The spindle: a dynamic assembly of microtubules and motors

focus on
cell division

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In all eukaryotes, a microtubule-based structure known as the spindle is responsible for accurate chromosome segregation during cell division. Spindle assembly and function require localized regulation of microtubule dynamics and the activity of a variety of microtubule-based motor proteins. Recent work has begun to uncover the molecular mechanisms that underpin this process. Here we describe the structural and dynamic properties of the spindle, and introduce the current concepts regarding how a bipolar spindle is assembled and how it functions to segregate chromosomes.

A defining characteristic of living organisms is their ability to proliferate. At a fundamental level, this ability is reflected in the division of single cells to generate daughter cells with exact complements of the parental genetic material. In all eukaryotic cells, faithful segregation of the genetic material is accomplished by a bipolar, microtubule-based structure, the spindle. Our aim in this review is to describe the complex and dynamic nature of the spindle and to enumerate the many different, and partly redundant, proteins and pathways that cooperate in its assembly and function. We first introduce spindle structure and dynamics, and then briefly address the mechanisms for spindle assembly and chromosome segregation. For more in-depth coverage of the functions of specific proteins in cell division and on the various subprocesses involved in spindle assembly we urge the reader to consult several other recent reviews[1–9].

Spindle structure

The primary structural element of the spindle is an antiparallel array of microtubules with their minus ends focused and anchored at the spindle poles and their plus ends projecting towards the chromosomes (Fig. 1). Microtubules are rigid polar polymers of αβ-tubulin dimers with a slow-growing minus end and a fast-growing plus end. Two properties of the microtubule lattice are exploited to build a spindle. First, microtubule ends undergo stochastic changes from a polymerizing to a depolymerizing state, a phenomenon known as dynamic instability (reviewed in ref. 10). Hydrolysis of GTP by β-tubulin associated with microtubule assembly fuels this non-equilibrium property and generates the potential for polymerization dynamics to carry out useful mechanical work in the cell. Second, the polar microtubule lattice serves as a track for the mechanochemical motors of the dynein and kinesin superfamilies; these proteins convert energy from ATP hydrolysis into spatial displacement along microtubules (reviewed in refs 11–13).

Chromosomes constitute the second principal structural element of spindles and are not just passive 'corpses at the funeral', but rather are active participants in generating spindle structure and dynamics. The kinetochore, a complex protein machine that assembles on the centromere, forms a localized, high-affinity site for the attachment of spindle microtubules to chromosomes. At least three morphologically distinct sub-populations of microtubules exist within mitotic spindles: first, kinetochore microtubules that terminate end-on in the outer region of the kinetochore and which connect chromosomes to spindle poles; second, interpolar microtubules that originate from opposite poles and interact in an antiparallel fashion, and which stabilize the bipolarity of the spindle; and third, astral microtubules that extend away from centrosomes into the cytoplasm and which act as 'handles' for orientating and positioning the spindle within the cell (Fig. 1A). The latter category of microtubules is absent in anastral spindles of the kind that are formed, for instance, in many meiotic cells.

This view of spindle structure has been derived from a combination of electron microscopy and analysis of the differential stability of spindle-microtubule subpopulations. Detailed three-dimensional reconstructions derived from serial sections of spindles have been produced for vertebrate tissue-culture cells[14] and in budding and fission yeast (reviewed in ref. 15, this issue). Although such high-resolution structural information is lacking for other popular experimental systems such as *Xenopus* egg extracts and syncitial *Drosophila* embryos, less comprehensive ultrastructural analysis of spindles in a wide variety of eukaryotic species has revealed fundamental similarities in spindle structure[16].

Spindle dynamics

The static appearance of spindles in immunofluorescence images of the type shown in Fig. 1B is highly deceptive. Spindle microtubules are very dynamic and turn over with a half-life of 60–90 s (refs 17, 18). The rapid turnover kinetics of metaphase spindle microtubules seems to be conserved throughout the eukaryotic kingdom and have been observed in fungal, invertebrate, vertebrate and plant cells[19,20]. The mechanisms underlying this rapid turnover have been primarily investigated in cytoplasmic extracts prepared from *Xenopus* eggs that can be stably arrested in either an interphase or a mitotic state. Comparison of the dynamic parameters of individual microtubules between the two extract states has indicated that rapid turnover of mitotic microtubules is driven primarily by an increase in the catastrophe rate, that is, the rate of transition of microtubule ends from a polymerizing to a depolymerizing state. This hypothesis has been supported by mathematical modelling showing that microtubule distributions are exquisitely sensitive to changes in the catastrophe rate[21]. In addition, comparison of the parameters of microtubule dynamics in extracts to those measured with purified tubulins indicates that distinct factors must exist in cells not only to accelerate microtubule growth but also to promote microtubule catastrophes (reviewed in refs 10, 22).

