Oscillatory Movements of Monooriented Chromosomes and Their Position Relative to the Spindle Pole Result from the Ejection Properties of the Aster and Half-Spindle

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Abstract. During mitosis a monooriented chromosome oscillates toward and away from its associated spindle pole and may be positioned many micrometers from the pole at the time of anaphase. We tested the hypothesis of Pickett-Heaps et al. (Pickett-Heaps, J. D., D. H. Tippit, and K. R. Porter, 1982, *Cell*, 29:729–744) that this behavior is generated by the sister kinetochores of a chromosome interacting with, and moving in opposite direction along, the same set of polar microtubules. When the sister chromatids of a monooriented chromosome split at the onset of anaphase in newt lung cells, the proximal chromatid remains stationary or moves closer to the pole, with the kinetochore leading. During this time the distal chromatid moves a variable distance radially away from the pole, with one or both chromatid arms leading. Subsequent electron microscopy of these cells revealed that the kinetochore on the distal chromatid is free of microtubules. These results suggest that the distal kinetochore is not involved in the positioning of a monooriented chromosome relative to the spindle pole or in its oscillatory movements. To test this conclusion we used laser microsurgery to create monooriented chromosomes containing one kinetochore. Correlative light and electron microscopy revealed that chromosomes containing one kinetochore continue to undergo normal oscillations. Additional observations on normal and laser-irradiated monooriented chromosomes indicated that the chromosome does not change shape, and that the kinetochore region is not deformed, during movement away from the pole. Thus movement away from the pole during an oscillation does not appear to arise from a push generated by the single pole-facing kinetochore fiber, as postulated (Bajer, A. S., 1982, *J. Cell Biol.*, 93:33–48). When the chromatid arms of a monooriented chromosome are cut free of the kinetochore, they are immediately ejected radially outward from the spindle pole at a constant velocity of 2 μm/min. This ejection velocity is similar to that of the outward movement of an oscillating chromosome.

We conclude that the oscillations of a monooriented chromosome and its position relative to the spindle pole result from an imbalance between poleward pulling forces acting at the proximal kinetochore and an ejection force acting along the chromosome, which is generated within the aster and half-spindle.

THE movement of prometaphase chromosomes to the equator of a bipolar spindle is hypothesized to be the result of poleward pulling forces applied at sister kinetochores by kinetochore fibers (K-fibers)[1] oriented to opposite spindle poles (e.g., 9, 16, 19, 20). Since the poleward force on a kinetochore appears to be a linear function of K-fiber length (9), and since the length of a K-fiber appears to change in response to an applied force (9, 17), chromosomes move (i.e., congress) to the spindle equator where the opposing antagonistic poleward forces are balanced.

A recent in vivo analysis (4) of monooriented chromosome

1. *Abbreviations used in this paper*: K-fibers, kinetochore fibers; MTs, microtubules; NLCs, newt lung cells.

behavior in newt lung cells (NLCs) reveals that each moves toward and away from the single pole with which it is associated. The movement away from the pole is rapid and extensive enough to suggest the existence of a previously neglected active component in chromosome movement. Bajer (4) hypothesized that these oscillatory movements arise from an elongation and shortening of the single K-fiber which is attached to the pole-facing kinetochore. His model is supported by the fact that the distal kinetochore on a monooriented chromosome invariably lacks microtubules (MTs) (reviewed in reference 24) and by the observation that MT elongation, induced by taxol, can exert a pushing force on chromosomes (5).

A major implication of Bajer's (4) hypothesis is that the movement of a monooriented chromosome away from its associated pole, and the maintenance of a position often many micrometers distal to the polar region (as in sea urchins [11], crane flies [7], or newts [30]), does not require antagonistic pulling forces acting on opposite sister kinetochores. A single K-fiber can presumably generate force for chromosome transport both toward and away from the pole.

In contrast, Pickett-Heaps et al. (21) feel that the movement of a monooriented chromosome away from its associated pole occurs by a mechanism that is consistent with the accepted model of chromosome congression, i.e., that the proximal kinetochore is responsible for poleward movement, while the distal kinetochore is responsible for movement away from that pole. Support for this hypothesis comes largely from an analysis of prometaphase chromosome movements in diatom spindles and from rare observations on the movement of monooriented NLC chromosomes which enter anaphase before achieving a normal bipolar attachment. In these latter cases the proximal chromatid undergoes anaphase movement into the polar area, while the distal chromatid moves radially away from that pole into the cytoplasm, with its kinetochore leading the way. These observations suggested to Pickett-Heaps et al. (21) that the kinetochore on the distal chromatid can interact with and move radially along astral MTs and that the oscillatory movements of monooriented prometaphase chromosomes are simply the result of this interaction. Indeed, this hypothesis is consistent with the observation that bipolar-oriented metaphase NLC chromosomes, in which both kinetochores are active (i.e., both possess associated MTs), show oscillations similar to those of monooriented chromosomes (4). However, one of the major observations used to support the model, that the distal anaphase chromatid of a monooriented chromosome moves radially away from the polar region with its kinetochore leading the way, can be interpreted in many ways. For example, it is possible that these "monooriented" chromosomes are in reality bipolar-oriented between the obvious primary spindle pole and a not-so-obvious additional ectopic spindle pole.

We reasoned that the model proposed by Pickett-Heaps et al. (21) could be initially evaluated by a careful light and electron microscopic analysis of anaphase in monooriented chromosomes and then directly tested by using laser microsurgery to create monooriented chromosomes containing a single kinetochore. Our high-resolution correlative light and high-voltage electron microscopic observations of normal and irradiated chromosomes, which are presented here, clearly demonstrate that the oscillatory movement of a monooriented chromosome does not involve the distal kinetochore. Additional observations on oscillating chromosomes and acentric chromosome fragments generated by laser microsurgery suggest that these movements are not the result of a push-pull generated by the single pole-facing K-fiber. Rather, our data are consistent with the hypothesis that the oscillations of a monooriented chromosome, and the positioning of these chromosomes relative to the polar region, result from an imbalance between a poleward pulling force acting at the single active (proximal) kinetochore and an outward force, associated with the polarized array of astral MTs, which acts along the chromosome to eject it from the polar region.

Materials and Methods

Tissue Culture and Light Microscopy

Primary lung cultures from the newt *Taricha granulosa* were prepared as previously described (22, 23). Briefly, minced lung fragments were washed in 0.5-strength PO_4-buffered saline, trypsinized (20 min, 20°C) in saline, and washed in 0.6-strength L-15 medium supplemented with 10% fetal calf serum, 5% whole egg ultrafiltrate, 5 mM Hepes (pH 7.1), and antibiotics. The fragments were cultured in L-15 medium at 23°C in Rose chambers. By the eighth to tenth day divisions could be found in the epithelial monolayer. Selected mitotic cells, containing monopolar spindles or monooriented chromosomes on bipolar spindles, were followed in vivo with either (a) a Nikon Diaphot microscope equipped with phase-contrast optics and a UFX 35-mm automatic exposure system or (b) a Zeiss Axiomat microscope system with phase-contrast optics (100×; numerical aperture [NA] 1.3 objective) and video image-processing instrumentation (located at the National Institutes of Health Laser Microbeam Program Biotechnological Resource Facility, University of California, Irvine; see reference 13 for details).

