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Self-Organization and Forces in the Mitotic Spindle

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Abstract

At the onset of division, the cell forms a spindle, a precise self-constructed micromachine composed of microtubules and the associated proteins, which divides the chromosomes between the two nascent daughter cells. The spindle arises from self-organization of microtubules and chromosomes, whose different types of motion help them explore the space and eventually approach and interact with each other. Once the interactions between the chromosomes and the microtubules have been established, the chromosomes are moved to the equatorial plane of the spindle and ultimately toward the opposite spindle poles. These transport processes rely on directed forces that are precisely regulated in space and time. In this review, we discuss how microtubule dynamics and their rotational movement drive spindle self-organization, as well as how the forces acting in the spindle are generated, balanced, and regulated.

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INTRODUCTION

During cell division, the mitotic spindle divides the genetic material into two equal parts. This complex dynamic micromachine is composed of microtubules, chromosomes, and a variety of accessory proteins. The mitotic spindle has a well-defined shape, as reflected by its name, and a characteristic arrangement of microtubules, which extend from the opposite spindle poles and interact with each other. These interactions give rise to antiparallel bundles of microtubules known as interpolar, or overlap, microtubules (29, 71) (**Figure 1*a*,*b*,*c***). Microtubules that extend from the same pole can also interact with each other, forming parallel bundles (29, 74) (**Figure 1*a*,*b*,*c***). The most prominent parallel bundles are k-fibers, which are attached to chromosomes via kinetochores, protein complexes on the chromosome (19). The forces exerted by k-fibers on the chromosomes drive their segregation toward the opposite spindle poles.

To understand how the mitotic spindle functions, it is necessary to discuss two concepts: self-organization of the spindle and the forces acting in the spindle. The spindle arises from self-organization of microtubules and chromosomes, where the dynamics of the components help them explore the space and eventually get near each other so that they may interact. In the first part of this review, we discuss how microtubule dynamics and their rotational movement, as well as the diffusion of chromosomes, drive spindle self-organization.

Once the interactions between the chromosomes and the microtubules have been established, the chromosomes need to be transported to the equatorial plane of the spindle, and after the sister chromatids separate, they need to be moved toward the opposite spindle poles. While the cell progresses through mitosis, the spindle changes its shape and size. Chromosome transport and changes in the spindle rely on directed forces that are precisely regulated in space and time. These forces are the focus of the second part of the review. Although the self-organization and force-driven movements in the spindle act together and to a large extent simultaneously, we discuss them separately to dissect the mechanisms underlying each of the two concepts.

SELF-ORGANIZATION OF THE MITOTIC SPINDLE

Self-organization of the spindle occurs through two main processes: formation of microtubule bundles and kinetochore capture by microtubules. During microtubule bundle formation, two microtubules that are initially far apart ultimately approach each other and interact via

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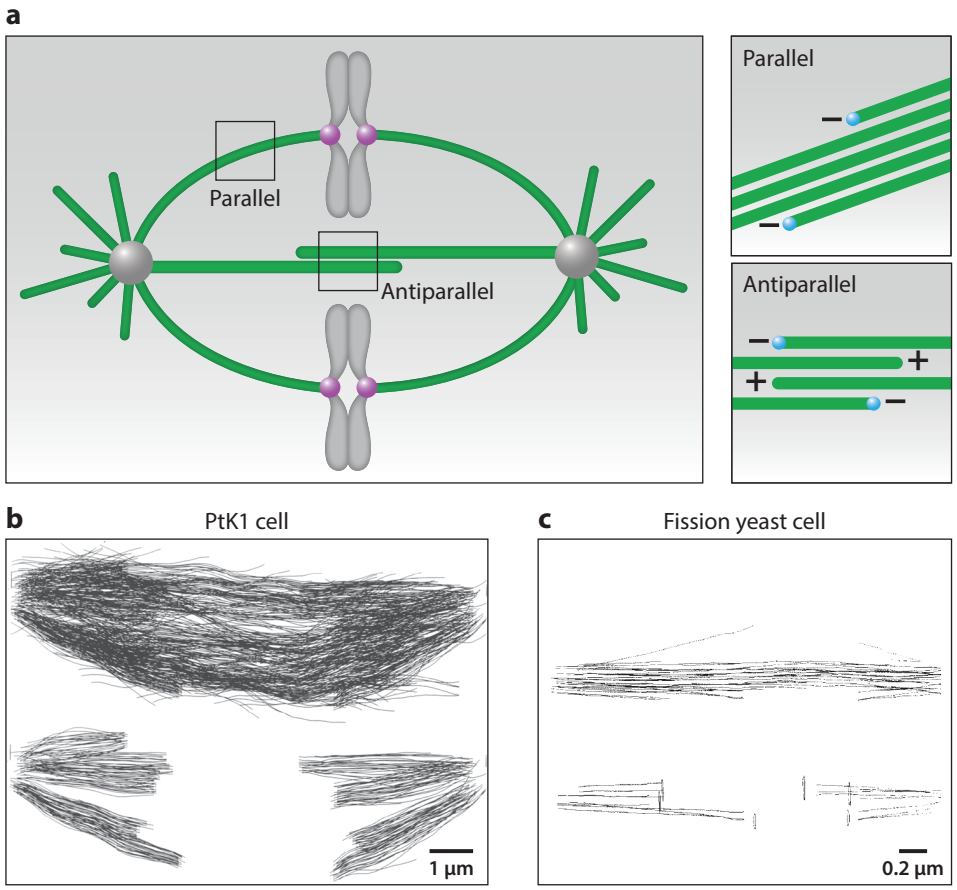

Figure 1

The mitotic spindle is composed of microtubule bundles. (*a*) Schematic representation of the spindle showing microtubule bundles (*green*) extending from the spindle poles (*gray spheres*). K-fibers are bundles of parallel microtubules attached to the kinetochore (*magenta*), a protein complex on the chromosome (*elongated gray shapes*). Interpolar, or overlap, microtubules form antiparallel bundles. Insets: Enlarged segment of a parallel bundle and an antiparallel bundle, with plus (+) and minus (−) ends of the microtubules marked accordingly. Blue spheres denote microtubule nucleation sites. (*b*) Reconstruction of spindle microtubules from electron micrographs of mammalian PtK1 cells in early anaphase. (*Top*) Spindle microtubules; (*bottom*) microtubules associated with nine kinetochores. Images reproduced with permission from Reference 71. (*c*) Reconstruction of spindle microtubules from electron micrographs of fission yeast cells. (*Top*) Spindle microtubules; (*bottom*) kinetochore microtubules and kinetochores. Images reproduced with permission from Reference 29.

cross-linking proteins. Similarly, to integrate a chromosome into the spindle, the kinetochore on the chromosome and a microtubule must get near each other for kinetochore capture to occur.

