

Motile Kinetochores and Polar Ejection Forces Dictate Chromosome Position on the Vertebrate Mitotic Spindle

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THE view still found in most cell biology textbooks for how chromosomes move to the equator of the forming mitotic spindle (i.e., congress), and then to the poles in anaphase, is based on a "traction fiber" model initially developed by Ostergren (reviewed in reference 54). In this model chromosomes are pulled towards opposite poles by the motions of their sister kinetochore fibers. To explain congression, the strength of the pulling force on a kinetochore was proposed to increase with kinetochore fiber length. Under this condition congression results from a "tug-of-war" between sister kinetochore fibers pulling towards opposite poles. Movement to the spindle equator occurs spontaneously because that is the position where the lengths of the kinetochore fibers, and thus the antagonistic pulling forces, are equal. When chromosome disjunction occurs at the onset of anaphase, the separated chromosomes are pulled poleward (anaphase A) by the same kinetochore fiber-based forces that produced congression.

Over the years a number of mechanisms have been proposed to explain the force-versus-kinetochore fiber length relationship central to Ostergren's model. These include the concepts, e.g., that kinetochore microtubules disassemble along their length (30); that kinetochore fibers contain a stretched elastic "collar" component (55); that contractile forces are produced along the fiber by actin and myosin (19); that kinetochore microtubules move poleward by treadmilling while disassembling at the poles (38); or that kinetochore microtubules are studded along their length with pole-directed microtubule motor molecules that act against the spindle matrix (64) or non-kinetochore fiber spindle microtubules (25, 41). Because kinetochore fiber microtubules are more stable than other spindle microtubules, the latter concept requires only that the cargo carrying tail end of a microtubule minus-end-directed motor (e.g., cytoplasmic dynein) binds more slowly to microtubules than the motor end (41).

Although such a "traction fiber" model for chromosome positioning is supported by some experimental evidence (e.g., 21, 24, 25, 52), new information over the past several years has shown that it is not accurate for mitosis in vertebrate cells. Here we review this new evidence and discuss

its ramifications for understanding chromosome congression and segregation during vertebrate mitosis. This evidence strongly favors the view that chromosome movements are generated by the motility of kinetochores along relatively stationary kinetochore microtubules and by pushing forces associated with the polar spindle microtubule arrays.

For brevity we have not addressed the mechanisms which establish and maintain the separation of spindle poles (for review see reference 74), or attempted to compare congression in vertebrates with non-vertebrate systems (where, with the exception of insect spermatocytes, there is little detailed information). Space limitations also precluded us from citing many of the primary references on which this review is based. Related information beyond the scope of this commentary can be found in recent papers on the mechanism of microtubule dynamic instability (e.g., 17); spindle microtubule dynamics (e.g., 61); potential roles of microtubule motors in spindle assembly and function (e.g., 41, 63); kinetochore (e.g., 13, 39) and centrosome (e.g., 32, 72) reproduction, structure and composition; non-microtubule components of the spindle (e.g., 34, 40); and cell cycle control of mitotic progression and chromosome disjunction (e.g., 16, 26, 68).

Spindle Structure and Chromosome Behavior during Vertebrate Mitosis

The primary structural components of the vertebrate spindle (reviewed in 57, 61) include two poles (i.e., centrosomes), the chromosomes with replicated "sister" kinetochores (one per chromatid), microtubules, and a large number of additional poorly characterized proteins. The spindle pole is defined by a radial (astral) array of dynamically unstable microtubules that are nucleated by the centrosome and grow and shorten by tubulin subunit addition and removal at their "plus" ends distal to the centrosome (Fig. 1). The kinetochore is defined as a multi-protein, plate-shaped complex, intimately associated with the centromeric DNA, that attaches the chromosome to spindle microtubules (e.g., 13, 39, 56). Spindle formation is initiated at the onset of prometaphase when nuclear envelope breakdown allows the kinetochores to interact with, and attach to, polar microtubules (reviewed in 58).

As a rule, chromosomes centrally located between the two poles at nuclear envelope breakdown usually become bi-

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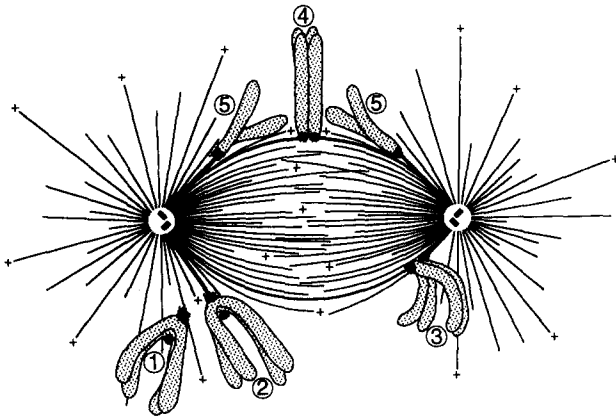


Figure 1. Schematic illustrating spindle microtubule polarity and chromosome behavior during mitosis in a vertebrate cell. The bipolar spindle is formed from two overlapping polar microtubule arrays each oriented with their microtubule “plus” (+) ends distal to the pole. Shown in the diagram are: (1) an attaching chromosome in which one kinetochore is exhibiting rapid poleward movement (gliding) along the surface of a polar microtubule; (2) a monooriented chromosome oscillating between poleward and away from the pole phases of motion; (3) a bioriented chromosome, near one pole which is initiating congression; (4) a fully congressed metaphase chromosome that is oscillating around the spindle equator; and (5) anaphase chromosomes that are undergoing poleward motion. The bundles of microtubules which tether kinetochores to the spindle poles are represented by the thick black lines.

oriented very rapidly, i.e., the chromosome acquires a bipolar attachment in which the sister kinetochores become attached to microtubules from opposing spindle poles (Fig. 1). In contrast, chromosomes closer to one pole at nuclear envelope breakdown usually become monooriented and move towards that pole as one kinetochore becomes attached to microtubules from the pole, while the other kinetochore remains unattached (e.g., reviewed in 56, 58, 60; Fig. 1). During the initial stages of attachment the kinetochore may move rapidly poleward, along the wall of a single microtubule, at velocities (25–50 $\mu\text{m}/\text{min}$) typical of cytoplasmic vesicles driven along microtubules by cytoplasmic dynein (reviewed in reference 1).

As the chromosome moves poleward, the kinetochore captures the plus ends of polar microtubules and motion slows to velocities typical of congression and segregation (1–3 $\mu\text{m}/\text{min}$). The outer kinetochore plate ultimately becomes saturated with polar microtubule plus ends (reviewed in 58; Fig. 1). Neighboring polar microtubules cluster around kinetochore microtubules to form the kinetochore fiber, and the microtubules within the kinetochore fiber are more stable than other spindle microtubules to treatments that disrupt microtubules (reviewed in references 56, 61). The enhanced stability of kinetochore fiber microtubules depends directly on the kinetochore since they rapidly disassemble or disperse if the kinetochore is pulled off the end of its associated microtubules (51), or if the kinetochore fiber is severed in the middle (69).

The kinetochore fiber and polar microtubules associated with a spindle pole define a half-spindle in which the great majority of microtubules possess the same growth polarity (plus ends distal to the centrosome; Fig. 1). Strong argu-

ments can be made that the bipolar animal cell spindle is simply two opposing half-spindles, tethered to one another by interactions between the opposing astral microtubule arrays and by the kinetochore fibers associated with bioriented chromosomes (reviewed in reference 11).

Congression is initiated for each chromosome after it attaches to the spindle, and its completion may require the entire period of spindle formation. Chromosome biorientation is considered to be a prerequisite for congression. Therefore, in order for a monooriented chromosome to congress, it must first acquire a bipolar connection. This likely occurs by the same mechanism that produces monoorientation, i.e., the unattached kinetochore encounters a microtubule growing from the opposite pole (Fig. 1). Once all of the chromosomes have congressed, the cell is considered to be at metaphase. In somatic cells metaphase is usually brief and is terminated when the sister chromatids separate and move towards opposite spindle poles during anaphase (Fig. 1). There is some evidence that tissue cells temporarily delay the onset of anaphase until all of the chromosomes have bioriented and fully congressed to form a “metaphase plate.” As a result, congression is considered to be monitored by a cell cycle checkpoint control as discussed in detail elsewhere (e.g., see references 16, 68).

