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Mechanisms and Molecules of the Mitotic Spindle

Review

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In all eukaryotes, morphogenesis of the microtubule cytoskeleton into a bipolar spindle is required for the faithful transmission of the genome to the two daughter cells during division. This process is facilitated by the intrinsic polarity and dynamic properties of microtubules and involves many proteins that modulate microtubule organization and stability. Recent work has begun to uncover the molecular mechanisms behind these dynamic events. Here we describe current models and discuss some of the complex repertoire of factors required for spindle assembly and chromosome segregation.

Introduction

Essential to the process of cell division is the mitotic spindle, which partitions a complete set of chromosomes to each daughter cell. The spindle consists of microtubules, polar dynamic fibers that polymerize from tubulin subunits, as well as hundreds of other proteins that function together to orchestrate chromosome segregation. These include a large set of microtubule-based motor proteins that use ATP hydrolysis to generate movement, or alter microtubule dynamics.

While the basic steps of spindle assembly and anaphase chromosome segregation have been documented since the emergence of light microscopy (Figure 1), pioneering techniques have continued to tell us new things about spindle microtubule dynamics. Molecular approaches, empowered by complete genome sequences, are continuing to identify the proteins responsible for the phenomena observed. In this review, we highlight some of the latest techniques developed and molecules identified that shed light on how the spindle assembles and functions to segregate chromosomes.

Spindle Anatomy and Steps of Assembly

Organizing a specific arrangement of microtubules and chromosomes within the spindle is central to how the process works (Figure 1A). Microtubules must be arranged into a bipolar array, such that each half spindle contains uniformly oriented microtubules, with their minus-ends at the pole and their plus-ends extending away. Each duplicated chromosome has a pair of specialized structures at its centromere, called kinetochores, which function to attach sister chromatids to microtubules from opposite spindle poles, to allow for directed translocation of chromosomes within the spindle [1].

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Microtubule nucleating sites exert a major influence on spindle assembly. Most animal cells contain a single microtubule nucleating structure, the centrosome, which consists of a pair of centrioles surrounded by amorphous material that harbors templates for microtubule nucleation. The polarity of microtubule growth from centrosomes, with their minus-ends tethered and their plus-ends extending outward, facilitates proper organization of the spindle.

How is the spindle set up? By the onset of mitosis, at prophase, the centrosome and the chromosomes have duplicated and a cascade of events occurs, including nuclear envelope breakdown, chromosome condensation and centrosome separation (Figure 1B). An increase in the frequency of microtubule shrinkage events, called catastrophes [2], and a decrease in events rescuing growth [3] contribute to the dismantling of the interphase array, thus allowing interaction between dynamic microtubule plus-ends and the condensed chromosomes. During prometaphase, some microtubules emanating from one centrosome attach to the kinetochore of one of the duplicated chromatids. Subsequent attachment of the sister kinetochore to microtubules growing from the other centrosome results in the bi-orientation of the chromosome and its eventual congression to the center of the antiparallel microtubule array. Once all of the chromosomes are bi-oriented and aligned, the cell is in metaphase. In addition to the kinetochore fibers, other populations of microtubules also contribute to the bipolar structure, including the interpolar microtubules that overlap to form an antiparallel array, and the astral microtubules, that extend from each centrosome away from the spindle where they can interact with the cell cortex (Figure 1A).

When the chromosomes are aligned and oriented, a cellular checkpoint is satisfied, and anaphase A ensues as sister chromosomes separate and move toward opposite spindle poles with their kinetochores leading (Figure 1B). Anaphase B also contributes to chromosome segregation, as spindle poles separate and the central spindle forms. Telophase marks the reformation of the nuclear envelopes around daughter cell nuclei as the cytokinetic furrow pinches the cell into two.

Although memorizing of the phases of mitosis has tortured students for decades, understanding how these events actually occur continues to occupy cell biologists, as a complete molecular model has yet to be obtained. Although Figure 1 is reasonably accurate in depicting a static view of progression through mitosis, it does not convey the dynamic nature of the spindle. Furthermore, the canonical diagram does not take into account the exceptions to the rules, which have been extremely instructive in elucidating the principles underlying spindle assembly. Below we describe some models of spindle dynamics, and then launch into a description of the molecules that underlie the behaviors seen.

