

Anaphase Spindle Mechanics Prevent Mis-Segregation of Merotelically Oriented Chromosomes

Daniela Cimini,* Lisa A. Cameron, and E.D. Salmon
 Department of Biology
 CB#3280
 University of North Carolina at Chapel Hill
 Chapel Hill, North Carolina 27599

Summary

Merotelic kinetochore orientation is a kinetochore misattachment in which a single kinetochore is attached to microtubules from both spindle poles instead of just one. It can be favored in specific circumstances [1–5], is not detected by the mitotic checkpoint, and induces lagging chromosomes in anaphase [6, 7]. In mammalian cells, it occurs at high frequency in early mitosis [5], but few anaphase cells show lagging chromosomes [5]. We developed live-cell imaging methods to determine whether and how the mitotic spindle prevents merotelic kinetochores from producing lagging chromosomes. We found that merotelic kinetochores entering anaphase never lost attachment to the spindle poles; they remained attached to both microtubule bundles, but this did not prevent them from segregating correctly. The two microtubule bundles usually showed different fluorescence intensities, the brighter bundle connecting the merotelic kinetochore to the correct pole. During anaphase, the dimmer bundle lengthened much more than the brighter bundle as spindle elongation occurred. This resulted in correct segregation of the merotelically oriented chromosome. We propose a model based on the ratios of microtubules to the correct versus incorrect pole for how anaphase spindle dynamics and microtubule polymerization at kinetochores prevent potential segregation errors deriving from merotelic kinetochore orientation.

Results and Discussion

In this study, we tested the hypothesis that an anaphase correction mechanism, based on the number of microtubules to the correct versus incorrect pole, prevents mis-segregation of merotelically oriented chromosomes [5]. In the few cases in which the ratio of microtubules to opposite poles is nearly one (same number of microtubules to both poles), the merotelically oriented chromosome remains near the spindle equator (lagging chromosome), thus producing a segregation error. Conversely, when the number of microtubules to opposite poles is sufficiently different, the merotelically oriented chromosome moves away from the equator. We determined the mechanics of correction/prevention by using live cell imaging of PtK1 cells microinjected with X-rhodamine-labeled tubulin (microtubules) and a nonperturbing concentration of Alexa 488 anti-CENP-F antibody (labeling

of kinetochores and spindle poles). We identified pro-metaphase/metaphase cells possessing one or more merotelic kinetochores by taking a through-focus image series of the spindle and finding kinetochores with fluorescent fibers toward opposite poles. These cells were imaged by time-lapse spinning-disk confocal microscopy [8, 9]. The collected images were analyzed, and both spindle poles and one or more kinetochore pairs were tracked to determine kinetochore positioning and movement in metaphase and/or anaphase. We identified 54 merotelically oriented kinetochores (in 50 cells), and 25 of these were followed into anaphase.

Merotelically oriented kinetochores in metaphase exhibited several distinctive structural and kinetic features compared to those of normally oriented kinetochores. In PtK1 cells, the outer face of a kinetochore is 0.3–0.5 μm in diameter and contains about 24 kinetochore microtubules bound end-on into a 40 nm-thick outer plate [10]. The outer face of normally attached kinetochores at metaphase was perpendicular to the sister kinetochore axis and perpendicular to a robust fluorescent kinetochore fiber (Figures 1A and 1C, arrowheads). In contrast, merotelic kinetochores appeared tilted relative to this axis and sometimes stretched laterally (relative to their outer face) toward the incorrect pole by the fluorescent kinetochore fiber to that pole (“K2m” in Figures 1A and 1C). In addition, at metaphase, normally oriented sister kinetochore pairs showed an interkinetochore stretch of $2.64 \pm 0.78 \mu\text{m}$ ($n = 29$) with on average one sister on one side and the other on the other side of the spindle equator [5, 11] (Figures 1A and 1C). In chromosomes with one merotelically oriented sister, the interkinetochore stretch was reduced to 2.02 μm (standard deviation = 0.73 μm ; $n = 16$; *t* test, $p < 0.05$), and in most cases (80%) the merotelic sister kinetochore was shifted closer to the spindle equator, between the average positions of normally oriented sisters (Figures 1A–1D). Finally, most merotelic kinetochores were positioned at the periphery of the spindle (Figures 1A, 1C, and 1E). We speculate that the attachment of kinetochore microtubules from opposite poles favors the peripheral positioning of chromosomes with a merotelic kinetochore because of the larger angle of kinetochore microtubules to the spindle interpolar axis and hence easier accessibility to the merotelic kinetochore for microtubules coming from opposite directions.

Normally oriented sister kinetochore pairs in the middle of the spindle oscillated back and forth about the equator because of their kinetochore directional instability [12–15], whereas those at the periphery oscillated less (L.A.C., unpublished data). Likewise, the peripherally localized merotelic kinetochores oscillated less (Figure 1B). However, merotelically oriented kinetochores were relatively stationary even when the normally oriented sister oscillated between poleward movement, which stretched its centromere, and antipoleward movement, which relieved this stretch (Figure 1D; see also Movie 1, available with this article’s Supplemental Data online). The frequency of this oscillation was higher