A Kinetochore Can Undergo Motion away from Its Associated Pole in the Absence of a Poleward Force Acting on Its Sister: Kinetochores Exhibit Directional Instability

Most models of congression, including traction fiber models, assume that the force responsible for moving a kinetochore away from its attached pole is generated entirely by the pulling force on the other kinetochore towards the opposite pole (reviewed in 37, 43, 55, 61). However, it is now clearly established for vertebrates that a kinetochore frequently moves away from the pole to which it is attached even when its sister kinetochore is unattached (or absent as in anaphase). Indeed, monooriented chromosomes in most, if not all, vertebrates (e.g., chick [36]; rat kangaroo [60]; salamanders [65]; chimpanzee [Rieder, C. L., unpublished results]) oscillate conspicuously, moving both towards and away from the pole to which they are attached (Fig. 2, A and B). These oscillations can cover substantial distances, persist throughout all stages of mitosis including anaphase (reviewed in 67; Fig. 2 C), and continue on monooriented chromosomes when the distal sister kinetochore is destroyed by laser microsurgery (59).

The motion of a kinetochore away from its associate pole, in the absence of an antagonistic poleward pulling force on its sister, is not confined to monooriented chromosomes in mitosis. In many organisms the initial attachment of a meiosis I bivalent to the forming spindle results in rapid and forceful motion of the opposing kinetochores towards opposite poles, stretching the chromosome apart. In some cases, e.g., mantids and phasmids, this stretch may cover 2/3 or more of the spindle length (27). With respect to the mechanism of congression, it is significant that each kinetochore region on a stretched bivalent exhibits oscillatory movements towards and away from its respective pole, as it moves progressively closer to the forming metaphase plate. These kinetochore oscillations often occur independently of one

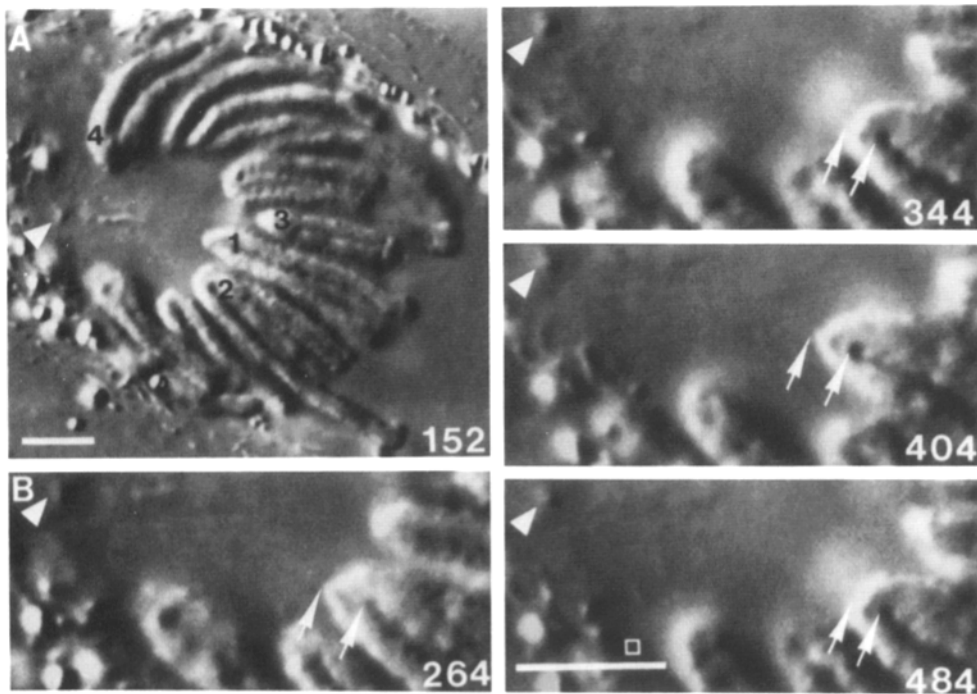
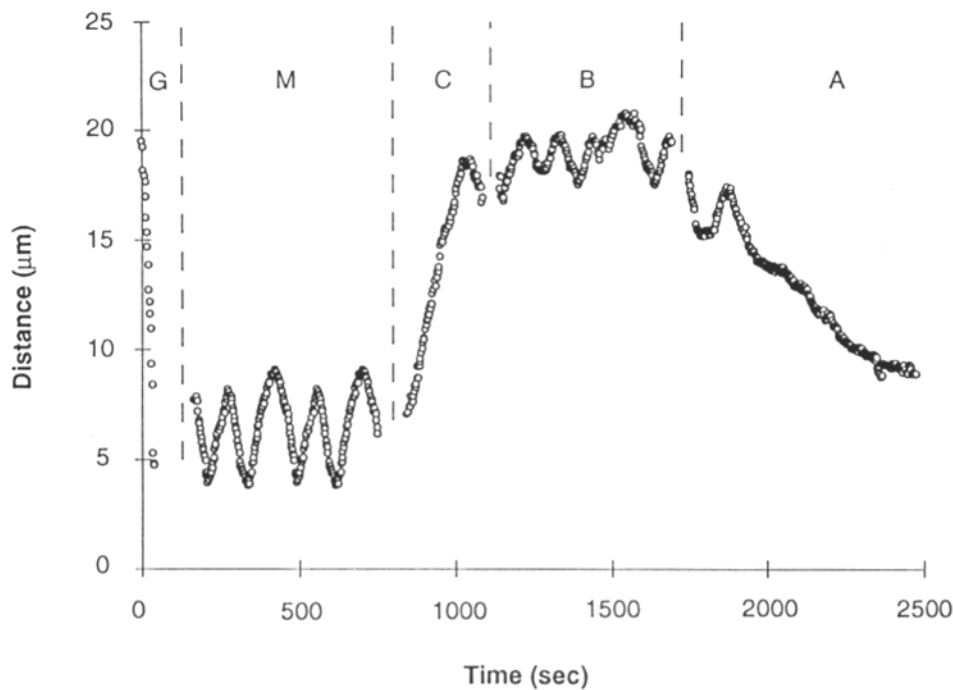


Figure 2. Video-enhanced DIC images of chromosome oscillations and kinetochore directional instability on a newt lung cell monopolar spindle. (A) Low-magnification view of a monopolar spindle. Some of the mono-oriented chromosomes are positioned many micrometers from the single spindle pole while others are closer. (B) Sequential high-magnification views of centromere deformation on chromosome 2 produced by the pulling and pushing forces associated with kinetochore directional instability. The narrow arrowheads mark the positions of the proximal and distal kinetochore regions, while the thick arrowhead marks the position of the pole. The box represents the size of the 8×8 pixel cursor used for tracking the motion of the centrosome and kinetochore regions. Time in sec in lower right hand corner (from Cassimeris et al. [11]). (C) Velocity versus duration plot of the movements a single kinetochore may exhibit throughout mitosis. Note that the history plot was generated by concatenating several files of actual kinetochore movements onto one time axis and aligning the resultant plots to represent the stages of movement one kinetochore could make throughout mitosis. *G*, gliding; *M*, monooriented; *C*, congression of a bioriented chromosome towards the equator; *B*, bioriented near the equator; and *A*, poleward motion during anaphase A (from Skibbens et al. [67]). Bars, $10 \mu\text{m}$.



another (20, 65), i.e., the “antagonistic fibers of a bivalent can operate in or out of phase” (20).

Recently Skibbens et al. (67) used high-resolution video microscopy and computer-assisted tracking techniques to measure the motion of individual kinetochores in living newt lung cells. They found that attached kinetochores switched abruptly (within periods of 6 s or less) between persistent phases of constant velocity poleward and away from the pole motion (on average $1.7 \mu\text{m}/\text{min}$; Fig. 2, B and C). During poleward motion the centromere was typically stretched poleward, while during motion away from the pole it became flattened or indented. For bioriented chromosomes, the

centromere became maximally stretched when both sister kinetochores were moving poleward, and maximally compressed when both were moving towards the spindle equator. Using deformation to infer the state of force applied to the centromere, Skibbens et al. (67) concluded for both mono- and bioriented chromosomes that the abrupt change from poleward to away from the pole motion involved a switch between a state in which the kinetochore pulled the chromosome poleward and a state in which the kinetochore was either actively pushing or at the very least moving away from its pole as fast or faster than the centromere region. This characteristic tendency of a kinetochore to autonomously

and abruptly switch between persistent phases of poleward and away from the pole movement was termed "directional instability". Significant displacement of the centromere on the spindle, as occurs during congression, was seen only when one kinetochore was in poleward motion pulling the centromere toward the equator while the other kinetochore was in away from the pole motion, pushing or not resisting centromere motion toward the equator.

