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# Asymmetric spindle positioning

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When a spindle is positioned asymmetrically in a dividing cell, the resulting daughter cells are unequal in size. Asymmetric spindle positioning is driven by regulated forces that can pull or push a spindle. The physical and molecular mechanisms that can position spindles asymmetrically have been studied in several systems, and some themes have begun to emerge from recent research. Recent work in budding yeast has presented a model for how cytoskeletal motors and cortical capture molecules can function in orienting and positioning a spindle. The temporal regulation of microtubule-based pulling forces that move a spindle has been examined in one animal system. Although the spindle positioning force generators have not been identified in most animal systems, the forces have been found to be regulated by both PAR polarity proteins and G-protein signaling pathways in more than one animal system.

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## Introduction

When a mitotic spindle is positioned asymmetrically within a cell, cell division results in daughter cells that are unequal in size. Such asymmetry in spindle position occurs commonly, for example in budding yeast mitotic divisions and in countless developmental cell divisions. In animal development, asymmetric divisions like these often have an additional, important role in unequally partitioning cell-fate determinants. Asymmetry in size of cells alone is likely to be important to partition such determinants precisely [1] and to allow large stem cells to divide repeatedly without becoming depleted of cytoplasm [2]. Asymmetric spindle positioning was first seen over a century ago (Figure 1), yet the mechanisms involved are only now beginning to be elucidated [3]. Here, we discuss the physical forces that asymmetrically position spindles, the molecular machinery that may generate and regulate these forces, and the checkpoints

that can monitor spindle position in some systems. We will highlight some recent findings that have shed light on the molecular mechanisms of asymmetric spindle positioning.

## Forces that asymmetrically position a spindle

In a symmetrically dividing cell, passive mechanisms locate the spindle at the center of the cell [4]. Certain cell shapes alone can dictate asymmetric division planes [5,6], but in most cases of asymmetric division, it is likely that forces are actively exerted on a spindle from one or more specialized sites on the cell cortex. To assess the regional sources of these pulling and pushing forces, researchers have cut spindles in half, or eliminated one side of a spindle, and followed the subsequent movement of the experimentally isolated spindle parts [7,8\*,9]. Such experiments have been performed to date on only one type of asymmetrically dividing cell, the relatively large (50  $\mu\text{m}$  long) one-cell stage *C. elegans* embryo (Figure 2) [8\*,10]. Here, experiments have demonstrated that microtubule pulling forces are pervasive throughout the cell cortex, and that these pulling forces are stronger on one side of the cell — at the posterior cortex — causing the spindle to shift from the center of the embryo towards the posterior. These posterior pulling forces are generated early in mitosis, even before the spindle is completely assembled. Spindles are not shifted this early in the cell cycle, however, as astral microtubules tether the spindle to the anterior cortex until near the time that spindle assembly is completed [8\*]. Determining whether pulling forces dominate similarly in other asymmetrically dividing cells awaits experiments in other systems.

