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Mechanisms of spindle positioning: cortical force generators in the limelight

Sachin Kotak and Pierre Gönczy

Correct positioning of the spindle governs placement of the cytokinesis furrow and thus plays a crucial role in the partitioning of fate determinants and the disposition of daughter cells in a tissue. Converging evidence indicates that spindle positioning is often dictated by interactions between the plus-end of astral microtubules that emanate from the spindle poles and an evolutionary conserved cortical machinery that serves to pull on them. At the heart of this machinery lies a ternary complex (LIN-5/GPR-1/2/Gα in *Caenorhabditis elegans* and NuMA/LGN/Gαi in *Homo sapiens*) that promotes the presence of the motor protein dynein at the cell cortex. In this review, we discuss how the above components contribute to spindle positioning and how the underlying mechanisms are precisely regulated to ensure the proper execution of this crucial process in metazoan organisms

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Introduction

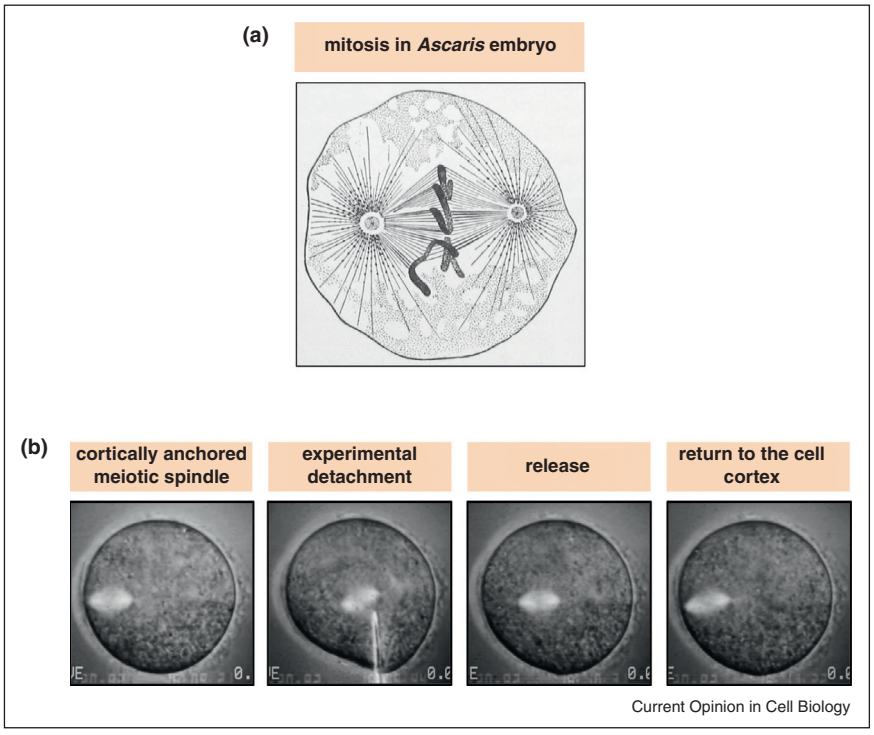
The mitotic spindle is a diamond-shaped microtubule-based structure that faithfully segregates sister chromatids during cell division. Several types of microtubules emanate from the spindle poles, including astral microtubules that reach out to the actin rich cortex located beneath the plasma membrane (Figure 1a). Pulling forces exerted on the plus-end of astral microtubules at the cell cortex are critical for accurately positioning the spindle with respect to cell-intrinsic or cell-extrinsic spatial cues. In turn, correct spindle positioning dictates placement of the cytokinesis furrow and is thus essential for determining the relative size and spatial disposition of the resulting daughter cells [2]. Accurate spindle positioning also ensures that cell fate determinants are appropriately segregated into daughter cells during development and in stem cell lineages [3].