*Correspondence: cimini@email.unc.edu

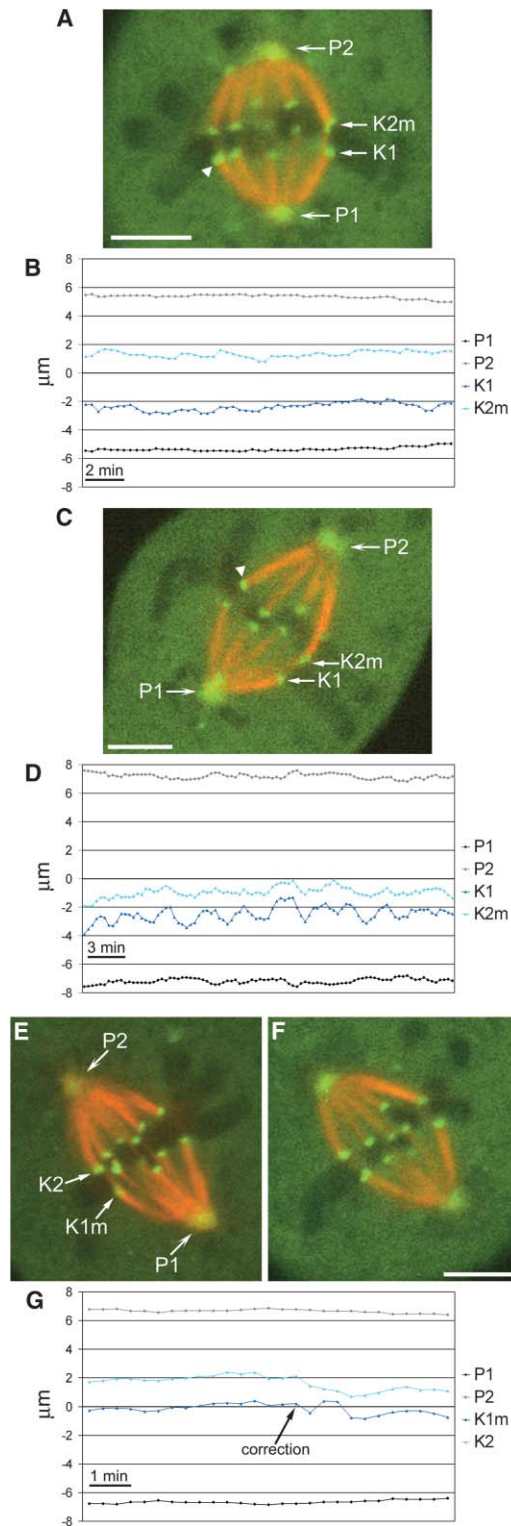


Figure 1. Merotelic Kinetochores in Metaphase Exhibit Distinctive Structural and Kinetic Features Compared to Those of Normally Oriented Kinetochores

(A) Metaphase cell with one merotelically oriented kinetochore (K2m). Normally oriented kinetochores are oriented perpendicularly to the inter-sister-kinetochore axis and perpendicularly to a robust fluorescent kinetochore fiber (arrowhead). The merotelic kinetochore appears tilted and stretched laterally (relative to its outer face) toward the incorrect pole.

than what occurred for the normally oriented kinetochores of chromosomes in the middle of the spindle. This is most likely because the merotelic kinetochore remained relatively stationary, whereas the oscillations of two normally oriented sisters were coordinated, so that when one kinetochore was moving poleward, its sister was following until they both switched direction.

We looked for evidence of merotelic correction in metaphase and found only eight examples out of 54 merotelic kinetochores analyzed. Correction occurred by loss of the dimmer fiber after the merotelic kinetochore had become aligned at the metaphase plate. This left the merotelic kinetochore attached to only one pole and produced a normal bipolar chromosome orientation (Figures 1E–1G). Before correction, the merotelic kinetochore was shifted closer to the spindle equator and did not show oscillation. After correction, the kinetochore oscillated back and forth, moving eventually away from the equator toward a position on average closer to its pole and establishing, in a short period of time, coordinated oscillations with its sister (Figure 1G). Our observation of correction in living cells shows that the mechanism that operates before anaphase to correct merotelic orientations can do so by loss of kinetochore microtubules to the incorrect pole. A role for mitotic centromere-associated kinesin (MCAK) in “preanaphase” correction can be hypothesized [16–18] because perturbations of its centromeric function in vertebrate cells produce kinetochore misattachments [18]. Correction of merotelic orientation, however, occurs while the chromosome remains at the spindle equator, as opposed to syntelic-orientation correction, which involves microtubule depolymerization and abrupt movement of the chromosome toward the spindle pole [19]. The preanaphase correction of merotelic orientation fails to correct many merotelic kinetochores (46/54), which persist until anaphase onset. The stability of merotelic kinetochores might seem surprising, considering that a half-life of 5 min has been reported for kinetochore microtubules in metaphase [20]. We argue that either microtubule at-

(B) The positions of the spindle poles (P1 and P2) and the two sister kinetochores (K1, K2m) of the cell in (A) were tracked over time (19 min). The merotelically oriented kinetochore was shifted closer to the equator than its sister (average: $1.3 \pm 0.2 \mu\text{m}$ versus $2.4 \pm 0.3 \mu\text{m}$). The sister kinetochore pair was positioned at the periphery of the spindle, and the two kinetochores did not show oscillations back and forth about the spindle equator.

(C) Metaphase cell with one merotelically oriented kinetochore (K2m). The merotelic kinetochore appears shifted, tilted, and stretched laterally toward the incorrect pole, as opposed to normally oriented kinetochores (arrowhead).