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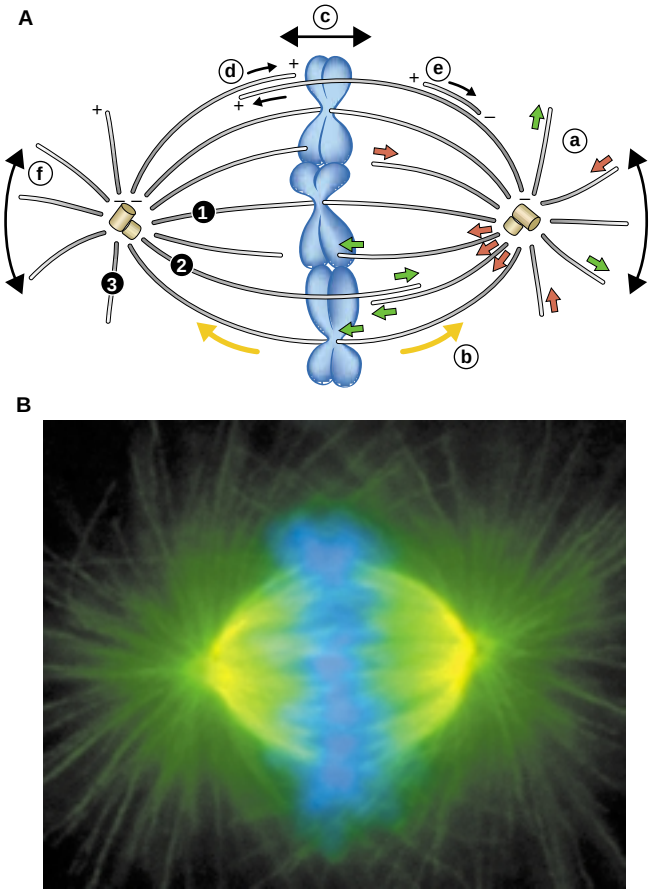

Figure 1 **Spindle structure and dynamics. A**, The hidden, dynamic nature of the spindle. The spindle contains different subpopulations of microtubules — kinetochore microtubules (1), interpolar microtubules (2) and astral microtubules (3). A variety of dynamic processes occur in the spindle, including microtubules undergoing dynamic instability (**a**, green and red arrows represent growing and shrinking microtubules, respectively); poleward microtubule flux (**b**, yellow arrows); chromosome movements (**c**); motor-driven antiparallel microtubule sliding (**d**); dynein-dependent, minus-end-directed microtubule transport (**e**); and orientation movements of the spindle poles (**f**). **B**, Static immunofluorescence photograph of a mitotic spindle in a tissue-culture cell. Green, microtubules; blue, chromosomes; red, TPX2 (a spindle-pole component). Overlapping immunostaining for spindle microtubules and TPX2 appears yellow.

A search for factors that promote microtubule catastrophes led to the identification of Op18/stathmin[23], a small, heat-stable protein, and XKCM1, a kinesin with an internal catalytic domain (Kin I)[24]. Op18 forms a ternary complex with two tubulin dimers and modulates the GTPase activity of β-tubulin[25–27], but the mechanism by which it induces catastrophes remains controversial. Op18 has been proposed to induce catastrophes indirectly by sequestering tubulin dimers, as well as directly by an unknown mechanism at microtubule ends[26,28–30]. Kin I kinesins do not function as motor proteins but are directly targeted to microtubule ends, where they induce catastrophes by disrupting protofilament interactions[31].