Pulling and Pushing Forces That Act on the Kinetochore Are Generated Primarily at the Kinetochore: The Motion of Kinetochore Fiber Microtubules Makes Only a Minor Contribution

There are two extreme models for the site at which the force is produced for chromosome motion: it is produced along the length of the kinetochore fiber or at the poles (traction fiber models) or at the kinetochore (kinetochore motor models; reviewed in references 43, 58, 62). If the magnitude of the poleward force on a kinetochore is related to kinetochore fiber length, one would expect that severing a metaphase kinetochore fiber should shift the chromosome a predictable distance towards the pole facing the undamaged kinetochore fiber. This experiment has been conducted on various cell types (insect spermatocytes, vertebrate somatic cells, plant cells), using various cutting methods (UV light and micro-needles), with similar outcomes—the chromosome either does not shift (18, 49) or shifts only slightly towards the attached pole (15, 31, 69). The fact that only a short segment of the kinetochore fiber is needed to maintain the position of a metaphase chromosome near the spindle equator clearly reveals that the poleward force on a kinetochore is not proportional to kinetochore fiber length. The results of these cutting experiments are also inconsistent with the proposal that congression is mediated by an elastic material that becomes stretched between the pole and kinetochore as the chromosome attaches (e.g., 55; and reviewed in reference 43). Two of these studies (49, 69) also found that kinetochore poleward motion during anaphase does not stop when the kinetochore fiber is severed. The mechanism by which such a shortened kinetochore fiber supports the position and continued motion of the chromosome is unknown, but it likely involves interactions between the kinetochore-associated kinetochore fiber remnant and other spindle microtubules or spindle matrix components (reviewed in references 34, 40).

Kinetochore microtubule marking studies have also shown that kinetochores move at the plus ends of relatively stationary kinetochore microtubules during poleward and away from the pole motion. When a bioriented chromosome moves toward one pole, the kinetochore microtubules associated with that pole must shorten, while those microtubules on the sister kinetochore moving away from its pole must elongate. After labeled tubulin is injected into metaphase (e.g., 46) or early anaphase (66) cells, it incorporates into elongating kinetochore microtubules only at the kinetochore. When kinetochores move poleward during metaphase and anaphase, photobleached (10, 12, 23) or photoactivated fluorescent (44, 45) marks on the lattice of kinetochore microtubules remain relatively stationary with respect to the pole—showing that the kinetochore is also the primary site of kinetochore microtubule disassembly. However, the pho-

toactivation studies do reveal that kinetochore microtubules continuously move or "flux" poleward, but not away from the pole, at about 0.5 $\mu\text{m}/\text{min}$ in metaphase PtK and newt cells. This flux rate is only 1/3 the rate (0.5 versus 1.7 $\mu\text{m}/\text{min}$) of the poleward motion exhibited by an oscillating kinetochore and does not contribute to away from the pole motion. There is also evidence that the motion of kinetochores along kinetochore microtubule ends stops in late anaphase, after which the chromosomes are slowly pulled poleward solely by the flux (45). Nevertheless, it can be concluded from these studies that during metaphase and early anaphase, 60–70% of kinetochore poleward motion, and all motion away from the pole, results from the motion of kinetochores at the plus ends of their kinetochore microtubules, i.e., kinetochore directional instability is a property of kinetochore motility at the kinetochore microtubule plus ends.

There is now solid behavioral evidence that the forces for kinetochore poleward and away from the pole motion, during prometaphase (reviewed in references 11, 58, 67) and anaphase (49, 67), are produced at the kinetochore. Indeed, both plus- and minus-end-directed microtubule motor molecules have been localized to the kinetochore by functional (e.g., 28, 29) and immunologic approaches (e.g., 75, 76). It is highly likely that these motors are involved in the production of kinetochore poleward motion and, in combination with the polar ejection forces (discussed below), kinetochore motion away from its associated pole. There is also evidence that kinetochore microtubule growth itself can contribute a pushing force (e.g., 7, 66) and that depolymerization of kinetochore microtubules at their plus ends can pull on the kinetochore (e.g., 14, 33).

In contrast to severing the kinetochore fiber, destroying one kinetochore on a bioriented chromosome always results in substantial chromosome motion towards the pole to which the undamaged kinetochore is attached (24, 31, 42), while destroying the kinetochore on an anaphase chromosome inhibits further poleward motion (5). If force production for poleward motion occurs primarily at the kinetochore, then the poleward force-producing potential of each sister kinetochore should be nearly the same (58). Thus, trivalents would be expected to acquire a final congression position closer to the pole to which two of the three kinetochores are attached but not, as interpreted by Östergren (52) and Hays et al. (25), because poleward force is proportional to kinetochore fiber length. Rather, it would be because poleward force production on the side of the chromosome containing two kinetochores is twice that produced on the other side. Similarly, partial (24) or complete (e.g., 42) destruction of a metaphase kinetochore would be expected to produce motion towards the pole to which the non-irradiated kinetochore is attached because the progressive destruction of force producers on one side of the chromosome creates an imbalance in forces. In all of these cases the length of the kinetochore fiber would be an outcome of congression and not its cause.

Chromosome Position Is Influenced by Half-Spindle Microtubule Arrays That Exert an Away from the Pole Ejection Force on the Chromosome

Monooriented chromosomes on monopolar and bipolar spindles can acquire an average distance relative to the spindle pole that approaches one-half the distance exhibited by

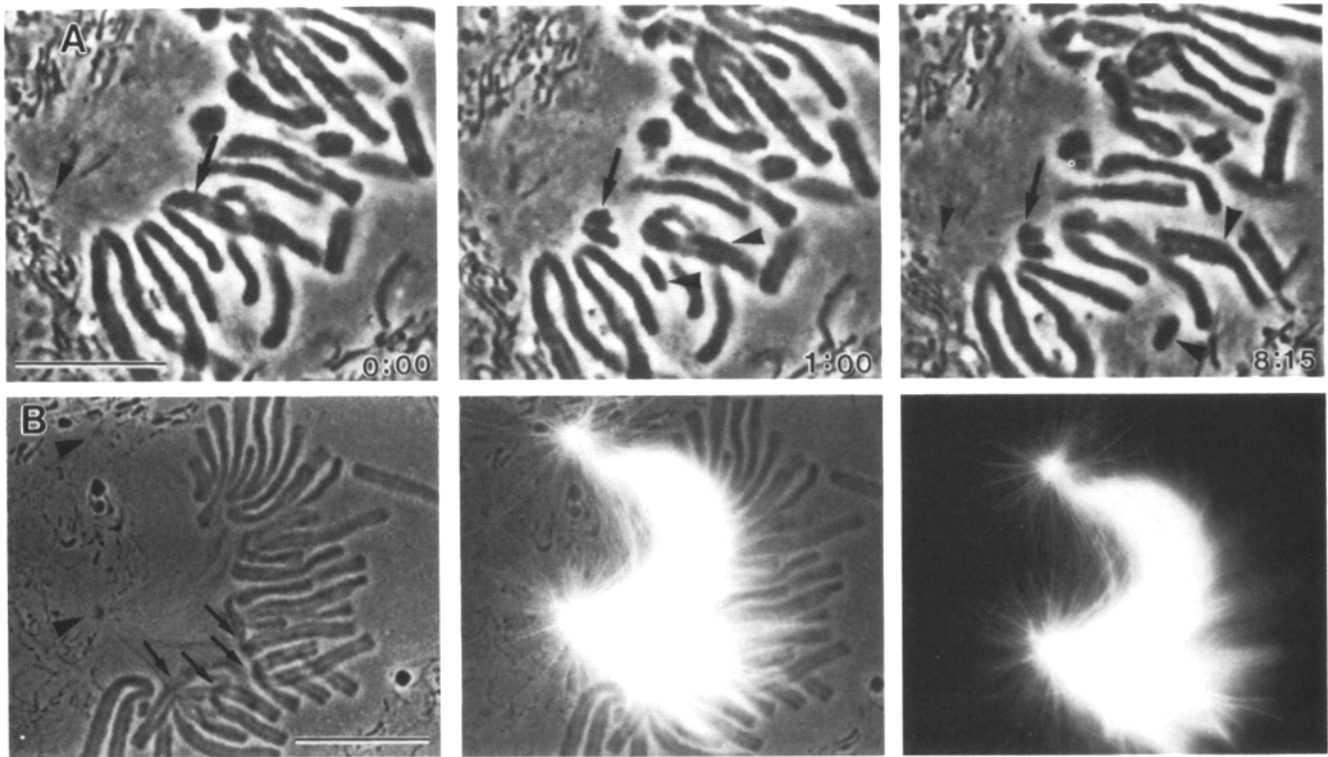


Figure 3. The behavior of laser-generated chromosome fragments and kinetochore regions (*A*), and the relationship between chromosome position and microtubule distribution (*B*), during prometaphase. (*A*) Three frames, from a phase-contrast time-lapse video series, depicting the behavior of a monooriented chromosome after its arms are severed from its centromere region by a laser microbeam. In the first frame the centrosome is noted by an arrowhead while the experimental chromosome, and the plane of the impending laser cut, is noted by the arrow. In the second frame the two acentric fragments generated by the cut (*arrowheads*) are being ejected away from the spindle pole as the centromere region moves closer to the centrosome. In the final frame the acentric fragments (*large arrowheads*) are fully ejected and reside at the spindle periphery, while the centromere region (*arrow*) has acquired a new average position closer to the pole (*small arrowhead*). Time in min/sec in lower right hand corner of each micrograph. (*B*) Phase-contrast (*left*), anti-tubulin immunofluorescent (*right*), and simultaneous phase-contrast/immunofluorescent (*middle*) micrographs of the same prometaphase newt spindle. The arrowheads in the phase micrograph mark the two spindle poles while the arrows note some of the chromosomes that are monooriented to the lower pole (cf. *A*). Note that these chromosomes are positioned well distal to the pole and that they reside at the peripheral regions of high microtubule density. Bar, 25 μm .