What features of the cell cortex allow interactions with astral microtubules to orchestrate spindle positioning? Specialized cortical sites are key. Their importance has been suggested for instance by elegant experiments in *Chaetopterus* oocytes, in which the meiotic spindle pulled away from its cortical attachment site using a micro-needle returns to that location once released (Figure 1b). Such experiments illustrate the existence of a mechanical link between the spindle and specialized cortical regions [4,5]. Laser microsurgery experiments in *Fusarium solani* or *C. elegans* suggested that this link corresponds to astral microtubules connecting the spindle poles with the cell cortex [6,7,8,9,10••]. Although not the focus of this review, there are instances where pulling forces are exerted along the length of astral microtubules instead of at their plus-end located at the cell cortex [11,12,13]. Work in several systems in recent years has increased our understanding of the basic principles governing spindle positioning and identified core molecular players and aspects of their mechanism of action. In this brief review, we will focus on cortically driven spindle positioning in one-cell *C. elegans* embryos and mammalian cells in culture. In doing so, we will discuss the nature of the ternary complex that anchors the motor protein dynein at the cell cortex and how dynein serves to generate pulling forces on astral microtubules. We will then review some of the mechanisms that regulate such cortical force generators and mention briefly the contribution of the actin cytoskeleton and of phosphatidylinositol lipids in spindle positioning. We will conclude by covering some of the exciting challenges that await the field.

The ternary complex: common players of an intricate game

Proteins governing spindle positioning in metazoan organisms have been identified notably through studies in the one-cell *C. elegans* embryo and in mammalian cells. In one-cell *C. elegans* embryos, the spindle assembles in the cell center, but is displaced under the influence of intrinsic anterior–posterior (A–P) polarity cues toward the posterior during metaphase and anaphase, resulting in unequal division (Figure 2a) [14]. Genetic and RNAi-based functional genomic screens have led to the identification of two partially redundant Gα subunits, GOA-1 and GPA-16 (collectively referred to as Gα hereafter), of two essentially identical TPR/GoLoco-domain proteins, GPR-1 and GPR-2 (hereafter

Figure 1



(a) Schematic of the first mitosis in the *Ascaris megalocephala* embryo. Note astral microtubules that emanate from the spindle poles and abut the cell cortex (from Theodor Boveri, adapted from Wilson [1]). **(b)** Images from time-lapse recording using polarized optics to follow the first meiotic spindle in the *Chaetopterus* oocyte in response to micromanipulation with a glass microneedle (visible in the second image from the left). Note that the spindle returns to its original cortical position after release from the microneedle. Adapted from *The Cell: An Image Library CIL-11961* and [5].

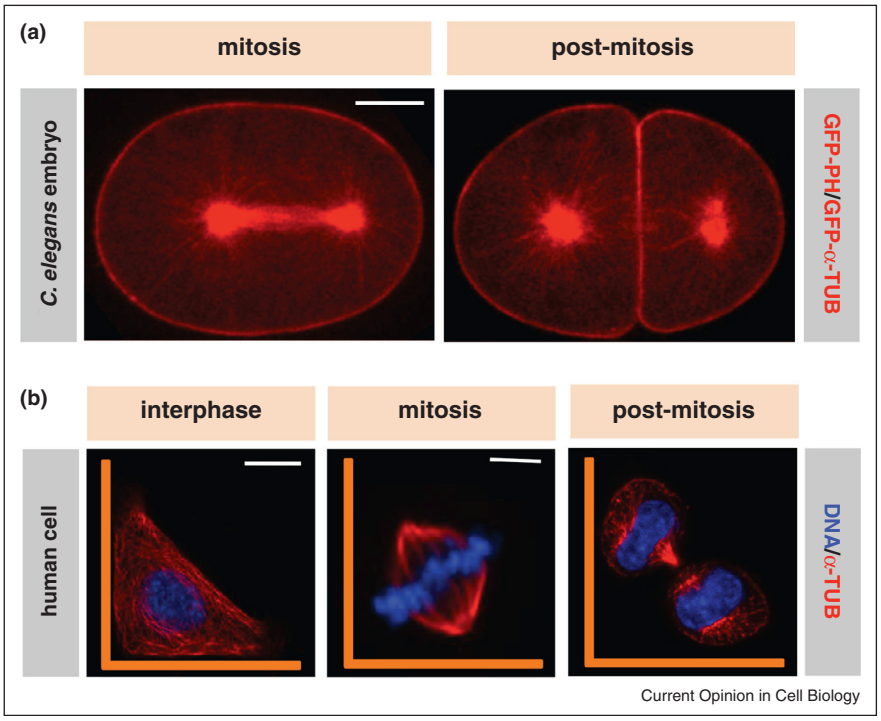
jointly referred to as GPR-1/2) and of the large coiled-coil protein LIN-5 as being essential for proper spindle positioning in the nematode [15,16,17,18,19]. Depletion of Gα, GPR-1/2 or LIN-5 results in the near absence of pulling forces on astral microtubules; as a consequence, the spindle remains centrally located and the first division is equal [16,17,15]. The distribution of cell fate determinants is not affected upon depletion of ternary complex components, indicating that polarity cues regulate spindle positioning and fate acquisition in a coordinated but partially independent manner [20]. Importantly, related components guide spindle positioning in non-polarized human cells, which undergo equal division. Such cells plated on uniform fibronectin or on fibronectin-based micropatterns position their spindle in a predictable manner in response to these extrinsic cues (Figure 2b) [21•,22•]. Mammalian homologs of Gα ($G\alpha i_{1-3}$), GPR-1/2 (LGN and AGS3) and LIN-5 (NuMA) are likewise essential for correct spindle positioning, suggesting that these proteins are at the core of this process across metazoan organisms [23,24,25].

