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The chromokinesin Kid is necessary for chromosome arm orientation and oscillation, but not congression, on mitotic spindles

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Chromokinesins have been postulated to provide the polar ejection force needed for chromosome congression during mitosis. We have evaluated that possibility by monitoring chromosome movement in vertebrate-cultured cells using time-lapse differential interference contrast microscopy after microinjection with antibodies specific for the chromokinesin Kid. 17.5% of cells injected with Kid-specific antibodies have one or more chromosomes that remain closely opposed to a spindle pole and fail to enter anaphase. In contrast, 82.5% of injected cells align chromosomes in metaphase, progress to anaphase, and display chromosome velocities not significantly different from control cells.

However, injected cells lack chromosome oscillations, and chromosome orientation is atypical because chromosome arms extend toward spindle poles during both congression and metaphase. Furthermore, chromosomes cluster into a mass and fail to oscillate when Kid is perturbed in cells containing monopolar spindles. These data indicate that Kid generates the polar ejection force that pushes chromosome arms away from spindle poles in vertebrate-cultured cells. This force increases the efficiency with which chromosomes make bipolar spindle attachments and regulates kinetochore activities necessary for chromosome oscillation, but is not essential for chromosome congression.

Introduction

Chromosome movement in vertebrate somatic cells has been extensively documented (Mitchison, 1989a; Gorbsky, 1992; Rieder and Salmon, 1994, 1998; Inoué and Salmon, 1995; Khodjakov et al., 1999). In general, chromosomes that are centrally located at the time of nuclear envelope breakdown are likely to become bioriented quickly and align early in prometaphase, whereas chromosomes that are closer to one pole are likely to become monooriented. Monooriented chromosomes remain closely associated with their attached pole and oscillate toward and away from the pole until microtubules emanating from the opposite pole contact the unattached sister kinetochore. Once the chromosome is bioriented it moves away from the pole toward the metaphase plate in a process called congression. Chromosomes aligned at the metaphase plate continue to move, undergoing oscillation about the spindle equator until anaphase onset results in loss of sister chromatid cohesion, thus leading to sister chromatid segregation (Skibbens et al., 1993).

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Chromosome alignment and segregation are driven by both poleward and away from the pole forces. Poleward forces are generated at kinetochores, and although the nature of this force is not entirely understood, it most likely involves the kinetochore-associated microtubule-based motor proteins MCAK/XKCM1, CENP-E, and cytoplasmic dynein (Pfarr et al., 1990; Steuer et al., 1990; Yen et al., 1991; Wordeman and Mitchison, 1995; Walczak et al., 1996; Schaar et al., 1997; Wood et al., 1997; Maney et al., 1998; Savoian et al., 2000; Sharp et al., 2000; McEwen et al., 2001) in addition to poleward microtubule flux (Mitchison, 1989b; Mitchison and Salmon, 1992; Waters et al., 1996). Away from the pole force is generated by an unknown mechanism acting along the length of chromosome arms. This force has been termed the polar ejection force and has been most clearly demonstrated through micromanipulation experiments in which the arm of a chromosome is physically separated from the kinetochore-containing region (Rieder et al., 1986; Ault et al., 1991; Khodjakov and Rieder, 1996). Arm fragments lacking kinetochores are ejected away from the nearest pole in a process that requires nonkinetochore microtubules. The polar ejection force is proposed to act continuously throughout mitosis and to vary in magnitude as a function of distance from the pole (Cassimeris et al., 1994; Rieder and Salmon, 1994).

In recent years, kinesin-like motors that localize to chromosome arms (chromokinesins) have been identified and postulated to generate the polar ejection force (Carpenter, 1991; Fuller, 1995; Vernos and Karsenti, 1995). Initial evidence supporting this postulate came from studies of mutations in the *nod* gene in *Drosophila*. Nod is a chromokinesin required for positioning and proper segregation of achiasmata chromosomes in metaphase I of female meiosis (Zhang et al., 1990; Theurkauf and Hawley, 1992; Afshar et al., 1995). It is proposed that Nod generates an away from the pole force necessary to counterbalance poleward kinetochore forces, and that this activity is essential for positioning nonexchange chromosomes because they lack chiasmata to hold homologues together. Consistent with this view, recent experiments have shown that Kid, the vertebrate homologue of Nod, localizes to chromosomes in mitosis (Tokai et al., 1996) and that depletion of Xkid from *Xenopus* egg extracts leads to misalignment of chromosomes at the metaphase plate (Antonio et al., 2000; Funabiki and Murray, 2000). Thus, these studies suggest that the chromokinesin Nod/Kid associates with chromosome arms and generates an

away from the pole force (i.e., polar ejection force) necessary for chromosome congression. However, the role of Kid in mitosis in somatic cells has not been tested, and away from the pole movements of chromosomes during congression have classically been defined in cultured somatic cells and not in either *Drosophila* oocytes or frog egg extracts. Thus, we tested the role of the chromokinesin Kid in chromosome movement in somatic cells using time-lapse video microscopy. Our results indicate that Kid is required for generating the polar ejection force that pushes chromosome arms away from the spindle poles, but that this force is not absolutely essential for chromosome congression.

Results

To investigate the role of the human chromokinesin Kid in chromosome movement in mammalian mitosis, we raised polyclonal antibodies against a 42-amino acid region of the protein (amino acids 549–590), which was found to have DNA binding activity in vitro (Tokai et al., 1996). The af-

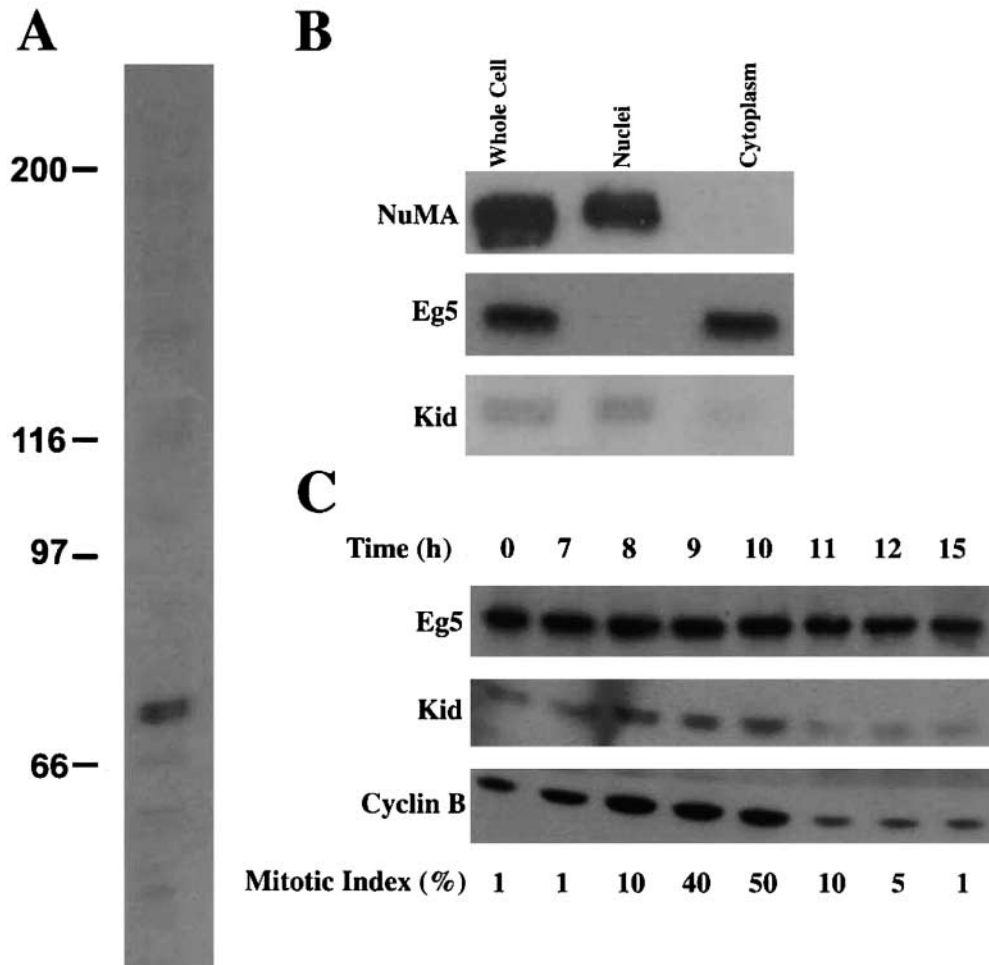


Figure 1. **Kid is a nuclear protein whose levels fluctuate in a cell cycle-dependent manner.** (A) Total HeLa cell protein was separated by SDS-PAGE and blotted with the affinity-purified Kid-specific antibody. Migration positions of myosin (200), β -galactosidase (116), phosphorylase B (97), and albumin (66) are shown in kD. (B) HeLa cells were separated into nuclear and cytoplasmic fractions and total cell, nuclear, and cytoplasmic proteins were separated by SDS-PAGE and blotted with NuMA-, Eg5-, and affinity-purified Kid-specific antibodies as indicated. (C) HeLa cells were synchronized in G1 by double thymidine block, released into fresh media, and harvested in SDS-PAGE sample buffer at indicated times. The approximate percent mitotic index was determined visually. The samples were subjected to immunoblot analysis using Eg5-, cyclin B-, and affinity-purified Kid-specific antibodies as indicated.