Formation of Microtubule Bundles

The pioneering idea that explains how microtubules from one centrosome find those from the other centrosome to form bundles is based on microtubule dynamics and isotropic microtubule

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nucleation at the centrosome (61). In this scenario, microtubules grow in random directions and shrink back to the centrosomes. Owing to this dynamic behavior, microtubules occasionally get close to those extending from the other centrosome. In a region where such microtubules overlap, they become cross-linked by specific proteins (**Figure 2*a***, left pathway).

The cross-linking proteins are divided into three classes. In the first class, motor proteins, such as kinesin-5 motors Cut7/Eg5/KIF11 (47, 58, 64, 101), slide the microtubules and thus the centrosomes apart by walking along the microtubules away from the centrosome, i.e., toward the plus end of the microtubule. The second class consists of motor proteins, such as kinesin-14 motors Ncd/HSET/KifC1 (13, 33, 73), that pull the centrosomes toward each other by walking along the microtubules toward the centrosome, i.e., toward the minus end of the microtubule. The third class of proteins, such as PRC1/Ase1 (5, 52, 81, 93), cross-links microtubules without walking along them in a directed manner.

Microtubule bundle formation in the context of spindle self-organization was explored in several theoretical studies relevant to different biological systems. Nédélec (85) investigated formation of antiparallel bundles of microtubules in somatic cells of higher eukaryotes using computer simulations, which include microtubules that grow in random directions from two spindle poles and motor proteins that link them. The author found that complexes made of two motors of opposite directionalities can lead to the formation of spindle-like structures with microtubules arranged in antiparallel bundles. Motors of opposite directionalities were also included in models for spindle assembly in a *Drosophila* embryo, in which the outward forces driving the separation of the spindle poles are exerted by cortical dynein and sliding of antiparallel microtubules by kinesin-5 motors, and the inward force on the poles is generated by sliding of antiparallel microtubules by kinesin-14 motors (24, 25, 119).

Recent work on budding yeast showed that cells lacking kinesin-14 motors have fewer antiparallel bundles in the spindle than wild-type cells do (48). This finding was explained by a model in which microtubules grow in arbitrary directions from the opposite spindle poles and become connected by kinesin-14 motors. These motors walk along one microtubule toward its minus end while bound to other microtubule, thereby aligning the two microtubules to form an antiparallel bundle (**Figure 2*a***, right pathway). Thus, in this model microtubules pivot, i.e., change their angle, to form antiparallel bundles. Indeed, experiments in fission yeast cells have shown that microtubules pivot around the spindle pole during mitosis (57).

Figure 2

Formation of microtubule bundles. (*a*) Two models for the formation of antiparallel bundles. In both models, the starting picture includes microtubules (*green*) extending in random directions from two separated spindle poles (*gray spheres*). The end result is an antiparallel bundle formed by microtubules extending from the opposite poles, cross-linked by motor proteins such as kinesin-5 (*red*) and kinesin-14 (*blue*), as well as by passive cross-linkers such as Ase1/PRC1 (*yellow*). In the first model (*left pathway*), microtubules extending from opposite spindle poles grow (*arrows*) and shrink and encounter each other by chance. In a region where such microtubules overlap, they become cross-linked by specific proteins. In the second model (*right pathway*), microtubules growing from opposite spindle poles are connected by kinesin-14 motors, which walk along one microtubule toward its minus end (*straight arrow*) while bound to other microtubule, thereby aligning the two microtubules into an antiparallel bundle (*curved arrow*). (*b*) Two models for the formation of parallel bundles. In the first model (*left pathway*), microtubules rotate (*arrows*) around the spindle pole. This motion helps the microtubules explore the space and approach one another, which in turn allows the cross-linking proteins (*yellow*) to connect the microtubules to form a stable bundle. In the second model (*right pathway*), a bundle is formed by the nucleation (*blue*) of a new microtubule and its growth (*arrow and dashed outline*) along the existing microtubule.

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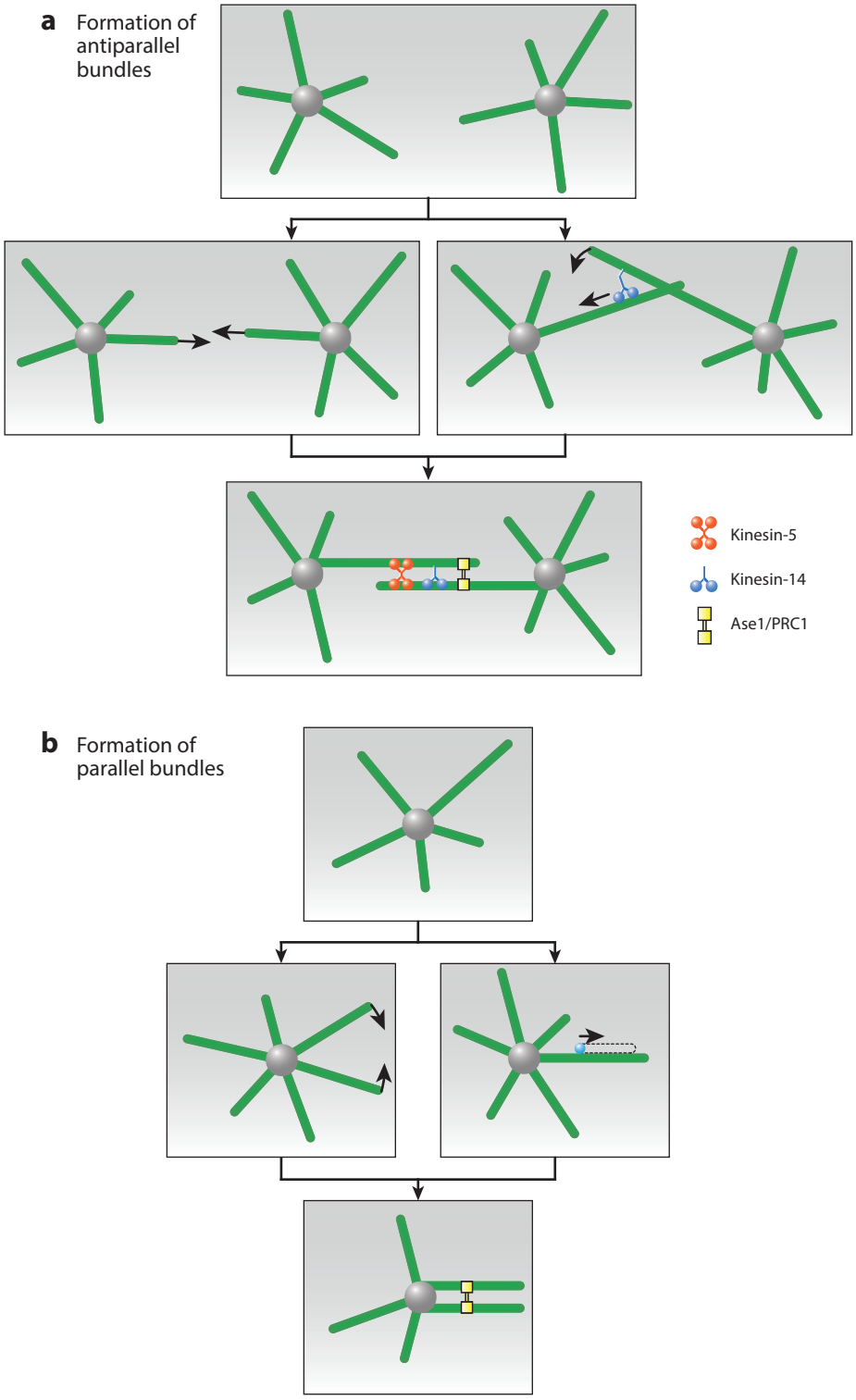