Counteracting the destabilizing effects of these two catastrophe factors are microtubule-associated proteins (MAPs) that bind to and stabilize microtubules (for a review on the role of MAPs in spindle assembly see ref. 1). A recently emerged class of MAPs, the Dis1 family, is essential for cell division in all eukaryotic species examined so far[32–36]. The *Xenopus* member, XMAP215, was purified biochemically as a MAP that specifically promoted the fast growth of microtubule plus ends in frog eggs[37]. Whereas structural MAPs such as tau, MAP2 and MAP4 stabilize microtubules by reducing the catastrophe rate, the effect of XMAP215 on tubulin polymerization is quite different. It increases the polymerization rate selectively at plus ends but does not seem to reduce the rate of catastrophes[38]. In contrast to its effect on pure tubulin, partial depletion of XMAP215 from frog-egg extracts markedly increases the catastrophe frequency, indicating that one important function of XMAP215 may be to control the susceptibility of microtubule ends to the action of catastrophe-promoting factors[39]. This hypothesis is supported by the discovery that inhibition of the catastrophe-promoting kinesin XKCM1 reverses the effect of partial XMAP215 depletion, revealing that the observed behaviour of a microtubule end in a cytoplasmic context reflects an integration of the antagonistic activities of these two molecules[39].

Cell-cycle-dependent regulation of these two proteins could explain the faster turnover of microtubules in mitosis relative to interphase. The activity of XMAP215 is indeed modulated by phosphorylation by cyclin-dependent kinase 1 (ref. 40), whereas XKCM1 seems to be active during both interphase and metaphase[39]. These studies have given rise to the simple speculative model in which rapid microtubule turnover during mitosis is driven by a phosphorylation-dependent change in the activity of an XMAP215-type MAP that reduces its ability to protect microtubule ends from the action of constitutively active catastrophe factors (Fig. 2a). Direct testing of this model by identifying and mutating XMAP215 phosphorylation sites as well as by reconstituting the mitotic behaviour of microtubules with purified components *in vitro* are two clear directions for future study.

In addition to rapid turnover, the entire ensemble of spindle microtubules undergoes concerted poleward movement. This was first demonstrated by photoactivation of caged fluorescent tubulins and more recently by fluorescent-speckle microscopy[41,42]. Poleward microtubule flux has been observed in the kinetochore microtubules of spindles from several different vertebrate cell types and in the bulk microtubule population of *Xenopus*-extract spindles[20,41,43,44], but has not been observed in photobleaching studies of spindles in budding and fission yeast[45,46]. During metaphase, when spindle length is constant, flux requires poleward translocation of spindle microtubules, continuous depolymerization of minus ends near the spindle poles, and polymerization of plus ends in the central spindle region. The mechanism that drives this complex behaviour remains mysterious. The only known inhibitor of flux is the non-hydrolyzable ATP analogue AMP-PNP, a broad-range inhibitor of kinesin-family motor proteins[43]. Inhibition of flux by AMP-PNP may reflect either involvement of kinesins in the mechanism of flux or a gain-of-function effect arising from its ability to induce a tightly bound rigor state of kinesins to microtubules. Depolymerization of microtubule minus ends may involve the microtubule-severing factor katanin. Katanin localizes to spindle poles, constitutes the principal source of microtubule-severing activity in mitotic *Xenopus* egg extracts, and is required for meitotic spindle assembly in *Caenorhabditis elegans*[47,48].

Microtubules also move polewards in a cytoplasmic-dynein-dependent manner that was initially observed using short, fluorescently labelled microtubule seeds added to spindles in *Xenopus* egg extracts[49]. However, seed movement appears to be distinct from microtubule flux, as flux is not disturbed by dynein inhibitors, and AMP-PNP does not inhibit seed movement[43,50].

In combination, these findings build a picture of the spindle as a remarkably dynamic structure (Fig. 1A). Large numbers of microtubules are rapidly polymerizing and depolymerizing while being continually translocated towards the poles. Highly dynamic spindle-microtubule ends are thought to be necessary to ensure that the small surface of the kinetochore encounters a microtubule. The function of poleward flux in the spindle remains less clear, although one possibility is that it is required during anaphase chromosome movement[51].

