DYNAMIC MICROTUBULES LEAD THE WAY FOR SPINDLE POSITIONING

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Coordination between the asymmetric partitioning of cell-fate determinants and equal partitioning of genetic material is crucial to the generation of diverse cell types in a developing organism, and to the maintenance of genomic integrity. The emerging model is of a highly organized and dynamic cellular landscape, the form of which is defined by polarized signals within the cell. Cytoskeletal elements are necessary to generate this landscape and to provide motive forces for proper spindle positioning. These forces are generated by interactions between microtubules and the cell cortex.

CENTROSOME
Also called the microtubule-organizing centre (MTOC) or spindle pole, this structure nucleates microtubules and is important for signalling processes.

SPINDLE
A bipolar microtubule array with microtubules organized from each spindle pole. The spindle is composed of polar, kinetochore and astral microtubules.

ANAPHASE
The period of mitosis during which duplicated chromosomes are segregated. In anaphase A, chromosomes move towards centrosomes; in anaphase B, the centrosomes are segregated.

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Asymmetric positioning of the nucleus and unequal cell division is essential for producing cellular diversity and for spatially organizing the embryo during development[1]. Such asymmetry is generated by intracellular differentiation, which requires a cascade of events that stem from an initial discontinuity and culminate in the establishment of polarized cytoskeletal elements. The initiating events range from a wound (which triggers a wound-healing response), to the penetration of an egg by a sperm, to the selection of sites for new bud growth. The cascade, in many systems, involves G proteins (such as CDC42) that signal the polarization of the actin cytoskeleton and the accumulation of protein assemblies that are involved in partitioning processes (such as the PAR proteins). CENTROSOMES might participate in the establishment phase and in interpreting polarity cues so as to position the mitotic SPINDLE and nucleus such that the segregation of chromosome sets and the segregation of polarized determinants can be coordinated.

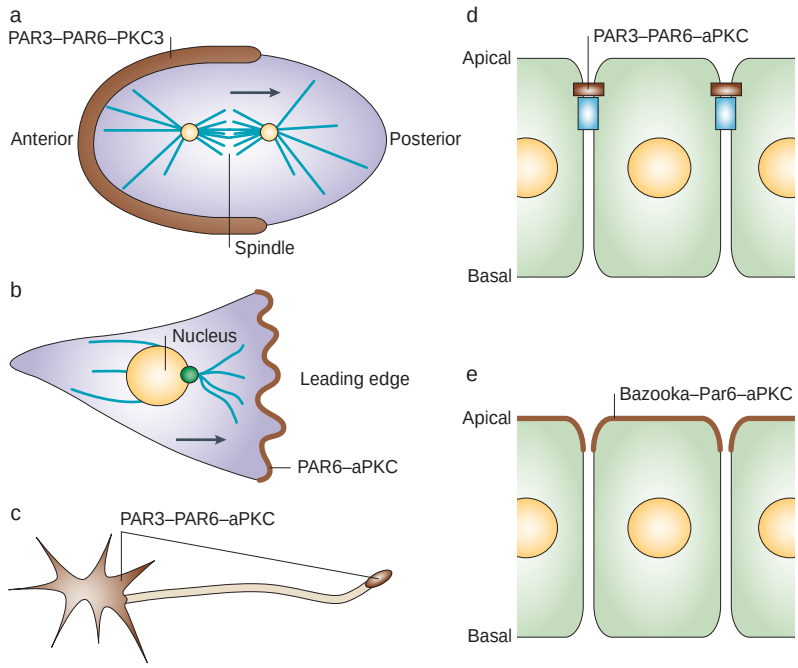
Dynamic microtubules are integral to the mechanisms that organize the mitotic spindle. They are also required to position and orientate the spindle to the site of cell division (such as in the budding yeast *Saccharomyces cerevisiae*), or to a site that is defined by the position of the spindle to be the cleavage plane (such as in the fission yeast *Schizosaccharomyces pombe* and in animal cells). Microtubules constantly search space, undergo transitions in their overall distribution and structure, and produce pushing and pulling forces on organelles within the cell (reviewed in REFS 2–5). The ability of dynamic microtubules to search space enables them to find specific regions of the cell into which to transport proteins, and within which to orientate centrosomes[6]. The pushing and pulling forces are coordinated to enable the spindle to be moved to, and positioned at, the site of cell division before ANAPHASE and CYTOKINESIS.

Understanding how microtubule dynamics are regulated, and how microtubules interact with the proteins that connect these dynamic filaments to an attachment site at the cell cortex, is therefore essential in elucidating the force-generating mechanisms that are required for proper spindle and nuclear positioning in a cell.

Polarity establishment

Initial signalling cascades. A SMALL GTPase, CDC42, has a central role in establishing and maintaining the polarity of fungal, plant and animal cells. CDC42 interacts with a number of effectors, including the formin Bni1 in *S. cerevisiae*. Bni1 stimulates the assembly of actin cables, and functions to control actin polymerization at sites of polarized growth[7–9]. The Diaphanous-related formin DIA1 — through CDC42 — also regulates cytoskeletal remodelling in mammalian cells[10,11]. Furthermore, CDC42 potentiates the activity of the atypical protein kinase C (aPKC; PKC3 in the nematode worm *Caenorhabditis elegans*), which binds the polarity protein PAR6 (REFS 12,13).

Box 1 | Polarized protein distribution



The proteins involved in polarization in *Caenorhabditis elegans*, *Drosophila melanogaster* and mammalian systems are homologous, and each shows a unique distribution pattern (reviewed in REF. 14). In one-celled *C. elegans* embryos (**a**), PAR3, PAR6 and protein kinase C-3 (PKC3) are polarized to the anterior of the cell. The spindle generates a pulling force towards the posterior to create an unequal segregation of polarity proteins upon cytokinesis. In migrating mammalian cells (**b**), CDC42, PAR6 and atypical PKC (aPKC; the mammalian PKC3 homologue) are polarized at the leading edge, towards which nuclei migrate[120]. Mammalian hippocampal neuronal cells (**c**) have PAR3, PAR6 and aPKC at the cell body and tip of the axon to polarize neurons[121]. Mammalian epithelial cells (**d**) polarize PAR3, PAR6 and aPKC to TIGHT JUNCTIONS (indicated by brown rectangle). Blue rectangle represents adherens junctions. In *D. melanogaster* epithelial cells (**e**), Bazooka (the homologue of PAR3), Par6 and atypical protein kinase C are asymmetrically localized to the apical cortex.

CYTOKINESIS
The separation of a cell into two, marked by ingression of the cleavage 'furrow' between two segregated masses of genomic DNA.

SMALL GTPases
Diverse cellular regulatory proteins that are controlled by the nature of bound nucleotide (active when bound to GTP; inactive when bound to GDP).

TIGHT JUNCTION
A seal between adjacent epithelial cells, just beneath their apical surface. PAR proteins migrate to tight junctions in mammalian cells.

MICROTUBULE-ORGANIZING CENTRE
(MTOC). Also called the centrosome or spindle-pole body, this structure nucleates and organizes microtubules.

As a result of CDC42 signalling and actin assembly, several polarity proteins become concentrated at localized sites (reviewed in REF. 14). For instance, in early *C. elegans* embryos, specific PAR proteins (PAR6 is one of six PAR proteins, all of which function in polarity establishment) become asymmetrically localized — PAR3, PAR6 and PKC3 to the anterior, and PAR2 to the posterior. They therefore define the anterior–posterior (A–P) axis (BOX 1). The protein-localization and polarity processes are quite different among different organisms and cell types (BOX 1); however, PAR proteins are conserved in *C. elegans*, the fruitfly *Drosophila melanogaster* and mammals[14]. Although defects in PAR function lead to defects in spindle orientation (reviewed in REF. 15), it is unclear how these cues are normally translated into proper spindle positioning.