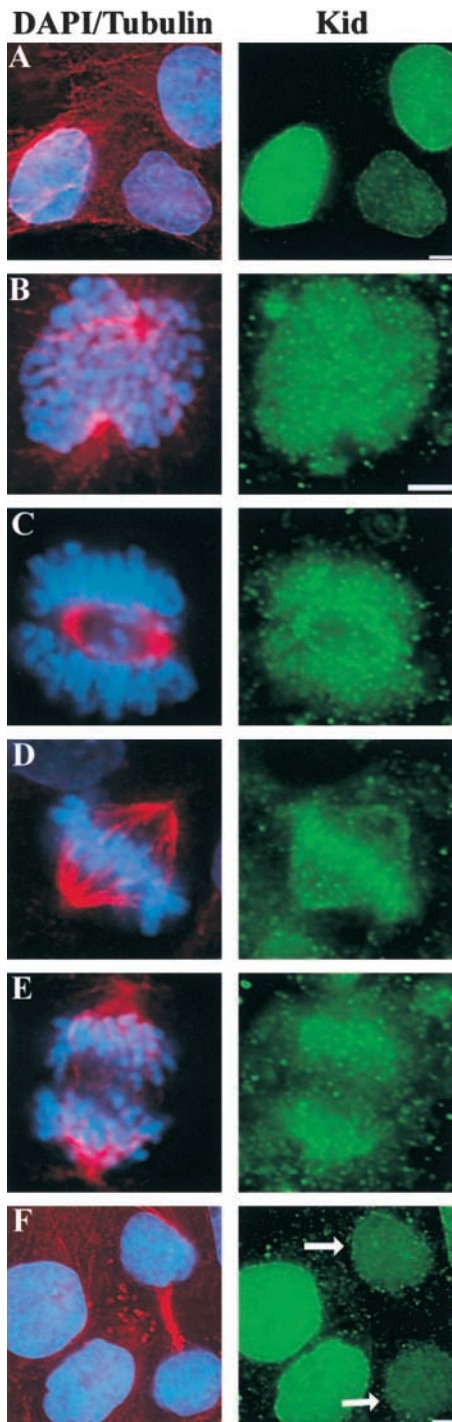


Figure 2. Kid localizes to the nucleus in interphase and to chromosomes and spindles in mitosis in human CFPAC-1 cells. Cultured CFPAC-1 cells were processed for immunofluorescence microscopy using Kid- (green) and tubulin-specific antibodies (red), and the DNA-specific dye DAPI (blue). Representative examples of cells in interphase (A and F), prophase (B), prometaphase (C), metaphase (D), anaphase (E), and telophase (F) are shown. Arrows indicate nuclei of telophase cells that were identified by a prominent midbody. Bars, 10 μ m.

finitly-purified antibody specifically recognized a doublet band of \sim 70 kD on immunoblots of total HeLa cell protein (Fig. 1 A). Immunoblot analysis of nuclear and cytoplasmic

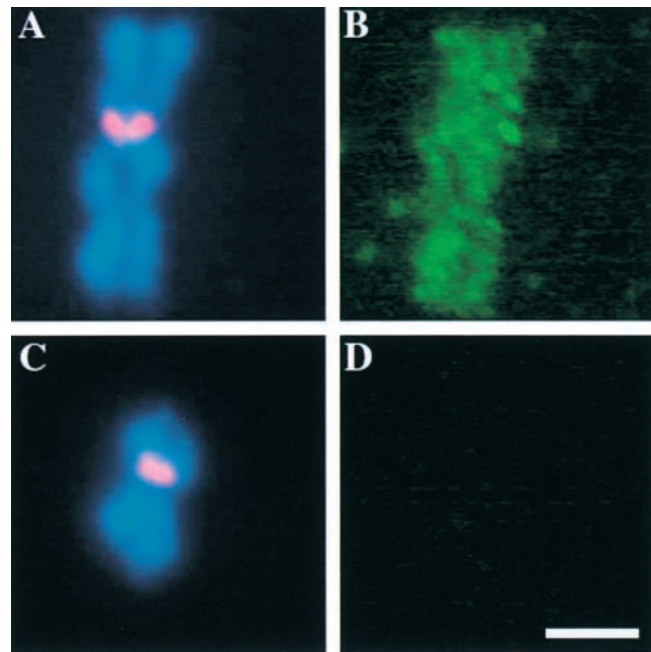


Figure 3. Kid displays a punctate staining pattern along chromosome arms. Chromosomes from mitotic HeLa cells were stained with the DNA-specific dye DAPI (blue, A and C), for kinetochores with human anticentromere antibody (red, A and C) and with either Kid-specific antibody (B) or secondary antibody alone (D). Bar, 5 μ m.

subcellular fractions showed that Kid was enriched in the nuclear fraction to a similar degree as NuMA, a control for nuclear enrichment in this fractionation experiment (Fig. 1 B). The kinesin-related protein Eg5 serves as a cytoplasmic control in this fractionation experiment and verifies that the nuclear and cytoplasmic fractions were efficiently separated. We also determined the cell cycle-dependent abundance of Kid by immunoblot analysis of cells synchronized in the cell cycle. Eg5 levels remained relatively stable throughout the time course and served as a loading control for this experiment (Fig. 1 C). As expected, Cyclin B levels peaked in G2/M (7–10 h) and dropped precipitously in G1 (11–15 h). The abundance of Kid fluctuated in a pattern similar to that of Cyclin B (Fig. 1 C), indicating that Kid levels are subject to cell cycle regulation.

We also localized Kid in human CFPAC-1 cells using immunofluorescence microscopy (Fig. 2). Kid localized to the nucleus of interphase cells, although the intensity of nuclear Kid staining varied significantly in different cells (Fig. 2 A). Nuclei of late telophase/early G1 cells (Fig. 2 F, arrows) stained very weakly for Kid, consistent with the cell cycle-dependent fluctuations in Kid abundance (Fig. 1 C). Upon entry into mitosis, Kid localized to the condensed chromosomes from prophase to anaphase (Fig. 2, B–E). Kid also localized to spindle poles in prometaphase and metaphase (Fig. 2, C and D) and displayed some punctate cytoplasmic staining, the nature of which is unknown. Immunofluorescence analysis of isolated human chromosomes showed that Kid is associated with chromosome arms in a punctate pattern (Fig. 3). Taken together, these results demonstrate that our polyclonal antibody is specific for the human Kid protein. Moreover, these results show that human Kid levels

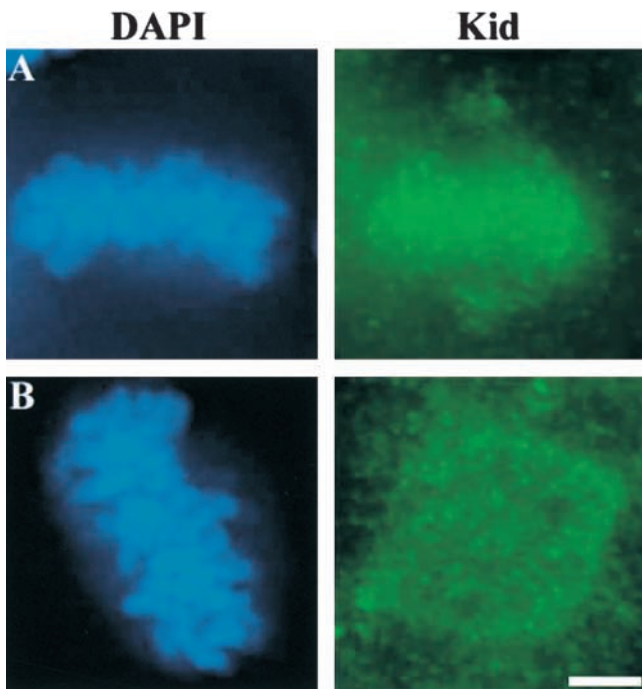


Figure 4. Microinjection of Kid-specific antibodies results in loss of Kid staining on chromosomes. Cultured CFPAC-1 cells were microinjected with either preimmune antibodies (A) or Kid-specific antibodies (B) and were processed for immunofluorescence using Kid-specific serum and the DNA-specific dye DAPI as indicated. Bar, 10 μm .

fluctuate in a cell cycle-dependent manner, and that Kid associates with chromosome arms throughout mitosis consistent with recent descriptions of Xkid in *Xenopus* (Antonio et al., 2000; Funabiki and Murray, 2000).

Chromosome movement on monopolar spindles in cells injected with Kid-specific antibodies

To determine Kid's role in chromosome movement in vertebrate-cultured cells we microinjected Kid-specific antibodies into human CFPAC-1 cells. Immunofluorescence analysis of cells that entered mitosis subsequent to injection with preimmune IgG showed Kid staining on chromosomes and spindles in metaphase (Fig. 4 A) similar to uninjected cells (Fig. 2 D). Cells that entered mitosis subsequent to injection with Kid-specific antibodies showed Kid staining on spindles similar to control cells, but significantly reduced Kid staining on chromosomes (Fig. 4 B). The average pixel intensity of Kid staining in the region of the spindle poles was not significantly different between cells injected with control (140 relative units) or Kid-specific (151 relative units) antibodies. In contrast, the average pixel intensity of Kid staining on chromosomes was reduced from 1,716 relative units in cells injected with control antibodies to 161 relative units in cells injected with Kid-specific antibodies. Thus, injection of G1 cells with antibodies targeting the putative DNA binding domain of Kid blocks the association of Kid with chromosomes in the ensuing mitosis and reduces the quantity of Kid associated with chromosome arms by at least 10-fold.