(D) Chart for the cell in (C). The chart shows that the merotelic kinetochore (K2m) is shifted closer to the spindle equator and does not oscillate, although the sister kinetochore is oscillating back and forth about the spindle equator (Movie 1). The cell was followed over a 29 min period.

(E) Example of merotelic kinetochore orientation (K1m) corrected before anaphase onset.

(F) Frame showing the cell in (E) after correction of the merotelic orientation.

(G) Chart showing how the merotelic kinetochore of the cell in (E) starts moving right after correction and reaches coordination with its sister kinetochore very quickly. The scale bars represent $5 \mu\text{m}$.

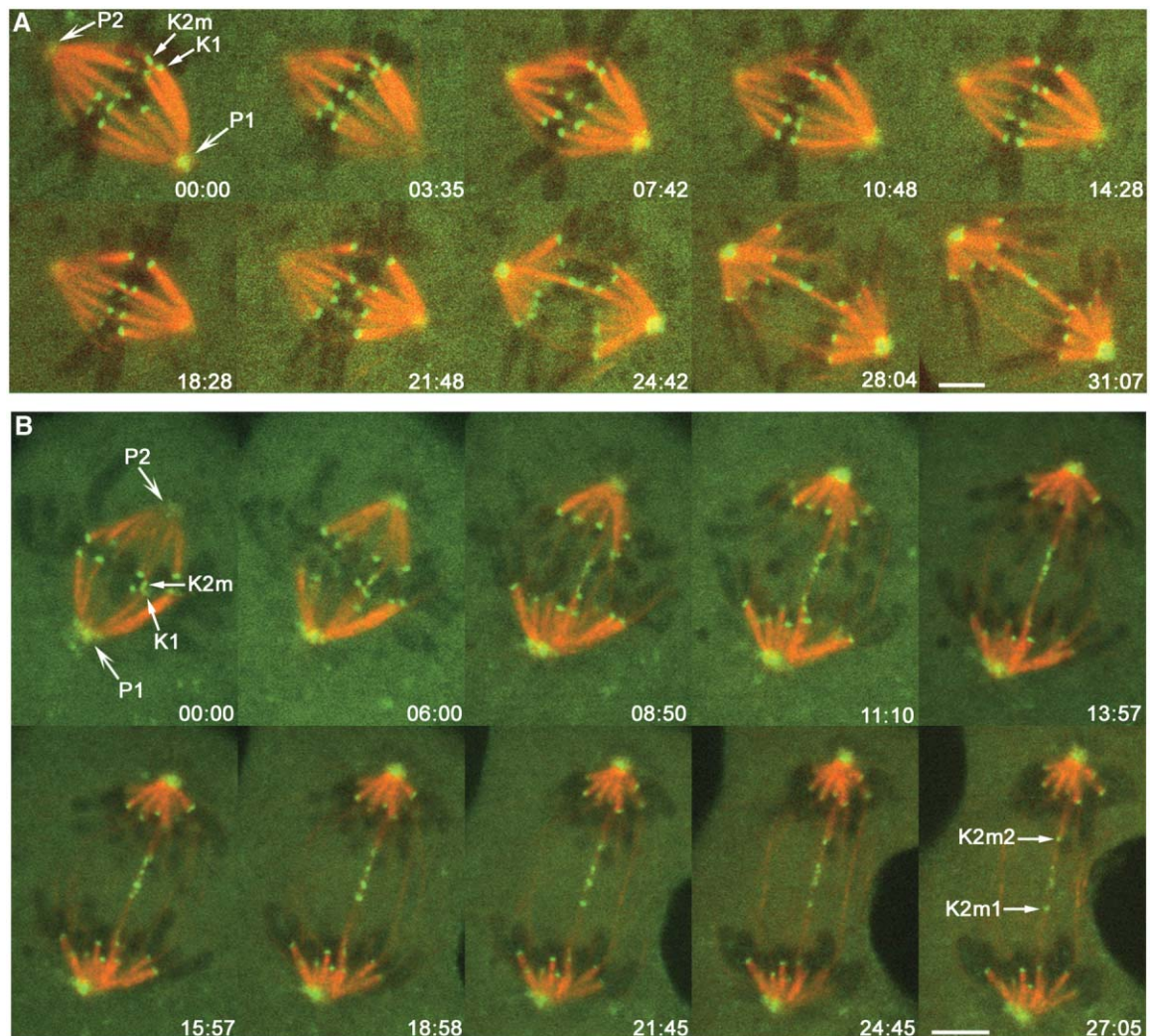


Figure 2. Anaphase Behavior of Merotelic Kinetochores Depends on the Ratio of Microtubules Attached to Opposite Poles

In the examples shown, fluorescence ratios of one (A) and two (B) induced very different behaviors.

(A) Example of merotelic kinetochore orientation producing an anaphase lagging chromosome. The merotelic kinetochore (K2m) is attached to microtubule bundles of approximately the same fluorescence intensity and does not move away from the spindle equator during anaphase (bottom row). Note that the chromosome moves from the periphery of the spindle toward the center. The ratio between the fluorescence intensities of the two microtubule bundles for this merotelic kinetochore was 1.05 (Movie 2).

(B) Example of merotelic kinetochore orientation (K2m) that does not produce a lagging chromosome (note the bulk of the chromatin moving away from the spindle equator) but does induce high kinetochore stretching (Movie 3). The ratio between the fluorescence intensities of the two microtubule bundles for this merotelic kinetochore was 1.99. Elapsed time is displayed as mm:ss. The scale bars represent 5 μm.

tachment is more stable for merotelic kinetochores or, more likely, that microtubule reattachment largely depends on the orientation of adjacent kinetochore microtubules.