a metaphase chromosome on a bipolar spindle (e.g., 3, 4, 11, 35, 59; Figs. 2 *A* and 3). The observation that a kinetochore exhibits frequent motions away from its associate pole in the absence of an antagonistic poleward pulling force (e.g., Fig. 2 *B*), and that monooriented chromosomes can become positioned many micrometers from their associated pole (e.g., Figs. 2 *A* and 3) are both fully consistent with the hypothesis that prometaphase chromosomes are influenced by two classes of forces: those associated with the kinetochores that exert poleward and away from the pole forces on the centromere, and those produced by half-spindle microtubule arrays that exert an away from the pole push on the chromosome as a whole.

There is considerable descriptive evidence suggesting that chromosomes experience forces that move them away from the pole independent of kinetochore function (e.g., reviewed in references 6, 47, 58). Rieder et al. (59) directly demonstrated, by severing chromosomes in living prometaphase cells with a laser microbeam, that chromosome fragments lacking kinetochores are actively transported away from the closest spindle pole at velocities approaching 2 $\mu\text{m}/\text{min}$ (Fig. 3 *A*). Because they lack kinetochores, the motion of these

fragments away from the pole cannot be due to forces produced by kinetochore fibers. Using the same laser-cutting approach it was further shown that when one or both arms were severed close to the kinetochore region of a monooriented chromosome, the kinetochore-containing fragment underwent persistent poleward motion until it achieved a new position closer to the pole (4; Fig. 3 *A*). In general, as the cross-sectional area perpendicular to the kinetochore fiber axis is reduced, the closer to the pole the fragment moves. Thus the position of a monooriented chromosome relative to its spindle pole is at least partly influenced by the size of the chromosomal surface area facing the pole.

It can be concluded from these observations that the polar arrays of aster and half-spindle microtubules generate ejection forces that both resist chromosome poleward movement and push chromosomes away from the poles, and that these polar ejection forces are partly responsible for positioning prometaphase chromosomes on the spindle (2, 4, 11, 59, 62). That microtubules are involved in producing these polar ejection forces is evident from several observations. First, unattached chromosomes and chromosome fragments do not experience displacements away from the poles when in an

environment devoid of microtubules, e.g., after colcemid treatment, when positioned outside of the spindle, or within the interzone of an anaphase-like prometaphase cell (e.g., reference 1). Second, a positive correlation exists between the number of astral or half-spindle polar microtubules within the vicinity of a monooriented chromosome and the kinetochore-to-pole distance (2, 11; Fig. 3 B). Finally, when cells containing monopolar spindles are treated with drugs (e.g., nocodazole or taxol) or temperature shifts that affect microtubule turnover, chromosome oscillations quickly cease and the chromosomes assume a new position relative to the pole characteristic of the drug treatment (4, 11). When chromosome arms in nocodazole- or taxol-treated monopolar spindles are severed from the kinetochore region by a laser, they do not move further from the pole (4). These findings demonstrate that the polar ejection forces generated in association with each aster and half-spindle are mediated by microtubules, and they further suggest that this force is dependent on the dynamically unstable nature of microtubule growth.

The nature of the ejection force remains to be determined, although we favor a mechanism based on the impact of growing aster and half-spindle microtubule plus ends against the chromosomes (4, 11, 58, 61). It has also been proposed that microtubule plus end (kinesin-like) motor molecules are distributed within the chromatin and interact with polar microtubules to provide the molecular basis for the ejection force (70, 77).

What Controls Kinetochore Switching to Allow Chromosome Congression followed by Anaphase Motion?

Skibbens et al. (67; see also references 47, 60) found that as a monooriented chromosome bioriented and congressed to the equator, the kinetochore attached to the closer pole persisted in motion away from that pole while the kinetochore facing the equator persisted at a similar velocity in poleward motion, until the centromere was near the equator (Fig. 2 C). Once near the equator, each sister kinetochore began switching between poleward and away from the pole phases of motion at frequencies and velocities similar to those exhibited by monooriented chromosomes (Fig. 2 C). Although each sister kinetochore sometimes switched between poleward and away from the pole motion independently of one another, switching was usually cooperative: 70% of the time when one kinetochore was in a poleward phase the other was in an away from the pole phase. By contrast, after anaphase onset the separated sister kinetochores persisted in poleward motion, only occasionally switching to motion away from the pole of short duration.

How is the cooperative switching of sister kinetochores on a congressing chromosome controlled so that one is pulled while the other is pushing (or at least passively following) the centromere towards the equator? A related question is how this control process is modified at anaphase onset so that each kinetochore moves progressively closer to the pole instead of simply achieving a new stable position, as do monooriented prometaphase chromosomes, which also have a single active kinetochore.

The answer to these questions requires the elucidation of two sets of interrelated mechanisms: one that causes the kinetochore to switch between poleward and away from the

pole motion, and another that senses whether a chromosome is mono- or bioriented and appropriately biases kinetochore switching as a function of distance from the poles. Since the growth and shortening of kinetochore microtubules in response to congression movements occurs primarily by subunit addition/deletion at the kinetochore, abrupt transitions between poleward and away from the pole motion must be accompanied by a cooperative switching between shortening and growth in all of the microtubule plus ends associated with the kinetochore. The switching of kinetochore microtubule ends is likely mediated by factors associated with the kinetochore that, e.g., control GTP hydrolysis in the terminal tubulin subunits. The observations that promoting kinetochore microtubule assembly induces kinetochores to switch into motion away from the pole (7, 66), while blocking assembly induces poleward motion (4, 10, 12), clearly reveals that the directional switching of kinetochores is somehow closely coupled to microtubule assembly/disassembly.

The kinetochore-associated plus and minus end microtubule motors described in the previous section are likely responsible for keeping the kinetochore attached to the plus ends of its associated microtubules as they shorten and elongate during chromosome motion. Any directional change in kinetochore motion must involve a cooperative and bulk switching between these antagonistic motors. Vale et al.'s (71) observation that microtubules spontaneously oscillate back and forth on surfaces coated with both dynein and kinesin raises the possibility that the directional instability of kinetochores somehow results from competing plus and minus end-directed kinetochore-associated microtubule motors. In this context, Hyman and Mitchison (28, 29) have shown that kinetochores on isolated chromosomes always move microtubules over their surface toward the microtubule minus end (poleward) when unphosphorylated, and toward the microtubule plus end (away from the pole) when phosphorylated.

Whether the directional instability of kinetochores is caused by abrupt changes in kinetochore microtubule assembly conditions and/or motor activity is not known. Regardless, the kinetochore must be "smart" as initially suggested by Mitchison (43) in that either of these potential mechanisms must, in some way, sense information within the spindle about kinetochore position relative to its pole and the activity of its sister. In response to this information, the kinetochore switches between its persistent states of poleward and away from the pole motion. How kinetochore directional instability is controlled within the spindle is not yet well understood, but it appears related to vectorial information provided by the density of the polar microtubule arrays and tension at the kinetochore microtubule attachment sites.

A simple observation demonstrates that polar microtubule density is a major factor in controlling chromosome position. Monooriented chromosomes in both monopolar and bipolar spindles achieve positions much closer to the pole in regions of low microtubule density than similar chromosomes in regions of higher microtubule density (4, 11, 59). Thus, models for controlling kinetochore motion based on microtubule-independent concentration gradients emanating from the spindle poles do not explain chromosome behavior within the spindle.

Controlling factors whose concentration depends in some

way solely on microtubule density can potentially explain kinetochore directional instability on monopolar spindles, but such gradient models still fail to explain congression. For example, consider a hypothesis for congression based on differential kinetochore phosphorylation where dephosphorylation induces poleward motion and phosphorylation induces motion away from the pole. For congression to spontaneously occur, the kinetochore further from the equator must persist in motion away from the pole (phosphorylated) while the kinetochore closer to the equator must persist in poleward motion (unphosphorylated). It is not clear how to define kinase/phosphatase activity gradients that generate the required differential phosphorylation of sister kinetochores, which are separated by only 1–2 μm , but situated anywhere from a few to 30 μm from the poles in newt lung cells. Remember, kinetochores on monooriented chromosomes situated 5–10 μm from the pole oscillate between poleward and away from the pole motions with similar durations and velocities as kinetochores on bioriented chromosomes at the spindle equator, 20–25 μm from the poles.