Centrosome and microtubule signalling. The centrosomes or associated microtubules themselves provide essential cues for specification of the A–P body axis in early *C. elegans* development[16,17]. For example, the position within the egg of the sperm pronucleus (the nucleus donated by the sperm), together with its associated microtubules and MICROTUBULE-ORGANIZING CENTRE (MTOC), defines the polarity axis. This axis is not fixed, but responds to the position of both external and internal cues. For instance, if the sperm entry position is altered, the A–P axis can be repositioned, and embryonic development proceeds as normal[18]. Alternatively, the position of the spindle axis provides an internal cue for the polarity axis. When SPN-4 (an RNA-binding protein) is mutated, P1 cells at the two-cell stage of embryogenesis do not rotate their spindle to align along the A–P axis as normal. Instead, these cells organize their asymmetrically positioned proteins to polarize cellular determinants along the unrotated spindle axis[19].

Likewise, the MTOC in budding yeast — more commonly known as the SPINDLE-POLE BODY, and the equivalent of the centrosome/spindle pole in other species — specifies the site of bud emergence in the first zygotic division following cell fusion[20]. In the early 1970s, Byers physically disrupted the position of the spindle-pole body by centrifugation, and showed that the first zygotic bud arose from the position of the displaced spindle-pole body[21,22]. These studies indicate that, at least in some developmental processes, the centrosome can dictate the position of polarity determinants.

In *C. elegans*, the close proximity of the pronucleus, centrosome and associated microtubules to the cortex possibly alters the localization of PAR3, which enables PAR2 to bind and subsequently facilitate establishment of the A–P axis[17,23]. Evidence that the centrosome or sperm ASTER, instead of the associated CHROMATIN and nuclear factors, might specifically contribute to the asymmetry of PAR3 localization stems from the fact that anucleate mutants exhibit normal A–P polarity[24]. Cuenca *et al.* have described the timing of pronuclear formation relative to the localization of green fluorescent protein (GFP)-tagged PAR6 and PAR2 in live *C. elegans* embryos[25]. Their evidence also indicates that the pronucleus or centrosome might restrict the PAR3–PAR6–PKC3 complex to the anterior, allowing PAR2 to accumulate in the posterior region — this region now being devoid of the ternary complex[25]. Further studies are now required to show directly how centrosome positioning is the crucial determinant in the asymmetric localization of PAR3–PAR6–PKC3. Nonetheless, these observations[25], together with the mutant analysis[24], indicate that sperm asters or the associated pronucleus might have a formative role in polarity establishment, by allowing PAR2 to accumulate at the posterior embryonic axis.

Protein delivery on microtubules. Microtubules that are nucleated from spindle poles deliver proteins that are required for polarity establishment. *S. pombe* grows from both ends (antipodal growth) to form an oblongate cell. Although the actin cytoskeleton functions to deposit cell-growth material, microtubules and their associated proteins are necessary to establish the polarized protein distribution that is required for cell growth. A key cell-end marker, Tea1 (tip-elongation aberrant-1), is carried on growing microtubule PLUS-ENDS from the

P1
The first cell division in *C. elegans* produces a large anterior blastemere, AB, a blastomere and a smaller posterior blastomere, P1.

SPINDLE-POLE BODY
The budding-yeast equivalent of the centrosome/spindle pole or MTOC. This structure, which is embedded in the nuclear envelope, nucleates both cytoplasmic and nuclear microtubules.

ASTER
An organized microtubule array, with the microtubule minus-ends focused at a point or centrosome, and the plus-ends emanating outwards.

CHROMATIN
Chromosomal DNA and associated proteins.

PLUS-END
The predominantly dynamic end of a microtubule, with β-tubulin exposed.

METAZOAN
Refers to the kingdom Animalia (animals) that comprises roughly 35 phyla of multicellular organisms.

CENTRIOLE
A short, barrel-like array of microtubules that organizes the centrosome and contributes to cytokinesis and cell-cycle progression.

TUBULIN
The basic subunit of microtubules. Tubulin comes in two forms, α- and β-tubulin, which form heterodimers that make up microtubules.

GTPase-ACTIVATING PROTEIN
(GAP). A protein that inactivates small GTP-binding proteins, such as RAS-family members, by increasing their rate of GTP hydrolysis.

ADENOMATOUS POLYPOSIS COLI
(APC). A protein that is mutated in many colorectal cancers. APC binds to microtubules and the microtubule regulator EB1.

CYCLINS
A family of binding partners for the main cell-cycle regulators, cyclin-dependent kinases. Cyclins are completely degraded and newly synthesized for progression through each cell cycle.

nucleus at the cell centre by a kinesin motor protein, Tea2 (Kip2 (kinesin-related protein 2) in *S. cerevisiae*), and is deposited at the tips of cells[26–29]. Tip1 (tip-elongation protein-1; CLIP170 in mammals and Bik1 (bilateral defects in karyogamy-1) in *S. cerevisiae*) and Mal3 (EB1 in mammals and Bim1 (binding to microtubules-1) in *S. cerevisiae*) are also found at growing microtubule plus-ends, and might promote microtubule plus-end polymerization to target microtubule growth to cell ends[28,30–33]. The anchoring of Tea1 at the cellular tips is dependent on a putative membrane-associated protein, Mod5 (REF. 34), and probably recruits the formin Bud6 to stimulate growth of the actin cytoskeleton and membrane deposition at sites of polarized growth[35]. So, as they deliver proteins to, or exclude them from, polarized sites within the cell, microtubules are integral to the mechanisms that are required for establishing polarized growth.

Establishment of spindle-pole fate

An asymmetry in the protein constituents and function of spindle poles might be necessary to achieve proper positioning and segregation of the spindle apparatus, such that each daughter cell receives one — and only one — spindle pole with its attached genome. Different strategies are used to ensure the segregation of spindle poles into each daughter cell. One strategy is to build the cell-division machinery between the spindle poles. In *S. pombe* and METAZOAN cells, spindle formation precedes and directs the assembly of proteins that are necessary for cell division. In *S. cerevisiae*, by contrast, the division site is defined before spindle formation.

Given the formative roles that centrosomes/spindle poles have in polarity establishment, these organelles probably contain more than instructions for microtubule growth. Although there are limited experimental data to support this view, important strides have been taken towards understanding spatial differentiation and protein dynamics within the centrosome itself.

Centrosome/spindle-pole differentiation. In mammalian systems, assembly of the microtubule core of the centrosome (the CENTRIOLE) seems to be conservative; the mother centriole maintains its existing TUBULIN whereas the daughter centriole incorporates newly synthesized tubulin[36]. Furthermore, there are additional mechanisms for differentiating centrosomes. The 'old', mother centriole migrates to the site of the cytokinetic furrow, which indicates that there might be a specified pattern of centrosome inheritance[37]. In addition, specific proteins such as PARP3 (poly(ADP-ribose) polymerase) are preferentially incorporated into the daughter centriole of mammalian cells[38]. The specific recruitment of proteins to one spindle pole and not the other has also been shown in *C. elegans*: the SAS-4 protein, which is required for spindle assembly, is specifically recruited to the daughter spindle pole[39,40].