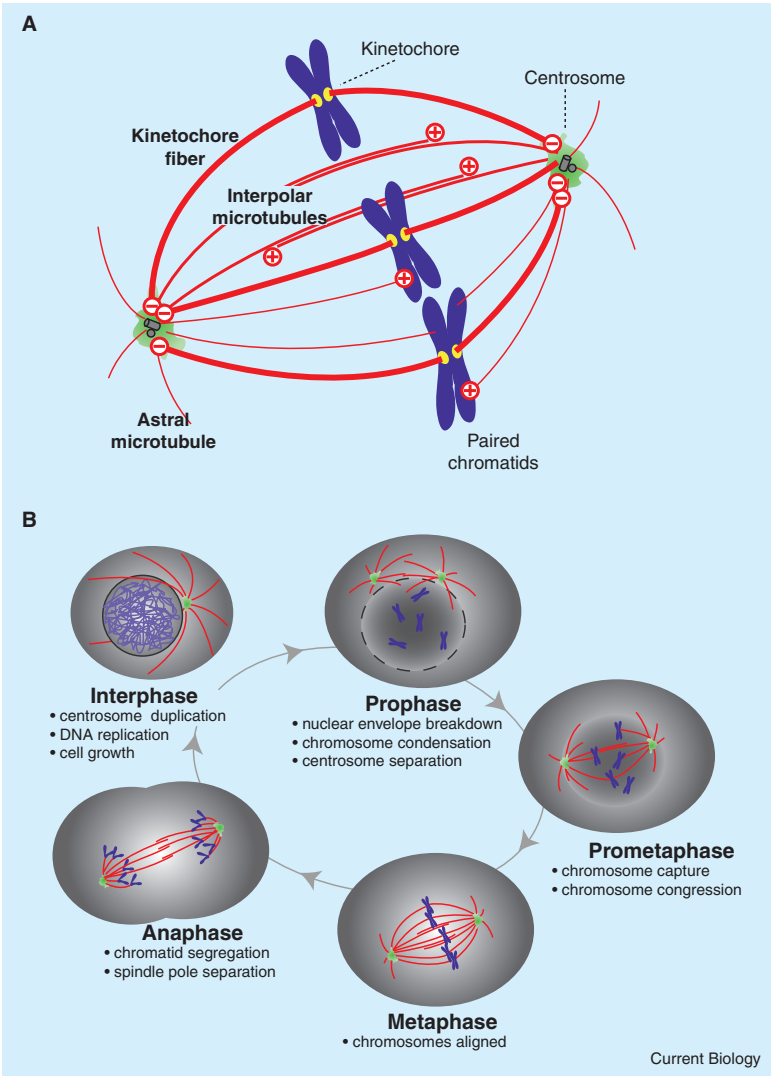

Figure 1. Spindle anatomy and the cell cycle.
(A) Features of the metaphase mitotic spindle. With their minus ends tethered at the spindle poles, microtubules extend either to the kinetochores of paired chromatids (kinetochore fibers), to the central spindle where they form an overlapping antiparallel array (interpolar microtubules), or away from the spindle towards the cell cortex (astral microtubules). (B) The stages of mitosis illustrating microtubule reorganization and chromosome translocation. During interphase, the chromosomes and centrosome are replicated. At prophase, chromosome condensation begins, centrosomes separate and the nuclear envelope breaks down. During prometaphase, chromosomes are captured by microtubules growing from the separated centrosomes and bi-orient, congressing to the center of the spindle at metaphase. Anaphase marks the loss of cohesion between sister chromatids and their movement to opposite spindle poles, which move apart to further separate daughter nuclei re-forming in telophase (not shown) prior to cytokinesis and the return to interphase.

Multiple Mechanisms at Work

One of the 'special' cases that has shed light on the process is the assembly of the female meiotic spindle, which occurs in the absence of centrosomes. Originally thought to be an anomaly, the mechanisms by which a bipolar microtubule array forms in this situation are now believed to be a general feature of spindle assembly. The predominant model of spindle assembly in the presence of centrosomes is based on microtubule dynamic instability and is known as the "search-and-capture" model [4]: Microtubules emanating from a centrosome undergo cycles of growth and shrinkage, randomly probing the cytoplasm until running into a kinetochore, with which they form a stable attachment (Figure 2A). Because microtubules from duplicated centrosomes encounter bivalent kinetochores, a bipolar spindle forms. In contrast, in the absence of centrosomes, microtubules polymerize in a disorganized fashion without focal nucleation sites and yet a spindle forms. Motor-dependent mechanisms must be invoked to sort these randomly oriented microtubules into a bipolar structure. The 'self-organization' model based on observations of acentrosomal spindle assembly (Figure 2B) [5], was not thought to apply to somatic cells harboring centrosomes. However, several lines of evidence have changed this view.

A major argument that self-organization is at work, even in the presence of centrosomes, is that spindle assembly can proceed after centrosome function has been abolished. For example, mutations have been identified in *Drosophila* that inactivate centrosomes, yet functional spindles still form [6–8], as they do in a related insect, *Sciara*, which can produce parthenogenic embryos lacking centrosomes [9]. When the centrosome is physically removed in vertebrate somatic cells using a laser beam or microsurgery, functional bipolar spindles form nevertheless [10–12]. The major effect on mitotic progression in the absence of centrosomes is that spindles are more often misoriented due to the loss of astral microtubules, which can decrease the fidelity of cytokinesis.