It has been proposed that not all merotelic kinetochores produce lagging chromosomes in anaphase because the forces that stretch merotelic kinetochores laterally toward opposite poles break the linkage to one pole either by detachment of kinetochore microtubules or breakage of the stretched kinetochore [1, 2, 4, 5]. Neither of these events occurred during anaphase for any of the merotelic kinetochores analyzed. During normal anaphase, microtubule attachment to kinetochores is very stable [20], and we found that attachments were

very stable even under severe lateral stretch of the kinetochore.

By live cell imaging, we were able to clearly follow and track the movements of 25 merotelic kinetochores after anaphase onset. Only 2 of 25 merotelic kinetochores produced lagging chromosomes (i.e., single chromatids that remained in the proximity of the spindle equator; note that after sister chromatid separation each sister chromatid becomes by definition an independent chromosome) during anaphase (Figure 2A; Figure S1; Movie 2). The ratio of microtubules to opposite poles was estimated by measuring the ratio between fluorescence intensities of the two microtubule bundles (see Supplemental Experimental Procedures for details). For

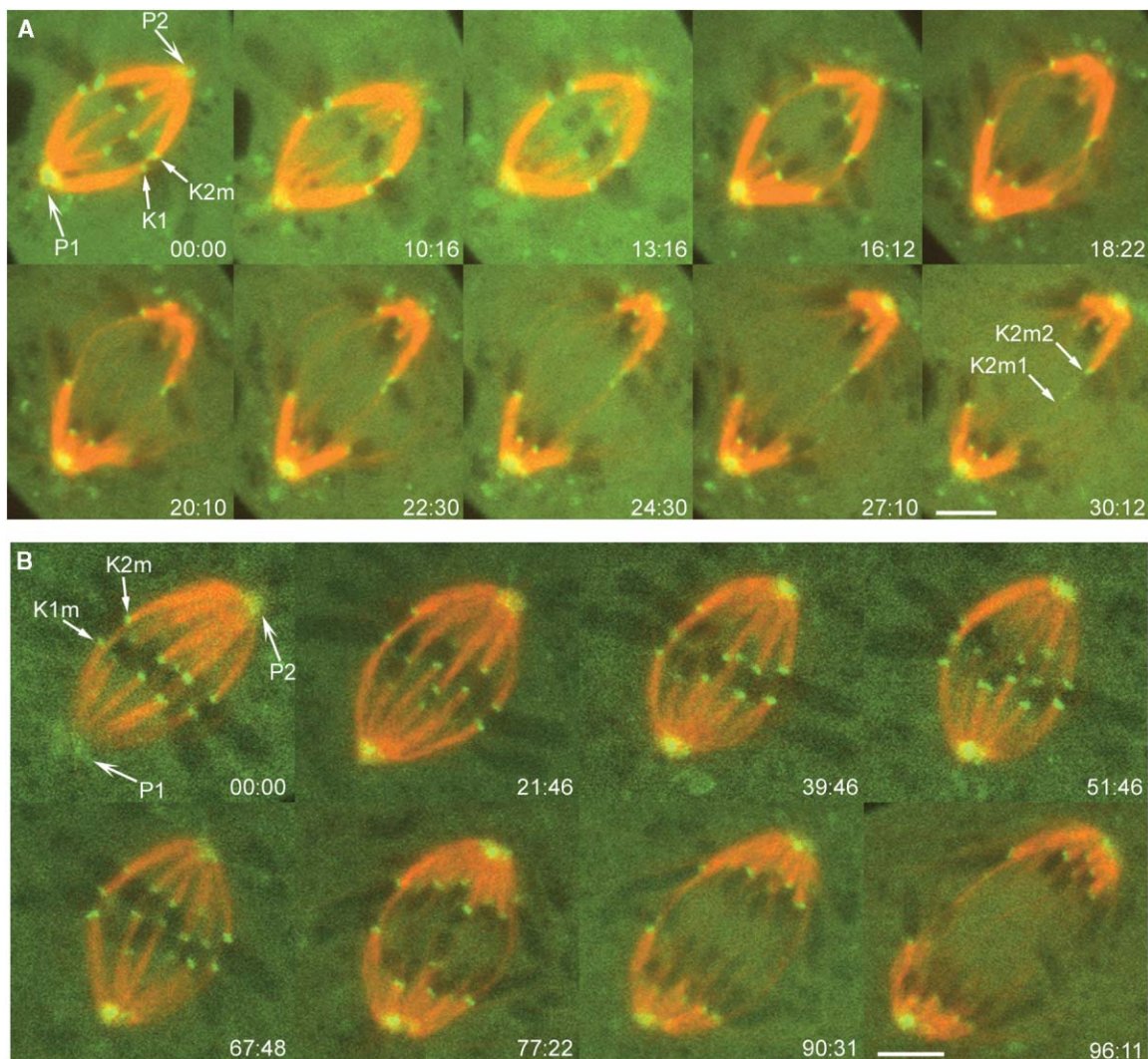


Figure 3. Merotelic Kinetochores with a Fluorescence Ratio Close to Three or Higher between the Two Microtubule Bundles Segregate to the Pole Attached to the Brighter Microtubule Bundle

(A) Example of merotelic kinetochore with a fluorescence ratio equal to 3.89 between the two microtubule bundles. The merotelically oriented chromosome moves away from the spindle equator (frames 4–10), and its kinetochore shows slight stretching (Movie 4).