As in mammals and *C. elegans*, certain components remain associated with the old spindle-pole body that enters the bud/daughter cell in *S. cerevisiae*[41]. However, the study of core spindle-pole-body proteins shows the structure to be more dynamic than was originally anticipated[42]: the spindle-pole-body protein Spc110 exchanges with the cytoplasmic pool and accumulates during late S phase and M phase[42]. Dynamic exchange and addition of spindle-pole components might be crucial for defining how microtubules are nucleated. *S. cerevisiae* might predispose the old spindle-pole body to the bud by maintaining pre-existing cytoplasmic microtubules at this spindle-pole body[41,43–45]. This allows dynamic microtubules that are destined for the bud/daughter cell to be captured by the bud-specific polarization machinery.

The mechanism for defining asymmetric protein distribution at spindle poles seems to be distinct from the regulatory mechanisms that ensure the cell cycle does not continue unless proper spindle positioning has taken place. The *S. cerevisiae* GTPase-ACTIVATING PROTEIN (GAP) Bfa1–Bub2 complex regulates exit from mitosis. Pereira *et al.* have proposed that the asymmetric localization of Bfa1 to the old spindle-pole body, which is now in the bud/daughter cell, is dependent on the interactions of cytoplasmic microtubules with the bud, rather than on the pre-determined fate of the spindle-pole body (see below)[41]. Although mother-cell (new) and daughter-cell (old) spindle-pole bodies are differentiated, their positions are key determinants in 'interpreting' polarity cues and ensuring that the mitotic spindle is properly positioned before cell division.

Asymmetric function of differentiated spindle poles. The mechanistic basis for spindle-pole-body differentiation in *S. cerevisiae* depends on the phosphorylation and asymmetric transport of proteins to microtubule plus-ends. A key protein that distinguishes the bud/daughter cell spindle-pole body from that of the mother cell is the polarity determinant for orientating spindle microtubules, Kar9 (karyogamy 9). Although Kar9 does not directly bind microtubules, it shares limited homology with the carboxy-terminal region of ADENOMATOUS POLYPOSIS COLI protein (APC)[46] — a microtubule-binding and -regulating protein that is modulated by the PAR6–GSK3–aPKC polarity complex (where GSK3 stands for glycogen-synthase kinase-3) in mammalian systems[47,48].

Kar9 phosphorylation by Clb4–Cdc28 — a CYCLIN–cyclin-dependent kinase (CDK) complex — is required for its spatial distribution to only one spindle-pole body[49]. Clb4 binds the spindle-pole body that is destined for the mother cell, thereby restricting unphosphorylated (active) Kar9 to the old, daughter-cell-bound spindle-pole body, because the Kar9 at the mother-cell spindle-pole body is 'inactivated' by phosphorylation (FIG. 1). The spindle-pole body is therefore a regulatory centre for spindle positioning: Kar9 delivery to specific microtubule plus-ends from one spindle-pole body guides these structures to their destination for proper spindle positioning. These data reflect a spatial feature that is inherent in differential regulatory cascades. Differentiated spindle-pole bodies might then generate signals and forces that interact with spatial cues within the cell for proper spindle positioning (described below).

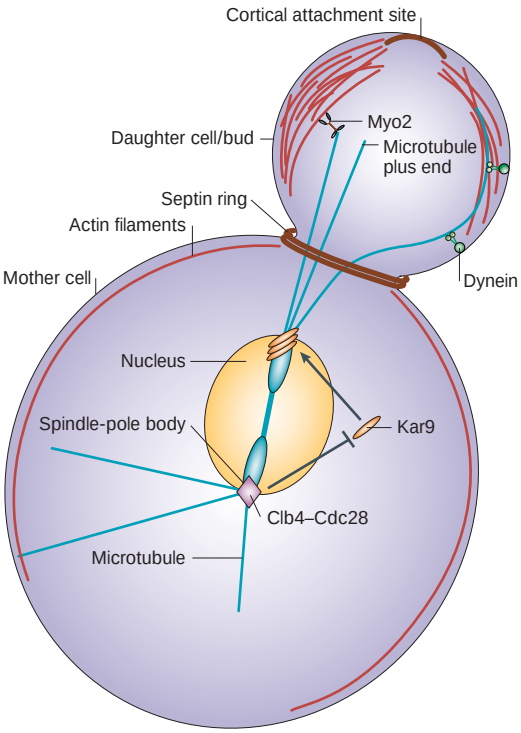


Figure 1 | **Asymmetric localization of Kar9 in *Saccharomyces cerevisiae*.** Kar9 (karyogamy 9) is asymmetrically loaded onto the spindle-pole body that is destined for the daughter cell. Kar9 is excluded from the mother spindle-pole body through phosphorylation by Clb4–Cdc28 (B-type-cyclin-4–cell-division-cycle-mutant-28). The asymmetric localization of Kar9 might allow for selective orientation of the bud-specific spindle-pole body towards the bud, by shuttling active Kar9 from the spindle-pole body to the microtubule plus-end for linking to the actin cytoskeleton. For more details see REF. 49.

Microtubule plus-ends for spindle positioning

In addition to their function in relaying information to and from the centrosome, microtubule plus-ends provide a tether between the centrosome and the cell cortex for both pushing and pulling forces. The repertoire of plus-end-targeted proteins includes microtubule-associated proteins (MAPs) and plus- and MINUS-END-directed microtubule-based motors (for reviews see REFS 3,5,50). Their functions are: to prepare the microtubule plus-end to grow, shorten or pause; to couple end-dynamics with force generation (see below and BOX 2); and to transport proteins (such as Kar9) for networking with the actin cytoskeleton or other polarity determinants. Spindle positioning is dependent not only on the function of microtubule-binding proteins, but also on the accurate placement of these proteins on specific microtubules.

MAPs at microtubule plus-ends. MAPs have classically been identified by their ability to bind to and regulate microtubule dynamics. There are several classes of MAP that associate with, and regulate, growing, shortening or both growing and shortening microtubule plus-ends. The molecular basis for how each of these proteins recognizes, is targeted to and regulates microtubule dynamics will provide an exciting avenue to a deeper understanding of how microtubule dynamics and binding proteins can generate forces.

Regulation of protein localization to plus-ends. In addition to specific association with growing and/or shortening microtubules, MAP targeting also occurs by different modes of delivery and maintenance, which generates a temporally and spatially regulated landscape of MAP localization. The plus-end-directed microtubule-based motors transport cargoes such as signalling components and other motor proteins to plus-ends. For instance, kinesin I transports the minus-end-directed motor cytoplasmic dynein to microtubule plus-ends at the posterior of *D. melanogaster* oocytes[51,52]. By contrast, dynein in *S. cerevisiae* is recruited directly to plus-ends from the cortex[53]. Once at plus-ends, dynein provides a vector for spindle orientation via cortical anchors to generate pulling forces for spindle movements (see below; FIGS 2e,3c). Likewise, the localization of the dynein-associated dynactin complex is regulated by protein kinase A (PKA) in mammalian tissue cells. PKA modulates the microtubule-binding capacity of p150^{GLUED} (a dynactin component) by phosphorylation, and might alter its plus-end-binding specificity[54].

The restriction of MAPs to a subset of microtubule plus-ends requires both microtubule motor proteins and local regulatory control. In *S. cerevisiae*, the plus-end-directed kinesin Kip2 is necessary for the transport of Kar9 (REF. 55) to microtubule plus-ends, to which Kar9 binds through its interaction with Bim1 (REFS 56,57). In yeast with mutations in both the Cdk Cdc28 and its cyclin partner Clb5, Kar9 spreads along the entire length of cytoplasmic microtubules[55],which results in premature migration of the entire spindle into the bud/daughter cell[45,55]. Clb5–Cdc28, therefore, through Kar9, regulates the selective attachment of microtubule plus-ends to the bud/daughter-cell cortex. So, whereas Clb4–Cdc28 confers tight spatial control of Kar9 to the bud/daughter-cell-bound spindle-pole body[49] (see above), Clb5–Cdc28 promotes Kar9 transport to cytoplasmic microtubule plus-ends that are destined for the bud/daughter cell[55] (FIGS 1,3). This spatial and cell-cycle-mediated control of Kar9 localization highlights the role of its transport mechanism in limiting the number and position of directed microtubules, and possibly in limiting the force that is necessary for proper spindle placement.