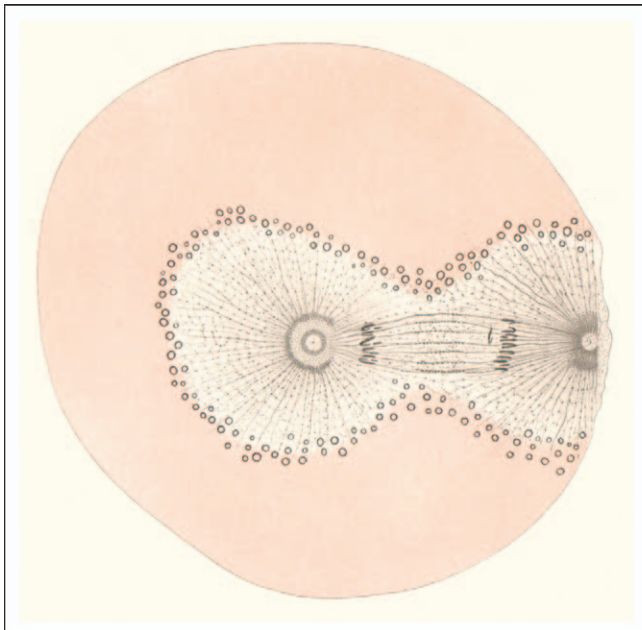
## Force-generating mechanisms

### Molecular motors

Early studies in *Chaetopterus* oocytes demonstrated the presence of a unique attachment site to which the spindle migrates when experimentally pulled away from the cortex [11], and morphologically unique sites in the cortex of certain sea urchin embryonic cells toward which spindles move [12]. Whether movement in either case is driven by molecular motors or by other mechanisms, such as microtubules depolymerizing [13] while maintaining continuous attachment to a cortical site, is not clear. These studies have been influential, however, in suggesting that regions of the cortex may be specialized for spindle attachment and spindle pulling.

Molecular motors that walk along microtubules or actin filaments can contribute to spindle positioning directly, by generating a pulling or pushing force, or indirectly, by transporting cargo proteins that contribute to spindle

Figure 1



Asymmetric spindle positioning in a mussel, a drawing from a 1901 publication based on staining of embryos with textile dyes used at the time by cytologists [3]. Several theories of how spindles are positioned asymmetrically already existed by this time. Reprinted with permission.

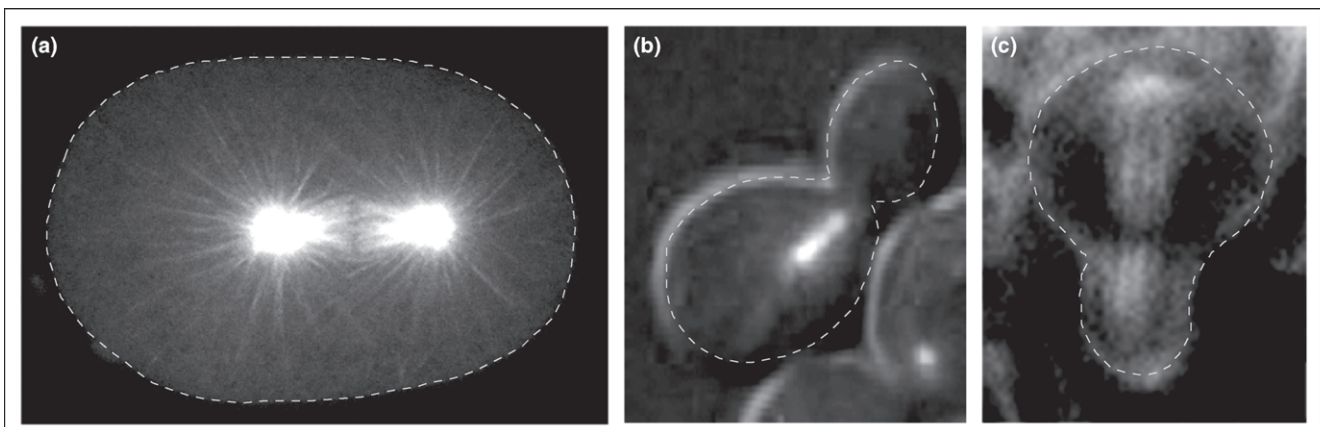
positioning. Budding yeast is one of the best-studied cases of motors functioning directly to position a spindle [14,15]. Spindle orientation is initially dependent on myosin, which functions through interactions with the plus-end microtubule binding proteins Kar9 and Bim1 to move microtubules along actin cables to the bud tip. Later, during anaphase, spindle positioning is dependent

on dynein, which binds microtubule plus ends and guides the spindle through the neck and into the daughter cell (Figure 3).

The posterior cell of the two-cell stage *C. elegans* embryo may undergo spindle rotation through attachment of microtubules to a cortical capture site enriched in actin, dynein and components of the dynactin complex, which are recruited to the cell division remnant of the previous one-cell stage division [16–18]. The use of conditional dynein mutants suggests that dynein is essential for spindle positioning in this cell, but is dispensable for spindle positioning at the one-cell stage [19]. These results might not completely rule out a role for dynein in positioning the spindle in the one-cell stage embryo, as whether dynein was completely nonfunctional was not clear. These disrupted dynein motors might still function in walking along a microtubule more slowly than normal and might inefficiently release upon reaching the minus end of a microtubule, since the authors observed a decrease in the rate of spindle positioning and an enrichment of dynein near centrosomes [19].

