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Motor Function in the Mitotic Spindle

Minireview

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The mitotic spindle is a complex and fascinating macromolecular machine that performs a crucial task: distributing an exact complement of chromosomes to each daughter cell during mitosis or meiosis. It is composed primarily of microtubules, dynamic cytoskeletal polymers of α/β tubulin subunits with an intrinsic structural polarity. The polymer lattice serves as a track for microtubule-based motor proteins, mechanochemical enzymes that couple ATP hydrolysis to directional movements of cargo along microtubules or of the microtubules themselves. A set of such motors is required for spindle assembly and function. Two papers in this issue of *Cell* by Funabiki and Murray (2000) and Antonio et al. (2000) explore the function of a chromosomally localized motor called Xkid (*Xenopus* kinesin-like DNA binding protein). Xkid participates in aligning chromosomes on the spindle and must be degraded for chromosomes to segregate during anaphase.

Multiple Organizational Forces Promote Spindle Assembly

The bipolar organization of spindle microtubules is a prerequisite for proper chromosome segregation. Microtubules are arranged into two arrays of uniform polarity, with their minus ends focused at each pole and their plus ends either interacting with chromosomes or overlapping in the center. This organization is promoted by distinct structural cues. In most animal cells, microtubules grow from the centrosome, an organelle that nucleates growth of microtubules with their plus ends extending outward. The centrosome duplicates before the onset of mitosis, providing two organizing centers that define the spindle poles. Another structural cue comes from the duplicated chromosomes, termed "sisters," which remain physically connected until their segregation in anaphase. Each sister possesses a kinetochore, the proteinaceous disk-like structure assembled at its primary constriction, the centromere (Maney et al., 2000). Duplicated kinetochores are oriented opposite one another, providing microtubule capture and attachment sites for microtubule plus ends extending from opposite spindle poles.

However, these paired cues are not sufficient for spindle formation. In addition, microtubule-based motor proteins provide essential forces for microtubule organization and chromosome movement. Motors found localized to the spindle include cytoplasmic dynein and seven different families of proteins related to kinesin (Kreis and Vale, 1999). A single isoform of dynein, in association with the dynactin complex, functions at spindle poles, at kinetochores, and in the cell cortex (Karki and Holzbaur, 1999). In contrast, the kinesin superfamily consists of multiple isoforms that perform distinct functions. Kinesin family members can differ in their oligomeric state and their directionality, which correlates with the relative position of the motor domain containing microtubule and nucleotide binding sites (Goldstein and Philp, 1999).

Motor Function in Spindle Organization

Why is motor function necessary for spindle formation? Despite its apparent symmetry and uniform appearance, the spindle is a dynamic structure, with a microtubule half-life in the range of 20 s to 1 min (Desai and Mitchison, 1997). This dramatic turnover is due to the dynamic instability of individual microtubules that undergo alternate phases of growth and shrinkage, and to the constant flux of tubulin subunits as they polymerize at plus ends and depolymerize at minus ends, causing the microtubule lattice to move poleward. The mechanism underlying spindle structural integrity appears to be a balance of opposing forces provided by cross-linking motors (Figure 1). This idea is largely derived from genetic studies in yeast, which gave rise to two basic principles: kinesins harboring N-terminal (KIN N) and C-terminal (KIN C) motor domains generate opposing forces in the spindle, and spindle motors often exhibit overlapping functions (Kreis and Vale, 1999). These tenets have held true in higher eukaryotes such as *Drosophila* and *Xenopus* (Walczak et al., 1998; Sharp et al., 2000).

At least three different motor activities exert forces influencing spindle pole separation (Figure 1A). Plus end–directed "bipolar" KIN N kinesins form tetramers with motor domains on each end. By cross-linking and walking along microtubules from opposite spindle poles, this motor type provides an expanding force that pushes spindle poles apart. Counteracting this force is a family of minus end–directed KIN C kinesins. A third type of force is applied to astral microtubules that extend from the pole to the cell cortex, where a fraction of cytoplasmic dynein could function to pull the poles apart.