The minus-end-directed motor protein Kar3, like Kar9, is spatially restricted to a subset of the plus-ends of microtubules in mating *S. cerevisiae* cells. Approximately three microtubules form a bundle that is directed towards the 'mating projection'[21], where they function to bring together the nuclei of mating cells[58]. Kar3 is preferentially localized to the plus-ends of shortening microtubules within this bundle, and is excluded from the ends of other microtubules in these cells[58,59]. The regulatory factors that allow Kar3 to discriminate between specific plus-ends by virtue of their subcellular localization remain to be elucidated.

MINUS-END
The predominantly stable end of a microtubule, which has exposed α-tubulin.

Box 2 | Structure and regulation of microtubule plus-ends

Microtubules are polymers that exhibit stochastic growth and shortening. These dynamic events are referred to as dynamic instability[122], which is characterized by the rate and frequency at which microtubule plus-ends undergo growth and shrinkage. Dynamic instability is intrinsically regulated by a stabilizing GTP 'cap', which is found at the GTP/GDP-exchange site of β-tubulin that is exposed on the plus-end of a microtubule (E-site)[122]. The GTP-bound nucleotide is hydrolysed on incorporation of β-tubulin into the microtubule lattice. After hydrolysis of GTP at the plus-end, microtubules become less stable, resulting in the release of energy that is stored within the microtubule lattice and polymer shortening[123] (reviewed in REFS 4,5).

Growing, shortening and paused microtubule plus-ends are also characterized by structural changes. Growing ends have a sheet of tubulin-dimer protofilaments that roll into the microtubule structure, whereas shortening ends show curling of individual protofilaments away from the microtubule axis[124] (reviewed in REF. 4). A transition state exists between these two events, which is defined as the paused or attenuated state. In this state, microtubules are not dynamic, and they have a 'blunt' end[125] (reviewed in REF. 4). Dynamic microtubules can search space, undergo transitions in their overall distribution and structure, and produce pushing and pulling forces on organelles within the cell — all these processes are essential for cell growth and division (reviewed in REF. 2).

Microtubule characteristics	Microtubule shortening	Transition (paused)	Microtubule growth	Treadmilling/flux
Plus-end structure	Curling protofilaments	Blunt	Rolling sheet	Not known
Force	Pulling	Static	Pushing	Pushing/pulling
Intrinsic regulation	Hydrolysis of GTP cap Force	Not known	Tubulin-dimer concentration	Not known
Regulation by microtubule-associated proteins (MAPs)	XMAP215/Dis1/Stu2 KinI OP18/Stathmin Katanin Kar3 Kip3	Not known	Mal3/Bim1/EB1 XMAP215/Dis1/Stu2 Neuronal MAPs (Tau, MAP1 and MAP2) Non-neuronal MAP (MAP4)	Not known

Regulating plus-end dynamics. In addition to their role in delivering proteins and maintaining an attachment to dynamic microtubules, a main function of microtubule-plus-end-targeting complexes is to regulate plus-end dynamics. Microtubule-binding proteins function by altering the parameters of microtubule growth and shrinkage events (BOX 1; reviewed in REFS 3,5). Two classes of plus-end-binding protein include those that promote growth and those that promote shortening.

The mammalian EB1 and CLIP170 proteins bind and stabilize microtubule plus-ends[5] and are involved in linking and/or transporting microtubule plus-ends to an attachment site. The budding-yeast EB1 protein, Bim1, probably mediates the attachment of microtubule plus-ends to the bud tip through Kar9 and the actin cytoskeleton[56,57,60,61] (FIG. 3). In addition, the association of Bim1 with growing microtubule plus-ends might facilitate efficient protein delivery. Mammalian EB1 is preferentially associated with growing plus-ends, as dramatically visualized at dynamic KINETOCHORES as they move away from the centrosome/spindle pole (growing plus-ends); it is lost from kinetochores that are moving towards the centrosome/spindle pole (shortening plus-ends)[62]. Therefore, EB1/Bim1 probably does not contribute to force-generation mechanisms that involve shortening microtubules. Alterations in EB1/Bim1 function might disrupt or attenuate microtubule-pulling forces, thereby leading to defects in proper spindle orientation.

By contrast, the preferential association of Kar3 with shortening microtubule plus-ends in *S. cerevisiae* KARYOGAMY led to the hypothesis that Kar3 might promote persistent microtubule depolymerization, thereby preventing microtubules from switching back to growth[59,63]. This hypothesis is supported by the *in vitro* demonstration that latex microspheres or beads that are coated with kinesin protein from HeLa cells persistently bind shortening microtubule plus-ends, and that microtubule depolymerization is enhanced under conditions that activate this kinesin motor[64]. It remains to be determined how these proteins are delivered to plus-ends. One possibility is plus-end-directed kinesin-based transport (as for Kip2 and Kar9, or kinesin I and dynein, described above).

In addition to plus-end-binding factors that are associated with depolymerizing plus-ends, 'CATASTROPHE factors' specifically regulate plus-end dynamics. The KinI family of kinesin-like proteins promotes ATP-dependent curling of PROTOFILAMENTS at microtubule plus-ends, thereby destabilizing the microtubule to induce catastrophe[65,66] (BOX 2; reviewed in REF. 5). The MAP OP18/Stathmin has been proposed to promote catastrophe events by sequestering tubulin dimers and limiting their addition to microtubules, or by inducing catastrophes (reviewed in REF. 67).

The XMAP215/Dis1/Stu2 family of MAPs can promote both growth and shortening of plus-ends, depending on the system and assay conditions[68–72]. This family is conserved from *S. cerevisiae* to humans[68–70,72,73]. *In vitro*, XMAP215 can promote microtubule dynamics[68] (BOX 2).

KINETOCHORE
A protein complex that provides a link between centromeric DNA and microtubules.

KARYOGAMY
The process in which two haploid nuclei come together and fuse to form a diploid nucleus during mating in *S. cerevisiae*.

CATASTROPHE
The transition from microtubule growth to shortening.

PROTOFILAMENT
Tubulin dimers aligned end-to-end make up protofilaments. Several protofilaments (usually 13) are organized into a tubular structure to form microtubules.