(B) Example of cell in which two sister kinetochores (K1m and K2m) are merotelically oriented. The fluorescence ratios for the two kinetochores were 4.23 and 3.82, respectively. In anaphase, both sister kinetochores moved away from the spindle equator, and neither one displayed any obvious stretching. Elapsed time is displayed as mm:ss. The scale bars represent 5 μm .

both examples of lagging chromosomes, the fluorescence ratio of microtubule bundles to opposite poles was close to one (1.05 and 1.06; Figure 2A). In both cases, the cell was followed through mid-anaphase, and the merotelically oriented chromosome did not move away from the spindle equator (Figure 2A; Figure S1; Movie 2). These results show that lagging chromosomes are produced by merotelic kinetochore orientation when the ratio of kinetochore microtubules to opposite poles is nearly one.

Most merotelic kinetochores (23 of 25) did not produce a lagging chromosome during anaphase (Figure 2B; Figures S2–S4). For these merotelic kinetochores, different behaviors were observed depending on the ratio of microtubules to opposite poles. In some instances, when the fluorescence ratio between the two

bundles was close to two, the kinetochore became highly stretched during anaphase as the bulk of the chromatin was shifted away from the spindle equator in the direction of the correct pole (Figure 2B; Figure S2; Movie 3). In one extreme example, the kinetochore became stretched up to 6.39 μm , extending from one side far into the other side of the spindle equator (Figure 2B; Figure S2). Note that in this example, the stretched region appeared as puncta separated by thin strands of CENP-F-stained material, as previously observed by CREST staining [7]. This extensive lateral stretch indicates that the microtubule attachment sites have stretched apart their supporting centromeric DNA by pulling apart the multiple DNA-protein subunits, as proposed by Zinkowski and coworkers [21].

Finally, when the fluorescence ratio between the two

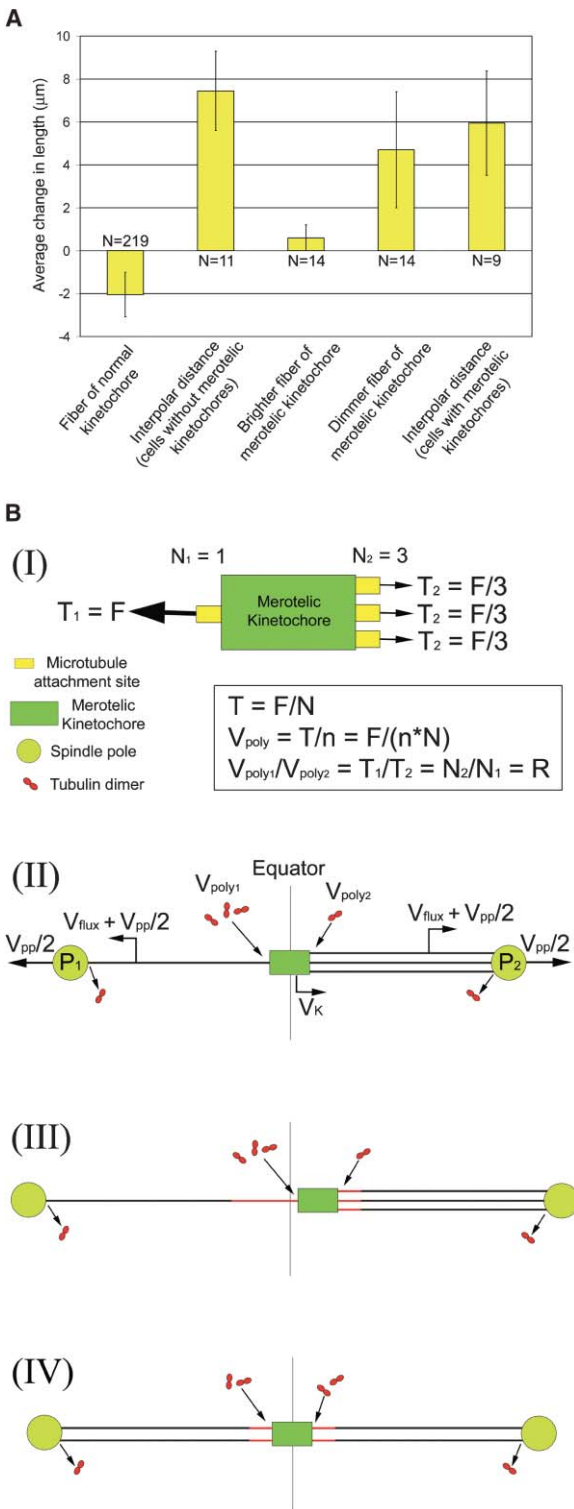


Figure 4. Anaphase B, but Not Anaphase A, Is Important for Correct Segregation of Merotelically Oriented Chromosomes