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We have recently studied the formation of parallel microtubules bundles. We introduce the pivot-and-bond model, which includes random angular motion of microtubules around the spindle pole (57) along with the attractive forces exerted by cross-linking proteins (**Figure 2*b***, left pathway) (M. Prelogović, L. Winters, A. Milas, I.M. Tolić, N. Pavin, unpublished manuscript). Our experimental results show that microtubules pivot around the spindle pole before aligning and forming a bundle. Bundle formation relies mostly on the cross-linking protein Ase1. Thus, in the pivot-and-bond model, microtubules explore the space by angular diffusion and eventually approach one another, which in turn allows the cross-linking proteins to connect the microtubules to form a stable bundle.

However, there are other ways to form parallel microtubule bundles. For example, some microtubules are nucleated at sites positioned along the preexisting mother microtubule (**Figure 2*b***, right pathway) (43, 67, 82). In meiotic *Xenopus* egg extracts, these new microtubules grow at small angles and with the same polarity as the mother microtubule, which makes them suitable to generate parallel microtubule bundles such as k-fibers (94).

How Microtubules Capture Kinetochores

At the onset of mitosis, the two centrosomes start to nucleate microtubules. At the same time, kinetochores are located away from the centrosomes. To get near the kinetochores, microtubules must explore the space. Several models have been proposed to explain how microtubules search for kinetochores (92).

The founding idea, termed search-and-capture, relies on microtubule dynamics and their nucleation at centrosomes (61). As a microtubule grows from the centrosome in a random direction, it probes the space while it searches for kinetochores. Even though a single microtubule probes only one direction, numerous directions are ultimately explored because a large number of microtubules grow from the centrosome (**Figure 3*a***). The first theoretical studies that investigated the search-and-capture model calculated the average capture time for microtubules searching for a single target at a certain distance (49, 50). When multiple kinetochores are included in the model, the time needed to capture all kinetochores grows logarithmically with the number of kinetochores (41, 120). Thus, the search-and-capture model predicts that in a human cell, which has 46 chromosomes, it would take roughly 100 min to capture all kinetochores. However, human cells capture their kinetochores within 20 min (120), which is five times faster than the theoretical prediction.

Several mechanisms have been proposed to accelerate the search for kinetochores (32, 80, 88). One reason why the kinetics of capture in the search-and-capture model is slow is because most microtubules grow toward regions of the cell devoid of chromosomes. To signal their location in the cell, chromosomes create a spatial gradient based on the GTPase Ran (21). In *Xenopus* egg extracts, GTP-bound Ran stabilizes microtubules and promotes spindle assembly (17, 55, 89, 116, 125). Because the factor that promotes exchange of GDP for GTP on Ran is bound to the chromosomes, a high local concentration of Ran-GTP is generated at the chromosomes (6, 17, 56). Ran-GTP provides a spatial signal that influences microtubule dynamics, by increasing microtubule rescue frequency and decreasing catastrophe frequency close to the chromosomes (16, 115). Thus, Ran-GTP provides microtubules that extend toward the kinetochore with an environment in which they can grow long enough to reach the kinetochore (**Figure 3*b***) (18). Consistent with this scenario, a theoretical study has shown that a bias in microtubule dynamics accelerates the capture of kinetochores (120).

Kinetochore capture can be accelerated also by increasing the number of microtubules that search for kinetochores. In addition to the microtubules nucleated at the centrosome, new

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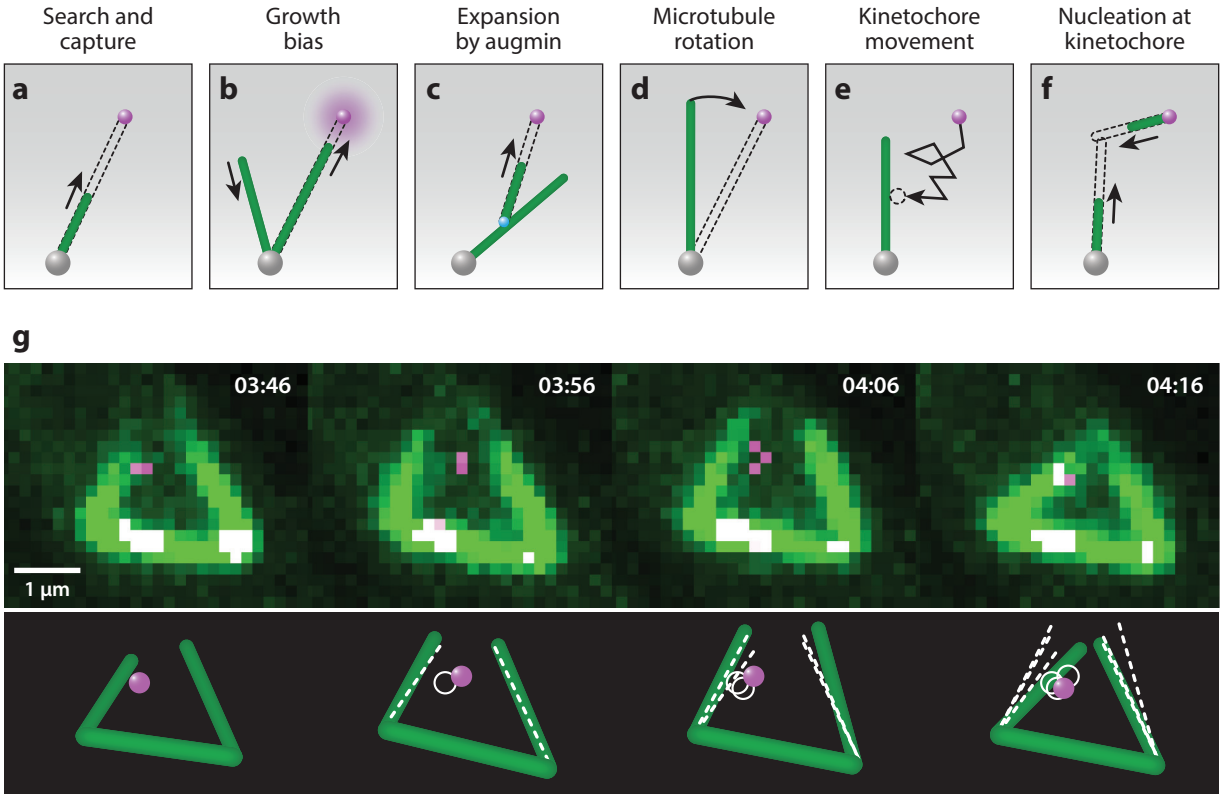