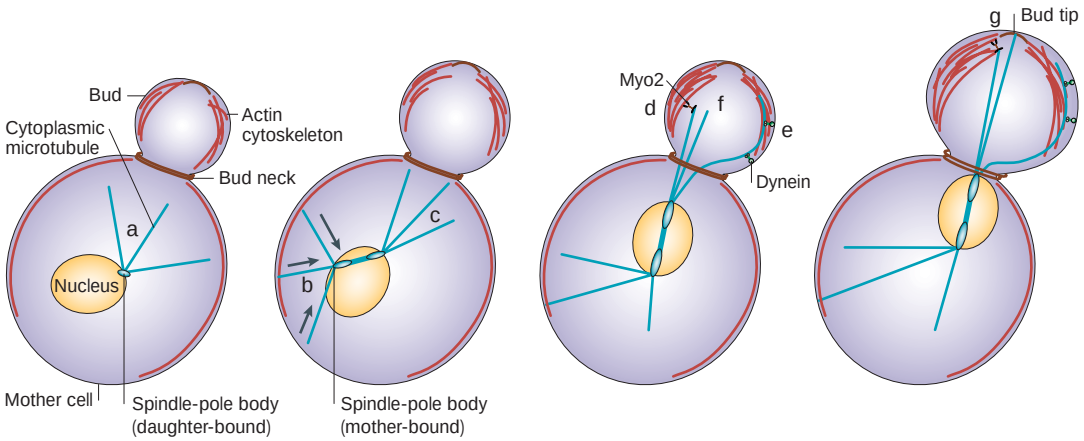


Figure 2 | **Stages involved in proper spindle positioning in *Saccharomyces cerevisiae*.** For the spindle to be positioned at the pre-defined site of cell division, dynamic microtubules use cortical cues and forces at the bud neck. **a** | Dynamic microtubules that are nucleated from the bud-bound spindle-pole body undergo dynamic instability to search the cytoplasmic space, to find and capture the bud neck. **b** | Forces (arrows) from the opposing, mother-cell-bound spindle-pole body help to push the spindle towards the bud neck. **c** | Dynamic microtubules are captured and stabilized by attachment to the bud neck[126,127]. This might be a loading site for the bud-specific forces that are necessary for spindle positioning. **d** | Microtubule plus-ends are translocated to the bud tip through translocation along polarized actin (red). This interaction and movement is facilitated by Bim1 (binding to microtubules-1), Kar9 (karyogamy-9) and Myo2 (a class-V myosin). **e** | Cortically localized dynein-dependent sliding of microtubules along the bud cortex directs microtubule plus-ends to the bud tip to be attached. **f** | Dynamically growing and shortening microtubules can search space to gain proper plus-end attachment at the bud tip. **g** | Microtubule attachment to the bud-tip or the mating-projection tip can maintain attachment to a microtubule that is growing and shortening[44,59,100]. Bud attachment and proper spindle positioning is necessary for equal segregation of the genome to mother and daughter cells.

However, in cell extracts from the African clawed frog *Xenopus laevis*, in *C. elegans* and in *D. melanogaster*, this family is important for promoting assembly of microtubules[3,69] (reviewed in REF. 74). In *S. cerevisiae*, Stu2 might promote microtubule catastrophe and dynamics by inhibiting dimer addition specifically at microtubule plus-ends[72]. Recently, the XMAP215 homologue in *C. elegans* — ZYG-9 — and the associated TAC-1 (transforming acidic coiled-coil-1) were shown to be important for regulating the length of spindle and ASTRAL MICROTUBULES[75–77]. Understanding how MAPs recognize microtubules and regulate their dynamics will be important for understanding the molecular mechanism of spindle positioning.

Dynamics and spindle positioning. Perturbations that affect microtubule dynamics reduce the fidelity of spindle positioning[30,31,78–81]. However, microtubule-plus-end binding proteins might perturb spindle positioning and orientation independently of their effects on microtubule dynamics. It is therefore important to ascertain whether alterations in the regulation of microtubule dynamics, or in microtubule-dependent functions (for example, the delivery of budding-yeast Kar9 or fission-yeast Tea1, Tea1 recruitment of the FORMINS For3 and Bud6, or microtubule translocation along actin cables), are the primary cause of any defects in spindle positioning and/or orientation.

For instance, although Bim1 binds growing microtubule plus-ends and facilitates microtubule transport along actin cables, loss of Bim1 also results in decreased cytoplasmic microtubule length and dynamics during G1 phase[31]. It is not clear whether the resulting spindle-positioning defects reflect alterations in microtubule length or dynamics, or alterations in the ability of Bim1 to translocate microtubule plus-ends to the bud site. In another example, in which the primary defect is uncertain, studies in *D. melanogaster* showed that RNA INTERFERENCE (RNAi) against the EB1 protein inhibited proper spindle positioning towards the cell centre[30]. Similarly, *C. elegans* mutants (such as *β-tubulin* or *zyg-9* mutants) with shorter astral microtubules that probably do not contact the cortex cannot undergo centrosome rotation in a one-cell embryo[81,82]. Inhibition of Stu2 in *S. cerevisiae* had a similar negative effect on spindle position, although cytoplasmic microtubule length remained the same[78]. Spindles in Stu2-depleted cells were randomly placed within the mother cell rather than aligned properly at the bud neck[78]. Like Bim1, Stu2 directly interacts with Kar9 (REF. 60). Stu2 might therefore contribute to the 'Kar9 pathway', which makes interpretation of the effect of microtubule dynamics on spindle positioning difficult[60].

To determine whether alterations in microtubule dynamics directly affect spindle positioning, Gupta *et al.* studied the consequences of specific mutations in β-tubulin that generated stable microtubules in *S. cerevisiae*[79]. They found that spindles were properly positioned at the bud neck, but that spindle orientation relative to the mother-cell–bud axis was defective. Dynamic microtubules in *S. cerevisiae* might therefore increase the fidelity of spindle positioning by promoting spindle orientation along the mother-cell–bud axis, but might not be as important for bringing the nucleus to the bud neck. However, β-tubulin mutations that stabilize

ASTRAL MICROTUBULE
A microtubule that is nucleated at the spindle pole and grows outwards towards the cell cortex; it is involved in spindle positioning.

FORMINS
A family of proteins that contain a formin homology-2 (FH2) domain. They are capable of promoting actin assembly.

RNA INTERFERENCE
(RNAi). A method to block the translation of RNA and thereby 'knock-down' the levels of specific proteins.