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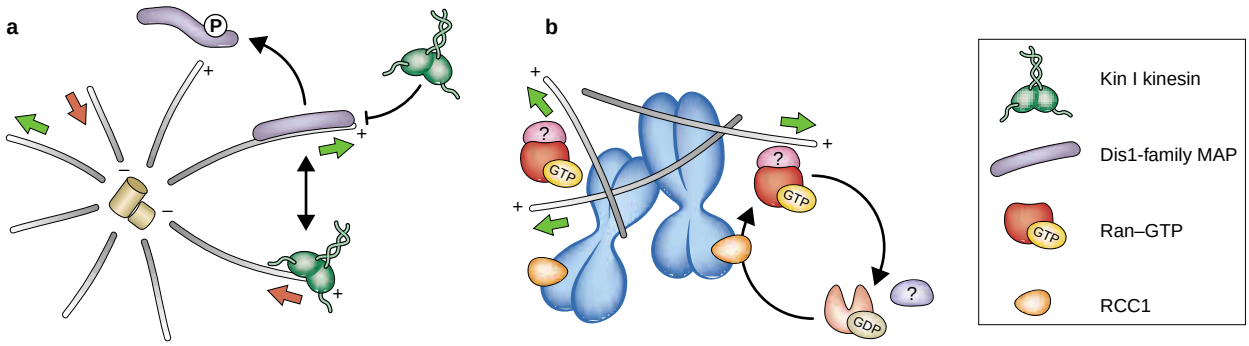

Figure 2 **Mechanisms that regulate microtubule dynamics during mitosis. a**, Within the spindle, microtubule dynamics seem to be regulated mainly by microtubule-associated proteins (MAPs) that stimulate microtubule polymerization and protect microtubule ends from catastrophe-promoting factors. The diagram depicts the recently documented antagonism between Dis1-family MAPs and kinesins of the internal-motor-domain family (Kin I) that depolymerize microtubules. Whereas Kin I kinesins seem to be constitutively active, the activity of Dis1-family MAPs may be regulated by phosphorylation. **b**, The chromatin-bound Ran guanine nucleotide exchange factor RCC1 creates a locally high concentration of Ran–GTP in the vicinity of chromosomes that leads to nucleation and/or stabilization of microtubules by an unknown mechanism. Green and red arrows represent growing and shrinking microtubules, respectively.

Establishing a bipolar microtubule array

Centrosomes, the principal centres for organization and nucleation of microtubules in animal cells, duplicate exactly once per cell cycle, around the time of DNA replication (reviewed in ref. 52). Separation of the two daughter centrosomes is thought to generate the first cue for the formation of a bipolar structure. The minus-end-directed motor cytoplasmic dynein, when anchored to either the nuclear envelope or the cell cortex, has been proposed to drive centrosome separation by pulling on the microtubules that are nucleated by the two daughter centrosomes[53–57] (Fig. 3). In the one-cell-stage *C. elegans* embryo, however, nuclei do not seem to be required for centrosome separation[58].

Cells of higher plants, as well as many meiotic cells, assemble bipolar spindles without centrosomes. Recent elegant experiments in which centrosomes were specifically destroyed by laser microsurgery have confirmed that animal cells that normally contain centrosomes can also utilize a centrosome-independent pathway to form bipolar spindles[59]. In metaphase-arrested *Xenopus* egg extracts, bipolar spindles readily form around DNA-coated beads[49]. These spindles do not contain centrosomes, because frogs, like most animal species, inherit their centrosome from the sperm. They also do not contain kinetochores, indicating that formation of a bipolar array does not require stabilization, mediated by capture of sister kinetochores, of oppositely orientated microtubules. The egg-extract system has been particularly useful for analysing the mechanism of spindle formation without centrosomes and kinetochores, and has revealed the importance of microtubule motor proteins in spindle assembly[60]. It has been proposed that when centrosomes are present they provide a dominant nucleation site that overrides the centrosome-independent pathway[50]. When only one of the two centrosomes in a tissue-culture cell is destroyed by laser microsurgery, however, the cell still assembles a bipolar spindle with a centrosome at only one pole[59]. This result indicates that there is no absolute difference in potency of the two assembly pathways in cells. A significant conceptual contribution of these studies has been to clearly distinguish between the centrosome and the spindle pole. The spindle pole does not require centrosomes for either its establishment or its maintenance but is rather a distinct structure that self-organizes during spindle assembly.