Figure 3

Models of kinetochore capture. (*a*) Search-and-capture. Microtubules (*green*) grow (*arrow*) in random directions from the spindle pole (*gray sphere*). The kinetochore (*magenta sphere*) is captured by the tip of a microtubule that by chance grows toward it (*dashed outline*). (*b*) Microtubules have growth bias toward the kinetochore, which is generated by the Ran-GTP gradient (*magenta-shaded area*). (*c*) The number of microtubules expands via nucleation along spindle microtubules by the augmin complexes (*blue sphere*). (*d*) Microtubules pivot (*arrow*) around the spindle pole, which allows them to explore the space more efficiently and approach the kinetochore. (*e*) Kinetochores move (*black path*), which helps them encounter microtubules. (*f*) Microtubules are nucleated at the kinetochores and interact with those extending from the spindle pole. (*g*) Kinetochore capture event in a fission yeast cell. Note that microtubules (*green*, tubulin-GFP) rotate, which corresponds to the model in panel *d*. At the same time, the kinetochore (*magenta*, Ndc80-tdTomato) moves, which corresponds to the model in panel *e*. Microtubule orientations and kinetochore positions are indicated by white dashed lines and white circles in the schemes, respectively. Time is given in minutes:seconds. Panel *g* is reproduced with permission from Reference 57.

microtubules can be nucleated along the preexisting microtubules in the spindle (**Figure 3*c***). Microtubule nucleation throughout the spindle has been observed in cells with and without centrosomes, and studies have shown that it depends on the augmin complex (10, 43, 67). In meiotic *Xenopus* egg extracts, new microtubules grow at small angles and with the same polarity as the mother microtubules (94). These daughter microtubules may assist kinetochore capture by interacting with a kinetochore, thereby connecting it to the mother microtubule.

All the mechanisms described above assume that as microtubules grow, they probe the space with their tips. On the contrary, if a microtubule moves laterally, it probes the space with its entire length (**Figure 3*d*,*g***). Our experiments in fission yeast cells have shown that microtubules pivot around the spindle pole, swiping through the cell and exploring the space laterally as they search for kinetochores located far away from the spindle pole (57). Microtubules pivot at random

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and are driven most likely by thermal forces because pivoting does not require ATP. A simple theoretical model based on random movement of microtubules and kinetochores reproduces the process of kinetochore capture. The model predicts that the speed of capture depends mainly on how fast microtubules pivot, which we confirmed experimentally by speeding up and slowing down microtubule pivoting (57).

Similarly, in *Drosophila* S2 cells, microtubules extending from the kinetochore have been observed to pivot (69). Kinetochore-bound microtubules are initially not oriented toward a spindle pole, but they change their orientation while growing and eventually become captured by the microtubules growing from the centrosome and integrated into the spindle. Finally, astral microtubules have been observed to pivot in budding yeast cells. This motion accelerates microtubule search for cortical anchor sites, thereby promoting the translocation of the spindle from the mother cell into the bud (3).

Microtubules may not be the only players probing the space. Namely, by moving around, the kinetochore may probe the space in search of microtubules (**Figure 3*e***). A theory based on the diffusion of kinetochores measured in human cancer cells and the known properties of microtubules shows that kinetochore diffusion and rotation accelerate spindle assembly, including capture of kinetochores (91).

Finally, microtubule assembly on the chromosome or at the kinetochore increases the efficiency at which chromosomes are incorporated into the spindle (**Figure 3*f***) (60). In the slide-and-cluster model, which is relevant for spindle formation in *Xenopus* egg extracts, microtubules are nucleated near chromosomes in a Ran-GTP-dependent manner, transported away from the center, and clustered together (11, 66). Similarly, nucleation of microtubules at kinetochores (26, 105, 117) may speed up kinetochore capture and spindle assembly (69, 110). Like centrosomes, mammalian and yeast kinetochores nucleate microtubules with distal plus ends (4, 62). This orientation must change because in a metaphase spindle kinetochores are attached to the plus ends of microtubules (35). The change occurs in such a manner that the kinetochore-nucleated microtubules disassemble after establishing contact with the centrosomal microtubules, leaving the kinetochore attached to the latter microtubules, whose plus ends are oriented toward the spindle midzone, allowing the kinetochore to reach the plus end (62). Computer simulations have shown that kinetochore-nucleated microtubules extend the target area that the dynamically unstable centrosomal microtubules must hit, thereby accelerating kinetochore capture (91).

In summary, kinetochores can be captured by numerous mechanisms, and one mechanism does not exclude contributions of others. For example, a single mechanism may explain the kinetics of kinetochore capture in fission yeast cells (57). In another example, typical capture times in human cells may be reproduced only by including several mechanisms (91).

FORCES IN THE MITOTIC SPINDLE

Once microtubule bundles have been formed between the two centrosomes and chromosomes have started to interact with microtubules, many tasks remain to be completed. Chromosomes must be moved to the equatorial plane of the spindle in metaphase and subsequently separated and moved to the opposite spindle poles in anaphase. Because chromosomes are large objects that need to be transported over large distances, their transport relies on directed and highly regulated forces generated by microtubules and motor proteins (109).

Oscillations of Mono-Oriented Chromosomes

During prometaphase, after one of the sister kinetochores has been captured by microtubules, the next step is to capture the other sister by the microtubules extending from the opposite spindle

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pole. This process is known as the transition from mono-orientation to bi-orientation. To describe this transition, we first discuss the forces that act on a mono-oriented chromosome.