Initially, we examined chromosome movement on monopolar spindles after perturbation of Kid. Chromosome

position and oscillation on monopolar spindles is determined by the antagonistic actions of poleward kinetochore forces and the polar ejection force (Cassimeris et al., 1994). In the absence of biorientation, the only force acting to push chromosomes away from the center of a monopolar spindle is the polar ejection force. Therefore, it stands to reason that perturbation of the polar ejection force on monopolar spindles would cause chromosomes to aggregate in the center of a monopolar spindle.

Cells injected with Eg5-specific antibodies form monopolar spindles due to a failure in centrosome separation (Blangy et al., 1995; Gaglio et al., 1996; Whitehead and Rattner, 1998; Sharp et al., 1999). Time-lapse video microscopy of cells injected with Eg5-specific antibodies showed that chromosomes formed a ring around the single pole (Fig. 5 A). There was a conspicuous chromosome-clear zone at the center of the monaster, and chromosomes were often oriented in a "V" shape with their kinetochore region pulled poleward and their arms pushed outward (Fig. 5 A, arrows). Furthermore, chromosomes oscillated toward and away from the pole, and were maintained $\sim 10 \mu\text{m}$ from the pole.

In contrast, time-lapse video microscopy of cells coinjected with Eg5- and Kid-specific antibodies showed that chromosomes moved poleward and approached very close to the center of the monaster forming a clump (Fig. 5 B). We could not detect a chromosome-clear zone at the center of these monopolar spindles and chromosomes did not oscillate (Fig. 5 B). Furthermore, it was difficult to identify individual chromosome arms in these cells, as chromosomes intermingled in the cluster and did not orient in a "V" shape as in cells injected with Eg5-specific antibodies alone.

To examine chromosome position on monopolar spindles in more detail we stained injected cells for centrosomes, chromosomes, and kinetochores (Fig. 6). Tubulin staining of cells injected with Eg5-specific antibodies or both Eg5- and Kid-specific antibodies showed monopolar spindles that were indistinguishable (unpublished data). Chromosomes on monopolar spindles induced by perturbation of Eg5 alone were arranged in a ring around the centrosome with their arms oriented away from the pole and the kinetochores proximal to the pole (Fig. 6 A). In contrast, chromosomes on monopolar spindles induced by perturbation of both Eg5 and Kid were tightly clustered adjacent to the centrosome (Fig. 6 B). In a given focal plane, chromosomes occupied an area of $\sim 660 \mu\text{m}^2$ in cells injected with both antibodies, compared with $\sim 1,200 \mu\text{m}^2$ in cells injected with Eg5 antibodies alone. Kinetochores in cells injected with both antibodies were nonuniformly arranged at variable distances from the centrosome. Although a few kinetochores were located very close to the centrosome, the variability in kinetochore location relative to the centrosome resulted in no significant difference in the average centromere to centrosome distances in cells injected with Eg5 antibodies alone, compared with cells injected with both Eg5 and Kid antibodies. Taken together, these data demonstrate that Kid generates an away from the pole force consistent with the properties of the polar ejection force. On monopolar spindles, the force generated by Kid is necessary to maintain the distance between a chromosome and the pole, presumably by antago-

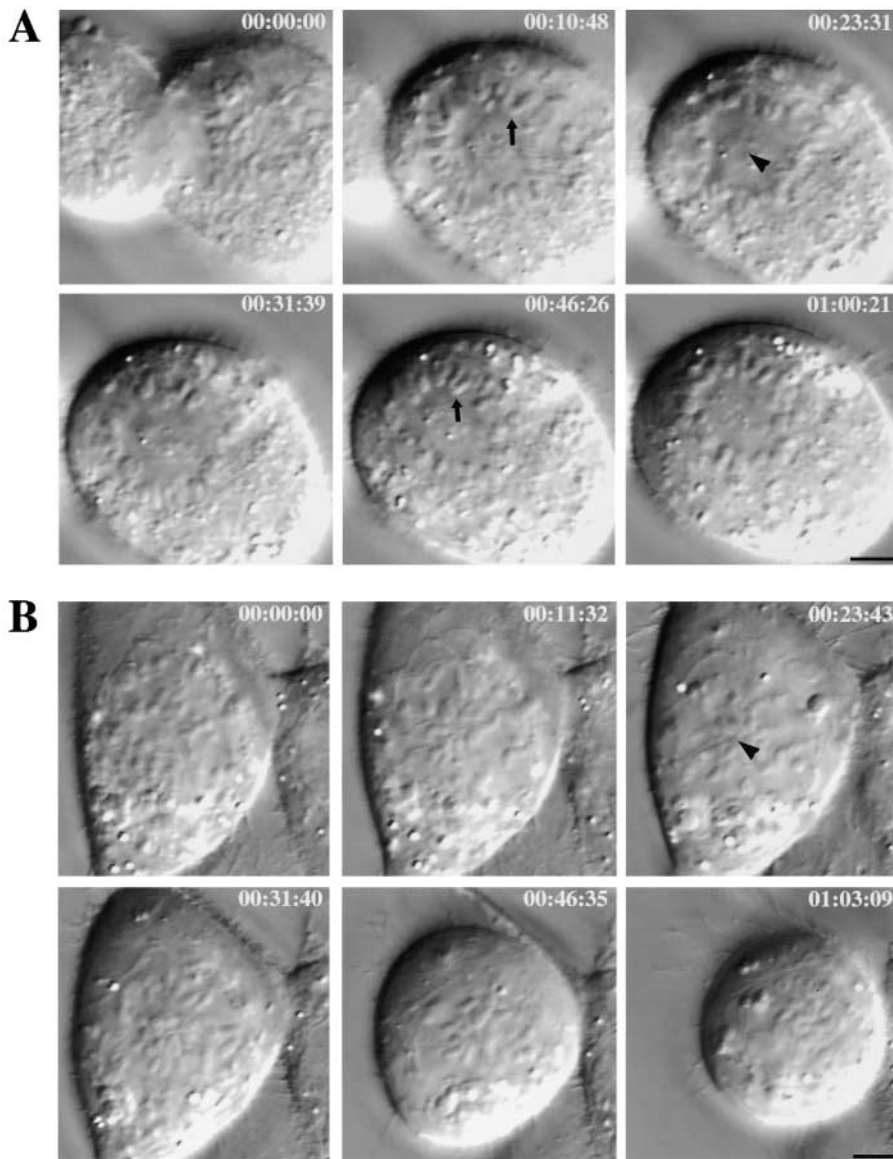


Figure 5. Chromosome movement on monopolar spindles in the presence or absence of Kid-specific antibodies. (A) Chromosome movement in a CFPAC-1 cell microinjected with antibodies to Eg5. Selected differential interference contrast images from a time-lapse record of a mitotic cell that has been microinjected with Eg5-specific antibodies. Times are indicated in hours:minutes:seconds. Arrows indicate chromosomes oriented with their arms pushed away from the pole and arrowhead indicates the center of the monopolar spindle (Video 1). (B) Chromosome movement in a CFPAC-1 cell microinjected with antibodies to both Eg5 and Kid. Selected differential interference contrast images from a time-lapse record of a mitotic cell that has been co-injected with Eg5-specific and Kid-specific antibodies. Times are indicated in hours:minutes:seconds. Arrowhead indicates the center of the monopolar spindle (Video 2). Bar, 10 μ m. Supplemental video is available online at <http://www.jcb.org/content/vol154/issue6/>.

nizing poleward kinetochore activity. Kid activity is also necessary to generate oscillatory chromosome movement.

Chromosome movement on bipolar spindles in cells injected with Kid-specific antibodies

To determine the role of the polar ejection force generated by Kid in chromosome positioning on bipolar spindles in vertebrate cultured cells, we performed time-lapse video microscopy on mitotic cells after microinjection with Kid-specific antibodies. Control cells injected with preimmune IgG displayed chromosome movements indistinguishable from uninjected cells and characteristic of other somatic cells described in the literature. Chromosomes attached to the spindle and congressed to the metaphase plate with their arms oriented perpendicular to the long axis of the spindle (Fig. 7, arrows). In general, chromosomes at the metaphase plate were oriented with their arms perpendicular to the long axis of the spindle and oscillated about the spindle equator. Under the conditions used here, control cells entered anaphase within ~ 1 h after nuclear envelope breakdown.