In addition to regulating the overlap of antiparallel microtubules in the central spindle, cross-linking motors also organize microtubules of the same polarity at spindle poles (Figure 1B). Although focal nucleating centers such as centrosomes contribute substantially to this organization, microtubules are frequently released, freeing their minus ends to depolymerize to allow poleward flux. Several motor activities function to maintain the organization of minus ends in spite of spindle dynamics. One well-characterized example is cytoplasmic dynein, disruption of which leads to splayed poles and abnormally long spindles in the *Xenopus* system (Heald et al., 1996). Dynein acts to focus spindle poles and to oppose the expanding force of bipolar KIN N motors (Merdes et al., 2000). In some systems, such as *Drosophila* embryos, KIN C motors function in conjunction with or instead of dynein in pole formation, indicating both functional redundancy and the importance of cross-linking minus end–directed motility in this process (Compton, 1998).

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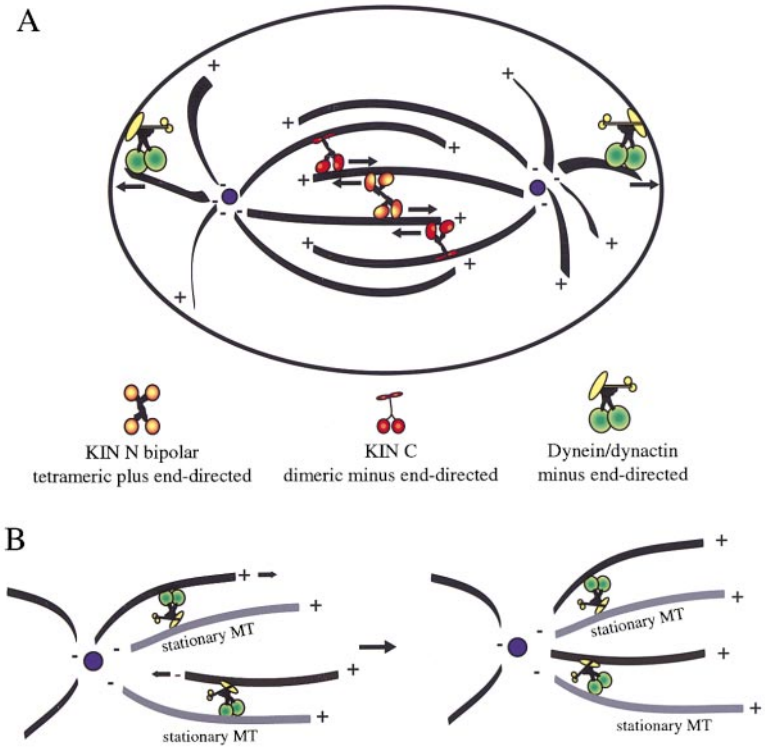

Figure 1. Motors that Generate Opposing Forces Organize the Spindle

Arrows indicate the direction of each force.
(A) Cross-linking motors that increase or decrease the overlap of antiparallel microtubules determine spindle pole separation. Cytoplasmic dynein in the cell cortex can contribute to spindle positioning and expansion (adapted from Sharp et al., 2000).
(B) Spindle pole organization by cytoplasmic dynein that can pull microtubules poleward or push microtubules toward the spindle equator.

Motor Function in Kinetochore Movement

Superimposed on the structural arrangement of microtubules in the spindle is their interaction with chromosomes. At the kinetochore of each sister, a bundle of 15–30 microtubules termed the kinetochore fiber (K fiber) forms an end-on attachment (Rieder and Salmon, 1998). Most of the force production for kinetochore movement appears to occur at the kinetochore itself, driven by coordinated polymerization and depolymerization at K fiber plus ends (Figure 2). Following their attachment to the spindle, kinetochores oscillate and the chromosome migrates to the spindle equator, a process termed "congression." Once all of the chromosomes have congressed, the cell is in metaphase and the chromosomes are said to lie on the metaphase plate. Kinetochores control the metaphase–anaphase transition by inhibiting sister separation until all the chromosomes are properly attached and aligned. At this point the linkage between sisters dissolves, and they move along shrinking microtubules to opposite spindle poles.