Cells used for the indirect immunofluorescence microscopy of MTs were prepared as described by Cassimeris et al. (6). Briefly, NLC cultures were rinsed in phosphate-buffered saline (PBS) and lysed for 15 s in an MT-stabilizing solution (pH 6.8) containing 80 mM Pipes, 5 mM EGTA, 1 mM $MgCl_2$, and 0.5% Triton X-100. They were then fixed for 10 min in 2% paraformaldehyde, 0.1% glutaraldehyde in PBS, pH 7.3. The coverslips were then rinsed with PBS, extracted in −20°C methanol (6 min) and −20°C acetone (1 min), rehydrated in PBS, rinsed in PBS with 1% bovine serum albumin (BSA) and 0.1% Tween 20, and finally rinsed again in PBS. They were then stained with a monoclonal antibody to B-tubulin followed by a secondary rhodamine-conjugated, goat anti-mouse IgG (Cappel Laboratories, Cochranville, PA).

Electron Microscopy

Untreated or experimentally treated cells were followed in vivo until the desired stage for fixation. They were then fixed by perfusion with 3% glutaraldehyde in 0.1 M PO_4 buffer (pH 6.9) for 30 min, osmicated in 1% OsO_4 in buffer (10 min, 4°C), stained en bloc with 1% aqueous uranyl acetate (3–12 h), dehydrated in ethanol, and embedded in Epon-Araldite. Cells previously followed in vivo were then serially thin- or semithick (0.25 μm)-sectioned, and the sections were stained as previously described (25). Thin sections were examined in a Philips 300 electron microscope with a 70-μm objective aperture. Semithick sections were examined in the Wadsworth Center's high-voltage electron microscope operated at 800 kV with an objective aperture of 30 μm.

Laser Ablation

The methods and instrumentation used to microsurgically remove kinetochores by laser microbeam irradiation were essentially those detailed by McNeil and Berns (13). A 0.3-μm-diam microbeam (λ = 532 nm, energy/pulse = 130 nJ, 10 pulses/s) was used to sever chromosome arms and kinetochore regions. This energy dose enabled us to cut chromatin selectively as the chromosome was translated over a 0.5-2-min period through the pulsing, in-focus laser beam. A 5-min irradiation of the centrosome or spindle fibers adjacent to the kinetochores, at the same pulse energy and rate used to cut chromosomes, produced no noticeable effect on chromosome or spindle behavior.

Cells were observed through a Zeiss Axiomat equipped with a Neofluor 100×/1.33 objective. Video images from a Video Standard camera (Sierra Scientific, Mountain View, CA) were recorded on ¾-in. U-matic tape using a Sony TVO 9000 time-lapse video cassette recorder. A rolling average of eight frames was used during image processing to suppress noise within the video image. This noise was generated within the video camera, in part, by effects of the high energy pulsing Neodynium-YAG (yttrium-aluminum-garnet) laser. The movements of kinetochores and chromatid arms were analyzed from video records on a Sony 5800H video cassette recorder with a video cursor generated by an Apple computer (Apple Computer Inc., Cupertino, CA) and home-made electronic interfacing. The experimental cells were processed for electron microscopy as outlined above.

Results

Oscillations

The oscillatory movements and positioning of monooriented NLC chromosomes relative to the single polar region have been described in detail by Molè-Bajer et al. (15) and Bajer (4). Those aspects directly relevant to and confirmed by our work are as follows.

The oscillations executed by monooriented chromosomes in bipolar spindles are identical to those of monooriented chromosomes in monopolar spindles. The most common oscillatory movements are saltations toward the spindle pole, with an amplitude of 0.3–1.5 μm, followed by saltatory movement away from the pole (for a detailed kinetic analysis see reference 4). In some cases oscillations with an amplitude of 5–10 μm are observed. Two types of oscillations can be distinguished: (a) short movements which involve the kinetochore region alone and (b) more extensive movements which involve the whole chromosome. Both types can occur any-

where within the half-spindle but are most common near the polar (i.e., astral) regions.

During the first type of movement the kinetochore and adjacent chromatin is stretched poleward without a corresponding positional change of the chromosome arms. Immediately thereafter the kinetochore and adjacent stretched chromatin returns to its original position in one smooth, constant motion.

During the second type of oscillation the kinetochore stretches poleward and the chromosome follows. The chromosome then stops and may either move closer to the pole, remain stationary, or move away from the pole. During movement away from the pole the chromosome does not noticeably change shape, nor is there a detectable deformation of the kinetochore region. Rather the entire chromosome appears to be pushed, along its length, by forces acting outward away from the pole (as emphasized in reference 4).