In interphase, centrosomes are the prinicpal microtubule-organizing and nucleating centres. So where do the microtubules originate that make up a spindle without centrosomes? Mitotic chromosomes have been hypothesized to generate a microtubule-nucleating and/or stabilizing activity, thus actively participating in spindle assembly[61,62]. Recent work in several laboratories has revealed that the small GTPase Ran, which is essential during interphase for nuclear import, can promote microtubule polymerization in its active, GTP-bound form[63,64]. Consistent with these observations is the fact that the Ran guanine nucleotide exchange factor RCC1 is required for microtubule polymerization in mitotic extracts[65]. RCC1 activates Ran by facilitating the exchange of bound GDP for GTP. Because RCC1 associates with chromatin, it has been proposed that chromatin-bound RCC1 induces a high concentration of Ran–GTP around mitotic chromosomes and locally promotes microtubule assembly[66] (Fig. 2b). How Ran–GTP promotes microtubule assembly and whether Ran is important for spindle assembly in cells that contain centrosomes remains to be determined.

In the absence of centrosomes, and probably also in their presence, microtubules that form around chromosomes in a random manner need to be sorted and bundled into an antiparallel, bipolar array (Fig. 3). Microtubule motor proteins have an important function in this self-organization process. They come in two different flavours, the kinesin superfamily, which includes both plus- and minus-end-directed motors[11–13], and cytoplasmic dynein, which, together with its activator dynactin, forms a large multi-protein assembly that moves towards microtubule minus ends[55,67]. In higher eukaryotes, at least seven subfamilies of kinesins, as well as cytoplasmic dynein, are involved in various aspects of spindle assembly. Early evidence that different kinesins cooperate and counteract with each other during spindle assembly came from elegant genetic studies in budding yeast[68]. Favourite candidate motors responsible for the sorting of microtubules into an antiparallel array are members of the BimC family of plus-end-directed kinesins. These tetrameric proteins have motor domains at both ends and can crosslink and slide antiparallel microtubules in opposite directions[69]. The importance of this class of kinesins in spindle function has been confirmed recently by the use of monastrol, a new chemical inhibitor of vertebrate BimC motors that was identified in a large-scale screen of a synthetic chemical library[70,71]. The mechanisms that lead to formation of a bipolar array of microtubules, which include the sliding of microtubules by BimC motors as well as pulling on astral microtubules by cortical dynein, seem to be antagonized by a minus-end-directed spindle kinesin with a carboxy-terminal motor domain (Kin C) that works to pull the poles together[56,68,72] (Fig. 3). Downregulation of the activity of this motor may be one way to increase the speed of pole separation during anaphase spindle elongation. In addition, chromosome-associated

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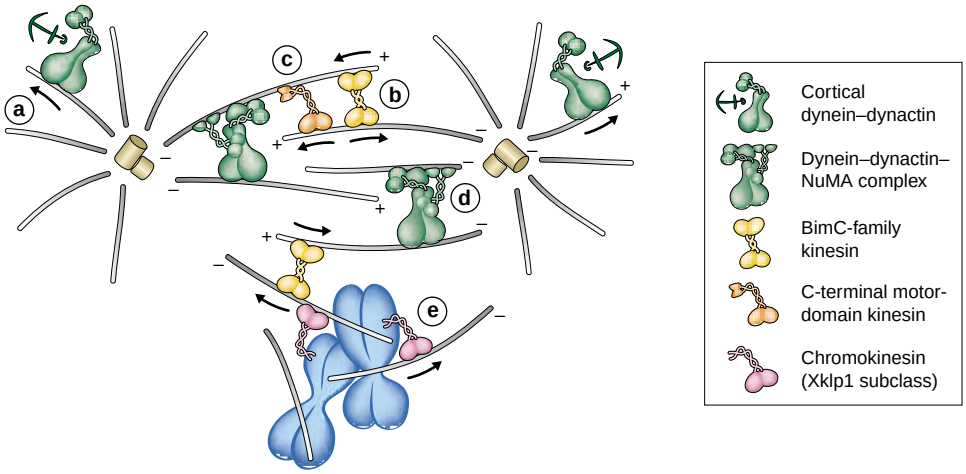

