JCB Mini-Review

Searching for the middle ground: mechanisms of chromosome alignment during mitosis

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The contributions of key molecules predicted to align chromosomes at the center of the mitotic spindle have been recently examined. New results dictate that models for how chromosomes align during the early stages of mitosis must be revised to integrate properties of microtubule-based motor proteins as well as microtubule dynamics.

Chromosome alignment at the spindle equator, or congression, is a remarkably conspicuous event during mitosis that defines the metaphase stage of the cell cycle. This movement of chromosomes to the spindle equator is necessary for accurate segregation of a cell's replicated DNA in organisms as diverse as plants, insects, and mammals (for review see Khodjakov et al., 1999). Results from more than a century of detailed observations of chromosomes in mitosis (particularly in vertebrate cells) have provided a well-scripted sequence for the steps involved in chromosome attachment to the spindle and subsequent congression to the spindle equator (for review see Rieder and Salmon, 1994). In addition, chromosome cutting experiments and microtubule marking experiments have revealed many, if not all, of the forces involved in driving chromosome movement (Gorbsky, 1992). However, a striking gap exists in our understanding of the mechanisms of chromosome movement due to our inability to identify specific molecules that drive chromosome movement or regulate chromosome alignment at the spindle equator. This review highlights recent results that begin to fill this gap and examines current models for chromosome congression in the context of this new data.

Microtubule–chromosome interactions occur primarily at kinetochores, specialized pairs of disc-shaped structures located on either side of the centromere. To congress to the spindle equator, a chromosome must biorient, i.e., attach to spindle microtubules with each kinetochore interacting with microtubules derived from one of the two spindle poles. Some chromosomes biorient immediately upon nuclear envelope breakdown and oscillate about the spindle equator, but do not tend to stray far from the spindle midzone (Fig. 1, b–e). Other chromosomes initially interact with microtubules at only one kinetochore. This leads to rapid chromosome movement toward the pole as it slides along the length of the microtubule in a manner similar to the transport of vesicles (Fig. 1 b) (Rieder and Alexander, 1990). Once near the spindle pole the kinetochore captures multiple microtubule plus-ends and builds a kinetochore fiber (Fig. 1 c). These monooriented chromosomes are positioned with their kinetochores pulled toward the pole and their arms pushed away from the pole and oscillate toward and away from their attached pole. During these oscillations, changes in kinetochore fiber length coincide with chromosome movement toward and away from the spindle pole. Eventually, a microtubule from the opposite pole will contact the unattached sister kinetochore establishing biorientation. The newly bioriented chromosome then moves in a directed manner (i.e., congresses) to the spindle equator (Fig. 1, d and e).

An appealing model for how chromosomes congress to the center of the spindle is based on ideas developed by Ostergren (1951). In this model, chromosomes attached to two spindle poles experience force toward each pole with the magnitude of each force being proportional to the length of the kinetochore fiber connecting the chromosome to the pole. Chromosomes align at the equator of the spindle where opposing poleward forces are equal and balanced. However, two key observations suggest that this model is not likely to be correct (for review see Rieder and Salmon, 1994). First, in many cell types chromosomes oscillate back and forth over substantial distances as they congress to the center of the spindle, indicating that forces acting on chromosomes do not change monotonically with distance from a spindle pole. Second, monooriented chromosomes display both poleward and away-from-the-pole movements, indicating that chromosomes experience force away from the pole independent of any attachment to the distal pole (the polar ejection force). These observations are more consistent with models that proposed "smart" kinetochores capable of integrating different signals and forces to determine their position in the spindle, as they dance toward its center (Mitchison, 1989a).