(A) Fibers of merotelically oriented kinetochores do not shorten during anaphase. The average change in length for kinetochore fibers of normally and merotelically oriented kinetochores is shown. For merotelically oriented kinetochores, microtubule fibers become longer during anaphase rather than shortening. However, the dimmer fiber lengthens much more than the brighter fiber. The histogram also shows spindle elongation, measured from anaphase onset to the beginning of furrow ingression, in cells with and without mero-

telic kinetochores. In cells possessing merotelically oriented kinetochores, the extent of spindle elongation is reduced but not significantly different as compared to that in cells without merotelic kinetochores. Error bars represent standard deviations. (B) Model showing how the ratio (R) of microtubules to opposite poles determines the movement of the merotelic kinetochore away from the spindle equator during anaphase. (I) For a merotelic kinetochore with unequal microtubule numbers to opposite poles, the tension (T) at each attachment site increases as the number of microtubules decreases. This determines different polymerization rates (V_{poly}). (II) The rate of movement of the merotelic kinetochore away from the spindle equator (V_K) is determined by the different polymerization rates for the two microtubule bundles (V_{poly1} and V_{poly2}). (III) Different polymerization rates during anaphase will lead to movement of the merotelic kinetochore away from the spindle equator, toward the pole with the higher number of kinetochore microtubules. (IV) If the number of microtubules to opposite poles is equal ($R = 1$), polymerization rates for the two microtubule bundles will be the same, and the merotelic kinetochore will not move away from the equator, thus producing a lagging chromosome. Refer to text for a more comprehensive discussion of the model. The following abbreviations were used: F, force; T, tension; R, ratio of microtubules to opposite poles; n, viscous coefficient; V_{poly} , polymerization rate; V_{flux} , flux rate; V_{pp} , rate of spindle pole separation; and V_K , rate of movement of the merotelic kinetochore away from the spindle equator.

microtubule bundles was close to three or higher, the merotelically oriented chromosome moved away from the equator in the direction of the pole connected to the brighter fiber (Figure 3; Figures S3 and S4). In such cases, the stretching of the merotelically oriented kinetochore was much less pronounced (Figure 3A; Figure S3; Movie 4) or almost nonexistent (Figure 3B; Figure S4). For most merotelic kinetochores, we could also identify and track the sister kinetochore before and after anaphase onset. When one of the two sisters was merotelically oriented and the fluorescence intensity of the two microtubule bundles was unbalanced, the brighter bundle was always connected to the correct pole (pole opposite the one to which the sister kinetochore was attached) (Figures 2B and 3; Figures S2–S4). These results show that merotelically oriented chromosomes segregate correctly when the ratio between kinetochore microtubules to the correct versus incorrect pole is sufficiently greater than one.

In higher eukaryotes, the segregation of chromosomes to opposite ends of the cell during mitosis is produced by two processes, defined as anaphase A and anaphase B. Anaphase A is chromosome poleward movement, which is coupled to shortening of kinetochore microtubules. Anaphase B is the movement of the spindle poles away from each other. To understand how much anaphase A and B contributed to the correct segregation of merotelically oriented chromosomes, we quantified the shortening of kinetochore fibers and the extent of spindle elongation after anaphase onset. We found that kinetochore fibers of normally oriented kinetochores became on average $2.05 \mu\text{m}$ shorter during anaphase (Figure 4A). However, most of the microtubule bundles that attached merotelic kinetochores to opposite poles became longer during anaphase (Figure 4A; see also Figure 2A, last three frames). For microtubule bundles with different fluorescence intensities, the dimmer fiber lengthened more than the brighter one ($4.2 \pm 2.5 \mu\text{m}$ versus $0.7 \pm 0.7 \mu\text{m}$) (Figure 4A; see also Figure

3A, frames 4–10). These results indicate that anaphase B, but not anaphase A, is extremely important for correct segregation of merotelically oriented chromosomes. Anaphase B spindle elongation pulled the merotelically oriented chromosome sufficiently far from the spindle equator that it could be included within the reforming nucleus in telophase. In spindles with merotelically oriented chromosomes, the velocity of interpolar spindle elongation was reduced to $0.42 \mu\text{m}/\text{min}$ (standard deviation = 0.15; $n = 9$), compared to $0.72 \mu\text{m}/\text{min}$ (standard deviation = 0.21; $n = 11$) in normal cells. However, the extent of spindle elongation, measured from anaphase onset to the beginning of furrow ingression, was not significantly different ($5.94 \pm 2.43 \mu\text{m}$ versus $7.44 \pm 1.85 \mu\text{m}$; t test, $p > 0.1$; Figure 4A). A decreased rate of spindle pole elongation had been previously observed in fission yeast cells possessing lagging chromosomes [22], but no description of the interaction between lagging chromosomes and spindle microtubules was provided in that study.

