Asymmetric spindle positioning
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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 exactly 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 μm 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 position at the cortex.
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 Ka9 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
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]. Disruption of Myo10 function results in defects in nuclear positioning, an event that normally requires microtubules [23,20], and in spindle structure and rotation, which normally requires actin filaments [20]. 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]. 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 γ-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 Ga/Gβγ complex when a regulator of this pathway induces the exchange of GDP for GTP on Ga, followed by the separation of Gβγ from Ga [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 Ga protein to induce pulling forces [59–61], in addition to being required for the cortical localization of a second Ga protein [62]. Another regulator of G-protein signaling, RGS-7, functions in stimulating the hydrolysis of GTP-Ga to GDP-Ga, 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*]. Neuroblasts lacking a functional Gβγ complex cannot correctly orient spindles [63*]. The activity of this G-protein signaling pathway is regulated by Pins (Partner of Inscuteable) and Loco (Locomotion defect), which localize apically along with Gα [64]. Pins and Loco function synergistically as guanine nucleotide dissociation inhibitors to facilitate the generation of free Gβγ [64,65], while Loco may have an additional function as a GTPase-activating protein regulating the equilibrium of GDP-Gα and GTP-Gα [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 Gα and Gβγ 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 Gα to NuMA, a microtubule binding protein [69*]. When either Gα or YFP:LGN is overexpressed, spindles in these cells have pronounced oscillations that are NuMA-dependent [69*], 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].

**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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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


This study reveals that the two sides of a spindle are subjected to different kinds of pulling forces in early *C. elegans* embryos even before the spindle begins to move, and that such forces are tethered by microtubules until the time of spindle assembly.


Cell structure and dynamics


This study of Drosophila neuroblast asymmetric spindle positioning identifies a Gα mutation that results in Pins-Gα mislocalization without mislocalizing other apical components. The results demonstrate that, as in C. elegans, G-protein signaling functions to position the spindle, and PAR protein homologs function in the localization of cell fate determinants.


These authors find that, in tissue culture cells, a conformational switch in LGN allows it to interact simultaneously with Gαs and the microtubule-binding protein NuMA, linking a spindle component to the cell cortex, where pulling forces may act. The authors propose that a similar mechanism may be used in asymmetrically dividing cells.