Figure 3 **Mechanisms for the establishment and maintenance of bipolarity.** Centrosome separation is maintained by the concerted action of cortical dynein (**a**) pulling on astral microtubules and plus-end-directed tetrameric kinesins of the BimC family (**b**) sliding antiparallel microtubules apart. BimC motors are also essential for the sorting of microtubules randomly growing around chromatin into an antiparallel array. Minus-end-directed kinesins of the C-terminal motor-domain family (Kin C) apply a force that counteracts pole separation and may be important for the regulation of the speed of spindle elongation (**c**). Cytoplasmic dynein, in complex with dynactin, NuMA and probably other spindle-pole proteins, crosslinks microtubules and as a result of its minus-end-directed movement leads to focusing of the poles (**d**). In addition, chromosome-associated, plus-end-directed kinesins mediate interactions between chromosome arms and spindle microtubules and push the spindle poles away from the chromosomes (**e**). Arrows indicate the forces applied on the microtubule lattice by the motor domains of the various motor proteins; note that these arrows point in the opposite direction to that of the motor directionality.

kinesins such as Xklp1 also contribute to the formation of a bipolar array by pushing the spindle poles away from the chromosomes[60,73].

Stabilization of the bipolar array also requires focusing of the minus ends of the sorted microtubules to form spindle poles. Oligomeric motor complexes that can crosslink and move along microtubules have been shown to be sufficient for the self-organization of pure tubulin asters *in vitro*[74] (Fig. 4). Thus, one way to generate a spindle-pole-focusing machine would be to oligomerize a minus-end-directed motor complex during mitosis. This indeed seems to be the case — cytoplasmic dynein–dynactin–NuMA complexes that coalesce dispersed microtubules into a focused pole are formed in mitotic, but not interphase, egg extracts[75,76]. This finding also explains why mitotic, but not interphase, cytoplasm is capable of forming self-organized microtubule asters[77,78].

Further stabilization of the focused microtubule ends is achieved by crosslinking them into a matrix-type structure at the spindle poles[79]. Although NuMA, a large coiled-coil oligomeric protein[80], is considered to be the principal crosslinker of microtubule minus ends at spindle poles, other proteins are involved in stabilizing poles. TPX2, a newly discovered spindle component that accumulates in nuclei during interphase, has been shown to translocate to the spindle poles in a dynein-dependent manner[81]. Depletion of TPX2 from mitotic *Xenopus* egg extracts resulted in spindles with a reduced density of microtubules and abnormal split poles. An excess of TPX2 led to the assembly of numerous asters not associated with chromosomes and of extremely dense monopolar spindles. Thus, TPX2 seems to regulate the density of spindle microtubules and spindle-pole structure. In addition, TPX2 is required for the accumulation of the plus-end-directed kinesin Xklp2 at spindle poles[77,81]. The function of Xklp2 in spindle assembly is not completely understood, but motors of the Xklp2 family may be required for some aspects of centrosome positioning during early mitosis[82,83].

Given that anaphase-competent spindles assemble in the absence of centrosomes, what do centrosomes actually do? Elegant studies using parthenogenetically activated *Sciara* embryos and *Drosophila* centrosomin mutants indicate that centrosomes act as 'handles' for orientating the spindle within the cell[84–86]. Spindle orientation determines the position of the cleavage plane and thus underlies many developmentally important asymmetric cell divisions. Spindle orientation depends on the interaction of astral microtubules with the cell cortex. The molecular mechanisms that underpin astral microtubule–cortex interactions are just beginning to be unravelled in budding yeast and seem to involve the actin cytoskeleton[87–89].

Alignment and segregation of chromosomes

The primary function of the mitotic spindle is to segregate chromosomes such that each half of a replicated chromosome ends up at opposite spindle poles. This involves a complex interplay between forces generated by motor proteins associated with kinetochores, chromosome arms and spindle microtubules, as well as those generated by the polymerization dynamics of spindle microtubules. Early in mitosis, the genetic material condenses to form chromosomes, physical packets that can be segregated within the spatial confines of the cell. During condensation, kinetochores assemble at the centromeric regions of the chromosomes and carry out three essential functions. First, they form a localized, high-affinity site on the chromosome to capture spindle microtubules; second, they ensure the necessary high fidelity of segregation by monitoring chromosome attachment to microtubules and regulating the metaphase–anaphase transition (for a recent review of mitotic checkpoints see ref. 90); and third, they act as central players in chromosome motility on the spindle (for detailed reviews of kinetochore function see refs 6, 7).