Monooriented chromosomes, whether in monopolar (Fig. 1) or bipolar (Fig. 2) spindles, can be many micrometers from the pole when the cell enters anaphase.
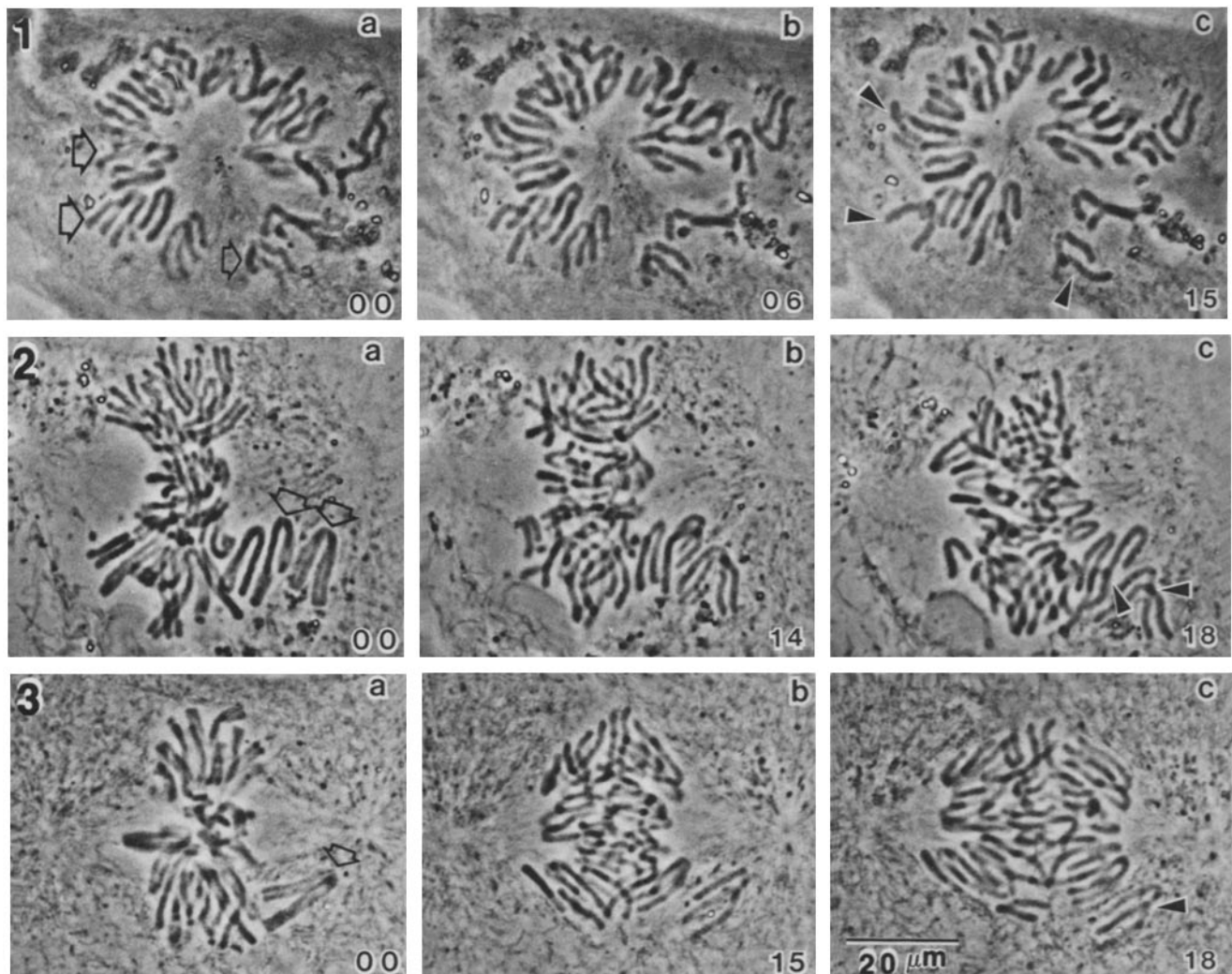
Figures 1–3. Anaphase in monopolar (Fig. 1) and bipolar (Figs. 2 and 3) NLC spindles containing one or more monooriented chromosomes. Open arrows in *a* show some distal chromatids (*solid arrows* in *c*) moving various distances away from the pole, with their kinetochores trailing the chromosome arms. In each example note the position of the monooriented chromosome(s), relative to the spindle pole, at the onset of anaphase. Time in minutes, relative to *a*, is in the lower right corner of *a–c*.

Entry into Anaphase before Bipolar Attachment

Monooriented chromosomes on bipolar NLC spindles usually become bipolar-oriented and congress to the metaphase plate before the initiation of anaphase. However, the model for monooriented chromosome oscillation proposed by Pickett-Heaps et al. (21) was based in large part on the behavior of monooriented chromosomes which enter anaphase before achieving a bipolar orientation. Our initial goal was therefore to examine in detail the behavior and structure of these chromosomes.

About 10% of NLCs entered anaphase before one or more monooriented chromosomes achieved a metaphase position.

The majority of these chromosomes were situated near the pole, with the arms of the chromosome pointing radially away from the pole (Figs. 3 and 4). As these chromosomes entered anaphase, one of two outcomes was observed. In most (90%) cases the proximal anaphase chromatid remained stationary or moved slightly closer to the polar region, while the distal chromatid moved a variable distance away from the pole, with the kinetochore region trailing one or both chromatid arms. This was true in both monopolar (e.g., Fig. 1, arrows) and bipolar (Figs. 2–4, arrows) spindles. An ultrastructural analysis of five of these cells revealed that in each case the kinetochore on the proximal chromatid
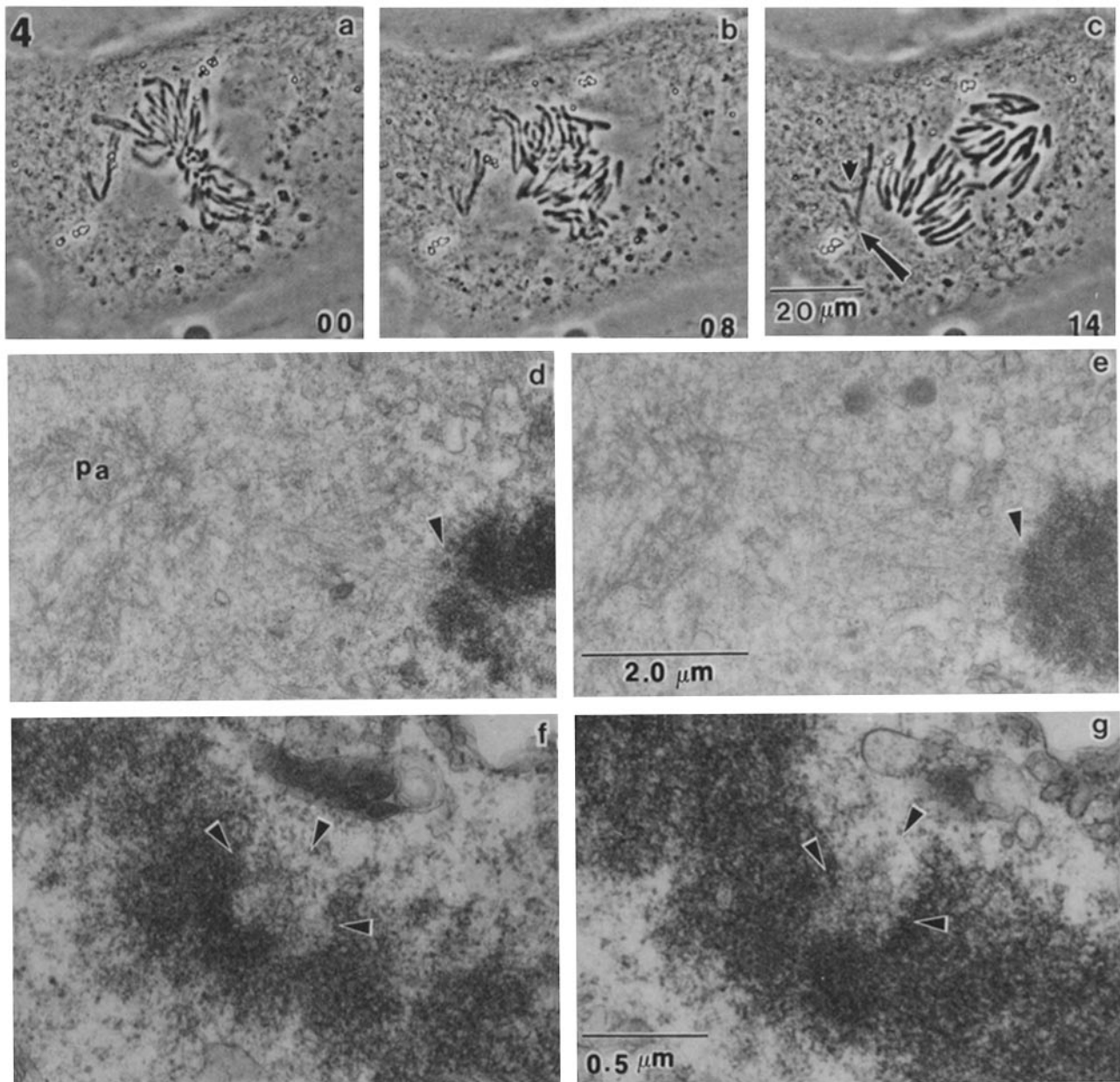