More evidence that mechanisms in addition to search-and-capture are at work comes from experiments showing that spindles can form in the absence of kinetochores, or even chromosomes. One system that has been particularly useful to directly compare

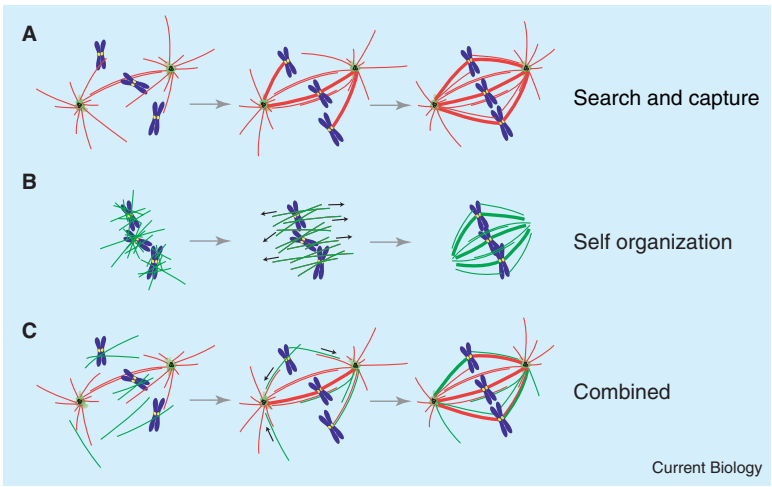

Figure 2. Models of spindle assembly.
(A) 'Search-and-Capture': microtubules nucleate from centrosomes and contact chromosomes and kinetochores by chance, and then become stabilized to form the spindle. (B) 'Self-Organization': randomly oriented microtubules nucleated in the absence of centrosomes are organized into a bipolar array by motor proteins. (C) Combined model: peripheral microtubules or those emanating from chromosomes are captured and incorporated into the centrosome-nucleated array to generate the spindle. Microtubules nucleated by the centrosome are labeled in red, microtubules that are not, are labeled in green.

spindle assembly pathways is *Xenopus* egg extract. A sperm nucleus added to this concentrated cytoplasm nucleates microtubules at its associated centrosome, which is duplicated along with the chromosomes as the extract cycles through interphase. Upon re-entry into mitosis, a bipolar spindle is set up [13]. In the absence of centrosomes and kinetochores, spindle assembly can also be induced by addition of DNA-coated beads to the extract. These beads recruit chromatin factors sufficient to promote bipolar spindle assembly in the absence of paired cues [14]. In some situations, spindles can even form in the complete absence of chromosomes. In *Drosophila*, some mutants have such severe defects in chromosome segregation during male meiosis that secondary spermatocytes develop completely lacking chromosomes. Nevertheless, these cells contain robust asters that form bipolar spindles, and even undergo a morphologically normal-looking anaphase [15].

These observations highlight the idea that multiple mechanisms promote bipolar spindle formation. While search-and-capture allows for essential attachments between chromosomes and microtubules, organizing forces are at work to promote bipolarity. Microtubule based motors are responsible for the generation of many of these forces, and are essential to establish the bipolar array in all cases. One example of such a motor is cytoplasmic dynein, a minus-end directed motor that associates in large complexes with several sites of the mitotic apparatus [16]. In *Xenopus* extracts, one of its major roles is to focus microtubule minus-ends to form the spindle poles. If cytoplasmic dynein is blocked, the poles splay apart regardless of whether a centrosome is present or not [17]. These and many other observations suggest that, through their unidirectional movement and ability to cross-link microtubules, motors sort populations of microtubules with regard to their polarity and orientation with respect to other spindle components, thus enforcing bipolarity [18].

Visualizing Spindle Dynamics

Major contributions to our understanding of spindle dynamics continue to come from imaging studies. In general, the basis of these approaches is to introduce fluorescently labeled tubulin subunits into cells, which become incorporated into the microtubule lattice, and then observe them using fluorescence time-lapse microscopy. Recent careful observations of cultured cells have helped to unify the search-and-capture and self-organization models, revealing motor dependent coalescence of different populations of microtubules during spindle assembly. One group showed that microtubule bundles are transported inward from the cell periphery in a dynein-dependent manner and incorporated into the spindle [19,20]. Another study revealed that kinetochore fibers form spontaneously, and that they can subsequently interact with microtubules emanating from a centrosome; these microtubules correct the improper orientation of the fiber and incorporate it into the spindle [21]. Thus, centrosomes are not the sole source of spindle microtubules, and a combination of capture and motor dependent activities generates the bipolar structure of the spindle (Figure 2C).

A twist on simply following the labeled microtubules is to mark the lattice. Early photobleaching and photoactivation experiments were used to study microtubule turnover and behavior in the spindle [22–25]. If a mark on the microtubule lattice is made, it only disappears when the tubulin subunits at that spot have been replaced with unmarked subunits. In addition, imaging the mark over time indicates how the microtubule lattice is moving, and where microtubules are polymerizing or depolymerizing. Such studies showed that polymers were turning over rapidly, and revealed a phenomenon called 'microtubule flux' [26]. Spindle microtubules are constantly polymerizing at their plus-ends and depolymerizing at their minus-ends, leading to a treadmilling effect and constant poleward movement of the lattice. The rate of flux varies in different cell types, and appears to be highest in embryonic systems such as *Xenopus* egg extracts [27].