At this time it appears much more likely that kinetochores obtain vectorial (magnitude and direction) information directly from the spindle microtubule arrays, i.e., positional information is encoded by the density as well as the orientation or growth direction of microtubules. The activity of sister kinetochores must also feedback on each other so that bioriented chromosomes spontaneously congress to the equator (one kinetochore in a poleward phase of motion while the other in an away from the pole phase), while separated chromatids move poleward following disjunction at anaphase. Thus far, two different vectorial models have been proposed. Hyman and Mitchison (29) suggest that the type of motor activity exhibited by a kinetochore, and thus its direction of motion, depends on the number of microtubules associated with the kinetochore. Under conditions of low kinetochore microtubule numbers it remains largely unphosphorylated and the poleward motor dominates, but as it acquires more kinetochore microtubules it becomes progressively phosphorylated favoring away from the pole motor activity. In their scheme the number of microtubules on a kinetochore is envisioned to depend on proximity to its associated pole; a kinetochore that is closer to its pole possesses more microtubules because it resides in a higher density of recruitable polar microtubules than its sister which is further from its pole (and its source of kinetochore microtubules). Chromosomes move to the spindle equator because that is the position where the density of polar microtubules emanating from opposite poles is similar. There, sister kinetochores would (and do, see reference 56) have similar numbers of kinetochore microtubules. This is an interesting hypothesis that is supported by some *in vitro* observations (28, 29, 71). Unfortunately, it is not consistent with chromosome behavior under experimental conditions. For example, instead of remaining stationary or moving away from the pole as predicted by the model, a metaphase kinetochore fully saturated with microtubules (and presumably phosphorylated) immediately initiates poleward motion when its sister is destroyed (e.g., 42).

Nicklas (reviewed in reference 48) showed that pulling a chromosome away from the pole with a microneedle induces the centromere to move away from the pole at a velocity limited by the elongation rate of the stretched fiber. This re-

veals that tension at the kinetochore/chromosome junction, caused by forces directed away from the pole, switches the stretched kinetochore into a phase in which its associated kinetochore fiber elongates allowing (or causing) the chromosome to shift away from the pole. Conversely, tension at the kinetochore of a monooriented or bioriented chromosome, caused by polar ejection forces on the arms or the pulling of a sister kinetochore, can be relieved by respectively severing the bulk of the arms (59; Fig. 3 A) or ablating one sister kinetochore with a laser (e.g., reference 42). Under both conditions decreasing those forces on the chromosome that are directed away from the pole decreases tension on the attached kinetochore and induce the chromosome to undergo poleward motion until it achieves a new position closer to its pole (e.g., Fig. 3 A). Finally, kinetochores on adjacent monooriented chromosomes switch between poleward and away from the pole phases of motion independently of one another whereas the switching of sister kinetochores on bioriented chromosomes near the spindle equator is highly coordinated as discussed above. This reveals that force changes at one kinetochore are transmitted to and influence the behavior of its sister. In the next section we discuss a model of vertebrate cell mitosis in which tension at the kinetochore microtubule attachment sites, generated by polar ejection forces on the chromosome arms and the activity of the sister kinetochore, provide the vectorial control of kinetochore directional instability necessary for chromosome congression and segregation to the poles.

A “Kinetochore Motor/Polar Ejection” Model for Congression and Anaphase A in Vertebrates

The model (Fig. 4) discussed here is based on kinetochore directional instability (11, 67) and polar ejection forces on the chromosome arms (4, 59), with the following proposed properties.

Kinetochore Directional Instability. (a) Poleward and away from the pole kinetochore motion is insensitive to changes in kinetochore tension, until switching occurs.

(b) The stochastic durations of kinetochore poleward and away from the pole phases of motion depend differently on the magnitude of tension at the kinetochore: kinetochores persist in poleward motion until the tension at the kinetochore makes the probability of switching to motion away from the pole likely. Kinetochores then persist in motion away from the pole until tension is sufficiently low or compression is sufficiently high to promote switching back to poleward motion. These concepts of kinetochore directional instability and the kinetochore as a tensiometer are based on a one-dimensional-biased random walk where tension or compression at the kinetochore microtubule attachment site biases the probabilities of switching between poleward and away from the pole phases of motion. However, since the poleward and away from the pole kinetochore states are distinctly different, they must have different probabilities of switching between directions of motion at any given kinetochore tension level (hysteresis in response to changes in tension).

(c) The switch in the kinetochore between poleward and away from the pole states may involve changes in kinetochore phosphorylation or other types of chemical reactions that control either motor activity or kinetochore microtubule dynamic instability. However, in our model these are down-

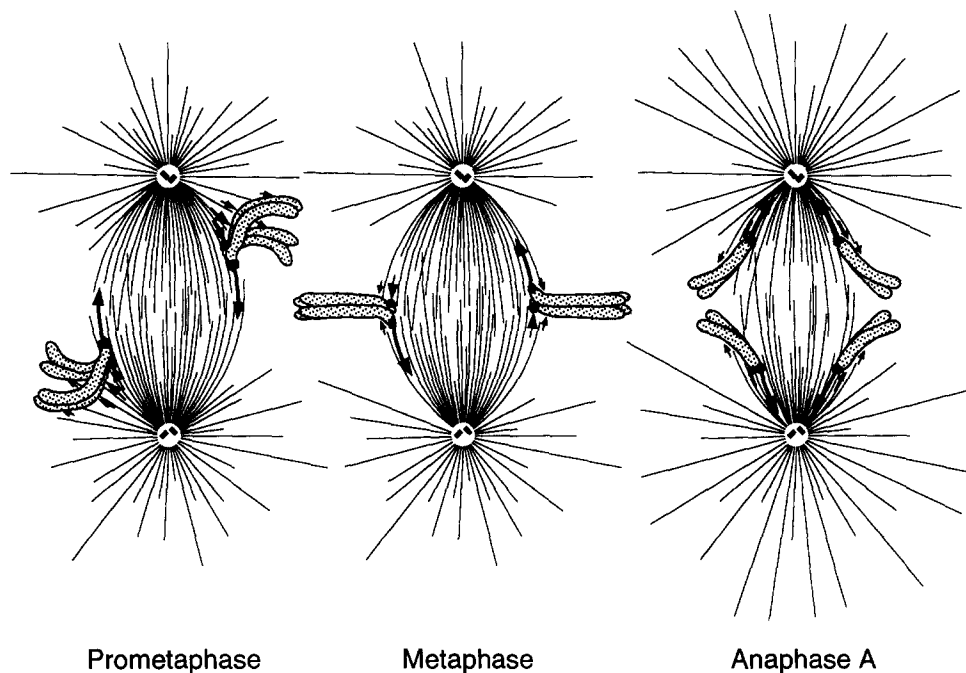


Figure 4. The kinetochore motor, polar ejection model views the kinetochore as an “active” and “smart” organelle capable of autonomously switching between distinctly different states of poleward and away from the pole motion along relatively stationary kinetochore fiber microtubules (kinetochore directional instability). In the drawing, arrows at the centromere indicate the direction of kinetochore motion and the strengths of kinetochore pulling or pushing forces on the centromere. For clarity, the bundles of kinetochore fiber microtubules are not drawn (see Fig. 1). This model also proposes that the polar arrays of spindle microtubules resist chromosome penetration towards the poles, and actively push the chromosomes away

from the pole in the direction of microtubule growth with a strength that is proportional to microtubule density, which increases closer to the poles within the central spindle. These polar ejection forces (represented by *arrows* on the chromosome arms) are envisioned to provide vectorial information to bias the relative duration of kinetochore poleward and away from the pole motion and push the chromosome arms to the periphery of the spindle. During congression the sister kinetochore facing the equator persists in pulling the chromosome towards its associated pole while the kinetochore facing away pushes the chromosome towards the equator by actively moving away from its associated pole. The polar ejection forces on the chromosome from the closer pole biases the proximal kinetochore into away from the pole motion and the distal kinetochore into poleward motion. The chromosomes oscillate back and forth near the equator because that is the position where the polar ejection forces from opposite poles are balanced. When chromosome disjunction occurs at the onset of anaphase, the relative motions of sister kinetochores no longer influence one another and kinetochores exhibit primarily poleward phase motion pulling the sister chromatids poleward in anaphase A.

stream biochemical events modulated by tension at the kinetochore.