The mechanisms by which kinetochores work remain largely obscure. Kinetochores seem to be the source of strong poleward forces but do not contribute significantly to movement of chromosomes away from the pole[91–93]. Chromosome movements on the spindle are coupled to changes in the length of microtubules attached in an end-on manner to the kinetochore[5]. An important breakthrough in the molecular analysis of kinetochore function came from the discoveries that cytoplasmic dynein and the kinesins

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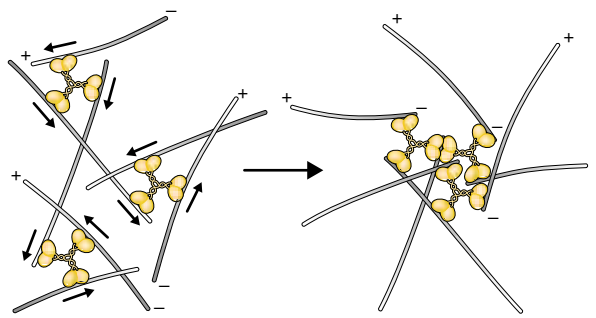

Figure 4 **Self-organization of microtubules and motors as a model for spindle-pole formation.** Oligomeric motor complexes can organize randomly growing microtubules into aster-like structures. Arrows indicate the forces applied on the microtubules by a minus-end-directed motor, transforming the random array of microtubules shown on the left into the aster on the right with the motor complexes accumulating in the centre. In the original experiments, plus-end-directed oligomeric kinesin was used, leading to the formation of inverted asters[74].

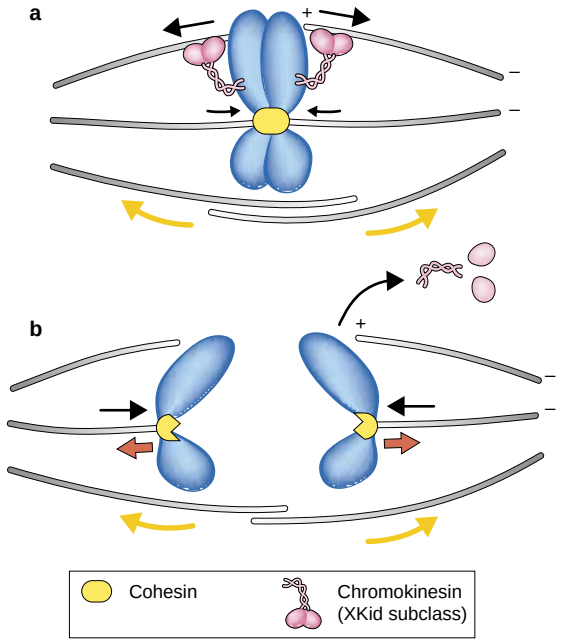

Figure 5 **A model for chromosome movement in *Xenopus*-extract spindles.** **a**, In metaphase, sister chromatids are held together by cohesin. Polar-ejection forces generated by plus-end-directed, chromosome-associated motors, as well as pulling forces that originate at the kinetochores, align the chromosomes on the metaphase plate. **b**, At the metaphase–anaphase transition, cohesin and the plus-end-directed chromokinesin are proteolytically degraded. As a consequence, both the kinetochore regions and the arms of the now-separated sister chromatids move polewards. In addition to pulling forces at the kinetochores, microtubule flux is thought to contribute to poleward chromosome movements in these spindles (yellow arrows). Red arrows represent microtubule depolymerization; black arrows indicate the forces applied on the microtubule lattice by chromosome-associated motors or microtubule depolymerization.

CENP-E, a plus-end-directed motor, and MCAK, a member of the microtubule-destabilizing Kin I subfamily, localize to kinetochores of vertebrate and invertebrate cells[94–97]. The precise functions of these proteins in kinetochore activity have been difficult to establish, although many studies have implicated them in various aspects of chromosome segregation (reviewed in ref. 6).

In brief, cytoplasmic dynein participates in the initial microtubule capture and fast-gliding motility that occurs before end-on attachment of microtubules to the kinetochore[92]. Cytoplasmic dynein is the only minus-end-directed motor protein that is localized to kinetochores, making it a strong candidate for driving poleward chromosome movement. However, the requirement for dynein in spindle assembly has made dissection of its function in chromosome movement rather difficult. Both early and recent studies of dynein in various cell types have revealed that the bulk of cytoplasmic dynein dissociates from kinetochores upon microtubule attachment, reducing its appeal as a candidate motor for poleward chromosome movement in anaphase[96–99]. However, recent functional analysis in *Drosophila* embryos and spermatocytes has resuscitated dynein as a candidate motor for poleward movement of chromosomes[100,101], although the relevance of these findings to other systems remains to be determined.