Experiments in which a mono-oriented chromosome created by laser ablation moved poleward have shown that microtubules pull the kinetochore toward the spindle pole (97). However, mono-oriented chromosomes do not end up at the spindle pole; they oscillate in its vicinity. Further experiments from the same study, in which chromatid arms of a mono-oriented chromosome were cut free of the kinetochore, showed that the cut chromosome fragments moved away from the spindle pole at a constant velocity of roughly 2 μm/min, which is similar to the velocity of the outward movement of a mono-oriented chromosome during its oscillations (97). Thus, the authors (97) concluded that the positioning of a mono-oriented chromosome results from a tug-of-war between poleward-pulling forces acting at the proximal kinetochore and the opposing polar ejection forces acting on the chromosome arms.

The poleward-pulling forces at the kinetochore are generated by motor proteins, including cytoplasmic dynein (102), and by microtubule depolymerization (44, 63), which is promoted by kinesin-13 family motors (122). The polar ejection forces are generated by chromokinesins (65), which are associated with the chromosome arms (107, 112) and move toward the plus end of the microtubules (123). In addition, force driving the chromosome away from the pole can be generated at the kinetochore by kinesin-7 family (CENP-E) motors, which also move toward the plus end of the microtubules (121).

A theoretical study (15) has shown that the oscillatory motion of mono-oriented chromosomes results from the tug-of-war between the poleward-pulling force at the kinetochore and the polar ejection force exerted by chromokinesins that push the chromosome arms away from the pole (**Figure 4*a***). Oscillations arise as a consequence of two properties of the system. First, the polar ejection force is larger when the chromosome is moving away than toward the pole, owing to the slower detachment and thus a larger number of attached chromokinesins in the former case. Second, the polar ejection force is larger close to the pole than it is far away from it, because microtubule density is higher near the pole. Even though the mechanism of the oscillations has been explored, their function is still unclear.

Establishment of Bi-Orientation and Congression to the Metaphase Plate

The transition from mono-orientation to bi-orientation is accompanied by chromosome congression to the metaphase plate. Congression is evolutionarily conserved and important for the correct segregation of chromosomes (87). Two scenarios propose how congression is related to bi-orientation. In the first scenario, a mono-oriented chromosome oscillates near the spindle pole, where the probability that it will be captured by the microtubules growing from the opposite spindle pole is rather low. To get closer to the opposite pole, such chromosomes are transported along spindle microtubules toward the spindle equator by kinesin-7 family (CENP-E) motor proteins (**Figure 4*b***, left pathway) (59). These motors recognize the microtubules that point toward the equator by their posttranslational detyrosination (2). In this scenario, congression is not a consequence of bi-orientation but rather a process that helps bi-orientation.

However, it is possible that bi-orientation occurs before congression. In the second scenario, microtubules capture first one and then the other sister kinetochore, resulting in bi-orientation. Subsequently, forces exerted by microtubules lead to congression of already bi-oriented kinetochores (**Figure 4*b***, right pathway) (98). A theoretical model has shown that bi-orientation can be established without congression in a biologically relevant time interval (91). This model relies on chromosome movement and rotation, on microtubules extending from the kinetochore, and on the dynamics of microtubules nucleated at the centrosome.

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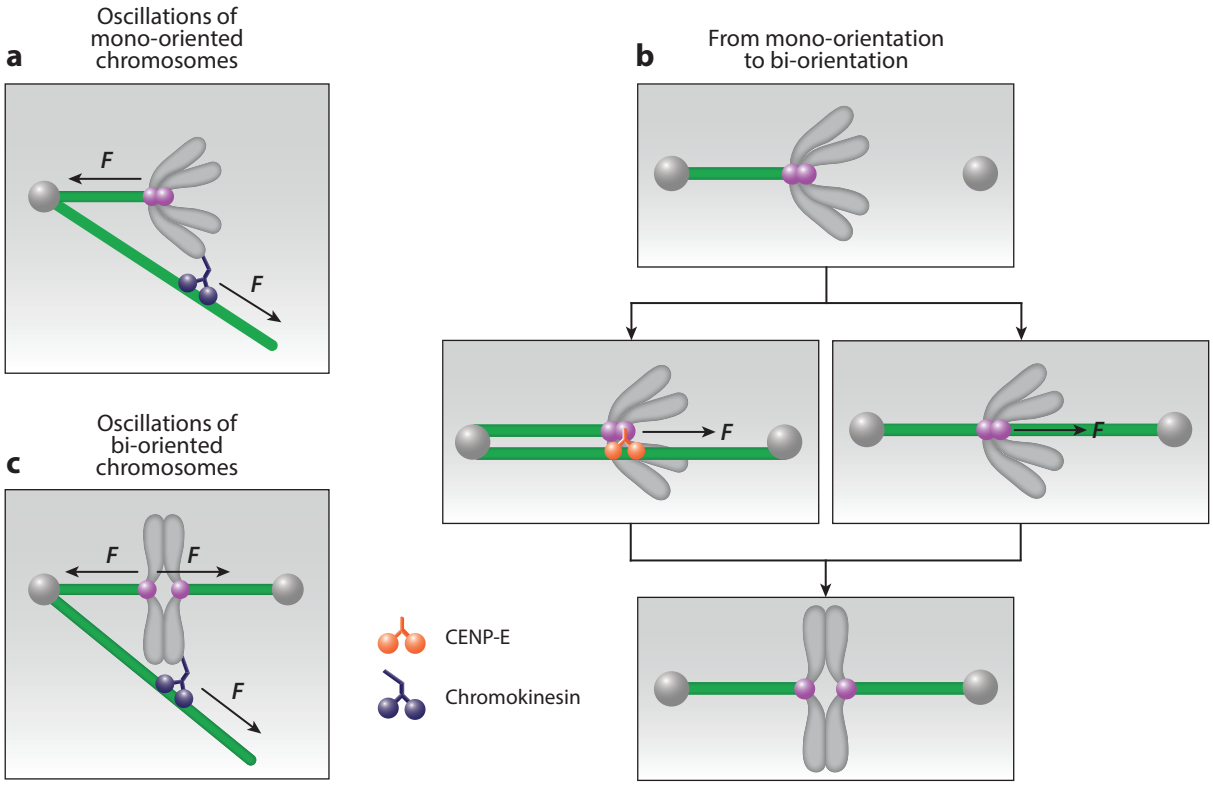