In Figure 4B we present a model illustrating how the ratio of microtubules to opposite poles governs the movement of merotelic kinetochores away from the equator during anaphase. In this model, we make the following assumptions: (1) Preanaphase spindle mechanisms such as polar ejection forces and other unknown mechanisms, which antagonize chromosome poleward movement [23], are inactivated in anaphase [24, 25]; (2) kinetochore microtubules of merotelic kinetochores, as for normally oriented kinetochores, maintain their minus-end anchorage at the spindle poles and their plus-end anchorage at kinetochores [15, 20, 26]; (3) poleward flux, which is coupled to minus-end depolymerization at the poles, occurs at the same rate for all microtubules (this is based on available data for normally attached kinetochores [8, 20], although we cannot exclude the possibility that microtubules attached to merotelic kinetochores behave differently); (4) the viscous resistance to chromosome movement is very low (<0.1 pN) at the slow velocities of merotelic kinetochores and much less than the force (100–400 pN) that can stall chromosome movement in anaphase [27, 28]; and (5) each microtubule attachment site is an independent unit capable of sensing tension, as suggested by our data and previous work by Khodjakov et al. [2]. On the basis of these assumptions, we postulate that the forces (F) in opposite directions on a merotelic kinetochore are equal, and the tension at each attachment site is equal to this force divided by the number of microtubules, so that the higher the number of microtubules, the lower the tension per attachment site (Figure 4B_i). Sister chromatid separation in anaphase induces a loss of tension on normally oriented kinetochores, and this loss of tension results in switching to a depolymerization state, producing much of the observed kinetochore fiber shortening (“Pac-Man” activity) [8, 14, 29]. But merotelic kinetochores in anaphase are under high tension; this prevents switching to depolymerization, and kinetochore microtubule plus ends persist in a polymerization state [8, 14, 30, 31]. As described by Maddox et al. [8], tension at polymerizing kinetochores is likely produced by attachment site resistance to the pole-directed velocity of the microtubule lattice. For merotelic kinetochores in ana-

phase, this velocity is the sum of the velocity of poleward flux ($\sim 0.2 \mu\text{m}/\text{min}$ [20]) plus half the velocity of interpolar spindle elongation, as diagrammed in Figure 4B_{ii}. In our model, tension at single attachment sites depends inversely on the number of kinetochore microtubules, and polymerization rate is proportional to tension [8] (Figures 4B_i and 4B_{ii}). For merotelic kinetochores with a microtubule ratio greater than one, at anaphase the microtubules of the smaller bundle will experience more tension and therefore polymerize faster (and thus become longer) than the bigger kinetochore bundle, whose microtubules are under less tension (Figures 4B_{ii} and 4B_{iii}). When the ratio of kinetochore microtubules to opposite poles is one, the rates of plus-end polymerization are equal for both the correct and incorrect attachments, and the chromosome remains near the spindle equator (Figure 4B_{iv}).

In summary, we propose that the difference in tension between kinetochore microtubule attachment sites facing opposite poles determines the direction and extent of movement of merotelic kinetochores away from the spindle equator in response to microtubule pulling forces produced primarily by spindle elongation. This model can also account for another major chromosome segregation error, in which separated sister chromatids in anaphase segregate to the same pole [32]. This would occur for merotelic kinetochores with more microtubules to the incorrect pole than to the correct pole (ratio < 1). We did not observe any of these kinetochores, probably because they were shifted further across the spindle equator into the region occupied by normally oriented kinetochores, and their identification was difficult. Our results and the model in Figure 4B also explain why single-kinetochore chromosomes produced by premature sister separation [4], defective DNA replication [1, 3], or laser severing from a sister [2] often move to one or the other spindle pole in anaphase after forming merotelic attachments and aligning near the spindle equator at metaphase. For microtubule ratios not equal to one in anaphase, they will migrate away from the equator in the direction of one or the other spindle pole with no need for detachment, as previously proposed [1–4].

Supplemental Data

Detailed Experimental Procedures, as well as several movies and figures, are available with this article online at <http://www.current-biology.com/cgi/content/full/14/23/2149/DC1/>.

Acknowledgments

We would like to thank Tsahai Tafari and Don Cleveland for the generous gift of labeled CENP-F antibodies, Alexey Khodjakov for critical reading of the manuscript, and members of the Salmon laboratory for helpful discussions and comments. L.A.C. is supported by American Cancer Society postdoctoral fellowship PF-02-095-01-CCG. This work was supported by National Institutes of Health grant GM-24364 to E.D.S.

Received: August 19, 2004

Revised: October 20, 2004

Accepted: October 21, 2004

Published: December 14, 2004

References

1. Brinkley, B.R., Zinkowski, R.P., Mollon, W.L., Davis, F.M., Pisegna, M.A., Pershouse, M., and Rao, P.N. (1988). Movement