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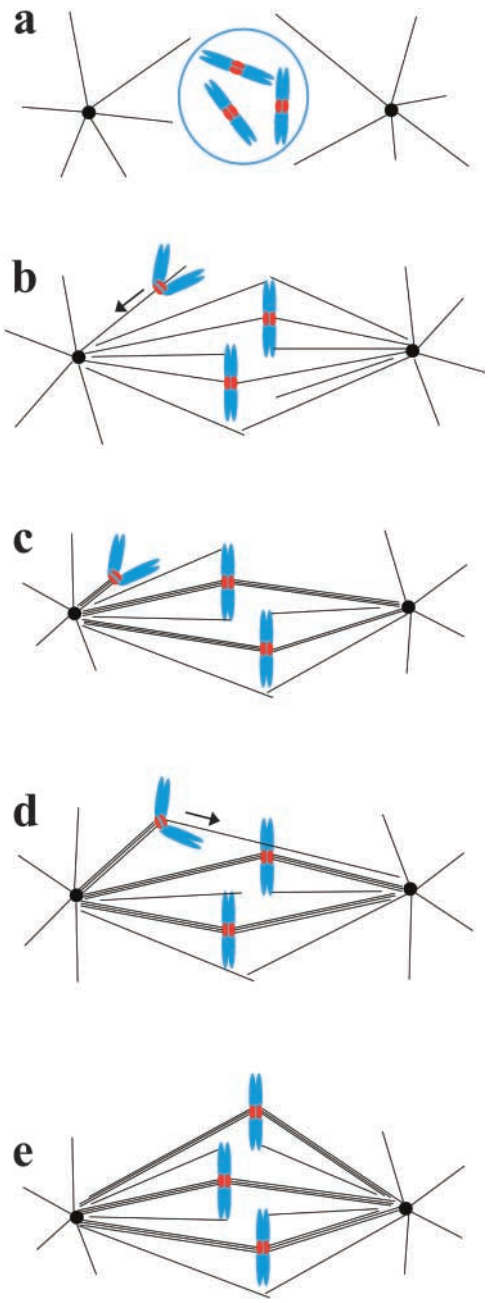

Figure 1. **Chromosome positioning on the mitotic spindle.** Schematic representation of a cell in prophase (a), prometaphase (b–d), and metaphase (e) of mitosis indicating the microtubules (black), chromosomes (blue), and kinetochores (red). Thin black lines represent individual microtubules and thick black lines represent bundles of 10–40 microtubules within kinetochore fibers. Arrows indicate the direction of chromosome movement.

Kinetochore directional instability

In 1993, Skibbens and Salmon published a study using high-resolution time-lapse video microscopy that provided crucial insight into how chromosomes move in living cells (Skibbens et al., 1993). Their four key observations were that (1) the rates of chromosome movement are the same at different positions on the spindle, (2) the transitions between poleward and away-from-pole movement are abrupt, (3) sister kinetochore movement is highly coordinated, and (4) chromosome congression is favored in prometaphase because kinetochores spend more time moving away from the pole than they do moving poleward. They termed this constellation of oscillatory behaviors kinetochore directional instability.

Based on these observations, the authors proposed that kinetochores toggle between states of poleward force generation and neutral (or pushing), and tension experienced by the kinetochore regulates the switching between those two states. Monooriented chromosomes are dragged poleward by their attached kinetochore. As poleward motion moves the chromosome progressively closer to the spindle pole, the leading kinetochore encounters increasing tension due to the antagonistic polar ejection force pushing the chromosome arms away from the spindle pole. That tension causes the leading kinetochore to cease poleward force production and shift into neutral permitting the polar ejection force to move the chromosome away from the pole. Kinetochores on bioriented chromosomes moving poleward experience tension derived from both the polar ejection force and the activity of the sister kinetochore pulling toward the opposite pole. High tension on the leading kinetochore will cause it to switch into neutral permitting the poleward force derived from the sister kinetochore, along with the ejection force from the proximal pole, to move the chromosome toward the spindle equator. Repeated iterations of these switches lead to congression because chromosomes spend more time moving away from the pole toward the spindle equator, and the spindle equator is the position where the polar ejection forces are equal—and, presumably, minimal—between the poles. This model provides explanations for chromosome oscillations on both bipolar and monopolar spindles in animal cells and for chromosome congression.