In recent years, imaging of the microtubule lattice has become more sophisticated with the development of the 'speckling' technique [28]. Speckles are generated by introduction of low levels of fluorescently labeled tubulin, which does not incorporate uniformly

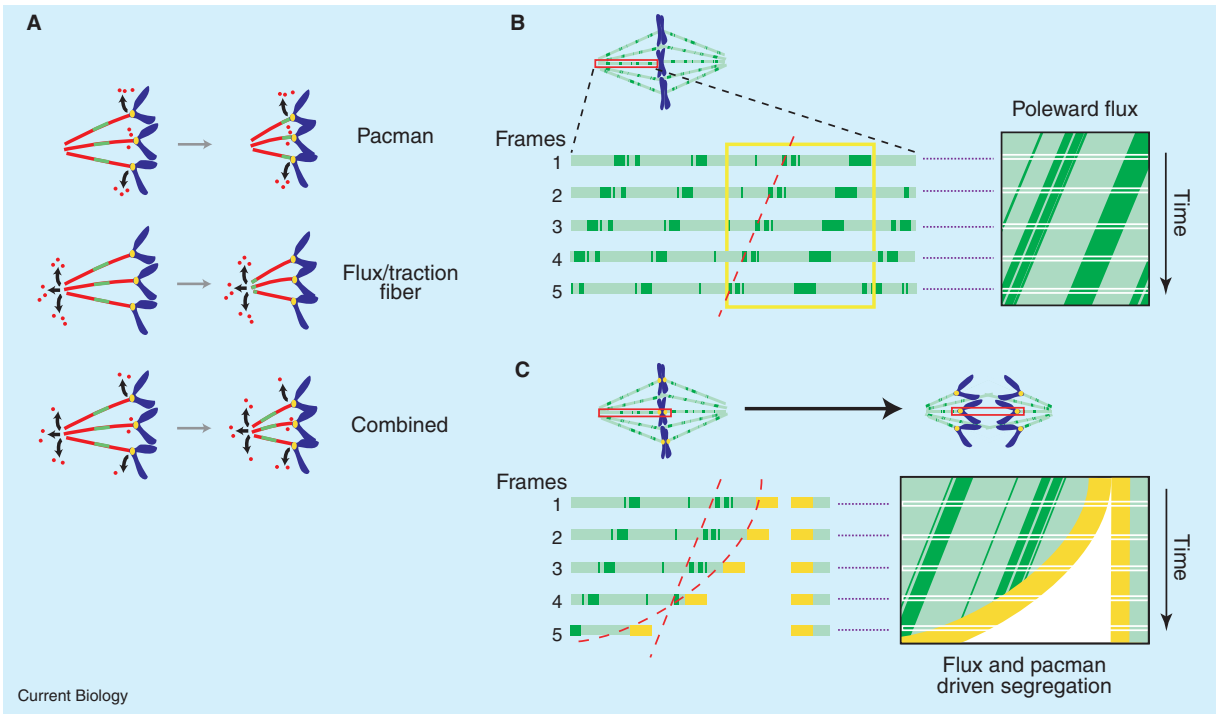

Figure 3. Speckling of spindle microtubules reveals mechanisms of chromatid-to-pole movement during anaphase.
(A) Proposed mechanisms of kinetochore microtubule depolymerization at the kinetochore plus-ends ('pacman') or the spindle pole minus-ends (flux/traction fiber), or both (combined). (B) A spindle containing low levels of fluorescent tubulin can be imaged over time to generate a kymograph, created by stacking one-pixel wide lines from video frames. The resulting 2-D stack illustrates movement of fluorescent marks in the observed line over time (descending in the *y*-axis). Flux velocity can be determined from the slope of speckle lines. In this example, the speckle lines have a constant slope, indicating a constant flux velocity. (C) Multiple fluorescent items in the same pixel line can move at different rates. In this example, the kinetochore (yellow) slope changes, indicating acceleration. The kinetochore overtakes microtubule speckles, indicating plus-end 'pacman' depolymerization of kinetochore microtubules. (Note that for alignment purposes, the right-hand kinetochore is fixed as a static point over time.)

into microtubules. This results in fiduciary marks — a bar code effect that allows specific regions of a microtubule to be recognized over time (Figure 3B). To better visualize speckle behavior, kymograph data are frequently presented, depicting a slice of the lattice from sequential frames, to help distinguish where microtubule polymerization and depolymerization are occurring, and whether the lattice itself is moving.