Figure 4. Phase-contrast micrographs (*a–c*) of a monooriented chromosome undergoing anaphase in vivo. This cell was fixed and processed for electron microscopy immediately after *c*. In this example the proximal chromatid (*arrow* in *c*) remained stationary throughout anaphase, while the distal chromatid (*arrowhead* in *c*) moved slowly away from the pole with its arms leading the way. Subsequent high-voltage electron microscopy of serial sections through the kinetochore on the proximal chromatid (*arrow* in *d* and *e*) revealed numerous K-fiber MTs directed towards the polar area (*Pa*). By contrast, the kinetochore on the distal chromatid (*arrowheads* in *f* and *g*) lacked associated MTs. Time in minutes, relative to *a*, is in the lower right corner of *a–c*.

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was attached to the polar region by numerous K-fiber MTs (Fig. 4, d and e), whereas the kinetochore on the distal chromatid lacked MTs (Fig. 4, f and g).

In the remaining 10% of NLCs containing a persistently monooriented chromosome the proximal chromatid moved towards the pole during anaphase, while the distal chromatid moved radially away from that pole ≥15 μm into the cytoplasm, with the kinetochore leading the way (Fig. 5). An ultrastructural analysis of four of these cells revealed that in each case the distal kinetochore had a well-developed K-fiber, which was attached to an ectopic and acentriolar spindle pole adjacent to the plasma membrane (Fig. 5, d and e). Thus the "monooriented" chromosomes in these cells were not truly monooriented; they were bipolar-oriented between one of the major spindle poles and a smaller additional pole within the cytoplasm.

The structure and behavior of the kinetochore on the distal chromatid of a truly monooriented chromosome undergoing anaphase suggested that this kinetochore is not under tension (i.e., it is inactive). However, these observations did not rule out the possibility that the oscillations of monooriented chromosomes arise from transient lateral interactions between the distal kinetochore and polar MTs.

Laser-created Monooriented Chromosomes

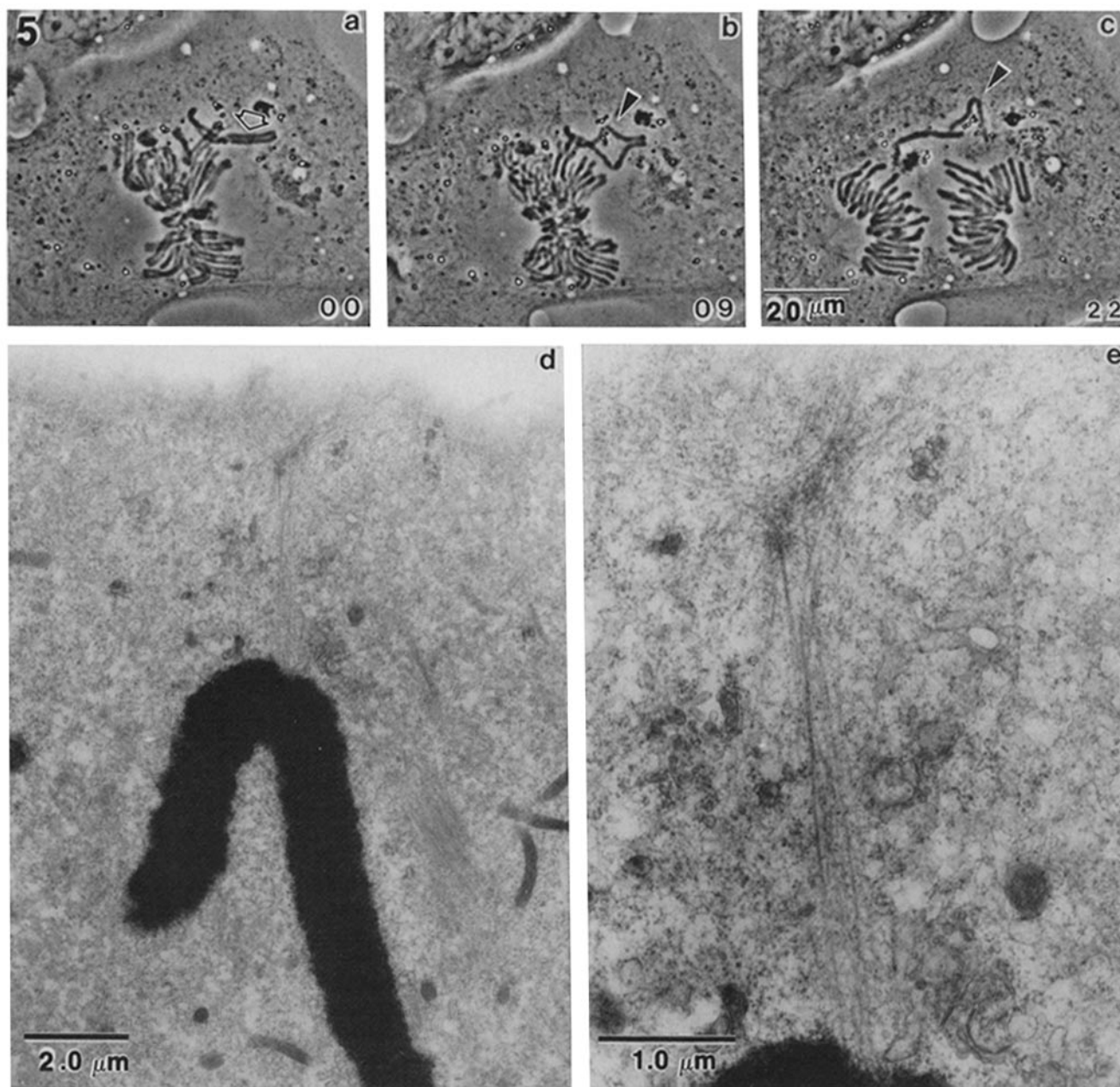To determine whether the oscillatory movements of mono-