Although we are still far from a molecular understanding of how kinetochores work, three motor activities have been localized to kinetochores that are thought to contribute to some aspect of their movement. Dynein/dynactin is thought to play a role in the initial capture of microtubules by kinetochores, which move rapidly poleward (Figure 2A). Once K fibers have formed, two different kinesins may coordinate microtubule dynamics with kinetochore movements (Figure 3A). The KIN N motor CENP-E is required for chromosome positioning at the metaphase plate. Because it is plus end–directed, CENP-E could move kinetochores away from the poles along K fibers as they polymerize and maintain association with K fiber plus ends as they depolymerize (Maney et al., 2000). KIN I (for internal) kinesin family members have also been found at kinetochores. Unlike the other kinesins discussed so far, KIN I motors do not appear to move along microtubules, but rather bind to their ends and cause depolymerization. Coordinated regulation of KIN I motor activity at K fiber plus ends could contribute to the coupled cycles of microtubule depolymerization/polymerization and kinetochore oscillation, as well as to K fiber shrinkage and poleward chromosome movements in anaphase.

Breaking Polar Wind

With their complex and coordinated behavior and their key role in regulating entry into anaphase, kinetochores are considered the brains of chromosomes in mitosis, directing their movements and orderly segregation. In contrast, chromosome arms have been referred to as baggage (Waters and Salmon, 1995). However, detailed analyses of mitosis indicate a necessary role for chromosome arms during congression. Kinetochores alone are not sufficient to move chromosomes to the metaphase plate because they only provide strong poleward forces and do not contribute significantly to movement away from the pole (Rieder and Salmon, 1994). Instead, chromosome arms are pushed away from spindle poles by forces generated at their surface by nonkinetochore microtubules, referred to as the polar ejection force or polar wind. This ejection force decreases with decreasing microtubule density and increasing distance from each pole, leading to a balanced position of the chromosome arms at the metaphase plate.

Mounting evidence supports the model that plus end–directed motors on chromosome arms are responsible for the polar ejection force. Chromosomally localized KIN N kinesins (chromokinesins) have been identified in

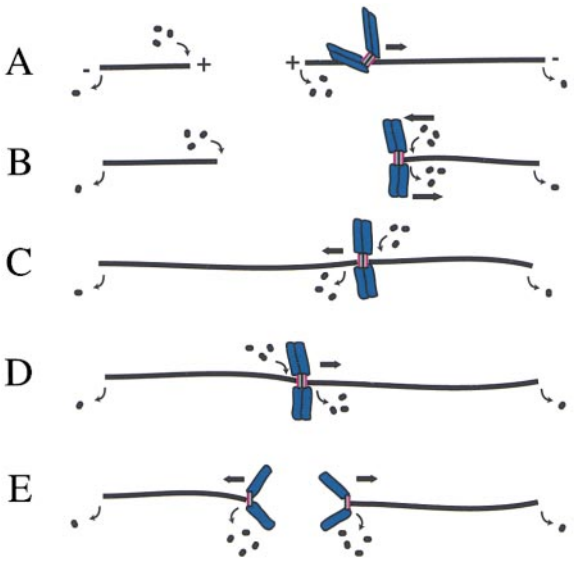

Figure 2. Kinetochore Movements during Chromosome Congression Are Coupled to K fiber Microtubule Growth and Shrinkage at Their Plus Ends

Constant depolymerization at microtubule minus ends generates slow poleward movement (flux) of the K fibers (adapted from Inoue and Salmon, 1995).

(A) Initial capture and rapid poleward kinetochore movement is thought to depend on cytoplasmic dynein localized to the kinetochore.