Microtubule speckling combined with kinetochore labeling has been used to definitively address a question about chromosome segregation. Two models have been proposed to explain chromosome transport to the pole during anaphase A (Figure 3A). After separation, the sister chromatids are transported to opposite spindle poles as kinetochore fibers shorten. A long-standing question concerned the site of microtubule depolymerization. One model, termed 'pacman' proposes that the kinetochore induces microtubule disassembly at the plus-ends, but maintains attachment as the fiber depolymerizes, thus chewing its way to the pole. In the other model, termed 'traction fiber', poleward microtubule flux is harnessed to move the chromosomes. If kinetochore microtubule polymerization ceases at the plus-ends, but depolymerization persists at the minus-ends, the chromosomes would be pulled towards the poles. A combination of microtubule speckling and kinetochore labeling techniques in *Xenopus* and *Drosophila* has revealed that both mechanisms contribute to the depolymerization of kinetochore microtubules [29,30] (Figure 3C).

The Molecules Behind the Mechanisms

Even though the phenomena of spindle assembly and behavior provide a rich source for modeling potential mechanisms, we have yet to obtain a molecular picture of how the complex dynamic events of mitosis occur. This is partly due to the large number of factors involved, on the order of hundreds, and their complex properties, interactions and regulation. Nevertheless, a molecular parts list is emerging that identifies some of the activities behind the observations.

An important principle is the molecular nature of the microtubule itself (Figure 4A, for reviews see [31,32]). Microtubules consist of parallel protofilaments of α-/β-tubulin heterodimers arranged head-to-tail that curve to form a tube. The polymer is highly dynamic and switches stochastically between growing and shrinking phases, *in vivo* as well as *in vitro*. This non-equilibrium behavior, known as 'dynamic instability', is based on the binding and hydrolysis of GTP at the nucleotide exchangeable site (E-site) in β-tubulin. Only dimers with GTP in their E-site can polymerize, but after polymerization this nucleotide is hydrolyzed and cannot be exchanged. The 'GTP cap' model proposes that the body of the microtubule, which consists of GDP-tubulin subunits, is unstable. The microtubule

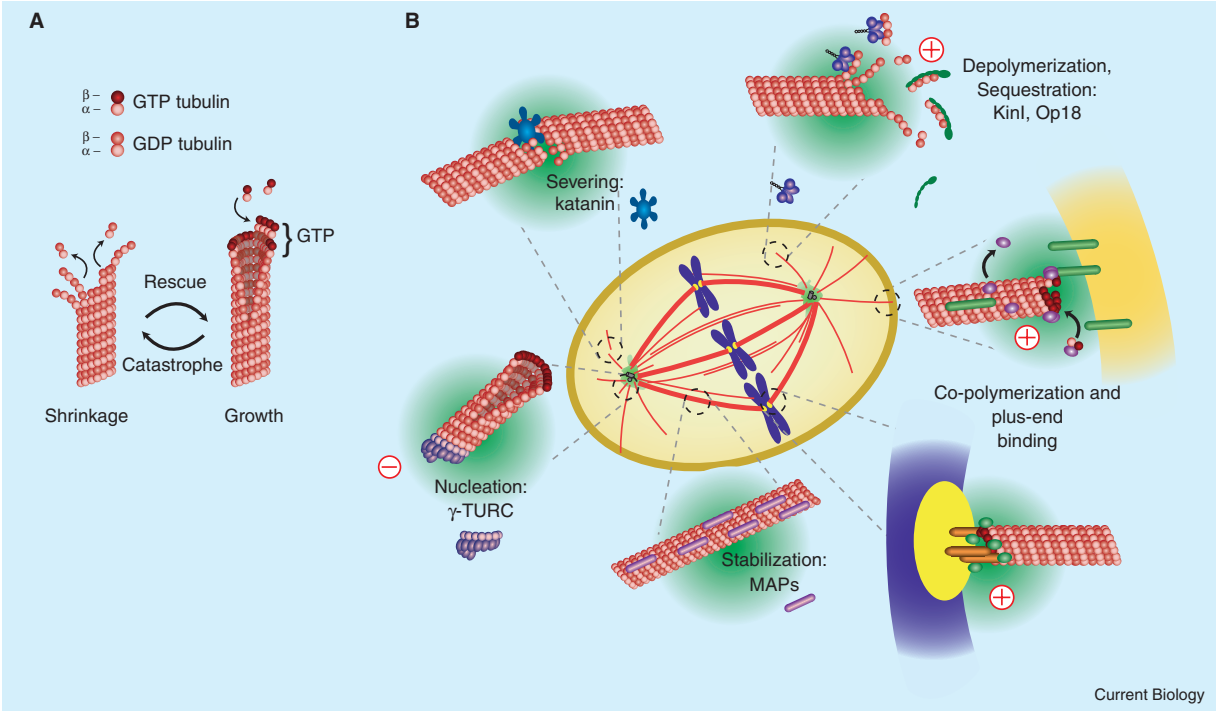

Figure 4. Regulation of microtubule dynamics.
(A) Microtubule dynamic instability is generated by GTP hydrolysis by tubulin subunits. (B) Various classes of cellular proteins mediating the nucleation (γ-tubulin ring complexes), stabilization (lattice-binding and end-binding MAPs), capture (end-binding MAPs and their partners), depolymerization (including kinI kinesins and Op18), and severing (katanin) of microtubules.

structure is stabilized by a 'cap' of GTP–tubulin subunits at the end that maintains association between neighboring protofilaments. When this cap is stochastically lost, the protofilaments peel outward and the microtubule rapidly depolymerizes.

