whether there might be a spindle positioning checkpoint in metazoan organisms. The existence of such a checkpoint is well established in *S. cerevisiae*, in which genetic and biochemical evidence supports a model in which Bub2p, Tem1p and Lte1p play a central role in monitoring spindle position [16,64]. Both Bub2p and Tem1p are associated preferentially with the spindle pole body that is destined for the bud, and Bub2p is a component of a GTPase-activating protein (GAP) that inactivates the Tem1p GTPase. By contrast, Lte1p is present exclusively in the bud and is a GTPase-exchange factor (GEF) that activates Tem1p. It is thought that mitotic exit is prevented by Bub2p until Tem1p comes into contact with Lte1p when the spindle is correctly positioned in the bud neck.

The available evidence does not support the existence of a strict spindle positioning checkpoint in *C. elegans* or *Drosophila*. Thus, cell division proceeds with apparently normal kinetics in embryos lacking *goa-1*/*gpa-16* or *let-99* function, despite severe spindle positioning defects [30,44]. However, the spindle assembly checkpoint is not functional either in early embryos of most organisms, raising the

possibility that a spindle positioning checkpoint might yet be discovered in somatic cells of *C. elegans* or *Drosophila*. Micromanipulation experiments in mammalian cells show that a misoriented spindle can delay anaphase onset [65]. It will be interesting to investigate to what extent a spindle positioning checkpoint may be widespread in metazoan organisms to ensure coordination of spatial and temporal aspects of cell division.

Concluding remarks

I have discussed a mechanism for spindle positioning in which interactions between cortical anchors and the plus ends of microtubules result in the generation of pulling forces acting on spindle poles. Exciting questions lie ahead. It will be important to elucidate the mechanical basis of force generation and to determine the molecular nature of cortical anchors. It will also be interesting to discover how cortical anchors and minus-end-directed motors such as cytoplasmic dynein can maintain interactions with depolymerizing microtubules, so as to generate pulling forces on spindle poles. Another major challenge lies in understanding how temporal and spatial regulation of microtubule dynamics might be used, perhaps under the control of polarity cues, to modulate the extent of pulling forces acting on spindle poles. Spindle position in some cells is influenced by cell-extrinsic factors and it will be of interest to unravel how the core machinery is altered in these cases. These and other advances will generate a more comprehensive understanding of the mechanisms underlying spatial cell division control.

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Ephrins in reverse, park and drive

Chad A. Cowan and Mark Henkemeyer

Eph receptors and their membrane-anchored ephrin ligands are thought to orchestrate cell movements by transducing bidirectional tyrosine-kinase-mediated signals into both cells expressing the receptors and cells expressing the ligands. Whether the resulting event is repulsion of an axonal growth cone, directing the orderly segmentation of hindbrain rhombomere cells or controlling angiogenic remodelling, such elaborate and diverse cell movements require intricate changes in the actin cytoskeleton, as well as precise regulation of cellular adhesion. Recent work by several groups has begun to link ephrin reverse signals to intracellular pathways that regulate actin dynamics and might help to explain how these ligands function as receptors to direct cell movement, adhesion and de-adhesion events.

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The development of multicellular organisms requires an exquisite interplay of cell proliferation, differentiation, adherence and movement. Fundamental to normal development are many different cell-anchored receptors that communicate information into their cell after exposure to the appropriate ligand. Whether soluble and acting at a distance, or anchored to the membrane and acting on cell–cell contact, most ligand–receptor systems transduce information unidirectionally from the ligand-expressing cell into the receptor-expressing cell.

Presenting a contrast to this model are the large family of Eph receptor tyrosine kinases and their membrane-anchored ephrin ligands. On cell–cell contact, these molecules transduce important signals bidirectionally into both the receptor-expressing cell and the ligand-expressing cell in what is known as 'forward' and 'reverse' signaling, respectively [1] (Fig. 1). In this way, the Eph receptors can also function as ligands and the ephrin ligands can also function as receptors. Bidirectional signaling mediated by Ephs and ephrins is thought to communicate signals that regulate the cytoskeleton during axon pathfinding, cell migration, cell adhesion and vascular remodelling (reviewed in [2–5]) and is now also linked to the regulation of fluid production and ion homeostasis in nonmotile epithelial cells [6]. As the known range of biological functions performed by this family of multitalented receptors and ligands continues to expand, focus is shifting to determining how these molecules transduce such important and diverse signals into the cell.

Ephs and ephrins can be divided into two classes on the basis of their structural features and binding affinities. A-subclass ephrins (ephrin-A1 to ephrin-A5) are attached to the outer leaflet of the plasma membrane by a glycosylphosphatidylinositol (GPI) linkage. They bind to and activate the A-subclass Eph receptors

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