What is the relationship between these three components? The Gα proteins are tethered to the plasma membrane through N-terminal myristoylation, and when bound to GDP, associate with the GoLoco domain of GPR-1/2/LGN, which in turn interacts with LIN-5/NuMA, thus forming a ternary complex [17,19,24,26,27] (Figure 3). By virtue of these molecular interactions, the entire ternary complex is anchored at the cell cortex below the plasma membrane. Although the related ternary complex components Gαi, Pins and Mud are essential for proper spindle positioning in *Drosophila*, a parallel pathway consisting of Pins/Dlg/Khc73 is also important in that system [28,29]. Whether Dlg/Khc73 relatives similarly contribute to spindle positioning in *C. elegans* and mammalian cells remains to be addressed. How does the cortical localization of the ternary complex enable positioning of the mitotic spindle? The answer lies in the ability of this complex to interact with the minus-end directed motor protein dynein, as discussed next.

Cortical dynein: a force-generating motor

Dynein is a multisubunit motor protein complex critical for many basic cellular processes [30,31]. In *C. elegans* embryos and human cells, co-immunoprecipitation experiments showed that LIN-5/NuMA associates with dynein [25,32•,33,34]. The region mediating this association has been mapped to an N-terminal region of NuMA [25], but the molecular nature of the partner protein on the side of the dynein complex is not known. The presence of dynein at the cell cortex is compromised

Figure 2



Spindle positioning in *C. elegans* embyos and human cells. **(a)** Images from time-lapse recording using spinning disc microscopy of *C. elegans* embryos expressing GFP-α-tubulin to mark microtubules and GFP-PH to mark the cell membrane (both pseudocolored in red); one-cell stage mitosis (left) and early two-cell stage (right) are shown. Note asymmetric spindle position along the anterior–posterior axis (a) leading to the unequal division into a larger anterior blastomere and a smaller posterior one **(b)**. Scale bar: 10 μm. (b) Human HeLa cells in interphase (left), mitosis (middle) and post mitosis (right) on L-shaped fibronectin micropattern coated coverslips, fixed and stained with antibodies against microtubules (red); DNA is visualized in blue. Note spindle position imparted by the micropattern. Scale bar: 10 μm.