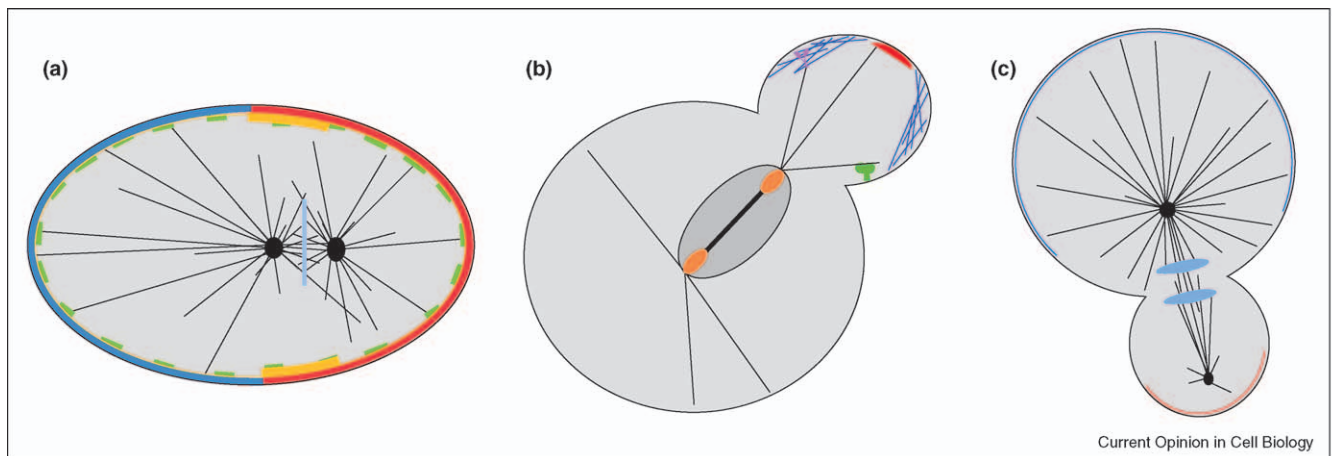
Meiotic divisions in animal eggs are extreme forms of asymmetric division, producing tiny polar bodies and large egg cells. Recent work on *C. elegans* and *Xenopus* meiotic division has identified motors required to position spindles: a microtubule-based motor in *C. elegans*, and an actin-based motor in *Xenopus* [20,21]. Yang and colleagues found a role for a kinesin motor in *C. elegans* meiotic spindle positioning. Meiotic spindles in *C. elegans* have defects in translocation to the cortex in oocytes lacking a kinesin-I homolog or its associated light chains, or a putative cargo protein that has been shown to interact with both the kinesin light chains and a heterochromatin binding protein in a yeast two-hybrid screen [22]. Meiotic

Figure 2



The mitotic spindle is positioned asymmetrically (a) closer to the posterior region of the *C. elegans* embryo, (b) at the bud neck in budding yeast cells and (c) along an apical–basal axis in *Drosophila* neuroblasts. Live-cell imaging of cells expressing tubulin:GFP or tau:GFP have allowed the analysis of changes in spindle position, orientation, structure, size and dynamics. Budding yeast image provided by J Molk and K Bloom. *Drosophila* image provided by A Brand.