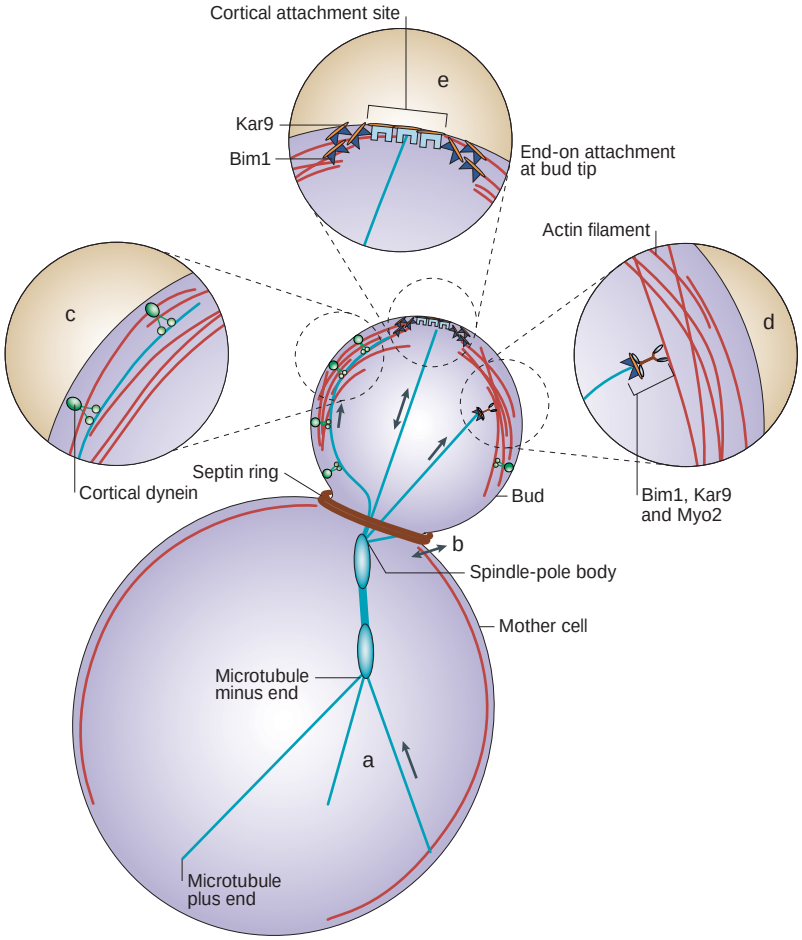


Figure 3 | **Balance of dynamic pushing and pulling forces in *Saccharomyces cerevisiae*.** To properly position the pre-anaphase spindle at the bud neck without moving the spindle into the bud, *Saccharomyces cerevisiae* provides a balance of pushing and pulling forces (arrows). **a** | Growing and shortening microtubules in the mother cell facilitate searching of the cytoplasmic space and establish pushing forces against the cortex to orientate the spindle to the bud neck. **b** | Stable attachment at the neck could provide a stabilizing force to limit pulling forces from the bud; it could also provide a loading site for actin-based transport of microtubules and dynein-dependent sliding, and/or it could maintain proper positioning at the bud neck. **c** | Minus-end-directed movement of cortically-anchored dynein provides a strong pulling force to bring the spindle to the bud neck. **d** | In a redundant pathway, microtubule plus-ends are linked through Bim1 (binding to microtubules-1) and Kar9 (karyogamy-9) to class-V myosin (Myo2) that is moving along polarized actin arrays, facilitating plus-end transport to the bud site. Additionally, transport might generate pulling forces to pull the spindle to the bud neck. **e** | Finally, end-on attachment can also generate force by maintaining attachment to both growing and shortening microtubule plus-ends.

GUANINE NUCLEOTIDE-DISSOCIATION INHIBITOR (GDI). A protein that inhibits the dissociation of GDP and therefore its replacement by GTP in a small GTPase, thereby maintaining the GTPase in an inactive state.

N-MYRISTOYLATION The chemical addition of the fatty acid myristate to the amino terminus of a protein, which enables that protein to become localized to a membrane.

microtubules in *C. elegans* inhibit centrosome rotation even though microtubule interactions with the cortex seem to occur. This implies that alterations of microtubule dynamics might have a greater impact on spindle positioning in other organisms[81]. Nonetheless, these β-tubulin mutations (in *S. cerevisiae* and *C. elegans*) might affect the binding of associated proteins that are involved in spindle positioning, as well as microtubule dynamics.

Microtubule-dependent spindle positioning in C. elegans. In *C. elegans*, GOA-1 and GPA-16 — α-subunits of heterotrimeric G-proteins — are required for asymmetric spindle positioning[83,84]. Moreover, a functional genomic screen for cell-division components in *C. elegans* identified two regulators of G-proteins, GPR-1 and GPR-2 (G-protein regulator-1 and -2)[85]. Severe spindle-positioning defects occur when these regulators are inactivated[86–88]. This regulation of heterotrimeric G proteins in the context of spindle positioning seems to be conserved, as the homologues of GPRs in *D. melanogaster* (Pins proteins) are also necessary for proper asymmetric cell division in neuroblasts[89]. GPR-1 and GPR-2 are highly identical at the sequence level (97% amino-acid identity) and function as GUANINE NUCLEOTIDE-DISSOCIATION INHIBITORS (GDIs)[86,88]. GOA-1 and GPA-16 contain *N*-MYRISTOYLATION sites that might tether these proteins to the cell membrane and establish the asymmetrical distribution of GPR-1/2 to the posterior cortex[86]. In elegant *in vivo* spindle-severing experiments, Grill *et al*. showed that the posterior spindle pole is subjected to greater pulling forces than the anterior pole[90]. Pulling forces are abrogated when GPR-1/2 or GOA-1/GPA-16 function is disrupted by RNAi[86,91]. The Gα proteins and GDIs therefore promote essential signals from localized sites for generating pulling forces on the spindle[91].

How are microtubule pulling forces regulated by G-proteins? One possibility is that the G-proteins regulate microtubule dynamics. Labbé *et al*. developed a new strategy to directly image microtubules at the cell surface (cortical imaging of microtubule stability)[80]. They found that, in wild-type cells, microtubules persist at the anterior cortex 15% longer than they do at the posterior cell surface. Inactivation of Gα proteins or of PAR proteins (PAR1, PAR2 or PAR3) using RNAi resulted in the equalization of microtubule dynamics *in vivo*[80]. These results provide important mechanistic clues as to how PAR proteins and Gα proteins generate asymmetric mechanical forces on the spindle. Differences in microtubule stability could promote an asymmetry in pulling forces if 'stable' microtubules in the anterior can resist a general pulling force at both the anterior and the posterior cortex. Pulling forces from attached and shortening microtubules (less persistent) might be antagonized in the anterior by stable microtubules (more persistent). In the posterior, more dynamic microtubules could pull the centrosome to the cortex. Polarity cues can therefore modulate microtubule-binding proteins and microtubule dynamics to facilitate spindle positioning.

Microtubule force generation

The involvement of microtubules in generating intracellular forces has been the focus of much research (reviewed in REFS 2,4,5,92,93). The positioning of the spindle by microtubules will probably involve both pushing and pulling forces.

Pushing forces. Dynamic microtubules are important to properly sense and push the cortical landscape. Microtubule pushing forces can result in substantial bending and buckling of microtubules[94,95], which, in turn, results in changes in the positioning of the spindle because of a loss of microtubule symmetry[94]. If the frequency of microtubule catastrophes increases, the

number of buckling events decreases, which allows the spindle to easily reorientate to the geometrical centre of the cell[96].

The change in residence time of microtubule plus-ends at a barrier, such as the cortex, is probably due to interactions of the plus-ends with microtubule regulators that are resident at that barrier. Alternatively, catastrophe events have also been shown to be induced by compression forces at the microtubule plus-end[97]. It is postulated that microtubule destabilization is a result of steric inhibition of tubulin-dimer addition[97]. Consistent with this, *in vivo* studies indicate that cells regulate the residence time at the cortex during a pushing event before undergoing a catastrophe[33,98]. In *S. pombe*, microtubules undergo a catastrophe event after approximately 1.5 minutes at the cell tip[98]. This allows for the nucleus to be pushed away from the cortex by the growing microtubule without the spindle completely translocating to the opposite end of the cell.

So, the cortical landscape, and possibly also microtubule-regulatory factors associated with it, is read by a simple microtubule-based, pushing-force-generating machine that is constantly probing the cortex and organelles within the cell. Forces that are generated by microtubule pushing against the cell cortex are dependent upon parameters of microtubule dynamics, and therefore specific perturbations of plus-end dynamics will probably have a substantial effect on microtubule pushing[96,98]. These experimental data, in combination with modelling evidence, indicate that the *S. pombe* system is excellent for studying the microtubule-pushing forces that control nuclear positioning[98].

Pulling forces. The searching of intracellular space by microtubule plus-ends is facilitated by both dynamic instability and active cellular machinery to increase the fidelity of microtubule capture by the bud cortical capture site (FIG. 2). In *S. cerevisiae*, a structure at the bud neck captures microtubules and maintains attachment to microtubule plus-ends (FIG. 2c). After attachment or localization of the microtubule to the neck, at least three pathways enable microtubule plus-ends to translocate into the bud (FIG. 2d–f). First, microtubule plus-ends move along polarized actin towards the bud tip (FIG. 2d). Second, microtubules interact laterally with anchored motor proteins that are 'walking' towards microtubule minus-ends (FIG. 2e). Finally, dynamic microtubule plus-ends can penetrate the bud neck during searching (FIG. 2f).