Cells injected with Kid-specific antibodies entered mitosis, built bipolar spindles, and displayed chromosome velocities poleward, away from the pole, and in anaphase not significantly different from uninjected and control injected cells (Table I and Fig. 8 A). 82.5% of these cells aligned chromosomes at the metaphase plate and underwent anaphase within ~ 1 h after nuclear envelope breakdown (Fig. 8 A). Electron microscopy showed that kinetochore fiber formation in injected cells was similar to control cells (unpublished data). Also, staining with an anticentromere antibody showed no significant difference in interkinetochore spacing between uninjected cells, cells injected with preimmune antibodies, and cells injected with Kid-specific antibodies (unpublished data). These results, when coupled with the fact that anaphase onset was not delayed and chromosome velocities were normal, demonstrate that most aspects of spindle assembly, such as kinetochore-microtubule interaction and checkpoint satisfaction, were not altered by injection of Kid-specific antibodies. However, chromosomes in these cells were not oriented properly on the

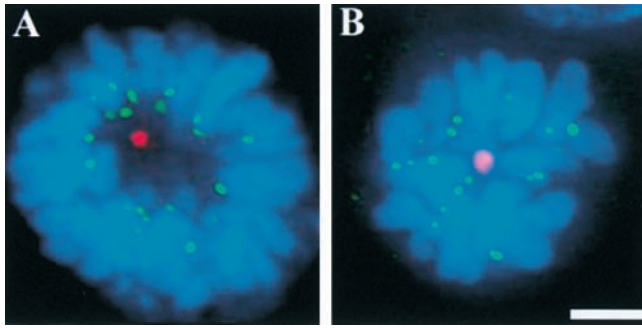


Figure 6. Kid function is required for away from the pole force on chromosomes on monopolar spindles. CFPAC-1 cells were microinjected with either Eg5-specific antibodies alone (A) or Eg5- and Kid-specific antibodies together (B). The cells were monitored until they entered mitosis and were processed for immunofluorescence using human anticentrosome antibodies (red), CENP-E-specific antibodies (green), and the DNA-specific dye DAPI (blue). Bar, 10 μm .

spindle. In contrast to control cells, chromosomes in cells injected with Kid-specific antibodies congressed to the metaphase plate with their arms trailing and oriented parallel to the long axis of the spindle (Fig. 8 A, arrows). Chromosomes at the metaphase plate were also oriented with their arms pointing conspicuously poleward, parallel to the long axis of the spindle (Fig. 8 A, arrowheads).

The remaining 17.5% of cells injected with Kid-specific antibodies built bipolar spindles but failed to enter anaphase during observation (up to 3 h). Immunofluorescence analysis of one such cell that had been in prometaphase for 2.75 h at the time of fixation indicated that whereas most chromosomes were efficiently aligned at the metaphase plate, a few chromosomes remained closely associated with the poles (Fig. 9). These data demonstrate that perturbation of the polar ejection force generated by Kid in cells containing bipolar spindles leads to disruption of chromosome arm orientation on the spindle, but only rarely disrupts chromosome congression.

We also observed that chromosome oscillations were significantly reduced in cells injected with Kid-specific antibodies (Fig. 8, B and C). On average, chromosomes made significantly more oscillations in a given interval in control cells compared with cells injected with Kid-specific antibodies (Fig. 8 C; *t* test, $P < 10^{-8}$). To represent this graphically, we tracked the position of individual chromosomes relative to the metaphase plate by measuring the distance of the centromere domain from the spindle equator over time (Fig. 8 B). A chromosome in an uninjected control cell oscillated about the cell equator achieving a maximal displacement of $\sim 6 \mu\text{m}$ in either direction. In contrast, a chromosome in a cell injected with Kid-specific antibodies remained relatively stationary, moving no more than $\sim 1 \mu\text{m}$ in either direction. Similar suppression of oscillatory chromosome movement was observed during prometaphase as chromosomes made smooth, uninterrupted poleward movement upon initial monoorientation (unpublished data) and congression upon biorientation (Fig. 8 A, arrow, and B). Thus, perturbation of Kid function in cultured human cells suppressed oscillatory chromosome movement.

Discussion

The polar ejection force has been most thoroughly characterized in cultured vertebrate cells and is a force that pushes chromosome arms away from spindle poles (Rieder and Salmon, 1994). The polar ejection force antagonizes poleward kinetochore force and is necessary to maintain the position of monooriented chromosomes some distance from the pole on both bipolar and monopolar spindles (Rieder et al., 1986; Cassimeris et al., 1994; Khodjakov and Rieder, 1996). Data presented here show that perturbation of Kid function causes chromosomes to aggregate near the center of monopolar spindles. In the absence of biorientation on monopolar spindles, the polar ejection force is the only mechanism available to push chromosomes away from the center of a monopolar spindle, and these results demonstrate that

Figure 7. Chromosome movement in a CFPAC-1 cell microinjected with pre-immune antibodies. Selected differential interference contrast images from a video record of a mitotic cell that has been microinjected with preimmune antibodies. Times are indicated in hours:minutes:seconds. The arrow indicates a chromosome undergoing congression to the metaphase plate with its arms oriented perpendicular to the long axis of the spindle (Video 3). Bar, 10 μm . Supplemental video is available online at <http://www.jcb.org/content/vol154/issue6/>.

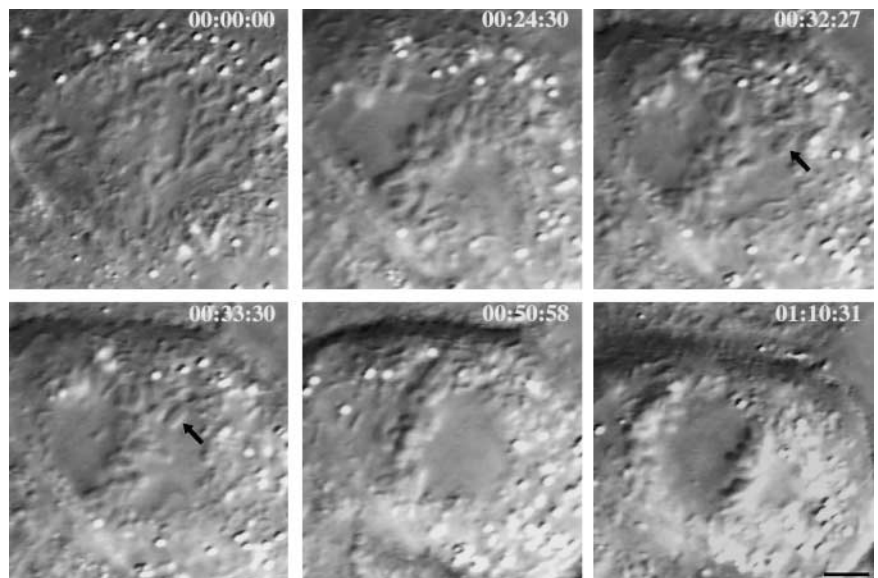


Table I. Chromosome velocities ($\mu\text{m}/\text{min}$)

Treatment	Poleward (n) ^a	Away from the pole (n) ^a	Anaphase A (n) ^b	Percentage of cells that complete mitosis (n) ^c
Uninjected	3.76 \pm 1.65 (37)	3.87 \pm 0.85 (41)	2.68 \pm 0.50 (16)	100 (16)
Control IgG injected	3.80 \pm 1.16 (24)	3.81 \pm 0.85 (29)	2.68 \pm 0.39 (10)	100 (10)
α -Kid injected	3.32 \pm 1.11 (37)	3.85 \pm 1.20 (43)	2.88 \pm 0.45 (10)	82.5 (12)

^aThe poleward and away from the pole velocities for uninjected and injected control cells include measurements of cells in prometaphase and metaphase. The values obtained during these two phases of mitosis were pooled because there was no significant difference between these rates during prometaphase and metaphase. The poleward and away from the pole velocities for α -Kid-injected cells include measurements of cells in prometaphase only, because metaphase cells did not show significant poleward and away from the pole chromosome movements.

^bThe rate of poleward movement at anaphase A was measured using the leading edge of the chromatid mass. Thus, the number of measurements refers to the number of cells observed.

^cThe percentage of cells that complete mitosis refers to the percentage of recorded cells that achieve a complete metaphase chromosome alignment, enter anaphase, and exit mitosis.

Kid provides that force. Thus, this is the first experiment that directly demonstrates that Kid generates polar ejection force because it eliminates any potentially confounding influences that arise when examining chromosome behavior on bipolar spindles. This experiment also indicates that our antibody injection efficiently perturbed Kid function, and that there may not be mechanisms for generating polar ejection force that act redundantly to Kid.

Our results also show that only a small proportion of cells with bipolar spindles failed to enter anaphase in a timely manner after disruption of Kid. Cells delayed in anaphase onset had one or more chromosomes closely opposed to a spindle pole, which is expected based on the proposal that the polar ejection force is necessary for chromosome congression (Rieder and Salmon, 1994). However, most chromosomes in these cells were aligned at the metaphase plate and most injected cells succeeded in alignment of all chromosomes and progression to anaphase. Furthermore, we directly observed chromosome congression to the metaphase plate in injected cells (Fig. 8 A). The likely scenario that explains these observations is that those chromosomes centrally located at the time of nuclear envelope breakdown capture microtubules at both kinetochores and achieve biorientation independently of the polar ejection force and are unaffected by perturbation of Kid function. Chromosomes located near one pole at the time of nuclear envelope breakdown most likely capture microtubules at the kinetochore facing that pole. Many of those monooriented chromosomes achieve biorientation and congress despite perturbation of Kid function as shown in Fig. 8 A. However, other monooriented chromosomes remain too close to (or behind) the pole in the absence of Kid function for the sister kinetochore to efficiently capture microtubules from the opposite pole, and cells containing these chronically monooriented chromosomes are significantly delayed in anaphase onset (Fig. 9). These observations demonstrate that the function of Kid (and, by inference, the polar ejection force) in vertebrate-cultured cells is to push chromosomes away from the pole in order to facilitate biorientation (Rieder et al., 1986; Rieder and Salmon, 1994).