Figure 3



The mitotic spindle is positioned asymmetrically in these cell divisions. **(a)** In the *C. elegans* one-cell stage embryo, the mitotic spindle is positioned close to the posterior cortex. This is dependent on the presence of cortical proteins (PAR-3 is blue, PAR-2 is red, LET-99 is orange) that regulate force generators, which might be dynein patches localized at the cortex (green). **(b)** In budding yeast, the spindle is oriented when myosin (purple) binds plus ends of microtubules to direct them to cortical proteins at the bud tip cortex (red), which may provide a pulling force. Dynein (green) positions the spindle into the bud neck during anaphase through interactions of astral microtubules at the cortex. **(c)** In *Drosophila* neuroblasts, the spindle is asymmetric during anaphase when the apical microtubules are able to grow longer than basal microtubules. Cortical complexes that are required for spindle orientation and cell fate determination include the PAR/aPKC and Pins/G $\alpha$  complexes, which are localized apically (dark blue), and Miranda, Prospero and Numb, which are localized basally (red).

spindles in *C. elegans* oocytes lack centrosomes and astral microtubules, and the potential link between kinesin-I and the meiotic spindle suggests a model in which the kinesin-I motor activity might directly translocate the spindle to the cortex. *Xenopus* meiotic spindles are positioned adjacent to the cortex by the interaction of microtubules and F-actin [23]. Recent work has found that an unconventional myosin, Myo10, interacts directly with microtubules [20<sup>••</sup>]. Disruption of Myo10 function results in defects in nuclear positioning, an event that normally requires microtubules [23,20<sup>••</sup>], and in spindle structure and rotation, which normally requires actin filaments [20<sup>••</sup>]. These results suggest a role for Myo10 in linking the actin and microtubule networks for their function in nuclear and spindle positioning.

Other motors function indirectly in spindle positioning, by transporting other motors or non-motor proteins that affect spindle movement. Recent studies in budding yeast have shown that Kip2 kinesin plays roles in transporting dynein and Bik1, a CLIP-170-related microtubule-stabilizing protein, to the plus ends of astral microtubules, from which dynein is presumably delivered to the cortex [24,25]. In asymmetrically dividing *Drosophila* neuroblasts, dynein has been shown to be required for apical localization of *inscuteable* mRNA, and thus of Insc protein, which plays a role in both spindle orientation and segregation of cell fate determinants [26,27]. Localization of basally localized proteins required for asymmetric division in this system requires the actin-based motors myosin VI and myosin II [28,29].

### Cortical capture of microtubules

A cortical capture mechanism can perform several jobs during cell division: microtubules that interact with the cortical capture site can orient the spindle along a specific axis, continued interaction with the site can maintain proper orientation, and cortical proteins that depolymerize microtubules can function in generating pulling forces to move a spindle to an asymmetric position. Cortical capture mechanisms may use microtubule motors, instead of microtubule depolymerizing proteins, to 'reel in' a spindle. Examples of this type of cortical capture include the *Chaetopterus* oocyte and the two-cell stage *C. elegans* embryo, as mentioned above.

In budding yeast, astral microtubules are captured at the bud tip cortex and function in positioning the spindle along the mother-bud axis [14]. Several recent studies have focused on understanding cortical capture in budding yeast and identifying proteins that may regulate microtubule dynamics at the capture site. Kar9 functions in linking microtubule plus ends, via Bim1, to Myo2, which guides microtubules along actin cables towards the bud tip. Live-cell imaging experiments in budding yeast have distinguished the roles of Kar9 and actin-associated Bud6 in microtubule cortical capture: Kar9 functions in delivery of microtubules along actin cables into the bud, while Bud6 functions in securing microtubule capture at the bud tip [30<sup>••</sup>]. It has also been shown that subunits of the type I phosphatase complex act via Bud14 to regulate the interaction of microtubules at the bud cortex, thus maintaining spindle position within the bud neck [31].

Other recent work has shown how an asymmetry in spindle pole bodies may contribute to cortical capture: the cyclin-dependent kinase Cdc28 and cyclin B Clb4 are localized to the bud-ward spindle pole body (SPB), and are translocated to the plus ends of astral microtubules in a manner dependent on Kar9 [32,33]. This complex regulates the interaction of microtubules with the bud cortex, although how Cdc28–Clb4 modifies cortically bound microtubules remains unknown. These findings are in contrast to a previous model, in which the Cdc28–Clb4 complex is associated with the mother cell SPB, where it prevents Kar9 binding [34].