Although growing microtubule plus-ends may contribute to the polar ejection force, recent evidence demonstrates that a majority of this force is generated by the Kid subfamily of kinesin-related proteins (Antonio et al., 2000; Funabiki and Murray, 2000; Levesque and Compton, 2001). Kid localizes along chromosome arms, and consistent with the kinetochore directional instability model, inhibition of Kid function in cultured cells abolished chromosome oscillation on both monopolar and bipolar spindles (Levesque and Compton, 2001). Moreover, in the absence of Kid function, chromosomes were unable to maintain their distance from monopolar spindle poles, suggesting that the poleward force at the kinetochore dominates in the absence of the polar ejection force and drags the chromosome into the pole. As chromosome oscillations were eliminated after Kid inhibition, it follows that the polar ejection force regulates switching of kinetochores between poleward and neutral states. However, the surprise was that bioriented chromosomes congressed normally in Kid-deficient cells despite the lack of oscillation. Thus, whereas biased durations of oscillatory motion are likely an important mechanism driving chromosome congression in animal cells, these new results suggest that another mechanism exists to provide positional cues to chromosomes and that this alternative mechanism can efficiently drive chromosome congression if the oscillation-based pathway is inoperative.

Kinetochore microtubule numbers

One potential source of positional information for chromosomes during early stages of mitosis may come from the number of microtubules attached to each sister kinetochore as the magnitude of kinetochore force, hence the direction of chromosome movement, may depend on the number of kinetochore microtubules (Hays and Salmon, 1990). McEwen and colleagues recently tested this model using correlative light and electron microscopy and observed no positive correlation between the number of microtubules bound to kinetochores and direction of chromosome movement (McEwen et al., 1997). Thus, these data argue against chromosome congression models in which the direction of chromosome movement is dependent, either directly or indirectly, on the number of microtubules bound to kinetochores.

Kinetochore motors

Another potential mechanism for chromosome congression could involve the precise regulation of kinetochore-associated microtubule motors. Microtubule marking experiments demonstrated that most poleward chromosome movement coincided with the disassembly of microtubule plus-ends at the kinetochore, indicating that in vertebrate cells chromosome movement may primarily be driven by kinetochore-associated motors (Gorbsky et al., 1988; Mitchison and Salmon, 1992). Based on these observations, it was proposed that forces generated by the kinetochore-associated motors dynein, CENP-E, and/or MCAK/XKCM1 could drive chromosome congression if appropriately regulated and coupled to microtubule plus-end dynamics (also known as the pac-man model) (Gorbsky et al., 1987). Regulation of the activity of these motors could occur through a variety of mechanisms including changes in the phosphorylation state or the abundance of the proteins at kinetochores (Hyman and Mitchison, 1991). Concordantly, CENP-E is known to undergo cell cycle–dependent phosphorylation, and the abundance of both CENP-E and dynein has been shown to be dependent on microtubule occupancy at kinetochores (Liao et al., 1994; King et al., 2000; Hoffman et al., 2001).

Recent experiments in cultured animal cells have tested the potential role of each of these motors in chromosome congression. Disruption of Kin I kinesin (MCAK) function in cultured cells using either antisense or overexpression of dominant–negative fragments caused defects in chromatid segregation at anaphase, but prior chromosome alignment at the spindle equator did not appear altered (Maney et al., 1998). Inhibition of cytoplasmic dynein activity impaired chromosome congression in fruit fly embryos (Sharp et al., 2000), but did not cause any detectable effect on the rate or extent of chromosome congression in cultured vertebrate cells (Howell et al., 2001). Finally, depletion of CENP-E from kinetochores by antibody injection caused cell cycle arrest with multiple chromosomes lying adjacent to the spindle poles instead of at the spindle equator (Schaar et al., 1997). Although suggestive of a failure in congression, careful analysis of these cells by electron microscopy demonstrated that the unaligned chromosomes failed to congress because they were monooriented (McEwen et al., 2001). Bioriented chromosomes in the same cells showed chromosome congression and oscillation indistinguishable from control cells even though the kinetochores lacked detectable CENP-E. Thus, although these data do not exclude the possibility that chromosome congression is driven by regulated kinetochore motor activity, the molecular mechanisms for regulating the activities of these proteins to determine the position of chromosomes in spindles have not been characterized.

Traction fiber

Another possible source of positional information in the spindle is based on the traction fiber model, perhaps the oldest and most widely discussed model for chromosome congression (Ostergren, 1951). Although the traction fiber model cannot explain the complex oscillatory movements of chromosomes, it offers an alternative to the oscillation-based mechanism to explain how chromosomes sense their position on spindles. In its developed form, this model proposes that kinetochore microtubules are translocated poleward generating a poleward force that is proportional to the length of the kinetochore fiber (Fig. 2) (for review see Rieder and Salmon, 1994; and for an alternative view see Pickett-Heaps et al., 1996). Hays and Salmon provided evidence that poleward force was proportional to the length of kinetochore microtubules in agreement with a traction fiber–based mechanism (Hays et al., 1982). However, the most compelling evidence that a traction fiber–based mechanism exists comes from the direct observation of the poleward translocation of kinetochore microtubules, referred to as poleward microtubule flux, in spindles in many different cell types (Mitchison, 1989b; Mitchison and Salmon, 2001).