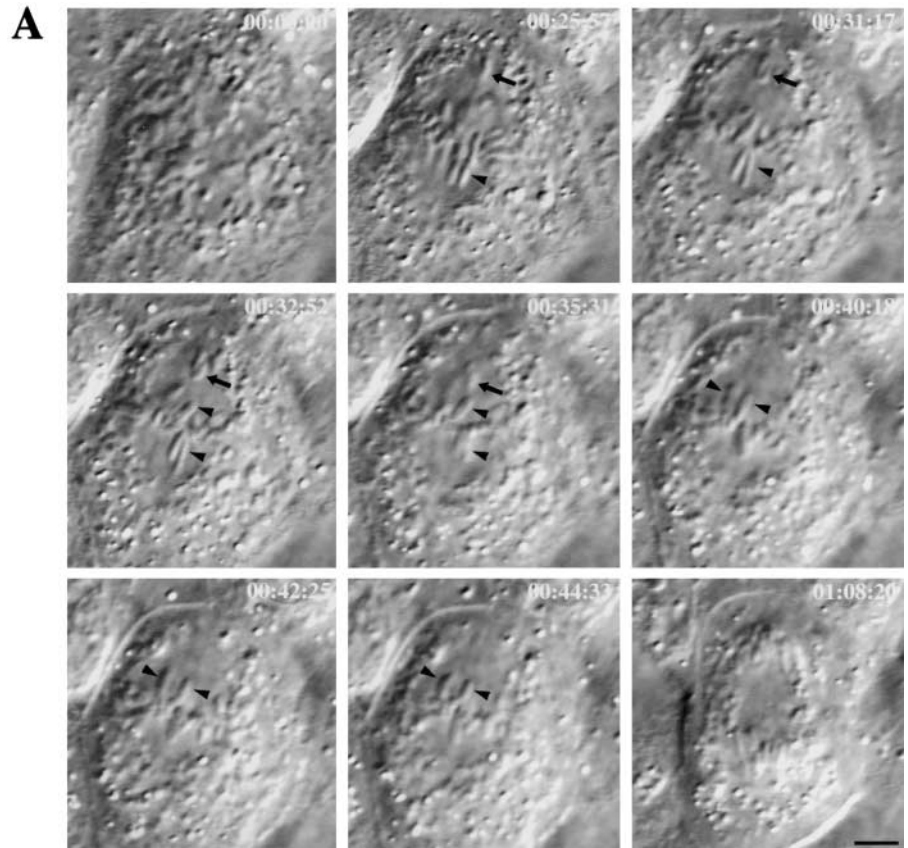
The demonstration that Kid generates polar ejection force in somatic cells is consistent with the function of Kid and its relative, Nod, in meiotic systems. In *Xenopus* egg extracts, Kid was shown to be necessary for accurate chromosome alignment on bipolar spindles (Antonio et al., 2000; Fu-

nabiki and Murray, 2000). Perturbation of Kid function caused chromosome arms to orient toward spindle poles and caused chromosome displacement from the metaphase plate despite the fact that initial chromosome alignment appeared normal and that 40 or 72% (depending on the particular study) of kinetochore regions remained properly positioned at the spindle equator. Similarly, the *nod* gene product in *Drosophila* has been shown to be essential for positioning of nonexchange chromosomes during female meiosis, and mutations in Nod cause segregation defects of nonexchange chromosomes because they are improperly positioned (Zhang and Hawley, 1990; Zhang et al., 1990; Theurkauf and Hawley, 1992). Results from both of these meiotic systems are consistent with Kid/Nod providing the polar ejection force. The major discrepancy between the data presented here and data from meiotic systems is that chromosome alignment defects observed after perturbation of Kid function on meiotic spindles are more severe than on mitotic spindles. We find that only 17.5% of cultured vertebrate cells failed to align all chromosomes and proceed to anaphase (Figs. 8 and 9), whereas 80% of spindles in frog egg extracts contained misaligned chromosomes and nonexchange chromosomes (i.e., chromosome 4) failed to position properly during 84% of female meiosis in fruit flies (Theurkauf and Hawley, 1992; Antonio et al., 2000; Funabiki and Murray, 2000). The most likely explanation for this difference is that meiotic spindles differ from mitotic spindles in the relative contribution that nonkinetochore microtubules make toward chromosome alignment. A definitive example of such differences between meiotic and mitotic systems is observed in *Drosophila*. The *nod* gene product is essential for nonexchange chromosome positioning during female meiosis, but is not necessary for chromosome alignment in mitosis or alignment of exchange chromosomes in female meiosis (Zhang and Hawley, 1990; Zhang et al., 1990; Carpenter, 1991; Theurkauf and Hawley, 1992).

Chromosome congression and oscillation

Current models for chromosome oscillation and positioning on spindles involve a combination of kinetochore directional instability and the polar ejection force (Murray and Mitchison, 1994; Rieder and Salmon, 1994, 1998). Chromosomes in many animal species show distinct oscillatory behavior on spindles, and these oscillations are generated by nonperiodic switching in kinetochore activity between modes of pole-

Figure 8. Chromosome movement in a CFPAC-1 cell microinjected with Kid-specific antibodies. (A) Selected differential interference contrast images from a video record of a mitotic cell that has been microinjected with Kid-specific antibodies. Times are indicated in hours:minutes:seconds. The arrow indicates a chromosome undergoing congression to the metaphase plate with its arms trailing toward the pole. Arrowheads indicate chromosomes at the metaphase plate with their arms oriented toward the pole, parallel to the long axis of the spindle (Video 4). (B) Chromosome oscillations are reduced in CFPAC-1 cells microinjected with antibodies to Kid. Traces of single representative chromosomes in an uninjected control cell and a cell injected with Kid-specific antibodies in metaphase and prometaphase as indicated. Distance (μm) of the centromeric region of the chromosome from the spindle equator is plotted versus time (s). (C) Chromosome oscillations were counted for 10 chromosomes each from uninjected control cells and cells injected with Kid-specific antibodies, and the average number of oscillations per 10-min time period is shown. Bar, 10 μm . Supplemental video is available online at <http://www.jcb.org/content/vol154/issue6/>.



ward force generation and neutral (Skibbens et al., 1993; Khodjakov and Rieder, 1996). The regulation of kinetochore switching is currently unknown, but is proposed to involve kinetochore tension, a depletable kinetochore component that requires a defined recharging period, or a combination of the two (Skibbens et al., 1993; Rieder and Salmon, 1998). Likewise, the mechanism of chromosome congression to the spindle equator is unknown and has been postulated to rely on the polar ejection force to either provide a position at the spindle equator where away from the pole forces are balanced (Rieder and Salmon, 1994; Khodjakov et al., 1999), or to generate tension that biases kinetochore switching such that chromosomes spend more time moving toward the spindle equator rather than toward the poles (Skibbens et al., 1993).

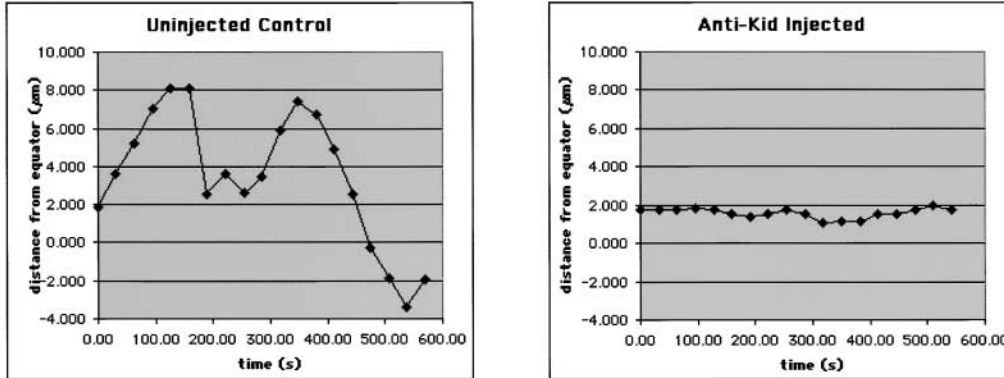
We show that disruption of the polar ejection force suppressed oscillatory chromosome movement on both monopolar and bipolar spindles. These data fit the model that the polar ejection force generated by Kid, either directly or indirectly, regulates the switching of kinetochores between poleward and neutral modes to generate chromosome oscillation in somatic cells. These results favor a tension-based mechanism for regulating kinetochore switching because perturbation of Kid eliminates the away from the pole force, which would generate tension by antagonizing poleward kinetochore force. However, the data cannot rule out the possibility of a combinatorial mechanism involving the tension-dependent depletion of a kinetochore component. Interestingly, perturbation of Kid function alters chromosome arm orientation as well as chromosome oscillation,

yielding chromosome behavior in cultured vertebrate cells that is remarkably similar to chromosome behavior in plant cells (Smirnova and Bajer, 1992). In plant cells, chromosomes do not oscillate, chromosome arms point toward spindle poles, and chromosome-severing experiments indicate an absence of polar ejection forces (Khodjakov et al., 1996). These similarities suggest that a primary difference in chromosome behavior between plant cells and vertebrate cells may be due solely to the presence or absence of the polar ejection force.