After capture of microtubules at the bud tip, end-on microtubule attachment, coupled with the force of depolymerizing microtubules, facilitates spindle movements (FIG. 2g). Although the force that is generated from growing and shortening microtubules at the bud tip is capable of moving the spindle, pushing and pulling forces might also be facilitated by microtubule motor proteins that are moving in either direction. In *S. cerevisiae*, both of these mechanisms might contribute to spindle positioning.

Dynein is anchored along the bud cortex, and its minus-end-directed activity brings the spindle towards the bud. As discussed above, dynein might contribute to the search-and-capture mechanism for microtubule end-on attachment by translocating or sliding plus-ends along the cortex to the bud tip where end-on binding sites exist (FIG. 2e,3c). Dynein sliding movements are also responsible for spindle movement to the bud neck[99,100]. Because dynein-generated sliding movements are a dominant force within the cell[100,101], antagonistic forces must be present to balance these forces and prevent premature transport into the bud (FIG. 3).

Similar to kinetochore–microtubule interactions, cortex–microtubule interactions might not be as simple as motor proteins pulling or pushing the microtubule lattice to properly position the spindle at the bud neck. Rather, pulling forces could be generated by shortening microtubules at either the plus-ends (plus-end catastrophe) or minus-ends (flux) while a complex that is anchored at the cortex maintains attachment (BOX 2; reviewed in REF. 2). As with pushing forces, tubulin-subunit gain and loss can also be crucial to the generation of pulling forces while maintaining an end-on attachment. The attachment complex probably involves proteins that promote microtubule disassembly (such as KinI kinesins or budding-yeast Stu2) and proteins that are responsible for maintaining attachment to dynamic microtubules. Coupling microtubule-polymerizing forces with spindle translocation will only occur when microtubules contact the cortex. Knowing the microtubule residence time, or frequency with which the cortical capture site loses attachment, is essential for understanding the contribution of this interaction to the generation of pulling forces.

In addition to facilitating proper plus-end capture at the bud tip, the translocation of microtubule plus-ends by polarized actin might provide an efficient mechanism for pulling the nucleus to the bud tip in *S. cerevisiae*. Actin cables provide a mechanical network for microtubule transport and therefore for the transport of centrosomes or spindle poles[102]. Microtubules direct the spindle towards and through the bud neck during anaphase in *S. cerevisiae*. Growing microtubule plus-ends are decorated with Bim1, which interacts with the class-V myosin Myo2 through Kar9 (REFS 56,57,60,83). Myo2 then transports (pulls) microtubules towards growth sites along the polarized actin cables[102]. On microtubule depolymerization at the bud tip, Bim1 is released. This cycle of Bim1 release, re-binding and Myo2-dependent translocation might be repeated to pull the microtubule and attached spindle, via actin cables, towards the bud.

Localization of anchors: lateral vs end-on attachments. For microtubule-based motors to facilitate spindle orientation, they must be able to generate force relative to an attachment site: simple translocation along the microtubule would not result in the net displacement of the spindle. This realization launched the quest to identify binding sites at the cell cortex that might anchor specific motor proteins. One of the first mutants that was found to be defective in nuclear migration in *S. cerevisiae* was called *num1* (nuclear migration 1). Num1 is an immobile cortical protein[103]. It contains a number of

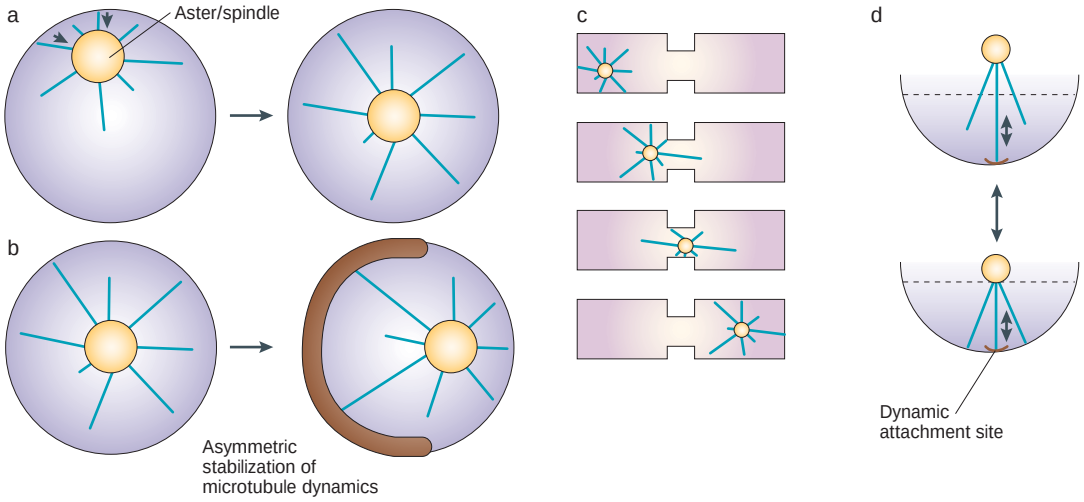


Figure 4 | **Organelle positioning by microtubule pushing and pulling forces. a** | An aster or spindle displaying dynamic instability within a constrained symmetrical space is able to generate equal pushing forces from all directions to facilitate 'centring' of an organelle (yellow) to the middle of the sphere[94]. **b** | By asymmetrically stabilizing microtubule dynamics (the brown area represents a microtubule-stabilizing region) the aster can be positioned away from the centre, finding a balance in pushing forces. **c** | Placing the aster in a chamber produces physical constraints that can also result in the displacement or movement of the aster[96]. The series of panels describes the movement of an aster that is nucleating dynamic microtubules, pushing itself through a chamber containing geometric constraints. Similar *in vivo* constraints are created by the bud neck of *Saccharomyces cerevisiae*. **d** | Proper aster position can also be created by a balance of both pulling and pushing forces (double-headed arrows). The middle microtubule maintains a dynamic attachment to the cortex, while surrounding microtubules provide a pushing force to keep the spindle from becoming juxtaposed to the cortex. The brown line represents a marker by which the position of the spindle in space can be defined.

tandem repeats of a 64-amino-acid polypeptide, a potential Ca^{2+}-binding site and a PLECKSTRIN-HOMOLOGY DOMAIN[103], and it binds an intermediate chain in the dynein complex[104]. Dynein, anchored to the bud cortex through Num1, facilitates sliding movements along the cortex to the bud tip[105].

Lateral microtubule interactions seem to be analogous to the rapid transport of kinetochores to the spindle pole that occurs during early mitosis (prophase) in mammalian cells[106,107]. Here, after rapid lateral transport, kinetochores attain 'end-on' attachment to microtubule fibres to produce the forces that are necessary for chromosome congression to the METAPHASE PLATE and subsequent anaphase onset. Interactions of motor proteins with the lattice of microtubules in polarity and chromosome movement might function to position microtubule plus-ends so that end-on attachments can generate the 'strong' forces that are necessary for movement. Different plus-end-translocation methods contribute to searching and translocation of the spindle to the bud neck. It remains to be determined how these 'translocation' forces relate to forces generated by plus-end attachment to the bud site (FIG. 3).

Geometrical shape — reading the landscape. How an organelle, such as the nucleus, migrates to a specific location within the cell is dependent on how that organelle senses and then responds to its surroundings. Although the deposition of polarity determinants is one mechanism for spindle orientation, the sensing processes are ultimately constrained by cellular geometry. In fact, a possible function of PAR3 in *C. elegans* might be to decrease geometrical constraints within the embryo that interfere with polarity establishment and microtubule-based processes[108].