Figure 4

Movements of mono-oriented and bi-oriented chromosomes. (*a*) Oscillations of mono-oriented chromosomes result from a tug-of-war between the poleward-pulling forces at the kinetochore (*magenta*) and the forces exerted by chromokinesins (*dark blue*) that push the chromosome arms (*gray elongated shapes*) along the microtubules (*green*) away from the spindle pole (*gray sphere*). (*b*) Two models for the transition from mono-orientation to bi-orientation and congression to the spindle center. In the first model (*left pathway*), a mono-oriented chromosome is brought to the spindle center by the kinetochore motor CENP-E/kinesin-7 (*orange*). Subsequently, bi-orientation is achieved when a microtubule extending from the opposite spindle pole interacts with the nonattached kinetochore. In the second model (*right pathway*), a mono-oriented chromosome first becomes bi-oriented by the interaction of a microtubule extending from the opposite spindle pole with the nonattached kinetochore. Subsequently, this microtubule generates a pulling force that brings the chromosome to the spindle center. (*c*) A model for the oscillations of bi-oriented chromosomes includes, in addition to the forces described in panel *a*, the forces exerted on the kinetochore by the viscoelastic cohesin connections between sister chromatids. Arrows marked with *F* denote the forces.

The two scenarios describing the relationship between congression and bi-orientation do not exclude each other, and not all kinetochores must follow the same scenario. For example, the kinetochores located away from the central spindle as the spindle assembles are transported by CENP-E, and those found between the centrosomes may be bi-oriented without motor-driven transport (1).

Oscillations of Bi-Oriented Chromosomes

When a pair of sister kinetochores establishes bi-orientation, they oscillate around the equatorial plane of the spindle (**Figure 4*c***). Kinetochores are attached to the plus ends of k-fiber microtubules; thus, their motion depends on the forces exerted by microtubules. Depolymerizing microtubules

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can generate pulling forces on kinetochores (44), whereas polymerizing microtubules can generate pushing forces (30). Thus, these forces can be controlled by regulating the switch from growth to shrinkage, termed catastrophe, and the opposite switch, termed rescue. In addition, motors that walk along the microtubule while attached to the kinetochore, such as dynein, which walks toward the minus end (102), and CENP-E/kinesin-7, which walks toward the plus end (121), may generate forces on kinetochores.

Kinetochore oscillations depend on kinesin-8. In mammalian cells, depletion of kinesin-8 results in oscillations with larger amplitude, whereas overexpression leads to a lack of oscillations (104). Similarly, fission yeast cells lacking kinesin-8 motors show more extensive movements of kinetochores on the mitotic spindle compared with wild-type cells (37, 114). Kinesin-8 motors increase the interkinetochore distance, suggesting that they regulate the tension between sister kinetochores (37, 72, 104, 114). The molecular mechanism underlying the effect of kinesin-8 on oscillations is attributed to the fact that kinesin-8 facilitates microtubule catastrophe and shrinkage, based on experiments in vitro (34, 45, 72, 111) and in vivo (46, 72, 106, 114). Kinesin-8 accumulates at the plus end of microtubules in a microtubule-length-dependent manner (111), resulting in more frequent catastrophes of longer microtubules (106).

An early model of oscillations in higher eukaryotic cells is based on force-dependent kinetics of microtubule attachment and detachment to and from the kinetochore (53). Studies of oscillations in budding yeast cells, where kinetochores interact with single microtubules, introduced force-dependent microtubule dynamics as a key microtubule property needed to generate oscillations (38, 103). Such force-dependent microtubule dynamics is incorporated in another model for higher eukaryotic cells, but this model also includes collective motor forces (20).

Contrary to the model in which tension at the microtubule–kinetochore interface promotes detachment of the microtubule (53), a recent study assumed that the absence of tension stimulates the microtubule to detach from the kinetochore, describing the activity of Aurora B kinase in fission yeast cells (39). This model was extended by including microtubule-length-dependent pulling forces acting on the kinetochore (70). This assumption is based on the observation that kinesin-8 accumulates at the plus end of the microtubule in a length-dependent manner during mitosis (70). However, the length-dependent effect of kinesin-8 on catastrophe may also be important for kinetochore oscillations and congression to the equatorial plane of the spindle, in a manner similar to how this effect helps the cell to find its own center (40).

In addition to regulating oscillations, kinesin-8 shows genetic interactions with proteins of the spindle checkpoint, a sensing mechanism that ensures proper attachment of all kinetochores before their separation in anaphase (83). Loss of kinesin-8 combined with a loss of the checkpoint protein Bub1 (114) or Bub3 (75) leads to cell death, whereas loss of only one of these proteins does not. The role of kinetochore oscillations in the spindle checkpoint is worthy of further exploration.

Forces that Shape the Spindle

Spindles vary in size, but their shape is remarkably similar among different species. Spindle shape reflects the spatial distribution of the microtubules. The outermost microtubules of the spindle are curved, giving, for example, HeLa cell spindles the shape of an orange. In general, microtubules are straight filaments that can curve when forces act on them. Thus, it is important to understand how forces shape the spindle.

To theoretically reproduce the spindle shape, Rubinstein et al. (100) introduced a model that takes into account the elastic properties of microtubule bundles. This model includes forces acting at the spindle poles, sliding forces acting in the overlap region of interpolar microtubules, and forces acting at the plus ends of microtubules interacting with chromosome arms. Their model

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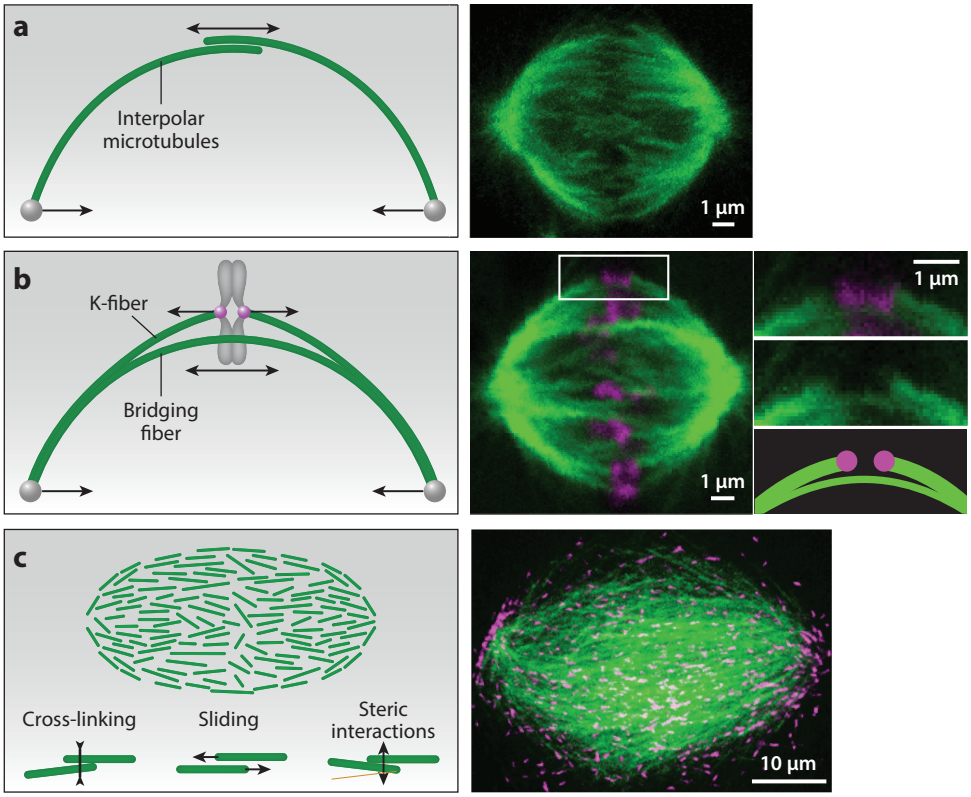