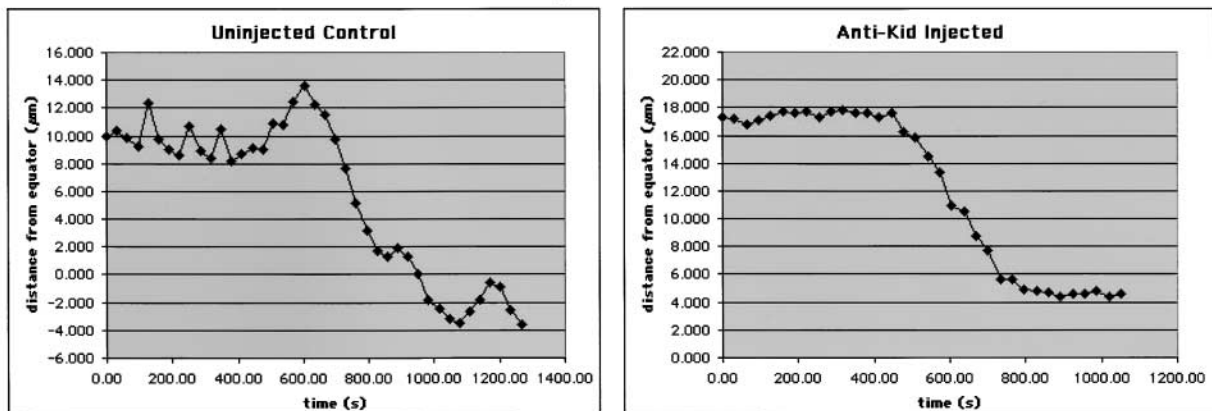
We also find that chromosome congression occurs efficiently in the absence of the polar ejection force. The polar ejection force is important to ensure that all chromosomes attain biorientation, but once sister kinetochores engage microtubules from opposite poles, chromosomes move to the spindle equator independently of the polar ejection force (Fig. 8). These results are in line with observations from mitosis with unreplicated genome cells. Those experiments showed that isolated kinetochores, dislocated from their associated chromatin and hence subject to little, if any, polar ejection force, retained the capacity to align at the spindle equator (Brinkley et al., 1988; Mitchison and Hyman, 1988; Zinkowski et al., 1991). Collectively, the data are consistent with the idea that kinetochores are "smart" (Mitchison, 1989a; Murray and Mitchison, 1994), and do not fit models for chromosome congression based on biased durations of oscillatory movement or balancing of away from the pole forces. Although we do not dispute the fact that the polar ejection force provides positional information, these results demonstrate that kineto-

Metaphase

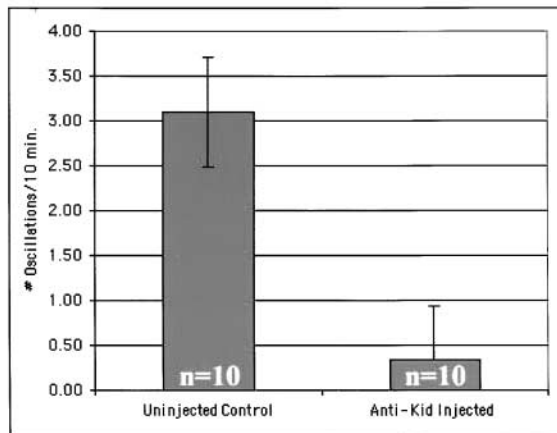
B



Prometaphase



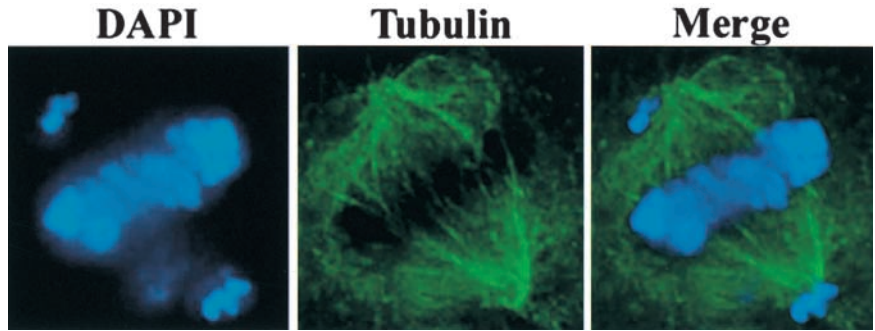
C



chores obtain information about their position on the spindle from other sources in addition to the polar ejection force. These additional positional cues are unknown at this time and numerous possibilities have been discussed in the literature (for review see Mitchison, 1989a; Rieder and Salmon, 1998). One possibility is that the poleward force exerted (or experienced) by kinetochores is directly proportional to the length of kinetochore fibers (Östergren, 1950). This “traction fiber” model has garnered experimental support (Hays et al., 1982; Hays and

Salmon, 1990) and is the only model for poleward chromosome movement that invokes microtubule disassembly at minus ends (Mitchison, 1989a). We favor this possibility because microtubule disassembly at minus ends, as a component of poleward microtubule flux (Mitchison, 1989b), is a consistent feature of spindles in metazoan cells (Mitchison and Salmon, 1992; Wilson et al., 1994; Desai et al., 1998) and can generate significant force (Waters et al., 1996). Whereas the rate of poleward microtubule flux is too slow to provide the primary mechanism for

Figure 9. Failure of chromosome congression in some cells injected with Kid-specific antibodies. An anti-Kid injected CFPAC-1 cell which had been in prometaphase for 2.75 h, was processed for immunofluorescence using tubulin-specific antibodies and the DNA-specific dye DAPI as indicated.



poleward chromosome movement in cultured vertebrate cells (Mitchison and Salmon, 1992), the force generated by flux may supply positional information to kinetochores to drive chromosome alignment.

Materials and methods

Cell culture

The human HeLa cell line was maintained in DME containing 10% FCS, 50 IU/ml penicillin, and 50 μ g/ml streptomycin. The human CFPAC-1 cell line was maintained in Iscove's modified DME containing 10% FCS, 50 IU/ml penicillin, and 50 μ g/ml streptomycin. Cells were grown at 37°C in a humidified incubator with a 5% CO₂ atmosphere.

Antibodies

Kid-specific antibodies were prepared by immunizing rabbits with a 42-amino acid segment of the Kid protein that spans the DNA binding domain (Tokai et al., 1996). A cDNA-encoding full-length Kid was obtained from the human genome sequencing consortium (GenBank/EMBL/DDJB accession no. R56446) and the region encoding the DNA binding domain was PCR amplified using the forward primer (CGGGATCCACATCCTGAAGAATAAAG) containing a BamHI site and the reverse primer (CCGCTC-GAGTTGGCGCCCATGAGC) containing an XhoI site. The PCR product was gel purified, digested with BamHI and XhoI, and inserted into the BamHI and XhoI sites of the PGEX-5X-3 vector. This construct results in the in frame fusion of glutathione S-transferase and amino acids 549–590 of Kid. Recombinant protein was expressed in BL21-Gold *Escherichia coli* by induction with 1 mM IPTG to liquid culture and purified by affinity chromatography using glutathione Sepharose-4B (Amersham Pharmacia Biotech). The column eluate was dialyzed against PBS and used to immunize two rabbits.

Kid-specific antibodies were affinity purified using the glutathione S-transferase–Kid DNA binding domain coupled to Affi-gel 10 (Bio-Rad Laboratories). Anti-Kid serum was adsorbed to the matrix for 30 min at room temperature. The gel was washed twice with TBS (10 mM Tris-HCl, pH 7.5, 150 mM NaCl) and Kid-specific antibodies were eluted with 5 ml of 0.5% acetic acid and 0.15 M NaCl. The acid was neutralized with 1 M Tris-HCl, pH 9, to a final pH of \sim 7, and the eluate was dialyzed overnight against PBS. The antibodies were then concentrated using Centricon spin columns (Millipore) to a final concentration of 20 mg/ml.

Other antibodies used in these experiments were as follows: NuMA was detected using a rabbit polyclonal antibody (Gaglio et al., 1995); Eg5 was detected using a rabbit polyclonal antibody raised against the central rod domain (Whitehead and Rattner, 1998); tubulin was detected using the mouse monoclonal antibody DM1 α (Sigma-Aldrich); cyclin B was detected using the mouse monoclonal antibody GNS1 (Santa Cruz Biotechnology); kinetochores were detected using either the human antacentromeric antibody ACA-m provided by Kevin Sullivan (Scripps Research Institute, San Diego, CA) or mouse monoclonal CENP-E antibodies provided by Tim Yen (Fox Chase Cancer Center, Philadelphia, PA); and centrosomes were detected with a human antacentrosome antibody provided by J.B. Rattner (University of Calgary, Calgary, Alberta).