Positioning an organelle in the centre of a spherical space is easily accomplished by pushing with equal efficiency from all directions on the surrounding surface (FIG. 4a). In the case of most biological systems, however, the landscape or intracellular space is not a sphere, but rather a shape that generates spatial limitations to how the nucleus can be positioned[94] (FIG. 4c). Steric hindrance of nuclear position is one mechanism of geometry-limited positioning. Microtubule pushing and pulling forces within a geometrically constrained environment become limited and/or directed by the surrounding environment (FIG. 4c). For example, a microtubule-cortical force that is pushing in all directions within a symmetrical 'cell' will position the spindle/aster in the middle of the cell[94] (FIG. 4a). If constraints such as the presence of a bud neck are placed within the cellular landscape, positioning by simple outward or pushing forces will no longer place spindles at the geometrical centre (FIG. 4c).

Balance of forces. A single, unregulated pulling or pushing force will obviously place the spindle at an extreme position within the cell, unless the force is regulated or counteracting forces contribute to placing the spindle at a defined region. In the case of spindle positioning in *S. cerevisiae*, there are pulling forces into the bud and 'away-from-bud' signals that keep the spindle from passing into the bud before anaphase (FIG. 3). Likewise,

PLECKSTRIN-HOMOLOGY DOMAIN
A protein domain that is typically made up of 100 amino-acid residues, and is found in many proteins that are involved in intracellular signalling. Named after pleckstrin, the main substrate of protein kinase C in platelets.

METAPHASE PLATE
During mitosis, chromosomes align at an equatorial plane between the two spindle poles, which is defined as the metaphase plate.

C. elegans might generate stable placement of the anterior spindle pole by balancing pulling and pushing forces from the mitotic spindle[80,90,91]. It is likely that, for most systems, a balance of force is required to properly position the spindle.

Both transient and stable interactions of microtubule plus-ends with the cortex can generate forces that are required for proper spindle positioning. As is the case for kinetochore–microtubule interactions, pulling forces are generally counteracted by an opposing force to accurately position chromosomes at the metaphase plate[109]. One such example with regard to spindle positioning would be a defined pulling force within a specific region of the cell (FIG. 4d). Pushing forces generated in the proximity of the pulling force could produce an antagonistic outward force, thereby balancing the outward and inward forces to place the spindle at a specific location (reviewed in REF. 15). Pushing and pulling forces can also be spatially regulated by maintaining regions of different microtubule dynamic parameters (FIG. 4b). These regions might be defined by physical constraints (such as the cell wall) or by differential localization of proteins (such as MAPs). This would create asymmetry in spindle positioning. Unlike microtubule–kinetochore interactions, microtubule interactions with the cortex seem to be more transient (1.5 minutes in *S. pombe*[98]; 3 minutes in *S. cerevisiae*[44,110]). These dynamic parameters might allow for effective probing and pushing of the cortical landscape.

Spindle-positioning checkpoint

The ability of a cell to track the temporal and spatial fidelity of key events during progression through the cell cycle is essential for its survival. Monitoring the position of the spindle is no exception. However, unlike damaged DNA, or unattached chromosomes, the actual event that is monitored by the spindle-positioning checkpoint is unknown. Yeh *et al*. found that, in cells that lack one of the key minus-end-directed motor proteins, dynein, the elongation of the anaphase spindle proceeded entirely within the mother cell[43]. The cells waited for up to three hours until the anaphase spindle migrated into the bud before progressing to mitotic exit and cytokinesis. On the basis of these findings, the authors suggested that cells monitor the position of the mitotic spindle and can delay cytokinesis until the both daughter and mother cells contain a complement of genomic DNA[43]. Subsequently, several mutants with defective spindle orientation have been found that delay cell-cycle progression (mitotic exit) until DNA is properly deposited into the future mother and daughter cells (reviewed in REFS 111,112). The signalling cascade that is responsible for initiating mitotic exit, dubbed the MITOTIC EXIT NETWORK (MEN), leads to the destruction of mitotic cyclin–CDK complexes only when the spindle-positioning checkpoint is satisfied (reviewed in REF. 112).

Microtubules themselves might be monitored. During early mitosis in *S. cerevisiae*, with the nucleus residing in the mother cell, astral microtubules traverse, and interact with, the bud neck (FIG. 2c–g). On spindle elongation in anaphase, spatial constraints limit astral-microtubule interactions with bud-neck proteins. The astral microtubules no longer contact the bud neck, and this possibly allows mitotic exit. Adames *et al*. have suggested that contact of astral microtubules with the bud-neck cortex suffices to restrain mitotic exit[113].

Alternatively, the spindle-pole body might deliver specific components to sites in the bud (monitoring by compartmentalization). Several components of the MEN are assembled at the spindle-pole bodies in discrete steps, in a manner that is dependent on spindle position and cell-cycle stage[114–116]. While the spindle resides in the mother cell, capture of MEN proteins at the spindle-pole body functions to sequester this regulatory complex from proteins in the bud. Upon spindle elongation, the spindle-pole body enters the bud, breaching the spatial separation. Contact of the bud-directed spindle-pole body, which bears the GTPase Tem1, with proteins in the bud cortex (such as Lte1) could trigger MEN activation during anaphase[114,117]. This hypothesis provides a mechanistic explanation for how the specific geometry of *S. cerevisiae* is exploited to activate a signal-transduction cascade that is required for mitotic exit.

A third idea is that Tem1 or another MEN component responds to forces or tension on the spindle-pole body as the mitotic spindle changes position during mitosis. Incorrectly positioned spindles, when re-positioned along the mother-cell–bud axis, accumulate Tem1 on the spindle-pole body that enters the daughter cell[115,118]. Pereira *et al*. suggest that contact between astral microtubules and the cell cortex gives rise to asymmetry in the GAP Bfa1–Bub2 complex as the spindle aligns along the mother-cell–bud axis[115,119]. This microtubule-mediated contact might also result in force or tension that pulls the spindle-pole body towards the bud neck.

Conclusions and perspectives

Spindle positioning and orientation is a highly regulated process that involves the coordination of cellular-polarity establishment, asymmetric protein deposition and forces generated by dynamic microtubules. A full appreciation of spindle positioning will require researchers to integrate signalling cascades, the dynamics of the cytoskeleton (actin and microtubules), the biophysical properties of these molecules and the spatial constraints that are defined by simple geometries.

Increasingly, cell biologists are using biophysical approaches to examine the dynamic properties of various elements *in vivo*, how intracellular forces are generated and how this translates into the highly ordered processes on which development and cell division rely. Although microtubules provide the basic machinery, the molecules that regulate their dynamics are bountiful and the mechanisms varied. Analysis of spindle orientation and positioning in model organisms such as *S. cerevisiae*, *S. pombe* and *C. elegans* shows that microtubule ends are decorated with a constellation of proteins, including motors, microtubule-binding proteins and linkers. So microtubule plus-ends themselves are differentiated, which provides the first step in transmitting spatial information.

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Competing interests statement
The authors declare that they have no competing financial interests.

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ADDENDUM

T-LOOPS AND THE ORIGIN OF TELOMERES

Titia de Lange

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The author wishes to make a short addition to the section 'The t-loop-evolution model'. In the last paragraph of this section, the author raises the possibility that the linear mitochondrial DNA of *Tetrahymena* sp. represents a 'living fossil' of the original t-loop-replication and -capping strategy. Unfortunately, the author omitted to highlight studies on the linear mitochondrial DNAs of several budding yeast species (for example, *Candida parapsilosis*), which, like *Tetrahymena* sp. mitochondrial DNA, contain tandem repeats at their ends. Importantly, electon-microsopy analysis has shown that the ends of the budding yeast mitochondrial DNAs are in a t-loop configuration[1].

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