- and segregation of kinetochores experimentally detached from mammalian chromosomes. *Nature* 336, 251–254.
2. Khodjakov, A., Cole, R.W., McEwen, B.F., Buttle, K.F., and Rieder, C.L. (1997). Chromosome fragments possessing only one kinetochore can congress to the spindle equator. *J. Cell Biol.* 136, 229–240.
 3. Wise, D.A., and Brinkley, B.R. (1997). Mitosis in cells with unreplicated genomes (MUGs): Spindle assembly and behavior of centromere fragments. *Cell Motil. Cytoskeleton* 36, 291–302.
 4. Yu, H.G., and Dawe, R.K. (2000). Functional redundancy in the maize meiotic kinetochore. *J. Cell Biol.* 151, 131–142.
 5. Cimini, D., Moree, B., Canman, J.C., and Salmon, E.D. (2003). Merotelic kinetochore orientation occurs frequently during early mitosis in mammalian tissue cells and error correction is achieved by two different mechanisms. *J. Cell Sci.* 116, 4213–4225.
 6. Cimini, D., Fioravanti, D., Salmon, E.D., and Degross, F. (2002). Merotelic kinetochore orientation versus chromosome mono-orientation in the origin of lagging chromosomes in human primary cells. *J. Cell Sci.* 115, 507–515.
 7. Cimini, D., Howell, B., Maddox, P., Khodjakov, A., Degross, F., and Salmon, E.D. (2001). Merotelic kinetochore orientation is a major mechanism of aneuploidy in mitotic mammalian tissue cells. *J. Cell Biol.* 153, 517–527.
 8. Maddox, P., Straight, A., Coughlin, P., Mitchison, T.J., and Salmon, E.D. (2003). Direct observation of microtubule dynamics at kinetochores in *Xenopus* extract spindles: Implications for spindle mechanics. *J. Cell Biol.* 162, 377–382.
 9. Maddox, P.S., Moree, B., Canman, J.C., and Salmon, E.D. (2003). Spinning disk confocal microscope system for rapid high-resolution, multimode, fluorescence speckle microscopy and green fluorescent protein imaging in living cells. *Methods Enzymol.* 360, 597–617.
 10. Rieder, C.L. (1982). The formation, structure, and composition of the mammalian kinetochore and kinetochore fiber. *Int. Rev. Cytol.* 79, 1–58.
 11. Waters, J.C., Chen, R.H., Murray, A.W., and Salmon, E.D. (1998). Localization of Mad2 to kinetochores depends on microtubule attachment, not tension. *J. Cell Biol.* 141, 1181–1191.
 12. Inoue, S., and Salmon, E.D. (1995). Force generation by microtubule assembly/disassembly in mitosis and related movements. *Mol. Biol. Cell* 6, 1619–1640.
 13. Khodjakov, A., and Rieder, C.L. (1996). Kinetochores moving away from their associated pole do not exert a significant pushing force on the chromosome. *J. Cell Biol.* 135, 315–327.
 14. Skibbens, R.V., Skeen, V.P., and Salmon, E.D. (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.
 15. Mitchison, T.J., and Salmon, E.D. (2001). Mitosis: A history of division. *Nat. Cell Biol.* 3, E17–E21.
 16. Gorbsky, G.J. (2004). Mitosis: MCAK under the aura of Aurora B. *Curr. Biol.* 14, R346–R348.
 17. Ohi, R., Coughlin, M.L., Lane, W.S., and Mitchison, T.J. (2003). An inner centromere protein that stimulates the microtubule depolymerizing activity of a KinI kinesin. *Dev. Cell* 5, 309–321.
 18. Kline-Smith, S.L., Khodjakov, A., Hergert, P., and Walczak, C.E. (2004). Depletion of centromeric MCAK leads to chromosome congression and segregation defects due to improper kinetochore attachments. *Mol. Biol. Cell* 15, 1146–1159.
 19. Lampson, M.A., Renduchitala, K., Khodjakov, A., and Kapoor, T.M. (2004). Correcting improper chromosome-spindle attachments during cell division. *Nat. Cell Biol.* 6, 232–237.
 20. Zhai, Y., Kronebusch, P.J., and Borisy, G.G. (1995). Kinetochore microtubule dynamics and the metaphase-anaphase transition. *J. Cell Biol.* 131, 721–734.
 21. Zinkowski, R.P., Meyne, J., and Brinkley, B.R. (1991). The centromere-kinetochore complex: A repeat subunit model. *J. Cell Biol.* 113, 1091–1110.
 22. Pidoux, A.L., Uzawa, S., Perry, P.E., Cande, W.Z., and Allshire, R.C. (2000). Live analysis of lagging chromosomes during anaphase and their effect on spindle elongation rate in fission yeast. *J. Cell Sci.* 113, 4177–4191.
 23. Levesque, A.A., and Compton, D.A. (2001). The chromokinesin Kid is necessary for chromosome arm orientation and oscillation, but not congression, on mitotic spindles. *J. Cell Biol.* 154, 1135–1146.
 24. Canman, J.C., Cameron, L.A., Maddox, P.S., Straight, A., Tirnauer, J.S., Mitchison, T.J., Fang, G., Kapoor, T.M., and Salmon, E.D. (2003). Determining the position of the cell division plane. *Nature* 424, 1074–1078.
 25. Funabiki, H., and Murray, A.W. (2000). The *Xenopus* chromokinesin Xkid is essential for metaphase chromosome alignment and must be degraded to allow anaphase chromosome movement. *Cell* 102, 411–424.
 26. Rieder, C.L., and Salmon, E.D. (1998). The vertebrate cell kinetochore and its roles during mitosis. *Trends Cell Biol.* 8, 310–318.
 27. Nicklas, R.B. (1988). The forces that move chromosomes in mitosis. *Annu. Rev. Biophys. Chem.* 17, 431–449.
 28. Nicklas, R.B. (1983). Measurements of the force produced by the mitotic spindle in anaphase. *J. Cell Biol.* 97, 542–548.
 29. Tirnauer, J.S., Canman, J.C., Salmon, E.D., and Mitchison, T.J. (2002). EB1 targets to kinetochores with attached, polymerizing microtubules. *Mol. Biol. Cell* 13, 4308–4316.
 30. Skibbens, R.V., Rieder, C.L., and Salmon, E.D. (1995). Kinetochore motility after severing between sister centromeres using laser microsurgery: Evidence that kinetochore directional instability and position is regulated by tension. *J. Cell Sci.* 108, 2537–2548.
 31. Skibbens, R.V., and Salmon, E.D. (1997). Micromanipulation of chromosomes in mitotic vertebrate tissue cells: Tension controls the state of kinetochore movement. *Exp. Cell Res.* 235, 314–324.
 32. Cimini, D., Tanzarella, C., and Degross, F. (1999). Differences in malsegregation rates obtained by scoring ana-telophases or binucleate cells. *Mutagenesis* 14, 563–568.