Subcellular fractionation

The method used to prepare nuclear and cytoplasmic subcellular fractions was modified from Mattagajasingh and Misra (1996). Unsynchronized HeLa cells were harvested with trypsin, washed three times with PBS, and resuspended in 10 ml of hypotonic buffer (10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 1.5 mM MgCl₂). The cells were incubated on ice for 15 min and were pel-

leted at 2,000 rpm, 4°C in an SS-34 rotor. The cells were resuspended in hypotonic buffer at 1.8×10^7 cells/ml and lysed by 8–10 strokes in a glass Dounce™ homogenizer. The homogenate was separated into a soluble cytoplasmic fraction and an insoluble crude nuclear fraction by centrifugation at 3,000 rpm, 4°C. The crude nuclear fraction was further enriched by resuspension in 5 ml of 10 mM Tris-HCl, pH 7.5, 250 mM sucrose, 3 mM MgCl₂, 1 mM PMSF, followed by centrifugation over 10 mM Tris-HCl, pH 7.5, 880 mM sucrose, 3 mM MgCl₂, and 1 mM PMSF at 3,500 rpm, 4°C. The resulting nuclear pellet was resuspended in a volume equal to the dounce volume.

Cell cycle timecourse

HeLa cells were synchronized by double block with 2 mM thymidine. The cells were washed with fresh media to release block and were harvested at various time points in SDS sample buffer. The approximate percent mitotic index was visually determined at each time point.

Immunoblotting

Cultured cells or subcellular fractions were solubilized with SDS-PAGE sample buffer. The proteins were then separated by size using SDS-PAGE (Laemmli, 1970) and transferred to polyvinylidene difluoride membrane (Millipore Corp.). The membranes were blocked in 5% milk TBS for 30 min at room temperature, and the primary antibody incubated for 3 h at room temperature in 1% milk TBS. Nonbound primary antibody was removed by washing five times for 3 min each in TBS, and bound antibody was detected using either HRP-conjugated protein A or HRP-conjugated goat anti-mouse (Bio-Rad Laboratories, Inc.). Nonbound secondary was washed away as above and the signal detected using chemiluminescence.

Chromosome spread

HeLa cells were synchronized by double block with 2 mM thymidine. After release from thymidine block, cells were allowed to grow for 6 h and nocodazole was added to a final concentration of 40 ng/ml. Mitotic cells were shaken off the bottom of the dish after 8 h, collected by centrifugation at 1,500 rpm, and washed twice in cold PBS. Cells were resuspended in 10 ml hypotonic buffer and incubated on ice for 15 min. The cells were broken by dounce homogenization, the homogenate seeded onto poly-L-lysine-coated glass coverslips, and the coverslips fixed and prepared for immunofluorescence as described below.

Antibody microinjection

CFPAC-1 cells growing on photo-etched alphanumeric glass coverslips (Bellco Glass Co.) were microinjected following the procedures of Compton and Cleveland (1993) and Capecchi (1980). For all experiments reported here, cells were arrested in late G1 by double block with 2 mM thymidine, injected in the nucleus, released from thymidine block, and analyzed 8–12 h later. Preimmune, Kid-specific, and Eg5-specific IgG's were purified from whole serum for microinjection by affinity chromatography using protein A-conjugated agarose (Roche). IgG fractions were placed into microinjection buffer (100 mM KCl, 10 mM KPO₄, pH 7.0) by gel filtration using PD-10 Sepharose (Amersham Pharmacia Biotech) and concentrated using Centricon spin columns (Millipore) to a final concentration of 20 mg/ml (anti-Kid and preimmune) and 5 mg/ml (anti-Eg5).

Time-lapse microscopy

Methods for time-lapse video microscopy were performed as described previously (Gordon et al., 2001) with the exception that cells were injected into the nucleus during G1 and monitored by time-lapse when they subsequently entered mitosis \sim 8–12 h later.

Chromosome velocities were obtained from the digital time-lapse record of each cell. The microscopy system used for time-lapse recordings

was calibrated using a stage micrometer under the same conditions used for image acquisition. Individual chromosome movement was tracked by frame-by-frame analysis of digital images using Openlab software (Improvision, Inc.). The straight line calibration tool in the Openlab software package was used to determine the distance traveled by an individual chromosome at the point of its centromere between different time points. Velocities were then calculated by dividing the total distance traveled (in μm) by the time interval in which the measurements were made (in minutes). The spindle equator was used as a frame of reference, and was assigned as the position where a bulk of the chromosomes were aligned. Chromosomes were judged to be making directed movements and/or oscillations when the chromosome was displaced by $\geq 2 \mu\text{m}$ in a linear fashion. Displacement of this magnitude is easily distinguishable from Brownian motion (Alexander and Rieder, 1991).

Indirect immunofluorescence

Fixation conditions varied depending on the specific experiment. For localization of Kid by indirect immunofluorescence microscopy (Figs. 2 and 4) and in the case of anti-Eg5 and anti-Eg5/anti-Kid injections (Fig. 5), CFPAC-1 cells were incubated in MTSB (4 mM glycerol, 100 mM Pipes, pH 6.9, 1 mM EGTA, and 5 mM MgCl_2) for 1 min, extracted in MTSB + 0.5% Triton X-100 for 2 min, followed by MTSB for 2 min. Cells were fixed in 3.5% paraformaldehyde for 5 min. When processing injected cells for immunofluorescence after time-lapse video microscopy (Fig. 9), cells were fixed in 3% paraformaldehyde + 0.05% glutaraldehyde for 5 min without prior extraction followed by permeabilization in TBS + 1% albumin containing 0.5% Triton X-100. Chromosome spread samples (Fig. 3) were fixed in 3.5% paraformaldehyde without prior extraction, followed by TBS + 1% albumin containing 0.5% Triton X-100. In all cases, samples were rehydrated in TBS containing 1% albumin for 5 min after fixation. The primary antibodies were incubated for 30 min in TBS + 1% albumin and detected using either fluorescein or Texas red–conjugated species-specific secondary antibodies at 1:500 (Vector Laboratories). DNA was detected using DAPI at 0.4 $\mu\text{g}/\text{ml}$ (Sigma-Aldrich). The coverslips were washed and mounted in Vectashield FITC-guard mounting medium (Vector Laboratories).

Fluorescent images were captured with a Hamamatsu Orca II cooled CCD camera mounted on a ZEISS Axioplan 2 microscope equipped for epifluorescence. A series of 0.5- μm optical sections were collected in the z plane for each channel (DAPI, fluorescein, and/or Texas red) and deconvolved using the Openlab software (Improvision Inc.) to eliminate extraneous fluorescence background.

Online supplemental material

Supplemental video material is available at <http://www.jcb.org/content/vol154/issue6/>. Time-lapse video microscopy reveals the role of the chromokinesin Kid during mitosis. When the microtubule motor protein Eg5 is perturbed, monopolar spindles form (Fig. 5 A), and time-lapse DIC microscopy shows that chromosomes continuously oscillate toward and away from the pole (Video 1). If the chromokinesin Kid is perturbed along with Eg5 (Fig. 5 B), the chromosomes coalesce into a mass adjacent to the pole of a monopolar spindle indicating that Kid generates the force that pushes chromosomes away from the pole on monopolar spindles (Video 2). In cells containing bipolar spindles (Fig. 7), time-lapse DIC microscopy shows that chromosomes move to the metaphase plate with their arms perpendicular to the spindle axis and continuously oscillate at the spindle equator (Video 3). When the chromokinesin Kid is perturbed (Fig. 8), time-lapse DIC microscopy shows that chromosomes congress to the metaphase plate with their arms trailing and do not oscillate (Video 4). These data indicate that the polar ejection force generated by the chromokinesin Kid is essential for chromosome arm orientation and oscillation on mitotic spindles, but that Kid is not absolutely required for chromosome congression to the metaphase plate, suggesting that kinetochores obtain positioning information from sources other than the polar ejection force.