Microtubules within cells grow more rapidly and undergo more catastrophes than microtubules polymerized from pure tubulin at the same concentration, suggesting that additional growth promoting and destabilizing factors are active *in vivo* (Figure 4B; reviewed in [33,34]). At the centrosome, microtubules are nucleated by an isoform of tubulin (γ-tubulin) in a large complex, the γ-tubulin Ring Complex (γ-TuRC), which is embedded in the pericentriolar material [35]. Classical stabilizing microtubule associated proteins (MAPs), like MAP2 or Tau, bind to the surface of the microtubule, bridging several tubulin subunits and possibly neutralizing the repulsive negative charge on the microtubule surface. Other MAPs, such as members of the highly conserved XMAP215/Stu2p/TOG family, appear to be enriched on spindle microtubules, but absent from astral microtubules. Microtubule end binding MAPs, such as CLIP-170 and EB1, may copolymerize with new tubulin subunits or selectively bind to a special conformation of the microtubule end; in addition, they might serve as attachments for growing or shortening microtubules to kinetochores or cellular membranes through interaction with proteins such as APC and CLASPs [36]. The microtubule destabilizing factor Katanin functions as a severing factor, generating new ends that lack a GTP cap [37], and may release microtubules from the centrosome. Depolymerizing kinesins of the KinI family, such as XKCM1 and MCAK, exist in several different pools in the cell, for instance at the kinetochores and spindle poles, where they bind to microtubule ends and distort the microtubule lattice such that protofilaments peel outward [38]. Op18/Stathmin has been proposed to sequester tubulin dimers and/or to promote GTP hydrolysis [39]. Thus, microtubule stabilizing and destabilizing factors function by a variety of mechanisms. Why are there so many modulating factors? In addition to global regulation, which establishes the mictrotubule dynamics that promote disassembly of the interphase array, there must be local regulation of microtubules to set up the spindle and generate the attachments that are necessary for chromosome movement and spindle positioning. The distinct localization of factors, and/or their local regulation, is key to this process.

Motor Proteins Are Essential for Spindle Organization

Superimposed on the global and local regulation of microtubules are the microtubule movements driven by motor proteins. These mechanochemical ATPases can move microtubules unidirectionally toward their plus- or minus-ends. The first motor proteins identified were the minus-end directed flagellar dynein and, twenty years later, the plus-end directed conventional kinesin. Over the next twenty years, this palette has expanded to include over a dozen classes of kinesins [40], many of which play roles in mitosis. Kinesin identification has been greatly accelerated by the complete genome sequences of a number of eukaryotes. Directed kinesin searches have been performed in multiple fungi [41],