Polar Ejection Forces. (a) Each of the two polar spindle microtubule arrays produce away from the pole ejection forces on the chromosome whose magnitude and direction depend, respectively, on the local density and orientation of polar microtubules. These polar ejection forces produce tension on attached kinetochores.

(b) There are two components that contribute to polar ejection forces. One is a “steric” resistance to chromosome penetration into regions of high microtubule density. The other is an away from the pole force (the “polar winds”) which push a chromosome away from the pole at a velocity which depends on kinetochore pulling/pushing forces and drag force on the chromosome.

(c) The polar ejection forces on the chromosome arms from opposite spindle poles are independent and antagonize each other.

In this model (Fig. 4) a kinetochore undergoes poleward motion when it first acquires kinetochore microtubules because it is initially under no or low tension. Under this condition the attaching kinetochore of a monoorienting chromosome persists in poleward motion, pulling the chromosome poleward against the polar ejection force within that half-spindle. Poleward motion continues until the chromosome moves into a region of microtubule density where the polar ejection force is high enough to raise the tension at the

kinetochore (seen by the extent of centromere stretching) to a level which makes the probability of switching to away from the pole motion likely. After switching to an away from the pole state, the kinetochore moves away from the pole at constant velocity. If this velocity is faster than the away from the pole chromosome velocity produced by the polar ejection forces, then the extent of centromere stretching decreases. When tension becomes low or the centromere is sufficiently compressed, the probability of switching back to poleward motion becomes likely. The non-periodic oscillatory motion of a monooriented chromosome is thus ascribed to both the stochastic nature of kinetochore directional instability and to fluctuations in the distance dependence of the ejection force magnitude within that region of the aster or half-spindle containing the chromosome. Since a monooriented chromosome on a bipolar spindle resides closer to its attached pole, than to the opposing distal pole, its position is influenced almost exclusively by the directional instability of one kinetochore and one polar ejection force.

Congression to the spindle equator involves interactions between sister kinetochore directional instabilities and ejection forces from opposite poles. The unattached kinetochore on a monooriented chromosome will exhibit a poleward phase motion when it first attaches to microtubules from the distal pole because it is initially under no tension. As it moves toward the equator, its motion will stretch the centromere, increasing the tension on the proximal kinetochore

and eventually inducing the proximal kinetochore to switch into or persist in motion away from its associated pole. The net polar ejection force on the chromosome is away from the proximal pole because it is the closest spindle pole. As a result, tension at the proximal kinetochore will be higher than at the distal kinetochore, biasing the distal kinetochore into poleward motion and the proximal kinetochore into motion away from the pole. This bias results in persistent chromosome motion towards the equator.

Bioriented chromosomes move to and oscillate about the equatorial plane because this is the position between the poles where the effects of opposing polar ejection forces on the directional instability of sister kinetochores are, on average, equal. At the equator sister kinetochores try to autonomously switch between poleward and away from the pole motion, but autonomous switching is biased by the tension produced at each kinetochore by the antagonistic activity of its sister's motion and the effects of unequal polar ejection forces when the centromere moves off the spindle equator.

When chromosomes disjoin at anaphase the linkage between sister kinetochores is broken. This abolishes the mutually antagonistic effects that sister kinetochores have on one another and, in effect, creates two monooriented chromosomes. On a bipolar spindle the distance between the equator and the poles is typically two or three times the average distance from the poles achieved by monooriented chromosomes (11; Figs. 2 *A* and 3 *A*). Thus, the magnitudes of the polar ejection forces at the equator are likely much lower than that required to induce the kinetochore on a monooriented chromosome to switch between poleward and away from the pole motion. As a result the kinetochore persists in poleward motion until the polar microtubule density increases the strength of the polar ejection force sufficiently to induce away from the pole motion and oscillations about positions near the pole. Transient switches to away from the pole motion may also be induced by various conditions that cause a transient elevation of kinetochore tension, e.g., by bridges between separating chromatid arms or obstructions within the cell.

Evaluating the Model

Some data appear, at a cursory level, to be inconsistent with our model. Monooriented chromosomes can become positioned very close to the pole to which they have attached (e.g., Figs. 2 and 3)—implying that the ejection force associated with that pole is too weak to be involved in congression. However, spindle formation in vertebrates is accompanied by rapid and dynamic rearrangements of astral microtubules which, in turn, lead to dramatic changes in the vectors along which the ejection forces are expected to work: the two prominent radial microtubule arrays are progressively converted into a compact, microtubule-rich, fusiform-shaped structure with greatly reduced asters (reviewed in 57, 72). It is not unusual during this process for the symmetry of each forming half-spindle to change so that it contains regions of high and low microtubule density, i.e., microtubule density changes not only with distance from the pole but also with azimuth and inclination from the pole-to-pole axis. When in a region of low microtubule density, which often arises where the spindle (or half-spindle) joins the aster (11, 59), monooriented chromosomes are able to

approach very close to the centrosome. By contrast, in regions of higher microtubule density (e.g., Fig. 3 *B*) such chromosomes would be expected to adopt a position substantially farther from the aster center. In this context it is noteworthy that monooriented chromosomes on vertebrate bipolar spindles do not reside within the microtubule-rich central spindle but are excluded by "transverse equilibria" forces (6, 50, 53) to the periphery of this region where microtubule density is lower (chromosomes 1 and 2 in Fig. 1). Although poorly defined, the transverse equilibria forces likely arise from the ejection properties of the aster and half-spindle and from the tendency of inclusions to be excluded between adjacent microtubules as they form ordered arrays. When the unattached kinetochore of a monooriented chromosome attaches to the distal pole, the resultant centripetal force generated by the poleward motion of the attaching kinetochore pulls the centromeric region of the chromosome into the polarized mass of microtubules that forms the central (half) spindle (e.g., chromosome #3 in Fig. 1). Under this condition the kinetochore positioned near the closer pole would be expected to switch into an away from the pole phase of motion as it experiences a sudden increase in the magnitude of the ejection force associated with that pole.

A similar concern involves an experiment where one kinetochore of a large metaphase chromosome is destroyed by laser irradiation (e.g., 31, 42). Under this condition the chromosome moves closer to the pole to which the non-irradiated kinetochore is attached—apparently in conflict with our tenet that the metaphase half-spindle exerts an away from the pole ejection force on the chromosome. However, as discussed above, the ejection force associated with a half-spindle can be substantially less at the spindle equator than the poleward pulling force at the kinetochore within that half-spindle. At the equator, the directional instability of sister kinetochores antagonize each other. Thus, when one kinetochore is destroyed the chromosome would be expected to initiate poleward motion in the half-spindle containing the undamaged kinetochore. Moreover, the now monooriented chromosome would also be expelled laterally from the microtubule-rich central spindle into the region of much lower microtubule density at the spindle periphery by the transverse equilibria forces in bipolar spindles. Once within this peripheral region, where ejection force is low, chromosomes with only one functional kinetochore would be expected to move very close to the pole.

Brinkley et al.'s (9) conclusion from fixed cells that kinetochores achieve a metaphase alignment even after being separated by caffeine treatment from the bulk of their chromatin, also appears inconsistent with our premise that away from the pole ejection forces are necessary for congression. However, most if not all of these kinetochores reside well within the central spindle where microtubule density, and thus the ejection forces, are expected to be the highest. Moreover, each has an appreciable surface area (79) over which the ejection forces can work.

Our model predicts that chromosomes should congress closer to one pole of the spindle, if the spindle is formed from two half-spindles that differ significantly in microtubule density. Such spindles rarely occur in nature, so this prediction has yet to be tested rigorously. However, Fig. 11 in Belar and Huth (8) depicts a late metaphase/early anaphase spindle in a worm oocyte in which one half-spindle is

clearly weaker than the other. In this figure the chromosomes are not positioned midway between the poles, but are shifted significantly closer to the pole in the smaller half-spindle.

Finally, anaphase chromosomes are monooriented yet they ultimately move much closer to their associated pole than many of the monooriented chromosomes on monopolar and bipolar prometaphase spindles. There are several possible non-mutually exclusive explanations for this behavior. The magnitude of the polar ejection forces on an anaphase chromosome may be less than on a prometaphase chromosome because former have a smaller cross-sectional area than the latter (62). Moreover, at anaphase onset dramatic changes could occur in the capacity of the cell to maintain the half-spindle ejection forces, e.g., changes that reduce microtubule assembly and/or turn off the away from the pole force producers. Indeed, at anaphase onset proteins in the spindle pole are dephosphorylated (72), the apparent γ tubulin content of this microtubule nucleating center decreases (78) and the cdc2/cdc28-cyclin B complex (MPF) is degraded (e.g., 26)—events that appear to radically change the assembly properties and length of polar microtubules (e.g., 73). As a result, the number and/or length of microtubules within the anaphase half-spindle progressively decrease with the greatest number of microtubule plus ends found just in front of the poleward moving chromosomes (e.g., 22).