Figure 5. As described for Fig. 4 except that the proximal chromatid moves into the pole during anaphase, while the distal chromatid moves into the cytoplasm, with its kinetochore leading the way. High-voltage electron microscopy of the distal chromatid (d and e) revealed that its kinetochore was attached, via a K-fiber, to an ectopic and acentriolar spindle pole adjacent to the plasma membrane. Time in minutes, relative to a, is in the lower right corner of a–c.
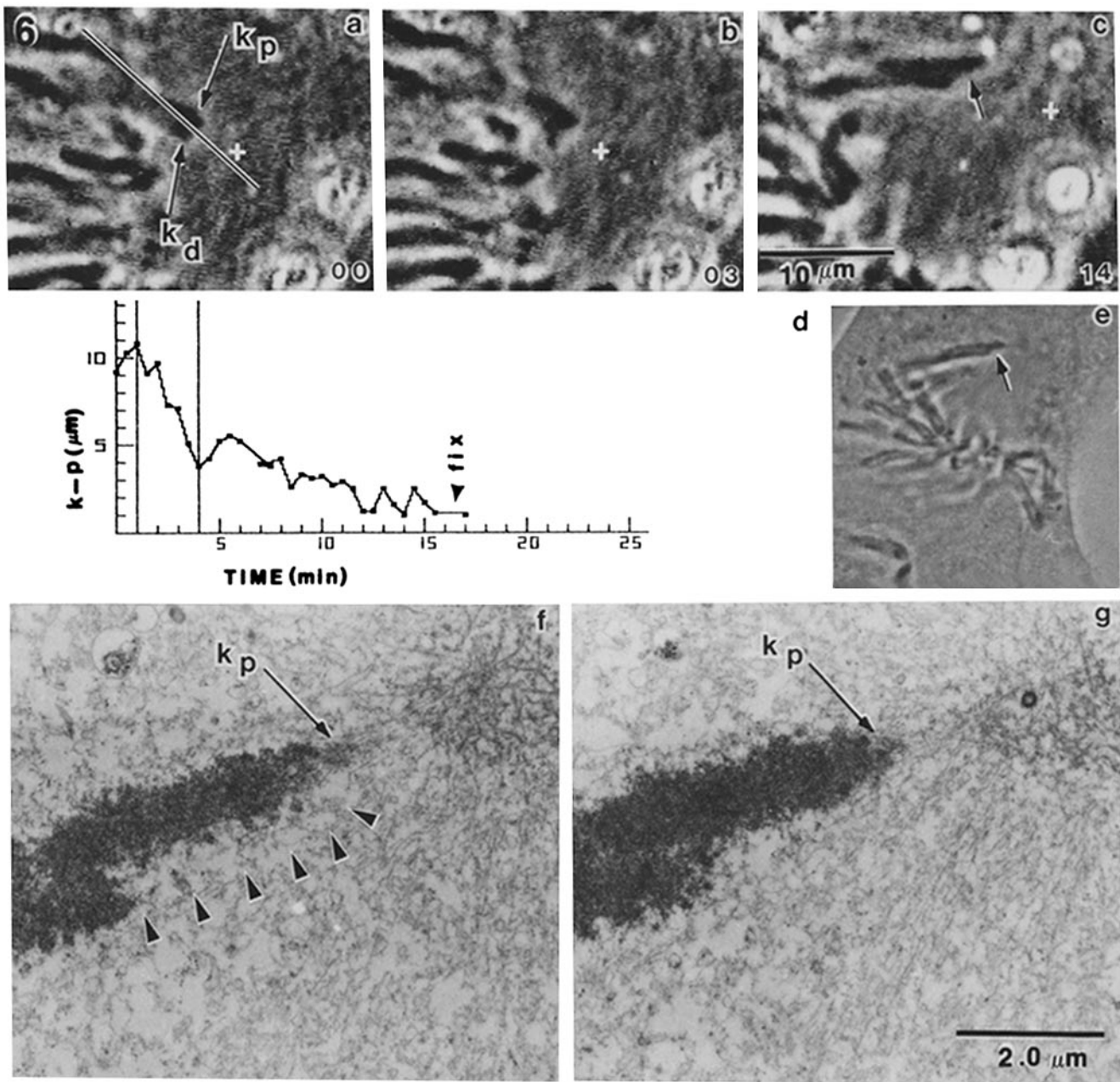
Figure 6. Phase-contrast photomicrographs (*a–c*), reproduced from the video screen, of a bipolar oriented chromosome from which a kinetochore is being microsurgically removed. In *a* the chromosome is congressing toward the metaphase plate, with its proximal (k_p) and distal (k_d) kinetochores in the same plane of focus. Over the next 4 min the distal kinetochore was cut from the chromosome (*b*), which then oscillated toward and away from the proximal pole and moved on average closer to the pole as it swung to the periphery of the spindle (*c*). These oscillations are plotted in *d*, where *k–p* is the distance from the kinetochore to the pole. Note that the chromosome continued to oscillate until it was fixed for electron microscopy. This same chromosome is pictured in *e* (*arrow*) in the embedded cell. Analysis of serial thin sections (*f* and *g*) confirmed that the chromosome contained a single kinetochore (k_p). The area of the chromosome removed by the laser is outlined by arrowheads in *f*. Time in minutes, relative to *a*, is in the lower right corner of *a–c*.

oriented chromosomes arise from a transient activity of the distal kinetochore, we used a laser to create monooriented chromosomes containing a single kinetochore. Our initial attempt to laser-ablate the distal kinetochore on monooriented chromosomes was quickly abandoned, since there was no way to assay functionally and structurally, with the light microscope, whether we had achieved our goal. Subsequently we found that we could make monooriented chromosomes that we knew contained a single kinetochore by using the la-

ser microbeam to cut off a single kinetochore region of bipolar-oriented chromosomes as they were congressing to the metaphase plate.

The first step was to select chromosomes on the surface of the spindle in which both kinetochores were clearly detected (i.e., as points on the chromosome that stretched toward opposite poles as the chromosome congressed). These chromosomes were cut, in the centromeric region and between the two kinetochores (i.e., parallel to the chromosome

arms), by slowly translating the chromosome through a rapidly pulsing laser microbeam (Fig. 6, a–c). In most cases the initial stage of cutting was accompanied by an increase in the distance between the two sister kinetochores, since they were now free to stretch toward their respective poles. This enhanced separation allowed us to microsurgically remove one of the kinetochores from the chromosome. Electron microscopy later confirmed that these chromosomes each contained a single kinetochore (Fig. 6, f and g).

Each of these laser-irradiated and now monooriented chromosomes immediately began moving toward the pole to which the single nondetached kinetochore faced (Fig. 6). Most moved laterally to the periphery of the spindle, and all showed repeated oscillations as they moved, over a period of time, closer to the pole. Their oscillatory movements were indistinguishable from those of nonirradiated monooriented chromosomes and did not appear to depend on the size of the chromosome containing the single functional kinetochore. Bipolar oriented prometaphase chromosomes were similarly cut in the centromeric region without removing a kinetochore. These controls congressed normally to the metaphase plate and showed normal metaphase oscillations.