(B–D) Both mono- and bioriented chromosomes oscillate between poleward and away from the pole movements as K fiber microtubules polymerize and depolymerize.

(E) Sister chromosomes separate at anaphase and follow shrinking microtubules poleward.

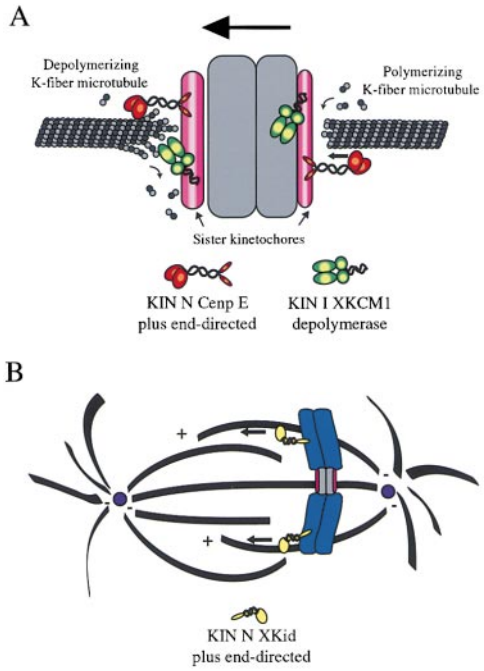

Figure 3. Kinesins Contribute to Chromosome Congression by Moving Kinetochores and Chromosome Arms

(A) At the leading kinetochore KIN I kinesin XKCM1 induces microtubule depolymerization while the KIN N CENP-E maintains attachment. XKCM1 activity is turned off at the lagging kinetochore where CENP-E moves toward the polymerizing plus end.

(B) The polar ejection force is generated by KIN N kinesins localized to chromosome arms that move along microtubules toward their plus ends.

several species, and the *Drosophila* chromokinesin Nod is required during female meiosis I for the alignment of chromosomes that have not undergone recombination (McKim and Hawley, 1995; Goldstein and Philp, 1999). Now, two papers in this issue support a more general role for a *Xenopus* chromokinesin, Xkid, in chromosome congression (Antonio et al., 2000; Funabiki and Murray, 2000). The authors show that Xkid activity is required to generate the polar ejection force, and that its regulation is also important for anaphase chromosome movements.

Both studies take advantage of synchronous cytoplasmic extracts prepared from *Xenopus* eggs that can reconstitute spindle assembly and anaphase in vitro, providing excellent cytology and allowing biochemical manipulations. This system permits specific depletion and add-back experiments, as well as manipulation of the cell cycle state. Both groups show that immunodepletion of Xkid does not inhibit bipolar spindle assembly but prevents proper metaphase alignment of sperm chromosomes, which stretch out in a disorganized fashion toward the poles. Interestingly, addition of anti-Xkid antibodies to preformed spindles also disrupted chromosome positioning, indicating that Xkid activity is required constitutively to maintain alignment of the arms. This mechanism may be required to counteract the effects of the constant poleward microtubule flux, which is likely to create a general minus end–directed dragging force on chromosomes. Although motility properties of Xkid have not been directly tested, its human homolog is plus end–directed. Therefore, Xkid is proposed to contribute to chromosome congression by moving chromosome arms along microtubules toward the spindle equator (Figure 3B).