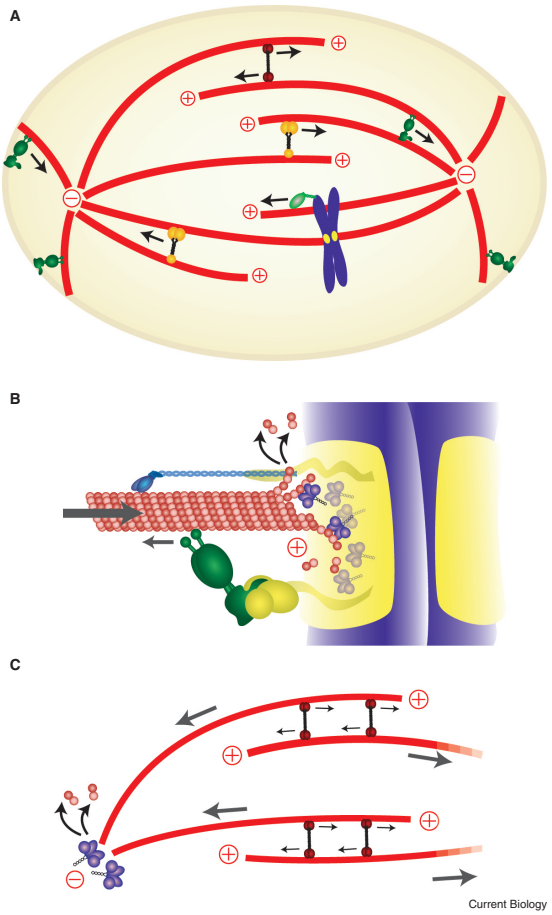

Figure 5. Diverse activities of motors in the spindle.
(A) Plus- (red) and minus-end (yellow) directed cross-linking motors that increase or decrease the overlap of antiparallel microtubules determine spindle pole separation. Cytoplasmic dynein (dark green) in the cortex can pull on astral microtubules or focus microtubule minus-ends into poles. Chromokinesins (light green) can mediate chromosome attachment and plus end-directed movement. (B) Coordinated kinesin motor and microtubule depolymerase activity at the kinetochore. The 'feeder' (dynein, green) helps deliver microtubules to the 'chipper' (KinI, purple) by moving microtubules with their plus ends leading. The plus-end directed motor CENP-E (light blue) is also shown. (C) Coordinated motor and microtubule depolymerase activity of kinesin at the spindle pole. Minus-end directed movement of microtubules driven by the flux motor 'feeder' (Eg5, red) is coordinated with minus end depolymerization by a pole-localized 'chipper' (KinI, purple).

Arabidopsis (61 kinesins; [42]), *C. elegans* (21 kinesins; [43]), *D. melanogaster* (25 kinesins; [44,45]), *Mus musculus* (45 kinesins) and *Homo sapiens* (44 kinesins; [46]). This allows for comprehensive screens for kinesins with mitotic roles by RNA interference (RNAi), which can be used to inhibit factors individually and in combination in model systems such as *Drosophila* and *C. elegans*, as well as in cultured vertebrate cells. For example, a directed RNAi-based screen in *Drosophila* yielded nine motors with mitotic functions [44]. While past models of mitotic functions have relied on hypothetical motors [47], we now have the complete toolbox of mitotic motors and the ability to inhibit them systematically.

Functional studies in several systems have shown a remarkable level of conservation among related motors (Figure 5A). The tetrameric BimC/Eg5-family of plus-end directed kinesins plays a fundamental role in spindle pole separation and spindle bipolarity [48–50]. This is thought to be due to cross-linking activity, which would bundle microtubules and push antiparallel microtubules apart [51,52]. Due to their opposite polarity, minus-end directed cross-linking motors appear to counteract the tetrameric kinesins, and function to focus microtubule minus-ends at the spindle poles [51,53–56]. Plus-end directed kinesins localized to chromosome arms contribute to chromosome attachment and movement toward the metaphase plate, while cytoplasmic dynein in the cortex can function to orient astral microtubules [57].

Importantly, a change in the balance of forces of spindle motors could contribute to spindle pole separation in anaphase B, although the molecular mechanisms behind this event are not understood. Several plus-end directed motors are capable of cross linking and generating antiparallel microtubule movements that could drive spindle pole separation, including Eg5, Mklp1/CHO1 and chromokinesin/KIF4 [52,58,59]. Though important for maintaining spindle bipolarity, Eg5 activity appears to be dispensable for anaphase pole extension in *Xenopus* egg extracts (Gadde and Heald, unpublished). Mklp1/CHO1 has a well-studied role in cytokinesis, but likewise is not required for anaphase B [60–62]. In line with its roles in prophase centrosome separation and bipolarity [55,63,64], the chromokinesin/KIF4 may be responsible for the antiparallel microtubule sliding of anaphase B, or another, uncharacterized motor may be required.

Feeders and Chippers

While a balance of opposing motor activities can drive spindle morphogenesis, it has been a long-standing question how motors contribute to the special dynamic properties of the spindle. For example, what are the molecules mediating poleward microtubule flux, and the 'pacman' behavior of the kinetochore, both of which contribute to chromosome movements? Based on recent discoveries in *Drosophila* and *Xenopus*, we propose a 'feeder and chipper' model (Figure 5B). Two newly examined *Drosophila* motors of the KinI family of kinesins are localized at the kinetochore and spindle poles, where they appear to promote depolymerization at the plus- and minus-ends, respectively [65]. Given that multiple KinI family members also exist in other systems, it is likely that these functions are conserved. Full-speed poleward movement of *Drosophila* chromatids requires, in addition to KinIs, kinetochore-localized dynein in its massive complex with dynactin, ZW10 and Rod [66,67]. Thus, at the *Drosophila* kinetochore, cytoplasmic dynein (a 'feeder') may drive the kinetochore microtubules in a plus-end direction toward the KinI MCAK (DmKLP59C, a 'chipper'). An analogous process could occur at the spindle poles, where microtubules are depolymerizing, as Eg5 (DmKLP61F) associated with the spindle could feed microtubules in a minus-end direction toward the KinI microtubule

chipper KIF2 (DmKLP10A). In support of this model, the plus-end directed kinesin Eg5 is required for microtubule flux in *Xenopus* egg extracts (D.T. Miyamoto, Z.E. Perlman, K.S. Burbank, A.C. Groen, T.J. Mitchison, unpublished data). Thus, we postulate that depolymerization of kinetochore microtubules requires a motor that continuously feeds them to an immobilized KinI depolymerase that 'chips away' at the microtubule ends. The combination of plus- and minus-end depolymerization of the kinetochore microtubules allows for robust movement of the chromatids toward the pole during anaphase A; this mechanism could also contribute to kinetochore movements during chromosome alignment.