Figure 5

Forces that shape the spindle. (*a*) Sliding forces acting in the overlap region of interpolar microtubules (*arrows at the top*) result in compressed microtubule bundles (*arrows at the gray spheres*). These forces determine the spindle shape. Image to the right shows an example of a spindle in a HeLa cell expressing GFP-tubulin. (*b*) In a model that includes bridging microtubules as a link between sister k-fibers, k-fibers are under tension near the kinetochore (*arrows at the magenta spheres*) and under compression near the spindle pole (*arrows at the gray spheres*). These forces are balanced by the compression in the bridging fiber (*arrows in the middle*). The compressive forces at the spindle pole generate the curved shape of the outermost k-fibers. Note the curved shape of the k-fibers of a spindle in a HeLa cell expressing tubulin-GFP (*green*) and mRFP-CENP-B (*magenta*, kinetochores) in the image to the right. Insets: Enlargements of the boxed region (top: merge; middle: GFP; bottom: scheme) show a bridging fiber connecting sister k-fibers. (*c*) The liquid crystal theory proposes that spindle organization and the resulting spindle shape are regulated at the local level by microtubules cross-linking, their sliding by motors, and steric interactions. This theory is relevant for, for example, metaphase spindles assembled in *Xenopus* egg extracts (image at the right; tubulin in *green* and EB1 in *magenta*). Panel *b* is reproduced with permission from Reference 54. The scheme in panel *c* is adapted from Reference 96; image in panel *c* courtesy of Simone Reber.

predicts that these forces result in compressed microtubule bundles (**Figure 5*a***). Although only the forces acting at the poles and at the spindle center are taken into account, the model reproduces the shapes of *Drosophila* embryo spindles.

Similar to interpolar microtubules, outermost k-fibers are also curved, which is indicative of compressive forces. However, the plus end of the k-fiber is under tension, which was inferred from the observed stretch of the elastic centromeric chromatin that connects sister kinetochores

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(7, 12, 95, 113). Tension between sister kinetochores is required to pass the spindle checkpoint (86). Interestingly, nonkinetochore microtubules between sister kinetochores have been observed in some electron micrographs (51, 71, 74, 90, 118), and our recent laser-cutting experiments have shown that these bridging microtubules connect sister k-fibers (54). To examine the role of the bridging fiber in the force balance on kinetochores, we have introduced a theoretical model that includes the bridging fiber as a link between sister k-fibers and takes into account the elastic properties of microtubule bundles as well as the forces acting at their ends (54). A macroscopic wooden model provides an intuitive understanding of this force balance (108). Our work shows that the bridging fiber, by linking sister k-fibers, balances the tension between sister kinetochores and the compression at the spindle pole (**Figure 5*b***). Thus, our model explains the paradox of the coexistence of tension and compression along a single k-fiber (see discussion in Reference 31). The compressive forces at the spindle pole generate the curved shape of the outermost k-fibers.

A different approach explains the shape of spindles in *Xenopus* egg extracts, which consist of many short microtubules and lack centrosomes. Application of the liquid crystal theory posits that the spindle organization and the resulting spindle shape are regulated at the local level by microtubule cross-linking, their sliding by motors, and steric interactions (**Figure 5*c***) (8, 96).

Mechanisms of Chromosome Separation in Anaphase

During anaphase, sister chromatids must separate far enough from the cytokinesis plane so that chromatids do not end up on the wrong side of this plane or get cut by it. Anaphase is divided into two stages. In anaphase A, sister chromatids approach the spindle pole to which they are attached. In anaphase B, the spindle poles, together with the attached chromosomes, move apart. Anaphase A and B occur simultaneously or sequentially in different organisms and contribute to chromosome separation to a different extent. For example, fission yeast cells separate their kinetochores in anaphase A by 3 μm in 1–2 min, which is followed by further separation up to 12 μm in 10 min in anaphase B (84).

The mechanisms Pac-Man and poleward flux drive chromosome movements in anaphase A. Sliding of overlap microtubules and pulling from the cortex on astral microtubules drive spindle pole separation, and thus further chromosome separation, in anaphase B. In anaphase A, k-fibers shorten, thereby bringing the chromosomes toward the poles. This shortening can occur at the plus or minus ends of kinetochore microtubules, which are at the kinetochore and near the spindle poles, respectively. Most organisms use a combination of both types of shortening. Different views of chromosome movement during anaphase that have been advanced for the past 100 years are discussed in an excellent review (68); here we focus only on the current paradigms of movement during anaphase.

To segregate sister kinetochores, spindle microtubules position them at the spindle equator in metaphase and pull them toward the opposite spindle poles in anaphase. Force on the kinetochores is generated largely by the Pac-Man mechanism, in which kinetochores stimulate the depolymerization of k-fibers at their plus ends and move toward the spindle pole by chewing up the k-fibers (**Figure 6**, left pathway) (42, 76). This movement may result from forces exerted by motor proteins, which pull the kinetochore along the k-fiber. Alternatively, movement may be generated by the depolymerization of the k-fiber (23). As the tubulin subunits dissociate from the plus end of the k-fiber, the kinetochore remains attached to the k-fiber and thus slides toward the spindle pole. In *Drosophila* cells, the kinesin-13 KLP59C depolymerizes kinetochore microtubules at their kinetochore-coupled plus ends, thereby contributing to the Pac-Man mechanism (99). In HeLa cells, movements of kinetochores in anaphase are accelerated by depletion of kinesin-8 and,