### Microtubule dynamics and length

Some asymmetric cell divisions may depend directly on microtubule dynamics to position a spindle. It is possible, for example, that by locally regulating the stability of microtubules, the duration of their interaction with microtubule motors can be controlled. It is also possible that locally stable microtubules that reach the cortex but do not interact with motors can occlude movement of a spindle. By imaging microtubules at the cortex in early *C. elegans* embryos, Labbé *et al.* [35] found that microtubules reaching the anterior cortex are more stable than those reaching the posterior cortex. Whether this difference is required to move the spindle asymmetrically is not yet known. Recent work in *C. elegans* has also examined the effects of specific mutations in tubulin isoforms on spindle positioning — certain dominant mutations of these tubulin isoforms affect microtubule dynamics [36] as well as spindle positioning events [37–39] — but how altered microtubule dynamics affect spindle positioning is unclear.

Microtubule length must also be regulated to correctly position a spindle, and this is especially apparent during the meiosis-to-mitosis transition in animal development. The *C. elegans* homologs of the microtubule-severing protein katanin are required to keep microtubules short during meiosis but must be downregulated in mitosis to allow the growth of a larger spindle that fills the one-cell-stage embryo [40–43]. Early *C. elegans* embryos lacking MBK-2, a member of the Dyrk family of protein kinases, have short microtubules and defects in spindle positioning [44]. This phenotype is rescued by knockdown of katanin, suggesting that MBK-2 protein normally functions to downregulate katanin, thereby controlling spindle size during mitosis [44]. Other proteins that affect microtubule length independently of the katanin pathway include the Doublecortin-related kinase ZYG-8 [45] and the TAC-1/ZYG-9 complex, members of the TACC family and the associated XMAP215 family, respectively [46–48].

Spindle positioning can occur by means of asymmetries in microtubule aster size, of which the most studied example is in *Drosophila* neuroblasts. In these cells, the spindle

is shifted basally, where the centrosome and associated microtubules are small compared to the apical centrosome and its microtubules (Figure 3) [49]. In an extreme case of spindle pole asymmetry, one-cell-stage embryos of the freshwater oligochaete *Tubifex* divide asymmetrically with only one spindle pole containing the microtubule-nucleating protein  $\gamma$ -tubulin and astral microtubules [50].

## Regulators of force-generating mechanisms

### Polarity establishment

*C. elegans* has been a well-studied model for polarity establishment (Figure 3). Recent research has aimed at understanding how polarity-establishing proteins function in controlling asymmetric spindle positioning. PAR proteins are essential for downstream events that may affect spindle positioning; these downstream events include the regulation of microtubule stability at the cortex [35], the generation of pulling forces [8\*,10], and the asymmetric localization of other proteins required for spindle positioning, such as LET-99 and GPR-1/2 [51–54]. *Drosophila* neuroblasts also localize a PAR protein complex to the apical cortex of the dividing cell [27]. It is important, then, to determine if similar polarity-establishing mechanisms are used in other asymmetrically dividing cell types. Recent work in mouse oocytes has revealed the localization of homologs of PAR6 and PAR3 to a cortical actin cap near the meiotic spindle [55\*,56\*]. Polarity establishment in animal cells may not always be regulated by the PAR proteins, however. HAM-1, for example, is localized asymmetrically and is required for asymmetric division in *C. elegans* neuroblasts [57].