Regulating the Regulators

While the molecular toolbox for spindle assembly continues to expand, our understanding of its regulation remains rudimentary. This is complicated by the fact that, remarkably, even a single factor may have multiple activities that seem contradictory. For example, XMAP215/dis1/TOG is a highly conserved MAP, originally purified due to its microtubule stabilizing activity [68]; however, recently it has been purified again in a truncated form as a microtubule destabilizing activity [69]. TPX2 is another vertebrate MAP implicated in microtubule nucleation and stabilization, which was originally identified due to its ability to target a kinesin to microtubules [70], and is now known to also regulate the mitotic kinase Aurora A [71]. Thus, there is a rich variety of activities, and we are still cataloging and characterizing them. Many factors are likely to be regulated temporally and spatially by associated proteins, such as kinases and phosphatases. An interesting example is that phosphorylation is reported to inactivate microtubule stabilizing MAPs [72], as well as the microtubule destabilizing protein Op18 [39], illustrating the complex choreography of regulation that takes place. The Aurora, Polo and NIMA families of kinases have emerged as important regulators of many mitotic events [73], and are currently being very actively studied. Perhaps the most complex site of regulation is at the kinetochore, which in addition to mediating chromosome movements, also serves to detect microtubule attachment and tension and transduces a checkpoint signal to ensure that all chromosomes are properly oriented before anaphase sister segregation. Recent studies show that Aurora B kinase controls the centromeric localization and catalytic activity of the microtubule depolymerase MCAK [74–76]. Physical tension due to microtubule attachment may influence the access of MCAK to Aurora B kinase and its, yet unidentified, opposing phosphatases. These findings emphasize that not only do a complex set of microtubule-modulating factors contribute to spindle dynamics, but their regulation is also precisely integrated with cell cycle progression.

In the last several years, another mode of regulation has been characterized in *Xenopus* egg extracts that involves the small GTPase Ran. During interphase, Ran is known to direct nucleocytoplasmic transport, as RanGTP is found exclusively in the nucleus, and is hydrolyzed to RanGDP in the cytoplasm [77]. Proteins containing a canonical nuclear localization sequence bind to import receptors in the cytoplasm and are transported through the nuclear pore. Upon encountering RanGTP, the cargo is released [78]. Interestingly, Ran also functions in a similar fashion during mitosis, at least in *Xenopus* egg extracts [79]. Despite nuclear envelope breakdown, a gradient of RanGTP and released cargoes persists around chromosomes. This is due to the chromatin association of the Ran guanine exchange factor RCC1 [80]. The RanGTP gradient releases cargoes that function in spindle assembly, including factors such as MAPs and motors [81–84]. While a mitotic role for Ran is emerging in a variety of systems, the generality of the gradient/cargo release mechanism is still under investigation. This pathway could be more important in large cells, such as eggs and early embryos, where a spatial cue may be more critical to demarcate the site of spindle assembly.

Are All the Elements and Principles Identified?

Experiments over the last several years indicate that, while we have identified many factors important for mitotic spindle assembly and function, we are still uncovering some basic principles. A current controversy in the field is whether another structural element exists, the so-called 'spindle matrix', which would constitute a scaffold distinct from microtubules [85]. In *Drosophila*, a protein named Skeletor has been identified that forms a spindle-shaped structure prior to the assembly of the microtubule-based spindle [86]. Fluorescent imaging of spindles formed in *Xenopus* extract revealed that the Eg5 motor is static relative to microtubules undergoing poleward flux, suggesting that Eg5 could constitute or be associated with a structural element distinct from the microtubules. [87]. These observations suggest that other structural components are present, but definitive proof has not yet emerged.

A prominent feature of spindle assembly and function is redundancy. Multiple mechanisms promote spindle morphogenesis, and different model systems emphasize different mechanisms. Reconstitution experiments represent a valuable approach, both *in vitro*, and by computer modeling of microtubule and spindle dynamics. These experiments can reveal the minimum activities required for behaviors observed, and the precise actions of specific protein ensembles. For example, experiments with pure tubulin have shown that the global parameters of microtubule growth rate and catastrophe frequency during mitosis can be largely mimicked by the addition of only two factors, XMAP215/Stu2p/TOG and the catastrophe inducing kinesin XKCM1/MCAK [88]. With respect to motor function, computer simulations have shown that complexes of two motors with opposite directionality leads to stable antiparallel interactions analogous to those in the spindle [89].

The wealth of approaches and systems makes spindle research an exciting area. Complete genome sequences, RNA interference techniques, and the development of small molecule inhibitors of specific factors have taken us to a higher level of investigation. In addition to molecular methods, high-resolution

imaging and micromanipulation techniques continue to advance our understanding, altogether making mitosis one of the most fascinating fields of cell biology.

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