Implications for Anaphase

The onset of anaphase triggers a dramatic change in the balance of forces on chromosomes. Sister chromosome cohesion is lost and K fibers shorten without detaching from kinetochores, leading sisters to opposite spindle poles. Anaphase is induced by activation of the anaphase promoting complex (APC), which induces ubiquitin-mediated proteolysis of several mitotic proteins (Morgan, 1999). Interestingly, Funabiki and Murray identified Xkid as a chromosomal protein specifically degraded during anaphase by the APC. Furthermore, addition of a nondegradable version of Xkid prevented chromosome segregation, indicating that the polar ejection force must be neutralized to allow poleward chromosome movement during anaphase in *Xenopus* egg extracts. How general is this mechanism? Unlike vertebrate somatic cells in which anaphase K fiber shortening occurs primarily at the kinetochore, K fiber depolymerization in *Xenopus* extracts occurs mostly at the poles. Anaphase chromosome movement is therefore largely generated by poleward microtubule flux, which is no longer balanced by plus end polymerization (Desai et

al., 1998). Analysis of Xkid homolog function and regulation in somatic cells will show whether other anaphase mechanisms also require neutralization of chromokinesin activity.

Even in the absence of Xkid, some sister kinetochores achieve an equatorial position despite disorganization of chromosome arms. To determine whether such chromosomes were still capable of segregating properly, Antonio et al. artificially induced anaphase in these extracts. These tangled chromosomes failed to segregate properly and never moved to the spindle poles. Therefore, Xkid must be both present and properly regulated to allow spindle function.

Two Classes of Chromokinesins?

Based on functional analyses, the chromokinesins identified to date can be divided into two main categories. Xkid/Nod family proteins are required for aspects of chromosome alignment, while *Xenopus* Xklp1, chicken chromokinesins, and *Drosophila* Klp38B may play a structural role in spindle assembly itself. A useful diagnostic assay is the analysis of spindle assembly around DNA-coated beads, which assemble into chromatin in *Xenopus* egg extracts, recruiting both Xkid and Xklp1. Bead spindles lack both centrosomes and kinetochores and form by a highly motor-dependent mechanism following polymerization of microtubules in their vicinity (Heald et al., 1996). Chromatin bead spindles are insensitive to disruption of Xkid, probably because unlike normal chromosomes the rigid nature of the beads makes them resistant to poleward forces. However, bead spindles fail to form when Xklp1 is inhibited, apparently as a result of defective chromatin–microtubule interactions (Walczak et al., 1998). Therefore, Xklp1-style chromokinesins may promote the dynamic association of microtubules with chromosomes necessary for organization by other motors, while Xkid may generate actual chromosome motility in the spindle.

Functional Multiplicity

Motor function is integral to spindle morphogenesis. Whereas the structural cues provided by centrosomes and kinetochores previously dominated our understanding of mitosis, the identification and characterization of spindle motors has illuminated less obvious mechanisms, which have only been appreciated since advancing technology has allowed a wider range of manipulations and visualization of spindle dynamics at high resolution. The more we learn, the more we realize that multiple overlapping and redundant forces contribute to the high fidelity of chromosome segregation, which is not surprising considering the devastating effects of errors in this process.

Studies in many different eukaryotic systems have led to models for the function of individual spindle motors based on their molecular features and the effects of their disruption, but have not always led to a consensus for the role of a particular motor. The lesson here is that different systems emphasize different motors and mechanisms. For example, while all eukaryotes require one or more bipolar KIN N kinesin for spindle assembly, yeast appear to lack chromokinesins as well as poleward microtubule flux (Mallavarapu et al., 1999). Is poleward microtubule flux an important component of spindle function in larger eukaryotic cells? Or simply a by-product of the many forces exerted on spindle microtubules? More complex reconstitution experiments using pure motors and dynamic microtubules may help address this question.

The next big step in understanding how spindle motors function requires studying their interacting proteins, their regulation and the integration of their activities. Some spindle proteins that play important roles in conjunction with motors have already been identified. For example, NuMA is transported to microtubule minus ends by dynein/dynactin where it plays a key role in spindle pole cohesion (Merdes et al., 2000). Interactions with motor accessory proteins, motility itself, and motor stability are likely to be further regulated by posttranslational modifications such as phosphorylation. As we have seen, changing the balance of motor activities can drive changes in spindle structure or chromosome movement, yet the integration of motor activities is poorly understood. Altogether, the many directions in this field promise to engage spindle enthusiasts for years to come.

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