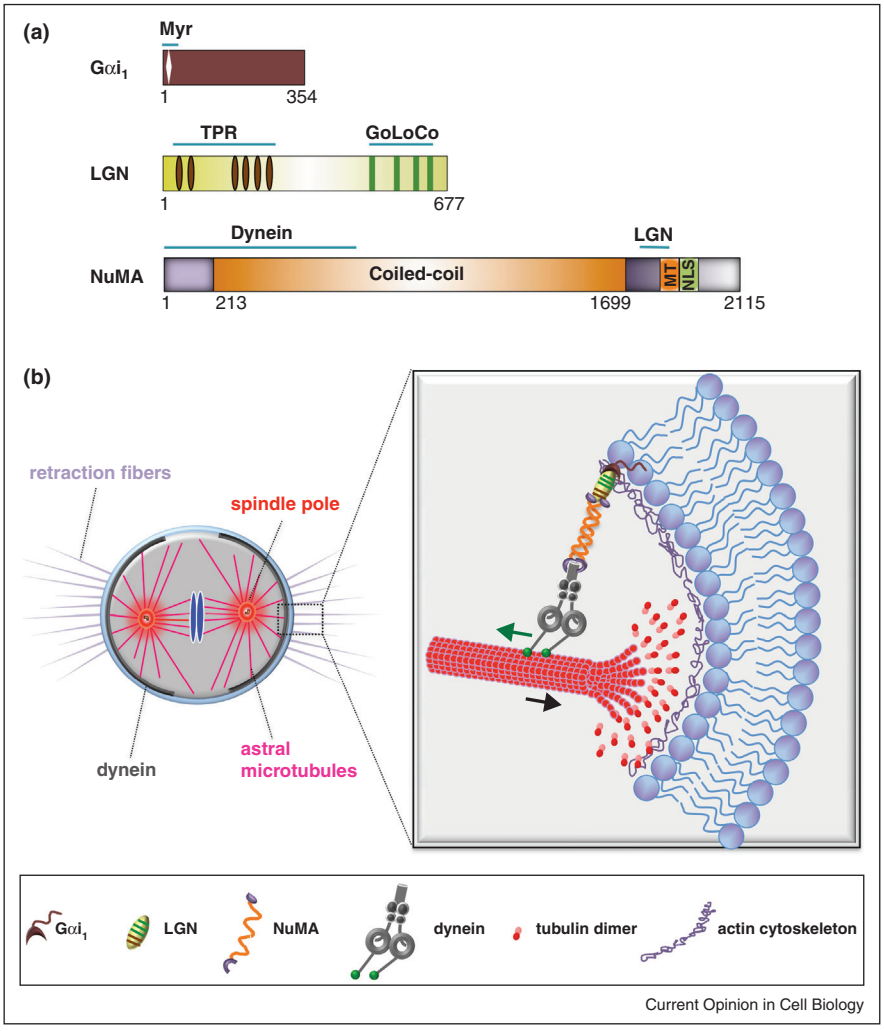
upon depletion of ternary complex components in *C. elegans* embryos and human cells [23–25,32•]. Conversely, increasing the levels of Gα or TPR/GoLoco proteins results in increased cortical dynein enrichment and concomitant augmentation of dynein-dependent spindle movements [25,35,36].

How does cortical dynein result in pulling forces on astral microtubules? The dynamicity of astral microtubules is part of the answer [32•]. In *C. elegans*, astral microtubules grow toward the cell cortex, where their plus-end resides for only ~1 second before undergoing catastrophe [37]. *In vitro*, shrinking microtubules can generate considerably more force (~50 pN) than that deployed by a single dynein motor (~7 pN) [38,39,37]. Because it has been estimated that a force in the order of ~50 pN pulls on individual astral microtubules during spindle positioning in *C. elegans* embryo [10••], it has been suggested that dynein may only serve to anchor depolymerizing microtubules to the cell cortex, with microtubule depolymerization generating the actual pulling force [37]. A variation on this theme is suggested by *in vitro* experiments in which a purified fragment of yeast dynein coated on the edges of microfabricated chambers interacts with the plus-end of microtubules and triggers their catastrophes, allowing positioning of a microtubule aster [40••]. Thus, the presence of dynein at the cell cortex may impact on the dynamics of astral microtubules and indirectly promote pulling forces in this manner. Alternatively, cortical dynein may exert pulling forces during spindle positioning directly through its motor activity. In this scenario, additional factors could enhance the force deployed by dynein *in vivo* or the motor protein may function in a cooperative manner to generate large collective forces as for phagosome motility [41•]. Regardless of the actual mechanism, given that the presence of cortical dynein is sufficient to generate pulling forces, what is the significance of having an intricate ternary complex to govern dynein localization? Emerging evidence suggests that cortical dynein must be tightly regulated, and that this is achieved in part by modulating ternary complex components, as described in the next section.

Balanced levels of cortical dynein are critical for proper spindle positioning

Alterations in ternary complex components have profound effects on cortical dynein levels and thus spindle positioning [25,35]. As mentioned earlier, GDP-bound

Figure 3



Membrane bound ternary complex anchors dynein to the cell cortex and thus mediates spindle positioning. **(a)** Schematic representation of $G\alpha i_1$, LGN and NuMA proteins (functional homologs of *C. elegans*, Gα, GPR-1/2 and LIN-5, respectively), with an indication of their size in amino acids. $G\alpha i_1$ harbors an N-terminal myristoylation signal important for membrane targeting (white diamond); the same holds for the partially redundant $G\alpha i_2$ and $G\alpha i_3$, which are not represented here for simplicity. LGN harbors N-terminal TPR motifs (dark brown oval) and C-terminal GoLoco domains (green rectangles), mediating interaction with NuMA and $G\alpha i_{1-3}$, respectively. NuMA is shown with its coil-coiled domain and the regions within the C-terminal part mediating interaction with LGN and microtubules (MT); the nuclear localization signal is also indicated (NLS), as is the domain needed for interaction with dynein. **(b)** Working model of spindle positioning in human cells. The ternary complex ($G\alpha i_{1-3}$/LGN/NuMA) is anchored below the plasma membrane and recruits the dynein motor complex. One possibility is that cortically anchored dynein attempts to move on astral microtubules by virtue of its minus end directed motility (green arrow), but instead pulls on the astral microtubule, resulting in the generation of a pulling force (black arrow). Alternatively, dynein may serve merely as an anchor to maintain a connection with the depolymerizing microtubule, which may be producing force in the context of spindle positioning. See text for additional details.