Summary

We argue that hypotheses for how chromosomes achieve a metaphase alignment, that are based solely on a tug-of-war between poleward pulling forces produced along the length of opposing kinetochore fibers, are no longer tenable for vertebrates. Instead, kinetochores move themselves and their attached chromosomes, poleward and away from the pole, on the ends of relatively stationary but shortening/elongating kinetochore fiber microtubules. Kinetochores are also “smart” (43) in that they switch between persistent constant-velocity phases of poleward and away from the pole motion, both autonomously and in response to information within the spindle. Several molecular mechanisms may contribute to this directional instability including kinetochore-associated microtubule motors and kinetochore microtubule dynamic instability. The control of kinetochore directional instability, to allow for congression and anaphase, is likely mediated by a vectorial mechanism whose magnitude and orientation depend on the density and orientation or growth of polar microtubules. Polar microtubule arrays have been shown to resist chromosome poleward motion and to push chromosomes away from the pole. These “polar ejection forces” appear to play a key role in regulating kinetochore directional instability, and hence, positions achieved by chromosomes on the spindle.

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References

- Alexander, S. P., and C. L. Rieder. 1991. Chromosome motion during attachment to the vertebrate spindle: initial saltatory-like behavior of chromosomes and a quantitative analysis of force production by nascent kinetochore fibers. *J. Cell Biol.* 113:805–815.
- Ault, J. G. 1986. Stable versus unstable orientations of sex chromosomes in two grasshopper species. *Chromosoma* 93:298–304.
- Ault, J. G., and C. L. Rieder. 1992. Chromosome malorientation during mitosis. *Cell Motil. Cytoskeleton.* 22:155–159.
- Ault, J. G., A. J. DeMarco, E. D. Salmon, and C. L. Rieder. 1991. Studies on the ejection properties of asters: astral microtubule turnover influences the oscillatory behavior and positioning of monooriented chromosomes. *J. Cell Sci.* 99:701–710.
- Bajer, A. S. 1972. Influence of UV microbeam on spindle fine structure and anaphase chromosome movements. *Chromosomes Today.* 3:63–69.
- Bajer, A. S., and J. Mole-Bajer. 1972. Spindle dynamics and chromosome movements. *Int. Rev. Cytol. Suppl.* 3: 271 pp.
- Bajer, A. S., C. Cypher, J. Mole-Bajer, and H. M. Howard. 1982. Taxol-induced anaphase reversal: evidence that elongating microtubules can exert a pushing force in living cells. *Proc. Natl. Acad. Sci. USA.* 79:6569–6573.
- Belar, K., and W. Huth. 1933. Zur teilungsautonomie der chromosomen. *Zeit. Zellforsch. Mik. Anat.* 17:51–67.
- Brinkley, B. R., R. P. Zinkowski, W. L. Mollon, F. M. Davis, M. A. Pisegna, M. Pershouse, and P. N. Rao. 1988. Movement and segregation of kinetochores experimentally detached from mammalian chromosomes. *Nature (Lond.)* 336:251–254.
- Cassimeris, L., and E. D. Salmon. 1991. Kinetochore microtubules shorten by loss of subunits at the kinetochores of prometaphase chromosomes. *J. Cell Sci.* 98:151–158.
- Cassimeris, L., C. L. Rieder, and E. D. Salmon. 1994. Microtubule assembly and kinetochore directional instability in vertebrate monopolar spindles: implications for the mechanism of chromosome congression. *J. Cell Sci.* In press.
- Centonze, V. E., and G. G. Borisy. 1991. Pole-to-chromosome movements induced at metaphase: sites of microtubule disassembly. *J. Cell Sci.* 100:205–211.
- Cooke, C. A., D. P. Bazett-Jones, W. C. Earnshaw, and J. B. Rattner. 1993. Mapping DNA within the mammalian kinetochore. *J. Cell Biol.* 120:1083–1091.
- Coue, M., Lombillo, V. A., and J. R. McIntosh. 1991. Microtubule depolymerization promotes particle and chromosome movement in vitro. *J. Cell Biol.* 112:1165–1175.
- Czaban, B. B., A. Forer, and A. S. Bajer. 1993. Ultraviolet microbeam irradiation of chromosomal spindle fibers in *Haemaphysalis katherinae* endosperm. I. Behavior of the irradiated region. *J. Cell Sci.* 105:571–578.
- Earnshaw, W. C., R. L. Bernat, C. A. Cooke, and N. F. Rothfield. 1991. Role of the centromere/kinetochore in cell cycle control. *Cold Spring Harbor Symp. Quant. Biol.* 56:675–685.
- Erickson, H. P., and E. T. O'Brien. 1992. Microtubule dynamic instability and GTP-hydrolysis. *Annu. Rev. Biophys. Biomol. Str.* 21:145–166.
- Forer, A. 1965. Local reduction of spindle fiber birefringence in living *Nephrotomasuturalis* (Loew) spermatocytes induced by ultraviolet microbeam irradiation. *J. Cell Biol.* 25:95–117.
- Forer, A. 1988. Do anaphase chromosomes chew their way to the pole or are they pulled by actin? *J. Cell Sci.* 91:449–453.
- Fuge, H. 1989. Rapid kinetochore movements in *Mesostoma ehrenbergii* spermatocytes: action of antagonistic chromosome fibers. *Cell Motil. Cytoskeleton* 13:212–220.
- Fuge, H. 1989. Traction fibres in chromosome movement: the pros and cons. *Biol. Cell.* 66:209–213.
- Fuseler, J. W. 1975. Temperature dependence of anaphase chromosome velocity and microtubule depolymerization. *J. Cell Biol.* 67:789–800.
- Gorbsky, G. J., P. J. Sammak, and G. G. Borisy. 1987. Chromosomes move poleward in anaphase along stationary microtubules that coordinately disassemble from their kinetochore ends. *J. Cell Biol.* 104:9–18.
- Hays, T. S., and E. D. Salmon. 1990. Poleward force at the kinetochore in metaphase depends on the number of kinetochore microtubules. *J. Cell Biol.* 110:391–404.
- Hays, T. S., D. Wise, and E. D. Salmon. 1982. Traction force on a kinetochore at metaphase acts as a linear function of kinetochore fiber length. *J. Cell Biol.* 93:374–382.
- Holloway, S. L., M. Glotzer, R. W. King, and A. W. Murray. 1993. Anaphase is initiated by proteolysis rather than by the inactivation of maturation-promoting factor. *Cell.* 73:1393–1402.
- Hughes-Schneider, S. 1946. The “pre-metaphase stretch” and kinetochore orientation in phasmids. *Chromosoma.* 3:1–21.
- Hyman, A. A., and T. J. Mitchison. 1991a. Two different microtubule-based motor activities with opposite polarities in kinetochores. *Nature (Lond.)* 351:206–211.
- Hyman, A. A., and T. J. Mitchison. 1991b. Regulation of the direction of chromosome movement. *Cold Spring Harbor Symp. Quant. Biol.* 56:745–750.
- Inoue, S. 1981. Cell division and the mitotic spindle. *J. Cell Biol.* 91:

- 131s-147s.
31. Izutsu, J. 1988. Changes of the chromosomal spindle (kinetochore) fibers and the behavior of bivalent chromosomes in grasshopper spermatocytes after irradiation of the kinetochores with an ultraviolet microbeam. *Protoplasma* (Suppl.) 1:122-132.
 32. Kalt, A., and M. Schliwa. 1993. Molecular components of the centrosome. *Trends Cell Biol.* 3:118-128.
 33. Koshland, D. E., T. J. Mitchison, and M. W. Kirschner. 1988. Polewards chromosome movement driven by microtubule depolymerization *in vitro*. *Nature (Lond.)* 331:499-504.
 34. Kuriyama, R., and C. Nislow. 1992. Molecular components of the mitotic spindle. *BioEssays* 14:81-88.
 35. Leslie, R. 1992. Chromosomes attain a metaphase position on half-spindles in the absence of an opposing spindle pole. *J. Cell Sci.* 103:125-130.
 36. Lewis, W. H. 1939. Changes in viscosity and cell activity. *Science (Wash. DC)* 89:400.
 37. Luyckx, P. 1970. Cellular mechanisms of chromosome distribution. *Internat. Rev. Cytol.* (Suppl.) 2:1-173.
 38. Margolis, R. L., and Wilson, L. 1981. Microtubule treadmills—possible molecular machinery. *Nature (Lond.)* 293:705-711.
 39. McEwen, B. F., J. T. Arena, J. Frank, and C. L. Rieder. 1993. Structure of the colcemid-treated PtK kinetochore outer plate as determined by high voltage electron microscopic tomography. *J. Cell Biol.* 120:301-312.
 40. McIntosh, J. R., and M. P. Koonce. 1989. Mitosis. *Science (Wash. DC)* 246:22-628.
 41. McIntosh, J. R., and C. M. Pfarr. 1991. Mitotic motors. *J. Cell Biol.* 115:577-586.
 42. McNeil, P., and M. W. Berns. 1981. Chromosome behavior after laser microirradiation of a single kinetochore in mitotic PtK₂ cells. *J. Cell Biol.* 88:543-553.
 43. Mitchison, T. J. 1989a. Chromosome alignment at mitotic metaphase: balanced forces or smart kinetochores? *In Cell Movement. Volume 2: Kinesin, Dynein, and Microtubule Dynamics.* F. D. Warner and J. R. McIntosh, editors. Alan R. Liss, Inc. New York. 421-430.
 44. Mitchison, T. J. 1989b. Polewards microtubule flux in the mitotic spindle: evidence from photoactivation of fluorescence. *J. Cell Biol.* 109:637-652.
 45. Mitchison, T. J., and E. D. Salmon. 1992. Poleward kinetochore fiber movement occurs during both metaphase and anaphase-A in newt lung cell mitosis. *J. Cell Biol.* 119:569-582.
 46. Mitchison, T. J., L. Evans, E. Schultz, and M. W. Kirschner. 1986. Sites of microtubule assembly and disassembly in the mitotic spindle. *Cell.* 45:515-527.
 47. Mole-Bajer, J., A. Bajer, and A. Owczarzak. 1975. Chromosome movements in prometaphase and aster transport in the newt. *Cytobios.* 13:45-65.
 48. Nicklas, R. B. 1977. Chromosome movement: facts and hypotheses. *In Mitosis Facts and Questions.* M. Little, N. Paweletz, C. Petzelt, H. Ponting, D. Schroeter, and H. P. Zimmerman, editors. Springer-Verlag, NY. 150-155.
 49. Nicklas, R. B. 1989. The motor for poleward chromosome movement in anaphase is in or near the kinetochore. *J. Cell Biol.* 109:2245-2255.
 50. Nicklas, R. B., and C. A. Koch. 1972. Chromosome micromanipulation. IV. Polarized motions within the spindle and models for mitosis. *Chromosoma.* 39:1-26.
 51. Nicklas, R. B., and D. F. Kubai. 1985. Microtubules, chromosome movement, and reorientation after chromosomes are detached from the spindle by micromanipulation. *Chromosoma* 92:313-324.
 52. Östergren, G. 1945a. Equilibrium of trivalents and the mechanism of chromosome movements. *Hereditas.* 31:498.
 53. Östergren, G. 1945b. Transverse equilibria on the spindle. *Bot. Not.* 4:467-468.
 54. Östergren, G. 1951. The mechanism of co-orientation in bivalents and multivalents. The theory of orientation by pulling. *Hereditas.* 37:85-156.
 55. Pickett-Heaps, J. D. 1986. Mitotic mechanisms: an alternative view. *Trends Biochem. Sci.* 11:504-508.
 56. Rieder, C. L. 1982. The formation, structure and composition of the mammalian kinetochore and kinetochore fiber. *Internat. Rev. Cytol.* 79:1-58.
 57. Rieder, C. L. 1990. Formation of the astral mitotic spindle: ultrastructural basis for the centrosome-kinetochore interaction. *Elec. Microsc. Rev.* 3:269-300.
 58. Rieder, C. L. 1991. Mitosis: towards a molecular understanding of chromosome behavior. *Curr. Opin. Cell Biol.* 3:59-66.
 59. Rieder, C. L., E. A. Davison, L. C. Jensen, L. Cassimeris, and E. D. Salmon. 1986. Oscillatory movements of monooriented chromosomes, and their position relative to the spindle pole, result from the ejection properties of the aster and half spindle. *J. Cell Biol.* 103:581-591.
 60. Roos, U.-P. 1976. Light and electron microscopy of rat kangaroo cells in mitosis. III. Patterns of chromosome behavior during prometaphase. *Chromosoma.* 54:363-385.
 61. Salmon, E. D. 1989a. Microtubule dynamics and chromosome movement. *In Mitosis: Molecules and Mechanisms.* J. S. Hyams, and B. R. Brinkley, editors. Academic Press, NY. 119-182.
 62. Salmon, E. D. 1989b. Metaphase chromosome congression and anaphase poleward movement. *In Cell Movement. Volume 2: Kinesin, Dynein, and Microtubule Dynamics.* F. D. Warner and J. R. McIntosh, editors. Alan R. Liss, Inc. NY. 431-440.
 63. Sawin, K. E., and S. A. Endow. 1993. Meiosis, mitosis and microtubule motors. *BioEssays.* 15:399-407.
 64. Sawin, K. E., K. LeGuellec, M. Phillippe, and T. J. Mitchison. 1992. Mitotic spindle organization by a plus-end-directed microtubule motor. *Nature (Lond.)* 359:540-543.
 65. Seto, T., J. Kezer, and C. M. Pomerat. 1969. A cinematographic study of meiosis in salamander spermatocytes *in vitro*. *Z. Zellforsch.* 94:407-424.
 66. Shelden, E., and P. Wadsworth. 1992. Microinjection of biotin-tubulin into anaphase cells induces transient elongation of kinetochore microtubules and reversal of chromosome-to-pole motion. *J. Cell Biol.* 116:1409-1420.
 67. Skibbens, R. V., V. P. Skeen, and E. D. Salmon. 1993. Directional instability of kinetochore motility during chromosome congression and segregation in mitotic newt lung cells: a push-pull mechanism. *J. Cell Biol.* 122:859-876.
 68. Sluder, G., and C. L. Rieder. 1993. The events and regulation of anaphase onset. *In Chromosome Segregation and Aneuploidy.* NATO-ASI Series. B. K. Vig, editor. Springer-Verlag, Berlin. H72:183-197.
 69. Spurck, T. P., O. G. Stonington, J. A. Snyder, J. D. Pickett-Heaps, A. Bajer, and J. Mole-Bajer. 1990. UV microbeam irradiations of the mitotic spindle. II. Spindle fiber dynamics and force production. *J. Cell Biol.* 111:1505-1518.
 70. Theurkauf, W. E., and R. S. Hawley. 1992. Meiotic spindle assembly in *Drosophila* females: behavior of nonexchange chromosomes and the effects of mutations in the nod kinesin-like protein. *J. Cell Biol.* 116:1167-1180.
 71. Vale, R. D., F. Malik, and D. Brown. 1992. Directional instability of microtubule transport in the presence of kinesin and dynein, two opposite polarity motor proteins. *J. Cell Biol.* 119:1589-1596.
 72. Vandre, D. D., and G. G. Borisy. 1989. The centrosome cycle in animal cells. *In Mitosis: Molecules and Mechanisms.* J. S. Hyams, and B. R. Brinkley, editors. Academic Press, NY. 39-76.
 73. Verde, F., M. Dogterom, E. Stelzer, E. Karsenti, and S. Leibler. 1992. Control of microtubule dynamics and length by cyclin A- and cyclin B-dependent kinases in *Xenopus* egg extracts. *J. Cell Biol.* 118:1097-1108.
 74. Waters, J. C., R. Cole, and C. L. Rieder. 1993. The force-producing mechanism for centrosome separation during spindle formation in vertebrates is intrinsic to each aster. *J. Cell Biol.* 122:361-372.
 75. Wordeman, L., E. R., Steuer, M. P. Sheetz, and T. Mitchison. 1991. Chemical subdomains within the kinetochore domain of isolated CHO mitotic chromosomes. *J. Cell Biol.* 114:285-294.
 76. Yen, T. J., G. Li, B. T. Scharf, I. Szilak, and D. W. Cleveland. 1992. CENP-E is a putative kinetochore motor that accumulates just before mitosis. *Nature (Lond.)* 359:536-539.
 77. Zhang, P., B. A. Knowles, L. S. B. Goldstein, and R. S. Hawley. 1990. A kinesin-like protein required for distributive chromosome segregating in *Drosophila*. *Cell.* 62:1053-1062.
 78. Zheng, Y., K. Jung, and B. R. Oakley. 1991. Gamma tubulin is present in *Drosophila melanogaster* and *homo sapiens* and is associated with the centrosome. *Cell.* 65:817-823.
 79. Zinkowski, R. P., J. Meyne, and B. R. Brinkley. 1991. The centromere-kinetochore complex: a repeat subunit model. *J. Cell Biol.* 113:1091-1110.