To explain how the microtubule lattice translocates toward the spindle pole, while keeping the length of the spindle constant, a nonmicrotubule mechanical ensemble, or spindle matrix, has been proposed (for reviews see McIntosh et al., 1969; Pickett-Heaps et al., 1982). Motor proteins may bind to this structure and generate force to drive microtubule translocation poleward. However, such a spindle component has not been biochemically characterized and the existence of the spindle matrix remains controversial. Recently, two observations have once again focused our attention on the possible existence of a spindle matrix. First, examining the localization of a protein "skeletor" in fixed *Drosophila* embryos suggests that a non-microtubule spindle–like structure exists (Walker et al., 2000). Whether this is in fact the long sought after spindle component is still unclear as the biochemical function of skeletor is unknown and homologous proteins in other cell types have not been identified or characterized. Second, examining the translocation and turnover of the BimC kinesin Eg5 in bipolar spindles using fluorescent speckle microscopy, it was found that the motor protein was static relative to spindle microtubules that fluxed polewards (Kapoor and Mitchison, 2001). An interpretation of this observation is that Eg5 is static while it interacts with a nonmicrotubule matrix in the spindle. However, other interpretations, including the possibility that the motor protein itself forms higher order oligomers with limited diffusion, cannot be ruled out. Validating a candidate spindle matrix component may be particularly challenging for at least two reasons. First, the matrix may not be a stable

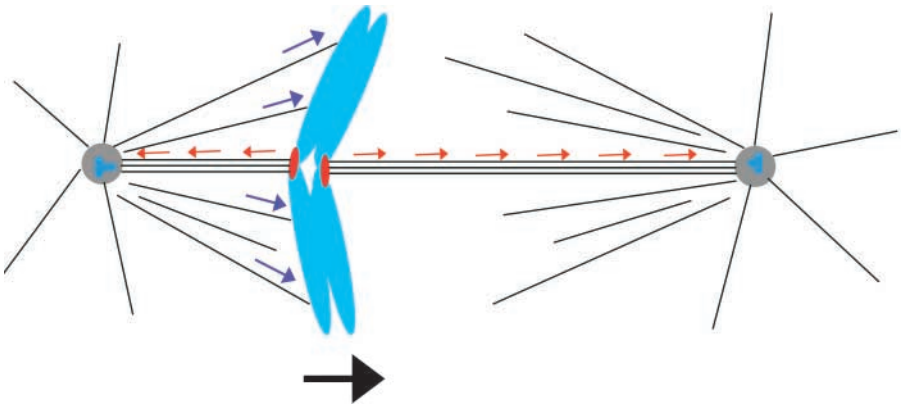

Figure 2. **Postional cues for chromosome congression may be derived by integrating two force gradients in the spindle, the polar ejection force and the traction fiber mechanism.** A chromosome (blue) moving from left to right is shown. Red arrows indicate translocation of the traction fiber with the number of arrows proportional to the length and therefore the forces acting along the kinetochore fiber. The blue arrows correspond to the polar ejection force. This force is predicted to decrease as the distance from the pole increases. The kinetochores (red) are under tension and stretched (pulled apart) due to forces acting on chromosomes. The magnitude of tension at kinetochores can regulate the movement of a chromosome.

framework but a dynamic assembly, consistent with the observation that fluorescent Eg5 speckles persist for few seconds and the protein rapidly exchanges in and out of the spindle (Kapoor and Mitchison, 2001). Second, the assembly of the spindle matrix and spindle microtubules may be interdependent.