Gα is the relevant Gα species in the context of spindle positioning [42]. In line with this, work in *C. elegans* indicates that the Gα guanine nucleotide exchange factor (GEF) RIC-8 is important for generating pulling forces [43,44]. Moreover, the Gα guanine nucleotide activating protein (GAP) RGS-7 also contributes to some extent, compatible with the Gα nucleotide cycle being important in spindle positioning [45]. Ric8 is also required for proper spindle positioning in non-polarized human cells [23]. In *C. elegans*, Gα·GDP levels are also modulated by Gβγ, since depletion of Gβγ results in excess pulling forces, presumably because excess Gα·GDP is available for interaction with GPR-1/2 [44,46]. Intriguingly, intracellular trafficking of the Gβ protein GPB-1 is modulated in time and space in *C. elegans* embryos in a manner consistent with it dictating cortical GPR-1/2 localization [47]. This raises the possibility that intracellular trafficking is crucial for proper spindle positioning, as is the case for other aspects of asymmetric cell division, for instance in *Drosophila* sensory organ precursor cells [48,49].

Other components, including kinases and phosphatases, can influence the availability of the ternary complex for interaction with cortical dynein. This is exemplified by the regulation of LIN-5 by the atypical protein kinase C PKC-3, which is part of the anterior PAR complex that establishes A-P polarity in *C. elegans* embryos. Using a quantitative mass-spectrometry approach as a starting point, LIN-5 was found to be phosphorylated by PKC-3; such phosphorylation negatively regulates LIN-5 function and thus contributes to lowering pulling forces on the embryo anterior [50]. In mammalian cells, atypical protein kinase C phosphorylates LGN, causing its loss from the apical cortex in polarized MDCK cells, thereby perturbing spindle positioning [51,52]. Studies in human cells have led to the identification of two other kinases involved in spindle positioning, ABL1 (Abelson kinase1) and PLK1 (Polo-like kinase1). ABL1 was identified in an RNAi-based screen in human cells grown on a uniform fibronectin substrate and then shown to phosphorylate NuMA and thus maintains its cortical localization [53•]. Intriguingly, ABL1 also enhances the presence of LGN at the cell cortex, suggestive of a positive feedback between NuMA and LGN. By contrast to ABL1, PLK1 negatively regulates cortical dynein, without impacting on the ternary complex [24]. PLK1 is enriched at the spindle poles, and it was proposed that there is a gradient of its kinase activity, being lowest at the cortical regions furthest from spindle poles, which thus escape this negative regulation, enabling cortical dynein accumulation. The molecular nature of PLK1 substrates involved in spindle positioning remains to be identified.

Other than the role of kinases, the contribution of phosphatases in spindle positioning has also begun to be unveiled. Thus, in *C. elegans*, the protein phosphatase 6 family member PPH-6 and its associated subunit SAPS-1 contribute to pulling forces by regulating the levels of GPR-1/2/LIN-5 [54]. The relevant target of PPH-6/SAPS-1 is also not known and it is furthermore unclear whether the related phosphatase contributes to spindle positioning in other systems. Interestingly, dynein can also negatively regulate cortical localization of the ternary complex by transporting LGN from the cortex to the spindle poles during metaphase in mammalian cells [35]. In summary, multiple regulatory steps can act on ternary complex components or perhaps directly on the dynein complex to fine-tune the localization and activity of cortical dynein during spindle positioning.