The laser microbeam could also be used to weaken the connection between the single active kinetochore and its associated monooriented chromosome by ablating both the distal kinetochore and large portions of the adjacent centromeric heterochromatin. Under these conditions the remaining single functional kinetochore underwent repeated oscillatory movements toward the pole (Fig. 7). As the kinetochore and adjacent chromatin stretched poleward, the remainder of the chromosome showed little movement. After each stretching the kinetochore moved back to its original position but never past that position. These oscillations looked, on the video screen, as if the chromatin were being stretched to its limit and elastically recoiling.

Ejection Fields

The above observations suggested that the poleward force acting on the proximal kinetochore of a monooriented chromosome was counteracted by an outward force acting on the chromosome arms to push them away from the pole. To test this hypothesis, we cut chromosome arms free of the kinetochore region and tracked their movements. The chromosome arms in monopolar spindles were embedded within, and aligned parallel to, the polar MTs (Fig. 8). A cut arm in these cells immediately moved radially outward to the periphery of the aster at a constant velocity of ~2 μm/min (n = 5; Fig. 8). For monooriented chromosomes in bipolar spindles, the outward transport of cut acentric arms occurred at a similar velocity (Figs. 9 and 10). These results demonstrate that each aster or half-spindle has associated with it an ejection field that pushes chromosomes radially away from the pole.

The orientation of chromosome arms in bipolar spindles reflects the resultant vectorial action of two opposing ejection fields each associated with an overlapping polar MT array. When the kinetochore region of a monooriented chromosome is close to the pole, the chromosome arms are aligned almost radially with the astral or half-spindle MTs of the nearby pole (Figs. 3–4). The arms of fully congressed chromosomes, at an equatorial position between the poles, are oriented perpendicular to the spindle axis and parallel to the
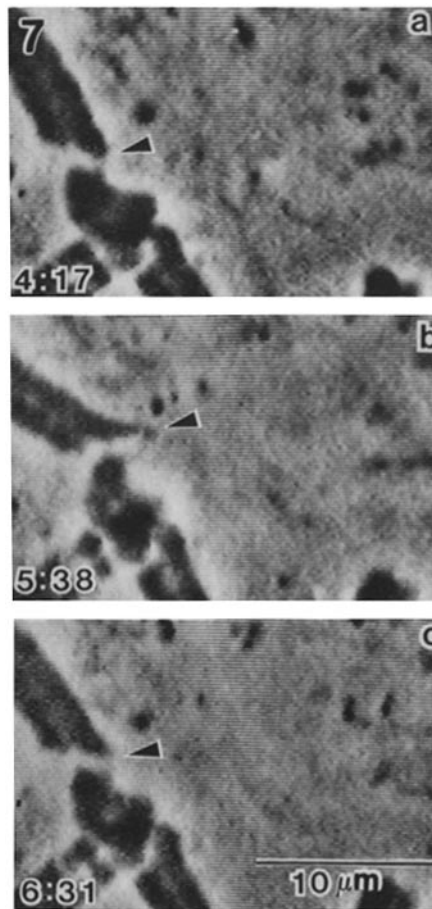

Figure 7. Phase-contrast photomicrographs, reproduced from the video screen, of an oscillating kinetochore (*arrowhead*). In this example the distal kinetochore and much of the centromeric region of the chromosome have been destroyed by laser irradiation. The kinetochore moves poleward (*b*) stretching the chromatin near the centromeric region, and then returns to its original position (*c*) with very little movement of the chromosome. Time in minutes/seconds is in the lower left corner of *a–c*.

metaphase plate (Figs. 3 and 4; also references 2 and 32). However, when a chromosome is positioned between a spindle pole and the metaphase plate its arms project in a direction intermediate between a radial and perpendicular orientation; the direction depending on the proximity of the chromosome to the pole (Fig. 2; also references 2 and 12). Finally, when a monopolar spindle develops into a bipolar spindle, the original pole splits into two, and the two poles move apart. In these cases the chromosome arms change their initial radial orientation, with respect to the original pole, consistent with a new opposing ejection field generated by the formation of a new polar array of MTs (not shown).

Discussion

Pickett-Heaps et al. (21) have argued that the oscillations of monooriented NLC chromosomes are consistent with the current view of how chromosomes congress, i.e., that the proximal and distal kinetochores of a monooriented chromosome are responsible for movement toward and away from that pole, respectively. To support this view they cited a
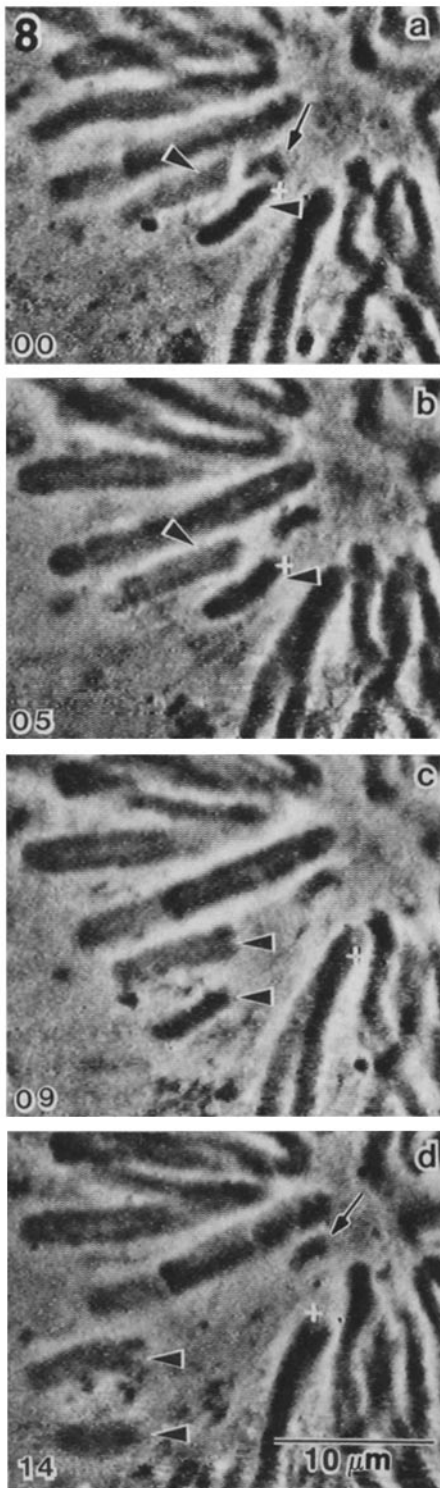
Figure 8. Chromosome fragments, generated by laser microsurgery, are ejected from the polar region of a monopolar spindle. Both arms (*arrowheads* in *a*) of a single chromosome are cut from the kinetochore region (*arrow*). These acentric fragments are transported away from the pole (*b–d*), as the kinetochore region continues to oscillate toward and away from the pole. Time in minutes, relative to *a*, is in the lower left corner of *a–d*.