### G-protein signaling

G-protein signaling is a major regulator of asymmetric spindle positioning in several systems including *C. elegans* and *Drosophila*. G protein signaling acts downstream of the PAR proteins, affecting spindle orientation without affecting the localization of cell fate determinants [58]. Spindle positioning is affected through the non-receptor-dependent  $G\alpha/G\beta\gamma$  complex when a regulator of this pathway induces the exchange of GDP for GTP on  $G\alpha$ , followed by the separation of  $G\beta\gamma$  from  $G\alpha$  [58]. Either of these subunits, or both, may promote downstream signaling. Recent work in *C. elegans* embryos has revealed that RIC-8 acts as a guanine nucleotide exchange factor, stimulating GTP binding to and activation of a  $G\alpha$  protein to induce pulling forces [59–61], in addition to being required for the cortical localization of a second  $G\alpha$  protein [62]. Another regulator of G-protein signaling, RGS-7, functions in stimulating the hydrolysis of GTP- $G\alpha$  to GDP- $G\alpha$ , modulating those forces [61]. While the PAR proteins are required for generating an asymmetry in pulling forces [8\*,10], G-protein signaling is required for generating strong pulling forces on both sides of the cell [52,53], indicating that PAR proteins differentially regulate forces that are strictly dependent on G protein signaling.

In *Drosophila* as in *C. elegans*, G-protein signaling functions in regulating the spindle orientation downstream of cell fate determinant segregation [27,63<sup>\*</sup>]. Neuroblasts lacking a functional G $\beta\gamma$  complex cannot correctly orient spindles [63<sup>\*</sup>]. The activity of this G-protein signaling pathway is regulated by Pins (Partner of Inscuteable) and Loco (Locomotion defect), which localize apically along with G $\alpha$  [64]. Pins and Loco function synergistically as guanine nucleotide dissociation inhibitors to facilitate the generation of free G $\beta\gamma$  [64,65], while Loco may have an additional function as a GTPase-activating protein regulating the equilibrium of GDP-G $\alpha$  and GTP-G $\alpha$  [64]. Recently, studies in *Drosophila* neuroblasts and sensory organ precursor cells demonstrated a role for Ric-8 in spindle positioning, in which Ric-8 regulated the cortical localization and activity of G $\alpha$  and G $\beta\gamma$  subunits [66–68].

How G-protein signaling causes an asymmetry in microtubule pulling forces is unknown in asymmetrically dividing cells. Recent work in mammalian cells has, however, suggested a model. Mammalian Pins, called LGN, links cortical G $\alpha$  to NuMA, a microtubule binding protein [69<sup>\*\*</sup>]. When either G $\alpha$  or YFP:LGN is overexpressed, spindles in these cells have pronounced oscillations that are NuMA-dependent [69<sup>\*\*</sup>], suggesting that these proteins regulate spindle positioning forces. It will be interesting to see if similar mechanisms are used in asymmetrically dividing cells, such as in *C. elegans* and *Drosophila*, where the LGN homologs GPR-1/2 and Pins become localized asymmetrically [27,52,53].

### Monitoring asymmetric spindle positioning

In budding yeast, spindle positioning is monitored, ensuring accurate chromosome segregation. The budding yeast spindle position checkpoint delays activation of the mitotic exit network (MEN) in cells with mispositioned spindles by activating the Bub2–Bfa1 complex [14]. Activation of this signaling pathway is triggered by changes in MEN protein dynamics at spindle poles upon penetration of the daughter-bound spindle pole into the bud [70]. Recent work by two groups describes how Kin4 kinase acts as part of this monitor by inhibiting MEN signaling in cells with mispositioned spindles [71,72]. The MEN signaling pathway ultimately triggers anaphase onset by regulating Cdc14 release from the nucleolus. Prior to this, a small wave of Cdc14 release occurs via the FEAR network (Cdc-fourteen early anaphase release), which triggers early anaphase events. The FEAR network has recently been demonstrated to play a role, via Cdc14, in ensuring proper nuclear position during anaphase [73]. Fission yeast cells, although they divide symmetrically, monitor spindle positioning by a checkpoint that also regulates anaphase onset timing [74–76]. Whether or not spindle position is monitored in animal cells or in other organisms is not yet clear.

### Conclusions

The movement of a spindle to an eccentric location is a complex process requiring motor activities that act at specific times in a cell. The examples cited here provide glimpses of the mechanisms by which this occurs. It will be interesting to determine to what extent these mechanisms function similarly in other systems. In addition, it will be interesting to see how the mechanisms that control positioning in asymmetric divisions are similar or different to those that function in symmetrically dividing cells.

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