Beyond the ternary complex

The role of the actin cytoskeleton and of associated motor proteins in spindle positioning has been extensively investigated. Here, we discuss only briefly the contribution of the actomyosin network in this process and refer readers to other recent reviews that offer a more extensive coverage of this aspect [55,56]. In human cells, the importance of actin is illustrated for instance by the fact that actin depolymerizing drugs or RNAi-mediated depletion of the actin-associated protein Moesin lead to spindle positioning defects [21•,22•,57]. Interestingly, experiments with fibronectin micro-patterns revealed that cells utilize focal adhesions and actin fibers established during interphase to impart spindle positioning during mitosis [21•]. Furthermore, laser ablation experiments reveal that such actin fibers are important also during mitosis [58•]. How does the actin cytoskeleton interface with the ternary complex and dynein? One possibility is that the actomyosin network modulates the cortical levels of these components and thereby influences spindle positioning. However, recent experiments in *C. elegans* speak against this hypothesis, as cortical localization of ternary complex components is not altered upon impairment of cortical actin [59]. An alternative hypothesis is that actin-associated proteins interact with the plus-end of astral microtubules and thus stabilize their interaction with the cell cortex [60]. Compatible with this view, depletion of the actin-associated protein MISP results in shorter astral microtubules and spindle positioning defects [61,62].

The lipid composition of the plasma membrane also plays a role in proper spindle positioning, as exemplified by the analysis of the casein kinase 1 CSNK-1 in nematodes [63•]. *C. elegans* embryos depleted of CSNK-1 exhibit excess cortical GPR-1/2 and LIN-5, as well as pulling forces. On the basis of an analogous relationship in budding yeast, CSNK-1 was proposed to negatively regulate the phosphatidylinositol-4,5-bisphosphate [$PI(4,5)P_2$] (PIP_2)-kinase PPK-1 and thereby keep PIP_2 levels low [63•]. Whether PIP_2 directly influences cortical GPR-1/2/LIN-5/dynein cortical distribution is unclear at this stage. Intriguingly, a related lipid, phosphatidylinositol-3,4,5-triphosphate [$PI(3,4,5)P_3$] (PIP_3) functions in human cells downstream of β1-integrin signaling and is essential for proper cortical dynein distribution and thus spindle positioning [64]. Inhibition of PI3K, a principal enzyme involved in PIP_3 biosynthesis, leads to randomization of dynein distribution at the cortex. Whether this PIP_3-mediated dynein localization pathway acts independently of the ternary complex is an important open question.

Future perspectives

Studies in a number of systems, including *C. elegans* embryos and human cells, have provided an initial understanding of the mechanisms governing spindle positioning. Exciting challenges lie ahead and novel insights are expected from several directions. Biophysical and structural analysis of proteins central to the force generating machinery is anticipated to be important. An illustration of such work is given by the recent atomic level characterization of the interaction between parts of LGN and NuMA [65,66]. Coupled with modeling and computer simulations of spindle positioning, this should yield an in depth molecular understanding of

the underlying mechanisms. Novel insights are also expected from analyzing the spatial and temporal regulation of the force generation machinery. For instance, it has been proposed that a chromosome-derived gradient of Ran·GTP prevents NuMA/LGN from localizing to the lateral cortex in metaphase human cells [24]. It will be interesting to expand on these findings, including in other systems. Moreover, dynein has been reported to transiently increase at the cell cortex during anaphase [67], and it will be important to investigate how ternary complex components are modulated as cells progress through mitosis. Another promising line of work entails the analysis of closely related species to explore how evolution may have acted on spindle positioning. For instance, differences in aster and spindle positioning between *C. elegans* and *Caenorhabditis briggsae* can be explained by alterations in the cortical distribution of ternary complex components between the two species [68•]. Further understanding is also expected from analyzing more complex instances of spindle positioning in developing organisms and in tissues, where extrinsic cues and geometrical constraints are bound to play an important role. For instance, Wnt signaling is important for proper spindle positioning at later stages during *C. elegans* embryogenesis [69], and the impact of Wnt signaling on spindle positioning has been recently reconstituted in a mammalian embryonic stem cell system [70•], suggestive of important progress to come in this area. In conclusion, because defective spindle positioning can result in tumorigenesis [71,72], it is anticipated that discoveries made regarding the fundamental mechanisms governing spindle positioning may also lead to the development of novel therapeutic tools.

Note added in proof

Two recent publications shed further light on the mechanisms of spindle positioning in human cells. One study revealed the role of cortical dynein and asymmetric membrane elongation in positioning the anaphase spindle [73], whereas the other one focused on the interplay between CDK1 kinase and PPP2CA phosphatase in dictating levels of cortical dynein during mitosis [74].

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