Figure 9. A monooriented chromosome (*open arrow* in *a*) on a bipolar spindle is severed (*b*) near its kinetochore region by laser irradiation. The lower acentric chromosome arm (*solid arrow* in *c–e*) is transported away from the pole and into the cytoplasm. The upper chromosome arm, which still contains a functional kinetochore, remains associated with the spindle pole until this kinetochore is destroyed by irradiation in *d*. The resultant acentric chromosome arm is then transported away from the pole (*e*; also Fig. 10). Time in minutes, relative to *a*, is in the lower left corner of *a–e*.

widely distributed film on NLC mitosis by Ohnuki and Sato (18). This film shows that as a monooriented chromosome enters anaphase, the proximal chromatid moves into the pole, while the distal 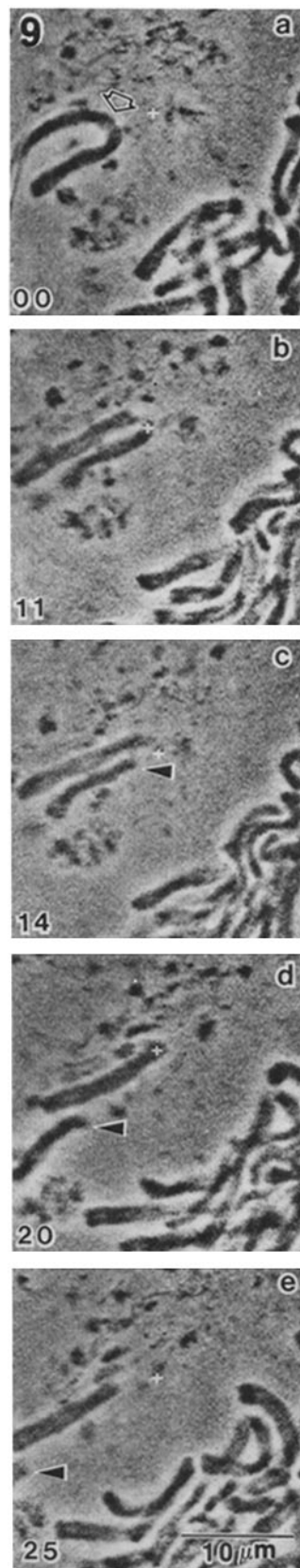chromatid moves 10–15 μm radially away from that pole into the cytoplasm, with its kinetochore leading the way. This observation was interpreted by Pickett-Heaps et al. (21) to indicate that a force was being applied to the distal kinetochore, generated perhaps by an interaction

between the kinetochore and astral MTs from the proximal pole.

Our correlative light and electron microscopic observations on anaphase in monooriented NLC chromosomes clearly show that when the kinetochore leads the distal chromatid radially into the cytoplasm, it does so because the kinetochore is attached via a K-fiber to an additional spindle pole. Thus these chromosomes, and presumably those seen in the Ohnuki and Sato (18) film, are not monooriented but amphioriented between the obvious primary spindle pole and a not so obvious additional ectopic spindle pole. Indeed, numerous small, asterlike MT arrays are frequently seen in the cytoplasm of mitotic NLCs after immunogold labeling of MTs (3; also Bajer, A. S., personal communication), and it appears that each of these can act as a functional spindle pole.

By contrast, during anaphase in a truly monooriented NLC chromosome, the kinetochore on the distal chromatid lacks MTs, and the chromatid moves out of the pole with its kinetochore region initially trailing one or both chromatid arms. Thus, this chromatid is clearly not transported away from the pole by forces acting on its kinetochore. This conclusion is consistent with the well-established absence of MTs on the distal kinetochore of monooriented chromosomes (reviewed in reference 24). Together these results imply that the distal kinetochore of a truly monooriented chromosome is not under tension.

Could movement away from a pole during an oscillation arise from transient interactions between the distal kinetochore and astral MTs from the proximal pole? To answer this question we used a laser to microsurgically remove one of the kinetochores from a chromosome. The fact that these experimentally produced monooriented chromosomes continued to oscillate normally demonstrates that the distal kinetochore is not required for the oscillatory movement of a monooriented chromosome away from its associated pole.

Our laser microsurgery results confirm Bajer's (4) contention that the oscillatory movements of a monooriented chromosome do not involve the distal kinetochore. However, his model predicts that movement away from the pole, which he envisioned to occur by a push from the elongation of the single pole-facing K-fiber, should deform the kinetochore region, i.e., a V-shaped chromosome should acquire a W shape as it moves away from the pole. No such deformation was found by Bajer (4), Molè-Bajer et al. (15), or ourselves. This lack of deformation prompted Molè-Bajer et al. (15) to suggest that movement away from the pole is "best explained by the transport properties of the asters."

We have shown that acentric chromosome fragments (and chromosome arms) are transported outward from the poles (see also reference 2). In the newt this ejection occurs, during prometaphase-metaphase, at a constant velocity of ∼2 μm/min, the same rate at which a monooriented chromosome oscillates away from its associated pole. The oscillatory movements of a monooriented chromosome could therefore be the result of an imbalance between a pulling force generated at the single active (proximal) kinetochore and a pushing force generated by the aster that acts along the chromosome. In this hypothesis the single pole-facing K-fiber is induced to elongate by a force acting on the chromosome to eject it from the aster. Elongation of the fiber allows the chromosome to move away from the pole but does not cause this movement.

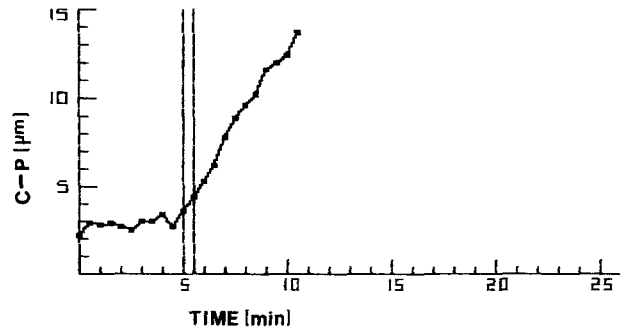The monooriented chromosomes in monopolar spindles in

Figure 10. Distance of the upper acentric chromosome fragment in Fig. 9 c from the pole as a function of time. After the kinetochore on this chromosome fragment was destroyed (Fig. 9 d), the fragment was ejected at a constant velocity from the spindle pole region.