The rate of poleward microtubule flux has been shown to be equal to that of poleward chromosome movement in anaphase in frog egg extracts, suggesting that it may be the primary poleward driving force in that system (Desai et al., 1998). However, the rate of poleward microtubule flux has been found to be significantly slower than the rate of poleward chromosome movement in many other cell types that have been examined (Mitchison and Salmon, 1992). Thus, contrary to the simple tug-of-war idea originally proposed in the traction fiber model, poleward microtubule flux may not be responsible for directly powering chromosome congression to the spindle equator in many cell types. However, this does not rule out the possibility that the force generated by poleward microtubule flux regulates kinetochore activity to appropriately position chromosomes at the spindle equator. If the findings of Hays and Salmon indicating that the magnitude of the force at the kinetochore were dependent on kinetochore microtubule length were confirmed in all cell types (Hays et al., 1982), then we envision that poleward microtubule flux may be a mechanism to bias chromosome movement to the spindle equator. This mechanism would most likely act independently of the polar ejection force and chromosomes may utilize both mechanisms to determine their position on the spindle. In this context, the traction fiber alone may provide all the positional information needed to correctly align chromosomes at the center of the spindle, which offers an explanation for how chromosomes congressed efficiently after perturbation of the polar ejection force generating motor Kid. However, it is currently unknown if the poleward translocation of spindle microtubules occurs during prometaphase, the critical period of mitosis for congression, and no direct test of this idea is possible at this time because no reagents are available to specifically inhibit poleward microtubule flux.

Force gradients: positional cues for chromosome alignment

A parallel exists between the mechanisms by which the polar ejection force and the traction fiber–based poleward microtubule flux could direct chromosome congression. These two forces are most likely manifested as force gradients within the spindle lattice with the magnitudes of force generated by polar ejection and poleward microtubule flux decreasing and increasing, respectively, as chromosomes move away from the spindle pole toward the spindle equator. It is appealing to speculate that both these force gradients influence kinetochore activity by generating tension at the kinetochore (Fig. 2). Tension has been shown to stabilize kinetochore-microtubule attachment and kinetochore motility in meiotic cells (Nicklas and Koch, 1969; Nicklas, 1977) and to influence kinetochore motility in mitotic cells (Skibbens et al., 1995). It has been shown that poleward microtubule flux can generate sufficient force to maintain interkinetochore stretching (Waters et al., 1996), an observation consistent with idea that a traction fiber mechanism could generate tension to regulate kinetochore activity (Mitchison, 1989a). Furthermore, the polar ejection force has been shown to regulate chromosome oscillations, presumably, through altering kinetochore tension (Levesque and Compton, 2001).

A key component of such tension-regulated mechanisms is the elastic properties of the centromeric DNA linking the kinetochore to the chromosome, a rather unexplored aspect of chromosome and spindle biology. Significant stretching of centromeric chromatin in response to spindle forces has been observed in both budding yeast and human cells (Shelby et al., 1996; Pearson et al., 2001). It has also been argued that the elastic properties of centromeric DNA influences the coordination between sister kinetochores during oscillatory chromosome movements (Skibbens et al., 1995; Pearson et al., 2001). Moreover, centromeric elasticity is most likely responsible for syntelic chromosome orientation, where both sister kinetochores attach to microtubules emanating from the same spindle pole (Rieder, 1982; Kapoor et al., 2000). It is not understood why kinetochore tension resulting from polar ejection forces is manifested as oscillatory chromosome movement, whereas tension generated at ki-

netochores by poleward microtubule flux is not. We speculate that this difference may result from the fact that the polar ejection force needs to be transduced from the chromosome arms through the elastic centromeric chromatin to the kinetochore, whereas the force exerted by poleward microtubule flux acts directly on the kinetochore, the likely location for the tension-sensitive mechanism.

Summary

Data from inhibition of molecules and examination of the dynamics of spindle components has begun to fill the gaps in our understanding of the process of chromosome congression. However, our complete understanding of congression may require the application of multiple experimental approaches as "something of a gulf exists between dynamics-centered and motor-centered views of spindle assembly and force generation" (Mitchison and Salmon, 2001). Thus, answers to outstanding questions, such as how does poleward microtubule flux contributes to chromosome congression, what is the tension-sensitive molecular switch that allows kinetochores to change direction of movement, and how can mechanisms of force generation be distinguished from sources of positional information, may only come through combining tools that perturb specific molecules with powerful new imaging technologies such as fluorescent speckle microscopy (Waterman-Storer et al., 1998).

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