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References

- Afshar, K., N.R. Barton, R.S. Hawley, and L.S.B. Goldstein. 1995. DNA binding and meiotic chromosomal localization of the *Drosophila* Nod kinesin-like protein. *Cell* 81:129–138.
- Alexander, S.P., and C.L. Rieder. 1991. Chromosome motion during attachment to the vertebrate spindle: initial saltatory-like behavior of chromosomes and quantitative analysis of force production by nascent kinetochore fibers. *J. Cell Biol.* 113:805–815.
- Antonio, C., I. Ferby, H. Wilhelm, M. Jones, E. Karsenti, A.R. Nebreda, and I. Vernos. 2000. Xkid, a chromokinesin required for chromosome alignment on the metaphase plate. *Cell* 102:425–435.
- 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 mono-oriented chromosomes. *J. Cell Sci.* 99:701–710.
- Blangy, A., H.A. Lane, P. d'Hérin, M. Harper, M. Kress, and E.A. Nigg. 1995. Phosphorylation by p34^{cdc2} regulates spindle association of human Eg5, a kinesin-related motor essential for bipolar spindle formation in vivo. *Cell* 83:1159–1169.
- Brinkley, B.R., R.P. Zinkowski, W.L. Mollon, F.M. Davis, M.A. Pisegna, M. Persehouse, and P.N. Rao. 1988. Movement and segregation of kinetochores experimentally detached from mammalian chromosomes. *Nature* 336:251–254.
- Capecchi, M.R. 1980. High efficiency transformation by direct microinjection of DNA into cultured mammalian cells. *Cell* 22:479–488.
- Carpenter, A.T.C. 1991. Distributive segregation: motors in the polar wind? *Cell* 64:885–890.
- 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.* 107:285–297.
- Compton, D.A., and D.W. Cleveland. 1993. NuMA is required for the proper completion of mitosis. *J. Cell Biol.* 120:947–957.
- Desai, A., P.S. Maddux, T.J. Mitchison, and E.D. Salmon. 1998. Anaphase A chromosome movement and poleward spindle microtubule flux occur at similar rates in *Xenopus* extract spindles. *J. Cell Biol.* 141:703–713.
- Fuller, M.T. 1995. Riding the polar winds: chromosomes motor down east. *Cell* 81:5–8.
- Funabiki, H., and A.W. Murray. 2000. The *Xenopus* chromokinesin Xkid is essential for metaphase chromosome alignment and must be degraded to allow anaphase chromosome movement. *Cell* 102:411–424.
- Gaglio, T., A. Saredi, and D.A. Compton. 1995. NuMA is required for the organization of microtubules into aster-like mitotic arrays. *J. Cell Biol.* 131:693–708.
- Gaglio, T., A. Saredi, J.B. Bingham, M.J. Hasbani, S.R. Gill, T.A. Shroer, and D.A. Compton. 1996. Opposing motor activities are required for the organization of the mammalian mitotic spindle pole. *J. Cell Biol.* 135:399–414.
- Gorbisky, G.J. 1992. Chromosome motion in mitosis. *Bioessays* 14:73–80.
- Gordon, M.B., L. Howard, and D.A. Compton. 2001. Chromosome movement in mitosis requires microtubule anchorage at spindle poles. *J. Cell Biol.* 152:425–434.
- 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.
- Inoué, S., and E.D. Salmon. 1995. Force generation by microtubule assembly/disassembly in mitosis and related movements. *Mol. Biol. Cell* 6:1619–1640.
- Khodjakov, A., and C.L. Rieder. 1996. Kinetochores moving away from their associated pole do not exert a significant pushing force on the chromosome. *J. Cell Biol.* 135:315–327.
- Khodjakov, A., R.W. Cole, A.S. Bajer, and C.L. Rieder. 1996. The force for poleward chromosome motion in *Haemaphysalis* cells acts along the length of the chromosome during metaphase but only at the kinetochore during anaphase. *J. Cell Biol.* 132:1093–1104.
- Khodjakov, K., I.S. Gabashvili, and C.L. Rieder. 1999. “Dumb” versus “smart” kinetochore models for chromosome congression during mitosis in vertebrate somatic cells. *Cell Motil. Cytoskeleton* 43:179–185.
- Laemmli, U.K. 1970. Cleavage of structural proteins during assembly at the head of the bacteriophage T4. *Nature* 227:680–682.
- Maney, T., A.W. Hunter, M. Wagenbach, and L. Wordeman. 1998. Mitotic cen-

- tromere-associated kinesin is important for anaphase chromosome segregation. *J. Cell Biol.* 142:787–801.
- Mattagajasingh, S.N., and H.P. Misra. 1996. Mechanisms of the carcinogenic chromium(VI)-induced DNA-protein cross-linking and their characterization in cultured intact human cells. *J. Biol. Chem.* 271:33550–33560.
- McEwen, B.F., G.K.T. Chan, B. Zubrowski, M.S. Savoian, M.T. Sauer, and T.J. Yen. 2001. CENP-E is essential for reliable bioriented spindle attachment, but chromosome alignment can be achieved via redundant mechanisms in mammalian cells. *Mol. Biol. Cell.* In press.
- Mitchison, T.J. 1989a. Chromosome alignment at mitotic metaphase: balanced forces or smart kinetochores? *In Cell Movement*. Vol. 2. Kinesin, Dynein, and Microtubule Dynamics. F.D. Warner and J.R. McIntosh, editors. Alan R. Liss, Inc. New York. 421–430.
- Mitchison, T.J. 1989b. Polewards microtubule flux in the mitotic spindle: evidence from photoactivation of fluorescence. *J. Cell Biol.* 109:637–652.
- Mitchison, T., and A. Hyman. 1988. Kinetochores on the move. *Nature.* 336:200–201.
- 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.
- Murray, A.W., and T.J. Mitchison. 1994. Kinetochores pass the IQ test. *Curr. Biol.* 4:38–41.
- Östergren, G. 1950. Considerations on some elementary features of mitosis. *Hereditas.* 36:1–19.
- Pfarr, C.M., M. Coue, P.M. Grissom, T.S. Hays, M.E. Porter, and J.R. McIntosh. 1990. Cytoplasmic dynein is localized to kinetochores during mitosis. *Nature.* 345:263–265.
- Rieder, C.L., and E.D. Salmon. 1994. Motile kinetochores and polar ejection forces dictate chromosome position on the vertebrate mitotic spindle. *J. Cell Biol.* 124:223–233.
- Rieder, C.L., and E.D. Salmon. 1998. The vertebrate cell kinetochore and its roles during mitosis. *Trends Cell Biol.* 8:310–318.
- Rieder, C.L., E.A. Davison, L.C.W. 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.
- Savoian, M.S., M.L. Goldberg, and C.L. Rieder. 2000. The rate of poleward chromosome motion is attenuated in *Drosophila zw10* and *rod* mutants. *Nat. Cell Biol.* 2:948–952.
- Schaar, B.T., G.K.T. Chan, P. Maddox, E.D. Salmon, and T.J. Yen. 1997. CENP-E function at kinetochores is essential for chromosome alignment. *J. Cell Biol.* 139:1373–1382.
- Sharp, D.J., K.R. Yu, J.C. Sisson, W. Sullivan, and J.M. Scholey. 1999. Antagonistic microtubule-sliding motors position mitotic centrosomes in *Drosophila* early embryos. *Nat. Cell Biol.* 1:51–54.
- Sharp, D.J., G.C. Rogers, and J.M. Scholey. 2000. Cytoplasmic dynein is required for poleward chromosome movement during mitosis in *Drosophila* embryos. *Nat. Cell Biol.* 2:922–930.
- 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.* 122:859–875.
- Smirnova, E.A., and A.S. Bajer. 1992. Spindle poles in higher plant mitosis. *Cell Motil. Cytoskeleton* 23:1–7.
- Steuer, E.R., L. Wordeman, T.A. Schroer, and M.P. Sheetz. 1990. Localization of cytoplasmic dynein to mitotic spindles and kinetochores. *Nature.* 345:266–268.
- 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.
- Tokai, N., A. Fujimoto-Nishiyama, Y. Toyoshima, S. Yonemura, S. Tsukita, J. Inoue, and T. Yamamoto. 1996. Kid, a novel kinesin-like DNA binding protein, is localized to chromosomes and the mitotic spindle. *EMBO J.* 15:457–467.
- Vernos, I., and E. Karsenti. 1995. Chromosomes take the lead in spindle assembly. *Trends Cell Biol.* 5:297–301.
- Walczak, C.E., T.J. Mitchison, and A. Desai. 1996. XKCM1: *Xenopus* kinesin-related protein that regulates microtubule dynamics during mitotic spindle assembly. *Cell.* 84:37–47.
- Waters, J.C., T.J. Mitchison, C.L. Rieder, and E.D. Salmon. 1996. The kinetochore microtubule minus-end disassembly associated with poleward flux produces a force that can do work. *Mol. Biol. Cell.* 7:1547–1558.
- Whitehead, C.M., and J.B. Rattner. 1998. Expanding the role of HsEg5 within the mitotic and post-mitotic phases of the cell cycle. *J. Cell Sci.* 111:2551–2561.
- Wilson, P.J., A. Forer, and C. Leggiadro. 1994. Evidence that kinetochore microtubules in crane-fly spermatocytes disassemble during anaphase primarily at the poleward end. *J. Cell Sci.* 107:3015–3027.
- Wood, K.W., R. Sakowicz, L.S.B. Goldstein, and D.W. Cleveland. 1997. CENP-E is a plus end-directed kinetochore motor required for metaphase chromosome alignment. *Cell.* 91:357–366.
- Wordeman, L., and T.J. Mitchison. 1995. Identification and partial characterization of mitotic centromere-associated kinesin, a kinesin-related protein that associates with centromeres during mitosis. *J. Cell Biol.* 128:95–105.
- Yen, T.J., D.A. Compton, D. Wise, R.P. Zinkowski, B.R. Brinkley, W.C. Earnshaw, and D.W. Cleveland. 1991. CENP-E, a novel human centromere-associated protein required for progression from metaphase to anaphase. *EMBO J.* 10:1245–1254.
- Zhang, P., and R.S. Hawley. 1990. The genetic analysis of distributive segregation in *Drosophila melanogaster*. II. Further genetic analysis of the *nod* locus. *Genetics.* 125:115–127.
- Zhang, P., B.A. Knowles, L.S.B. Goldstein, and R.S. Hawley. 1990. A kinesin-like protein required for distributive segregation in *Drosophila*. *Cell.* 62:1053–1062.
- Zinkowski, R.P., and J. Meyne, and B.R. Brinkley. 1991. The centromere-kinetochore complex: a repeat subunit model. *J. Cell Biol.* 113:1091–1110.