CENP-E, a kinesin of very large molecular mass that has plus-end-directed motor activity, has been implicated in kinetochore capture of spindle microtubules, congression to the metaphase plate and maintenance of congression[102–104]. In addition, CENP-E is part of a kinetochore-derived signalling pathway that monitors kinetochore–microtubule attachment and ensures high segregation fidelity[105,106]. *In vitro* studies have also indicated that CENP-E may be involved in moving chromosomes polewards, that is, towards microtubule minus ends, by acting as a coupling factor to depolymerizing microtubules[107]. MCAK is a putative microtubule-destabilizing kinesin that might function in depolymerizing microtubules proximal to the kinetochore as the chromosomes move towards the pole during anaphase[108]. The finding that kinesins, regardless of their inherent polarity, are capable of coupling movement of an object to depolymerizing microtubules was an important conceptual advance in understanding how the energy derived from GTP hydrolysis by tubulin can generate effective motility of objects such as chromosomes in the cell.

In contrast to kinetochores, chromosome arms are subject to a force directed away from the poles. This 'polar-ejection force' was first postulated on the basis of experiments to analyse the motility of laser-severed chromosome arms in the spindles of vertebrate somatic cells[93]. Chromosome-arm-associated, plus-end-directed kinesins, generically termed chromokinesins, have been proposed as one source of this force. This was first inferred from analysis of Nod, a *Drosophila* chromokinesin that is necessary for alignment of non-recombined female meiosis I chromosomes[109]. The most persuasive evidence that chromokinesins contribute to polar ejection comes from recent analysis of the *Xenopus* kinesin XKid[110,111]. In frog-extract spindles, XKid is required for the chromosome arms to congress to the middle of the spindle. XKid is a putative plus-end-directed motor, indicating that its activity may oppose a continual poleward force, possibly generated by the poleward flux of spindle microtubules (Fig. 5a).

The forces that act on chromosomes are thought to be fully established by metaphase, such that dissolution of sister-chromatid cohesion at the metaphase–anaphase transition would be sufficient to induce poleward chromosome movement in anaphase A. Recently, spectacular genetic and biochemical experiments in budding yeast have shown that cleavage of cohesin, the 'glue' that keeps the chromatids together, is indeed sufficient to induce anaphase[112–114]. In contrast, proteolytic destruction of XKid seems to be necessary for anaphase chromosome movement in frog-extract spindles[110]. The importance of XKid destruction may reflect a specialized mechanism for microtubule-flux-dominated systems in which depolymerization of kinetochore microtubules during poleward chromosome movement occurs distally to the kinetochore, in the vicinity of the spindle poles. In vertebrate somatic cells, the majority of poleward chromosome movement is associated with kinetochore-proximal microtubule depolymerization,

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and flux only provides a minor contribution[20,44,115]. Whether proteolysis of cohesin is sufficient for anaphase chromosome movement in vertebrate somatic cells or whether proteolysis of an XKid-type chromokinesin is also necessary remains to be determined (Fig. 5b).

Although more molecular detail about kinetochores and spindles continues to accumulate, the forces that move chromosomes within the spindle remain difficult to dissect[116]. Fundamentally, these forces can arise from the polymerization dynamics of the microtubules and from the action of motor proteins. Effective usage of polymerization dynamics requires coupling interfaces that can translate dynamics into motility. The discoveries that motor proteins can couple movement of objects to depolymerization of microtubules and that members of a centromere-localized family of kinesins are not motor proteins but rather are microtubule destabilizers significantly blurs the distinction between the two sources of force generation for chromosome movement. Spatially, forces may be highly localized, such as at the kinetochore, or distributed, such as those generated along the length of spindle microtubules or all along the chromosome arms. Spindle-wide flux-type mechanisms may also act to move chromosomes within the spindle. Given this complexity, functional perturbations combined with *in vitro* reconstitution approaches, high-resolution microscopy techniques and direct force measurements will be necessary to gain further insight into how chromosomes are physically segregated on the spindle. □

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