NLCs (Fig. 1), newt spermatocytes (30), crane fly spermatocytes (7), and sea urchin zygotes (11) maintain, for many hours, an average position 10–20 μm away from the single pole. Since the distal kinetochore is inactive (Figs. 1–4; also references 11, 15, and 24), this position is clearly not the result of a balance between antagonistic forces pulling on opposite kinetochores. What then keeps these chromosomes from moving into the polar region? What outward force balances the poleward force generated by the single K-fiber? Our results suggest that these chromosomes become positioned at that point where the poleward force generated by (or at) the single (proximal) kinetochore is balanced by an outward oriented force, generated in the aster and half-spindle, which ejects the chromosomes.

An ejection force generated in the half-spindle may also play a role in the positioning of chromosomes on the monopolar spindle in Sciara (1, 10). In this system the paternal chromosomes are connected via K-fibers to a single polar region, and they remain 12–14 μm from this pole throughout the division process. The Sciara half-spindle contains numerous nonkinetochore and polar MTs, and the shape of the paternal chromosomes suggested to Kubai that the K-fibers "are exerting at least some tension toward the spindle pole" (10). The K-fibers may thus "serve as anchors that impede poleward progress, preventing paternals from approaching the pole." However, our results suggest that the position of the paternals distal to the pole is established and maintained by an ejection force developed in the half-spindle.

The molecular mechanism responsible for the transport properties of asters and half-spindles is unknown, but it appears to be associated with MTs. The orientation of chromosome arms with respect to the polar region in a monopolar spindle, and the changes that occur in this orientation as a monopolar spindle becomes bipolarized, correlates well with the distribution and arrangement of spindle MTs (2, 3). Treatments that selectively disassemble nonkinetochore and astral MTs (e.g., cold, colcemid, nocodazole) immediately inhibit the oscillations of monooriented chromosomes and induce these chromosomes to move closer to the pole (24; also Rieder, C. L., and E. D. Salmon, unpublished observations). The well-characterized and rapid (∼1 μm/s) saltatory movement of small particles or organelles in and out of the aster probably involves MT transport proteins like kinesin or cytoplasmic dynein (28, 31). However, the ejection of large organelles and chromosome fragments is unidirectional and
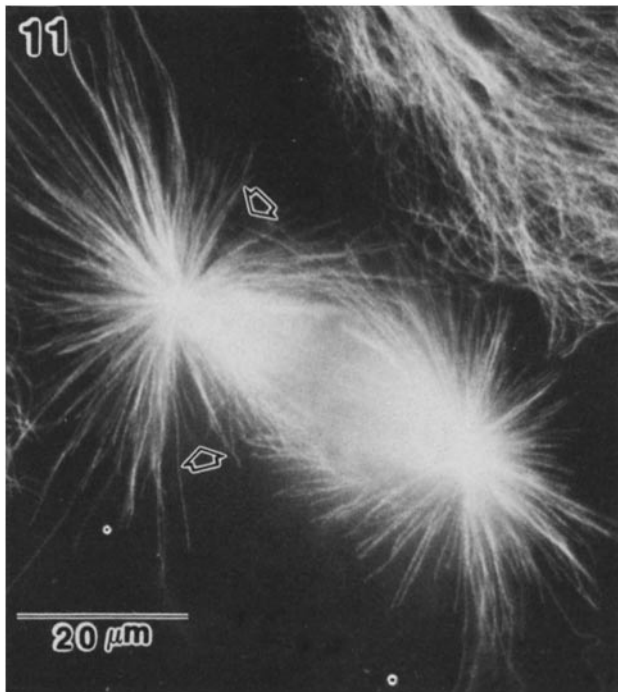
Figure 11. Anti-tubulin indirect immunofluorescent micrograph of a mid-prometaphase NLC spindle illustrating the cleft of low microtubule density (*open arrowheads*) between the central spindle and the aster.

30 times slower (i.e., 2 μm/min). It is possible that this ejection is the direct result of the dynamic instability of centrosomal MTs (14, 27). Since elongating MTs can exert a push (5, 8), the continuous nucleation, elongation, disassembly, and new nucleation of MTs at the centrosome could well expel large cellular components (e.g., chromosome fragments) to the periphery of the MT array. Indeed the in vivo elongation rate of centrosomal MTs in mammalian interphase cells at 37°C is ~3–4 μm/min (29), a rate comparable to the 2 μm/min ejection rate of NLC asters at room temperature.

It is currently unclear to what extent the amplitude of the aster ejection force changes as a function of mitotic stage. Our results do suggest, however, that the amplitude of this force decreases as the cell enters anaphase since at that time the proximal chromatids of monooriented chromosomes often approach closer to the pole, while the distal chromatids are not ejected to the same extent as acentric fragments in prometaphase or metaphase cells. We are currently investigating whether this change correlates with a change in astral MT density and/or dynamics.

Monooriented chromosomes on bipolar NLC and PtK₁ spindles are frequently positioned much closer to the pole than monooriented chromosomes in monopolar spindles (e.g., cf. Figs. 1 and 3), especially when they are produced by laser ablation of a single kinetochore (Fig. 6; and reference 13). A possible explanation for this observation may reside in the structure of bipolar NLC and PtK₁ spindles. As a rule once a long chromosome in these cells achieves a bipolar orientation it becomes positioned on the surface of the well-developed central spindle (Fig. 11). Cross-sections through these spindles therefore show a high density of po-

lar and kinetochore MTs, relative to the aster, with the kinetochore MTs positioned at the periphery of the spindle (22, 26). Since the long K-fibers gently curve toward the astral center the central spindle appears fusiform in shape. By contrast, those astral MTs that are not associated with the central spindle are straight and radially arranged around the astral center (Fig. 11). As a result a cleft of low astral MT density appears at the boundary between the curving central spindle and the astral MTs (e.g., *arrowheads* in Fig. 11). This cleft is most likely formed during spindle assembly as astral MTs are incorporated into the central spindle. Our hypothesis predicts that a monooriented chromosome, within such a region of low MT density, can approach very close to the pole. This explanation is consistent with our observation (unpublished) that monooriented chromosomes on the side of or behind the aster seldom approach as close to the pole as those on the surface of the central spindle. In addition, no central spindle is formed in monopolar NLCs and, although the chromosomes may be preferentially distributed on one side of the aster shortly after nuclear envelope breakdown (4), they achieve a radial orientation around its periphery over time (our Fig. 1). In these cases the astral MTs maintain an even radial density distribution around the astral center and the chromosomes are not allowed to approach as close to the single pole as they are in bipolar spindles. Finally, for technical reasons, bipolar oriented chromosomes are generally chosen for laser ablation of a single kinetochore. Since these kinetochores are already positioned on the surface of the spindle, they are adjacent to the cleft of low astral MT density. As a result when one of the kinetochores is destroyed, the chromosome is allowed to move, over a period of time, very close to the pole that the unirradiated kinetochore faces.

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