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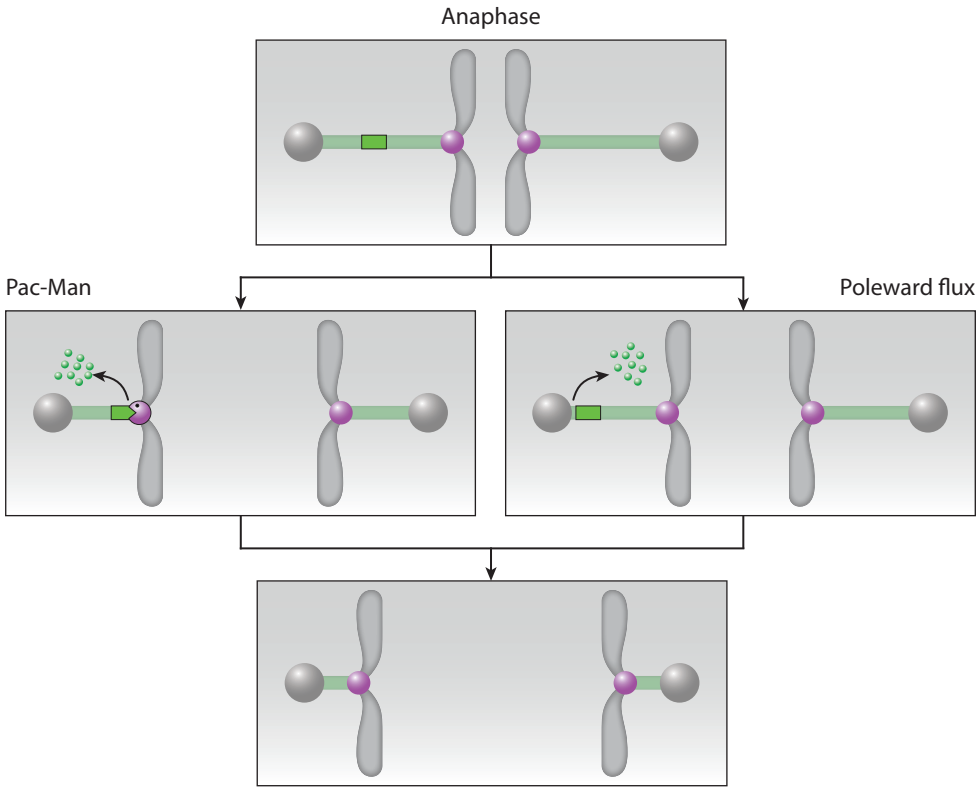

Figure 6

Models of chromosome segregation in anaphase. Two models are shown: Pac-Man (*left pathway*) and poleward flux (*right pathway*). In the Pac-Man model, k-fiber microtubules are disassembled at the kinetochore (*magenta*); small green spheres represent the resulting free tubulin. Shrinking microtubules bring each sister chromatid (*elongated gray shape*) toward the spindle pole (*gray sphere*) to which it is connected. In the poleward flux model, the whole k-fiber is translated toward the spindle pole, together with the attached kinetochore and chromatid. The two models can be distinguished by photoactivating a small region on the k-fiber (*bright green box*). In the Pac-Man model, the photoactivated region remains at a constant distance from the pole, while the distance to the kinetochore decreases. In the poleward flux model, the photoactivated region remains at a constant distance from the kinetochore, while the distance to the pole decreases. Most cell types employ a combination of both mechanisms to segregate the chromosomes in anaphase.

conversely, slowed down by its overexpression (104). Because the main contribution (~80%) to kinetochore movements in mammalian cells in anaphase comes from the Pac-Man mechanism (36, 124), these experiments suggest that kinesin-8 slows down Pac-Man by affecting tubulin release from the plus ends of kinetochore microtubules.

In addition to the Pac-Man mechanism, poleward flux of k-fibers contributes to the movement of the kinetochores (**Figure 6**, right pathway). Poleward flux is the translation of k-fibers toward the spindle pole, which resembles the movement of a conveyer belt and is accompanied by disassembly at the pole (77, 78). This movement was directly observed by following a fluorescent bar generated by local photoactivation of a photoactivatable fluorescent probe across the spindle microtubules (77). Poleward flux plays a major role in anaphase kinetochore separation in meiotic systems (27),

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whereas the contribution of the flux is small (~20%) in somatic tissue culture cell lines such as PtK1, newt lung, and HeLa. The latter conclusion is based on observations that flux in these cells is significantly slower than kinetochore movement (36, 78, 124).

Poleward flux requires kinesin-13 motors Kif2a in *Xenopus* egg extracts and mammalian cells and Klp10A in *Drosophila* cells (99). Because kinesin-13 motors depolymerize microtubules (28), they most likely contribute to poleward flux by depolymerizing microtubules at the spindle poles. In addition, kinesin-5 motors drive poleward flux by sliding microtubules apart in *Xenopus* egg extracts (79) and *Drosophila* embryos (9), but not in mammalian PtK1 cells (14).

CONCLUSION

The spindle, a fascinating structure fundamental to life, has been studied for more than a century. Today we know the identity of several hundreds of proteins involved in mitosis, but we still do not understand how they interact to form the spindle and generate precisely regulated forces on the chromosomes. The spindle arises from self-organization of microtubules and chromosomes, together with a variety of accessory proteins, whose different types of motions help them explore the space and interact with each other. In particular, microtubule dynamics and their rotational movement drive their interactions with each other, leading to the formation of parallel and antiparallel microtubule bundles that compose the spindle. Similarly, the same processes facilitate the capture of kinetochores by microtubules.

After the interactions between the chromosomes and the microtubules have been established, the chromosomes are moved to the equatorial plane of the spindle and ultimately toward opposite spindle poles. As chromosomes are large objects, their transport relies on directed and precisely regulated forces generated by microtubules and motor proteins. Moreover, forces in the spindle determine its shape.

A number of mechanisms underlying spindle self-organization and force generation have been uncovered. Different organisms and even different cell types of the same organism employ a combination of different mechanisms. Because of this multitude of mechanisms, a particular challenge for researchers is to uncover the contribution of each mechanism and of the relevant proteins in spindle mechanics and function. For example, knockdown of a single protein may have a minor effect on the spindle; thus, experiments with multiple knockdowns are needed, which are not always easy to design, perform, and interpret. Therefore, biophysical perturbations such as cutting, stretching, and squeezing, combined with the manipulation of protein expression or activity, provide important insights into spindle mechanics (22, 54, 69). Finally, expressing hypotheses through theoretical models offers testable predictions, which pose new challenges for experiments to uncover the biophysical secrets of spindles.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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